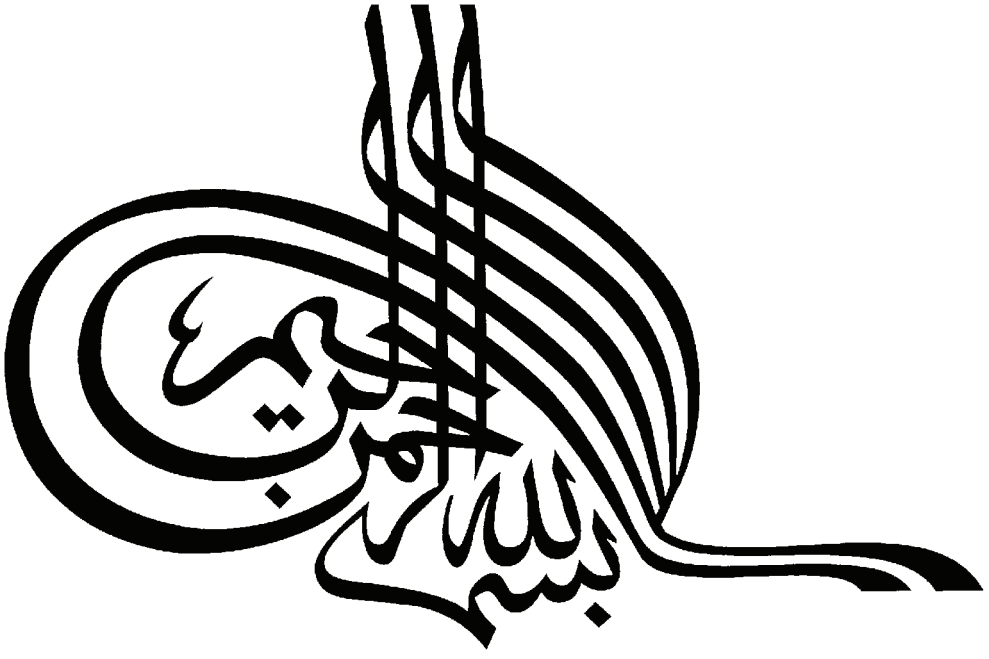
بسم الله الرحمن الرحيم

"...وَقُل رَّبِّ زِدْنِي عِلْمًا" سورة طه: 114

"*O my lord! Advance me in knowledge*"

The Glorious Qur'an: Taha 20: 114

FIMA
Year Book 2015

Federation of Islamic Medical Associations

الاتحاد العالمي للجمعيات الطبية الإسلامية

ENCYCLOPEDIA OF ISLAMIC MEDICAL ETHICS- PART II

موسوعة الأخلاقيات الطبية الإسلامية- الجزء الثاني

GENOMICS:
Scientific, Medical, Ethical and Islamic Perspectives

الجينوميات:

الأبعاد الطبية والأخلاقية والإسلامية

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Dear FIMA members
Assalamu Alaykum
Bismillah al-Rahman al-Rahim

Praise be to Allah the Most Merciful, the Most Beneficent. May Allah (ﷻ) shower His blessings and peace on the Prophet and Messenger Muhammad (ﷺ).

I begin by thanking the FIMA Executive Committee for honoring me with the responsibility of being the Editor-in-Chief again for this year's yearbook. I thank Allah (ﷻ) for giving me this opportunity and enabling me to accomplish this task. I pray to Allah (ﷻ) to accept my effort in His way and to reward all who participated in this effort.
This year we are publishing the second part of the Encyclopedia of Islamic Medical Ethics. This part is devoted to Genomics. It covers a very important and expansive topic that touches almost all aspects of biology and medicine. It is not possible to address all aspects of genetics , but attempts to mainstream the general principles of genetics, especially in relation to its impact on human health and disease, while examining the ethical, legal, and particularly the Islamic points of view on these issues. We tried to make the chosen topics appealing to scientists, researchers, and clinicians, by including basic science, as well as clinical applications, both diagnostic and therapeutic.
We understand that this field is new and progressing so rapidly that whatever information we provide here may not be the state of art in the forthcoming 5-10 years. We selected contributions from prominent experts on the various topics we selected and hope that the information provided will be both interesting and informative for our distinguished readers.

Professor Kasule addresses the general ethical principles of genetic research including preservation of human dignity, good intentions, obtaining the consent of the research subjects, and maintaining absolute confidentiality of the identity of the subjects and the research results.

Professor Mishal, in the chapter " Genomics: Contemporary Scientific, Ethical, and Islamic Perspectives "gives an overview of genetics to increase the understanding of scientific medical professionals, concerned scholars of ethics, and Islamic jurisprudence. He describes the basic structure of DNA, the building unit of the genome, the genetic code, and the evolution of the "Human Genome Project". He then discusses the role of genetics in the practice of medicine to include genetic examination, counseling and testing. He outlines the significance of presymptomatic genetic diagnosis and susceptibility testing as a tool for prevention or decreasing their severity while pointing out its pitfall of possible discrimination against those individuals who are presently healthy but may develop the disease later in life. He also discusses genetic engineering and its utility in producing large amounts of medications especially hormones and vaccines as well as its role in the study of diseases and possibly in their treatments. Professor Mishal also touches upon the

general principles of gene therapy, benefits and limitations such as its impermissibility in attempting modification of human germline or for genetic enhancement. He gives several examples of clinical trials of gene therapy such as in cystic fibrosis, beta thalassemia.He briefly discusses the production and use of Genetically Modified Foods (GMFs) and the related controversies. Professor Mishal also gives an account of the Islamic jurisprudential rulings on the HGP, genetic counseling, genetic testing, genetic engineering, gene therapy and GMFs, citing specifically the rulings by the two academies of Islamic *fiqh* and deliberations of the Islamic Organization of Medical Sciences (IOMS).

Professor El-Shanti, in the chapter "Role of Genetics and Genomics in the Practice of Medicine" writes that a new era of medicine "Personalized Medicine" has been ushered. It utilizes the genetic information and the additional available information to "maximize wellness through prediction, prevention and early treatment rather than the customary reactive medical practice". Genetic services used to diagnose and treat genetic diseases, now should provide genetic counseling, and testing to individuals at risk for conditions that have a genetic basis, including cancer. These services should provide reproductive genetic testing. He further states that genomic medicine bases the clinical care of the patient on the specific genomic variation the patient has. The author gives several examples of how successful this approach has been. Genomic information can also identify patients with risk of side effects from certain medications that can be avoided and conversely identify the patients who will benefit from a certain medication.In some case it may identify the recipient of a kidney transplant who will not need immunosuppression therapy.Personalized medicine is preventive. Women with BRCA1 and BRCA 2 mutations can be identified. They are at increased risk for breast and ovarian cancer. They need to undergo specific screening programs or preventive procedures that will minimize their risk.Another example is newborn screening which started approximately 50 years ago for one condition, phenylketonuria, can be extended now to screen for 29 disorders. Professor El-Shanti recommends that continuing education programs of physicians be enriched with genetics and genomic knowledge. He also recommends international standardization of genetic testing, genomic-based pharmaceutical products, and genomic based research and clinical tools to enhance the integration of genomic knowledge in clinical practice.

Professor Zahurin Mohamed of Malaysia defines pharmacogenomics as the scientific field that searches for the genetic basis of individual variability in the response to medications. Individualizing drug therapy with the use of pharmacogenomics has the potential of identifying patients for whom drugs will be both effective and safe. Variation in the response to warfarin, as an example, was found to be related to variation in 2 genes; CYP2C9 that codes for the enzyme responsible for warfarin metabolism, and VKORC1 gene the site of action of warfarin. Genetic tests are now available that will decrease the time it takes to titrate to the effective dose while minimizing the risk of bleeding events. Herceptin used in the treatment of breast cancer is only effective in women with a genetic defect which results in the overproduction of the HFR2 neu

receptor. Another example is the genetic test for HLA-B*5701 allele. HIV infected individuals who have this allele will have severe reactions to Abacavir and thus it cannot be used in them. The author reports several studies conducted by her group of the role of genetic variation in response to drugs used to treat epilepsy, schizophrenia, and in females with major depressive disorders.Accumulating evidence indicates that genomic polymorphisms in drug metabolizing enzymes, transporters, and other drug targets are linked to inter-individual differences in the efficacy and toxicity of many medications.

It has been known for some time that abnormal epigenetic factors can cause abnormal phenotype despite a normal genome. Professor Dauod discusses this topic. He defines it as the study of the factors that control the selection of the specific fraction of the genome that becomes activated in each cell type. It is also the study of factors that affect gene function primarily by turning them on and off. These modifications occur through a variety of mechanisms such as DNA methylation, post translational histone modification and non-coding RNA. Methylation tends to unwind DNA strands making them more or less accessible to RNA transcription. Histones are responsible for tight folding of DNA. In addition, there are start and stop codons that help regulate when to start and when to end transcription of DNA to mRNA. The author details these and other newly discovered mechanisms. He relates some examples of the importance of these factors. Global hypomethylation and regional hypermethylation of specific genes occurs during the aging process and may be associated with age-related diseases such as Alzheimer, autoimmunity, etc. Another example is the finding of increased incidence of Down's Syndrome (DS) as a result of lack of maternal folic acid supplementation. Also, children born to mothers who were in early pregnancy during the Dutch famine 1944-45, were at a significantly increased risk of cardiometabolic disorders in adulthood presumably due to epigenetic factors affecting the genome in utero. Epigenetic factors play a role in hypertension, and heart failure. He also states that epigenetic alterations are responsible for many cancers such as that of the colon, prostate, breast, and brain. Research may lead to targeted therapies that lead to re-expression of lost genes or down regulation of over-expressed genes in order to eradicate cancer. There are several studies of the molecular basis of brain functions, for example, memory, cognition, etc. and the putative role of epigenetic factors' dysfunction on brain pathology. It is important to find out how exposure to a wide scope of environmental factors such as drugs, abuse, infection, nutrition can affect epigenetic regulation of brain that ultimately translates to altered behavior. The importance of this accumulating information is the development of therapies based on modifying the abnormal epigenetic factors. The author reports two attempts at this; lysine methyl transferase inhibitors, and histone deacetylase inhibitors that may be promising in treating cancer. The author concludes by reminding us of the ethical guidelines embodied in the Helsinki Declaration and in the Islamic *Shari`ah's* principles. These need to be followed both in the basic genetic research and in the clinical trials.

Professor Mishal, in another chapter, discusses DNA fingerprinting in relation to the establishment and negation of paternity. He stresses the importance of lineage preservation in Islam and describes the *Shari`ah's* methods of establishing and negating paternity. Within the Islamic legal system, paternity is always limited to licit sexual relationship. Children born within the context of marriage are automatically attached to the husband who becomes the bona fide father. The basic principle is "the child belongs to *al-firash*". In cases where fulfillment of this principle is difficult to achieve, four other methods could be resorted to in this hierarchical order:1) Admission of paternity by the father (*Iqrar*). 2) *Bayyinah* (testimony). 3) Resemblance of physical features (*qiyafah*) 4) Lot casting. Negation of paternity according to the *Shari`ah* is only conducted by the mutual oath of condemnation (*li`an*).

DNA fingerprinting is almost 100 % accurate (except for human error). It became the standard method to resolve paternity disputes in the West.In Muslim majority countries there have been discussions about the utility of DNA fingerprinting viz-a-viz the *Shari`ah* rules in such disputes. The author cites the rulings of two major contemporary *Fiqh* councils; The Islamic *Fiqh* Academies and IOMS. The majority of participants in these councils favored the admission of DNA fingerprinting as the modern equivalent of *qiyafah,* but refrained from its substitution of *li'an* in negating paternity. However, some other modern Islamic jurists, on individual basis, believe that the new DNA technology can be used as a definitive way to establish and negate paternity. They consider it as a type of strong circumstantial evidence that denotes definitiveness. They believe that *Bayyinah* mentioned in the Quran and *Sunnah* is not limited to testimony. It includes all that is capable of exposing and proving the truth. In view of earlier precedents in Islamic legal tradition and the differences among the contemporary jurists, more jurist deliberations and *Ijtihad* are needed to resolve these questions.

Dr Khan discusses consanguineous marriage. It is defined as a union between individuals related as second cousins or closer. There is increased risk of autosomal recessive diseases. If both parents carry the recessive gene of a disease such as hemoglobinopathy and cystic fibrosis, each child will have a 25% risk of having the disease. Offspring of consanguineous marriages are also at increased risk for disorders of multifactorial inheritance. First cousin marriages increase the risk of significant birth defects in the offspring by only 1-3% above the general population. The absolute increase is insignificant unless there is family history of the disease. However, de novo mutation can quickly rise to a high frequency in a tribal sub community that practices endogamy. An otherwise rare disease can become relatively common, especially if families tend to have large number of children. On the other hand it is to be noted that consanguinity can weed out deleterious gene mutations resulting in overall healthier offspring.

It is recommended that couples undergo carrier screening for prevalent genetic diseases in the community and discourage union of couples who are both carriers of the same disease. In Saudi Arabia compulsory carrier testing for thalassemia and sickle cell anemia was started in 2004.When both the potential couple were found to be carriers, they were counseled but not obliged to cancel the plan for marriage. For the first two years of the program, 90% of those so identified

proceeded to marriage. However, over the next four years the frequency of voluntary marriage cancellation increased 5-fold and simultaneously the incidence of B-thalassemia has dramatically decreased.

It is noteworthy that while consanguinity is relatively common in Muslim majority countries, the practice is not advocated by Islam, although it may have been encouraged by inheritance laws.While there are examples of consanguineous marriages in the history of Islam, there are possible traditions, Prophetic Hadith and a statement by the second Caliph Omar encouraging marriage outside the family.

This policy of premarital genetic testing (PGT)has also been addressed by Professor Ebrahim who added testing for infections and blood transmitted diseases. In Bahrain, where this policy has been enforced, there was an increase of "separation before engagement", and 50% reduction in the incidence of sickle cell anemia. Professor Ebrahim explains concerns about mandating PGT especially from the Islamic point of view.There is violation of autonomy. There is concern about the possible breach of confidentiality especially in small tribal countries where people tend to know each other personally. There is also concerns about false security if the carried out tests are negative. In this case the couple may falsely believe they will have a perfectly healthy newborn. There are many diseases or defects that are not tested for. Some Muslim scholars accepted mandatory PGT because of its significant benefits. They support their opinion by i) the rule of obeying those in authority. ii) the Qur'anic guidance" do not make your hands contribute to your own destruction". iii) fulfillment of one of the goals of *Shari`ah* (preservation of progeny). iv) it is acceptable to compromise autonomy for the sake of achieving a pressing societal benefit based on two maxims: choosing the lesser of two evils, and that of blocking the means to an expected evil (*sadd al-dharai'a*). Scholars who object to PGT opine that adding a condition to the marriage contract, that is not in the *Shari`ah*, invalidates the marriage contract.

Dr Harira discusses prenatal screening and diagnosis. Initially it was primarily done for the diagnosis of Down's Syndrome and was based on maternal age (>35 at delivery). Later, several modalities were added, serum markers, ultrasound findings, and more recently by the direct examination of cell free fetal DNA (cFFDNA). Simultaneously the scope of screening increased to include other chromosomal anomalies, neural tube defects, and genetic diseases. When the screening test indicates a high risk, the diagnosis depends on examination of fetal cells obtained through chorionic villous sampling, amniotic fluid cells obtained through amniocentesis, fetal blood cells obtained by cord blood sampling or occasionally fetal tissue obtained by fetoscopy. The studies include fluorescent in vitro hybridization (FISH), karyotyping. DNA examination, enzymatic testing, etc. Dr. Harira describes the two most commonly used procedures; chorionic villus sampling and amniocentesis. Although many structural fetal anomalies can now be diagnosed by ultrasonography, only genetic diagnosis is addressed in this chapter.

The diagnosis of significant fetal anomalies gives the mother the chance to have an abortion. However, some of these anomalies can be treated in utero, medically or, in rare situations, surgically

with significantly improved neonatal outcome. It is pertinent to note that, Islamically, abortion is only allowed before 20 weeks and for lethal anomalies only. (for a more detailed discussion see reference 1).

Dr Harira describes the indications for prenatal screening and the various screening strategies. He discusses the most recent screening method, Non-invasive Prenatal Testing (NIPT). In this technique, free fetal DNA is obtained from maternal plasma and subjected to Massively Parallel Sequencing. DS and trisomies 18 and 13 and sex chromosomal anomalies can be detected. NIPT is highly accurate in detecting DS with both sensitivity and specificity of >99%,and non-reportable (failure) rate of only 0-4.9% The detection rates of T 18 and T 13 and sex chromosome anomalies are slightly less. Nevertheless when the results are positive they have to be confirmed by CVS or amniocentesis.

Genetic counseling is an important component that should be incorporated in the care of all pregnant women. It should be offered before screening tests are used and when the results are obtained. It is especially important if an invasive procedure is to be recommended. Its benefits, risks and all available options, if the result is abnormal: abortion, in utero treatment, neonatal treatment, palliative treatment and long term prognosis should be fully discussed with the patient before the procedure is performed.

Professor El-Zibdeh adds another aspect of prenatal diagnosis, diagnosis before the implantation of the blastocyst, Pre-implantation Genetic Diagnosis (PGD). It is performed in IVF centers. It can be done by a) Polar body biopsy. b) Cleavage stage biopsy where 1-2 blastomeres are removed on day 2-3 after fertilization. Or c) Blastocyst stage where 5-10 trophoectoderm cells are removed. He discusses the various cytogenetic and molecular genetic tests that can be done on these removed cells; FISH, Karyotyping and more recently, Comprehensive Chromosome Screening (CCS) or Array Comprehensive Genome Screening (aCCS). Only chromosomally normal blastocysts are implanted, which will result in higher successful implantation and probably higher pregnancy rates. It will allow the achievement of the goal of routine single embryo transfer (e SET) to avoid the complications of multifetal pregnancies. PGD is recommended for all candidates of IVF, especially those who are older than 40 years, those with repeated IVF failures, or with repeated miscarriages. PGD is indicated in couples with increased risk for an affected offspring such as women older than 35 years of age, couples with a previous child or with family history of a chromosomally abnormal fetus or one of the inherited genetic diseases, or if one of the parents has an autosomal dominant disease such as adult polycystic kidney, a disease when the fetus has a 50% risk. It can also be offered for a couple who had a child with combined immunologic deficiency, to select the implantation of an embryo that is HLA compatible with the affected sibling. PGD is also indicated in patients with familial cancer to select for implantation of only the embryos that are negative for the genetic mutation. In all these cases, the prospect of an invasive diagnostic test (CVS or Amniocentesis), later in the pregnancy, is avoided, and more importantly the need for an abortion in case the latter tests are positive for the abnormality. Another interesting use of PGD is sex selection. This is indicated in case of sex-linked diseases such as hemophilia. However sex selection for parental

preference of a particular sex is not indicated and is not favored ethically. The Islamic viewpoint on social sex selection has been discussed in a previous yearbook. Most jurists do not approve it.

Each human cell contains 1000-2000 mitochondria in the cytoplasm. Their function is to release ATP that provides energy to the cell and allows its survival. There are only 37 mitochondrial genes (mt DNA) which is distinct from nuclear DNA (nDNA).At fertilization, the ovum's cytoplasm becomes the cytoplasm of the zygote and of each dividing cell afterwards. Sperms do not contribute any mitochondria and the mitochondria are only maternally derived. A person may have all normal mitochondria or all abnormal mitochondria. In the latter case, death occurs either of the fetus (miscarriage) or of the newborn soon after birth. There are other individuals who carry both normal and abnormal mitochondria. It is only when the mutant load exceeds a certain threshold that the person will manifest one of the syndromes of mitochondrial genetic disorders, such as Leber Hereditary Optic Neuropathy. Sarah Salman addresses the newest techniques of assisted reproductive technologies that have been designed to allow normal offspring for female carriers of these syndromes. These are called Mitochondrial Replacement Therapy (MRT). She describes the two available techniques; pronuclear transfer (PNT) and Maternal spindle transfer (MST). A woman with healthy mitochondria is needed as an egg donor. In PNT the intended mother's ovum is fertilized with the sperm of the intended father. At the same time, the donor's egg is fertilized with the intended father's sperm. The two pronuclei from the donor –father combination are removed and discarded, leaving an enucleated cell with healthy cytoplasm. The two pronuclei from the intended parents are removed and placed in this cell. In MST the transfer occurs at the oocyte level. The maternal spindle is removed from the donor's egg with healthy mitochondria. It is also removed from the intended mother's egg and placed in the enucleated donor egg and then fertilized with the intended father's sperm. The resulting zygote is allowed to develop to the blastocyst stage and then implanted in the uterus of the intended mother.

In PNT fertilization occurs between the father's sperm and an egg of a woman that is not his wife, and a zygote is destroyed. On the other hand, in MST the donor's egg is not fertilized by the father's sperm and no zygotes are destroyed. The resulting baby has a trigenetic makeup (nDNA) from the intended parents and mtDNA from a donor. However, the genes relating to us as humans and in our traits are all dependent on nDNA. Therefore, there is no doubt about the lineage of the child. The child belongs to his biologic father and mother, the donor has no connection to his lineage.

In both PNT and MST the vertical transmission of donor mtDNA is inevitable. Any girl born will transmit this donor mtDNA to further generations. This will not be the case if boys are born. Therefore it has been advised to have only boys delivered with MRT until its complete safety is assured.

The MRT issue was not discussed in Jurisprudence councils, or in combined jurist-scientific medical seminars. Some contemporary Muslim jurists provided approval or rejection views, in the form of personal communications. Jurists and scientists are called upon to conduct proper deliberations to clarify *Shari`ah* opinion on this new and developing issue.

Haq et al wrote a comprehensive review of the molecular genetics of cancer. Cancer is believed to arise from a single cell referred to as the cancer stem cell with alteration of the genetic material which allows the cell to continue to divide resulting in cancer. Understanding the molecular mechanisms at play in this stem cell provides the tools necessary to interrupt these aberrant genetic pathways and successfully treating the disease. In normal cell division, DNA is replicated perfectly in the two daughter cells. If errors occur the cell is channeled to the apoptosis pathway and dies. If the regulatory mechanism malfunctions, a cell with altered genetic material survives and continues to divide leading to cancer. This altered genome could be over expression of a gene involved in growth, an oncogene, or suppression of a gene involved in apoptosis or a tumor suppressing gene (p53). Genetic alterations can be hereditary or result from environmental carcinogens (epigenetic factors) that damage DNA, such as radiation, or chemicals such as benzopyrene in tobacco smoke.

Oncoviruses, such as Human Papilloma Virus (HPV) and Epstien-Barr Virus (EBV), damage the DNA and cause cervical cancer, and B cell lymphoma, respectively. Hormones that naturally induce cell proliferation are also linked to cancer, for example testosterone and prostate cancer. The authors describe three primary proteins involved in DNA repair; mismatch repair, base excision repair, and nucleotide excision repair. If the genes producing these proteins are damaged, altered DNA remains in the cell and leads to malignant transformation. Epigenetic mechanisms which modify gene expression, rather than alter the genes themselves, also play a role. Hypermethylation of tumor suppressor genes render them inactive. Histones which are needed for the proper function of DNA can be altered by methylation or acetylation facilitating the development of cancer.

Scientists were able to sequence a large variety of cancer cells developing the cancer genome. Abnormalities include substitution of one base by another, insertion or deletion of small or large segments of DNA, rearrangements, copy number variation; increase or gene amplification or reduction, presence of exogenous DNA such as HPV, epigenetic changes, or alteration of mitochondrial genome.

An atlas of the cancer genome, which includes whole genome of sequencing of 1000 tumor samples, has been compiled. The authors also describe the various methods of studying molecular genetics. They classify the genetic abnormalities in cancer into: numerical and structural. They discuss in detail oncogenes and tumor suppressor genes, fusion genes, passenger, and driver mutations, metabolic enzymatic pathways. They discuss the concepts of metastasis, oncogenesis, apoptosis and drug resistance. The authors also discuss the molecular genetics of selected tumors; chronic myeloid leukemia, breast, and colorectal cancer. The authors tabulate the various targeted new drugs that have been developed as a result of the understanding of the molecular genetics of specific neoplasms and the FDA approved uses, for ex.Aftinib, for the treatment of non-small cell lung cancer. The authors believe that understanding of the molecular genetics will allow us to predict the probability of disease in healthy individuals who can be screened. Interventions can be developed before the disease manifests itself. Meanwhile they remind us of the potential drawback of this knowledge, primarily discrimination against these subjects.

The authors stress the Islamic perspective of studying the secrets of the universe and of human biology as a part of worship, and cite several Qur'anic verses in that regard. Also the Qur'an advocates us to advance our knowledge to save human lives.

Dr. Harun states that in addition to the genetic alterations mentioned above, evasion of immune destruction of malignant cells is another mechanism for the development and progression of cancer. Using genetic engineering based on these findings appears to be a new and innovative method of treating cancer.
Oncolytic viruses have the ability to selectively infect and replicate in cancer cells, killing them. They also have antiangiogenic and anti-vasculature properties which promote apoptosis of uninfected cancer cells. Genetic engineering of these viruses further potentiates them as cancer vaccines. Dr. Harun describes various techniques of genetic engineering to enhance their antitumor immunity and cytotoxicity. Monoclonal antibodies have been developed to target tumor antigens. Bispecific antibodies target both cellular and humoral immune responses. Immunotherapy has been used to manipulate the patient's own immune system to kill cancer cells. T cells can be genetically engineered to express T cell receptor (TCR) or a chimeric antigen receptor (CAR)specific for antigens expressed by the tumor. These T cells are able to recognize and kill the cancer cells. Dr. Harun cites in his chapter different clinical trials using this technology especially in leukemia therapy. He also discusses another modality of treatment that is genome editing, specifically gene-editing nucleases and Zinc finger nucleases which can also be used to treat other genetic based diseases. He also describes the use of transcription activator- like effector nucleases and the CRISPR-Cas 9 system. He believes that the novel genetic engineering technologies allow modification of the immune system and correction of cancer genome mutations thus offering the possibility of developing effective novel strategies to cure cancer.

Drs. Badr and al-Hendy discuss in detail the use of gene therapy in the field of gynecology and obstetrics. Gene therapy is a novel therapeutic approach that allows using recombinant DNA technology to create a functional gene-expressing unit. This unit can deliver DNA or RNA to a tissue and allows replacement of the absent DNA or integration with the "diseased". It transfroms the transcription function responsible for the pathologic expression. It either suppresses it or changes it to a non-pathologic one. Successful gene therapy trials have been reported in the treatment of cancer, neurodegenerative diseases, among others. The authors describe gene delivery methods to be either DNA (non-viral) delivery or through the use of viral vectors. They discuss ex vivo gene transfer as well as cell based gene therapy.
They describe applications of gene therapy in the treatment of gynecological diseases; fibroids, endometriosis, and premature ovarian failure. Gene therapy has also been used in the prevention of post-operative pelvic adhesions. The authors also discuss the various applications of gene therapy in the treatment of ovarian cancer and in the development of HPV vaccines used to prevent cervical carcinoma which remains one of the commonest malignancies in the world. The main application of gene therapy in obstetrics is the study and possible treatment of fetal growth retardation.

The authors stress the importance of moving from the basic science of gene therapy and its uses in animal studies to the all-important clinical trials. They report that more than 65% of gene therapy trials have been done in relation to cancer treatment. Out of 1843 cancer gene therapy trials, only 45 have reached phase III and only one in phase IV, that is the treatment of advanced thyroid cancer.
The authors list the challenges ahead to include safety concerns, and transgene expression. They encourage research to assess the long term gene expression following nanoparticle delivery and the application of induced pluripotent stem cells (iPSCs) combined with ex-vivo gene transfer to broaden the clinical application of cell-based gene therapy.

The genetic mutations in thyroid cancer are discussed in detail by Dr. Enas Younis. Thyroid cancer arising from follicular cells constitutes 95% of thyroid cancers. Many point mutations and translocations are involved in the genesis of differentiated thyroid cancer. These occur in genes particularly in the mitogen activated protein kinase (MAPK) pathway and the phosphatidyl inositol -3 kinase (P13K / AKT) signaling pathway. The majority of Medullary Thyroid Carcinomas (MTCs) are sporadic, but about 25% are inherited in an autosomal dominant pattern. The latter mostly occur in association with Multiple Endocrine Neoplasia (MEN) types 2A and 2B. Somatic or germline mutations of RET proto-oncogene plays an important role in the development of sporadic and familial MTCs. Genetic testing for this gene is important not only for the diagnosis but also for the prognosis of MTC. Other genetic mutations are also involved; TRK rearrangement, ALK rearrangement, PAX 8/ PPAR gamma rearrangement, RAS mutations, mutations in the P 13 K / AKT pathway, as well as BRAF gene and others. These findings have significant clinical applications particularly in relation to MTC. The diagnostic accuracy of thyroid nodules has been enhanced by testing for the different genetic abnormalities. The author describes in detail the different types of MTC and their association with MEN and the underlying genetic abnormalities that occur in both carriers and disease patients. One of the important clinical benefits of genetic testing is that RET genetic screening of patients with apparently sporadic MTC allows preclinical diagnosis and early treatment of unsuspected affected family members and allows the identification of a relevant percentage of hidden familial MTC (FMCT). Carriers of the mutated RFT gene should be offered prophylactic thyroidectomy. In case of MEN 2A / FMTC mutation of ATA level C risk, prophylactic thyroidectomy should be carried out before the age of 5.Those with RET mutations of ATA level A and B risk, prophylactic thyroidectomy may be delayed beyond the age of 5 years with close follow up.
The author details the therapeutic approaches subject to the underlying genetic abnormality. Further, the author describes the new molecular targeted therapy using novel small–molecule protease kinase inhibitors depending on the specific genetic abnormality.

Professor Mishal discusses the topic of stem cells and cloning. Significant advances have been made in these fields. They can help us better understand cell biology and offer great promise for

treating human diseases and specifically in regenerative medicine. He discusses the types of currently available stem cells. These are:

1. Human embryonic stem cells (hESCs) derived from the inner cell mass of excess embryos produced in IVF centers within the first 5 days of fertilization.. They have the capability of indefinite unlimited expansion in vitro. They are pluripotent. They can be induced to differentiate in any type of human cell and thus can potentially be used to replace diseased tissues. They can be genetically manipulated by transfecting them with DNA constructs for specific desired properties. They can also be derived from embryos with diagnosed mutations by PGD for research on the specific disease. While hESCs have great potential of curing disease and advancing regenerative medicine, their use raises ethical concerns because they cannot be obtained without destroying a human embryo.
2. Somatic cell nuclear transfer results in cloned embryos that can be used in similar ways to hESCs. They show pluripotent qualities and can also be made to differentiate into any type of cells so they can be used in regenerative therapy. They also raise the same ethical concerns.
3. Induced Pluripotent Stem Cells (iPSCs) are derived from somatic cells by reprogramming their genes so the somatic cell reverts to its embryonic counterpart and becomes pluripotent. These cells can be induced to differentiate in any cell type. Their use circumvents the ethical concerns associated with the use of hESCs. They can be used for the study of diseases and for cell therapy in many diseases.
4. Human adult stem cells: These are normally present in all body tissues but in very small numbers. Their use poses no ethical concerns.
5. Animal stem cells: They are usually obtained from mesenchymal pig stem cells and can be also used in regenerative medicine.

Hematopoietic stem cell transplantation is one of the most successful applications of stem cell therapy. It has been used in the treatment of many hematologic malignancies. It was later used in other diseases such as hemoglobinopathies, inborn errors of metabolism involving the hematopoietic system.

The Roman Catholic Church and some Protestant denominations, and Orthodox churches do not allow stem cell research or therapy. Other denominations and Islamic Councils have accepted their use as it is intended for curing human disease, as long as the source of the cells is duly permitted (excess human embryos at IVF centers that will not be used by the couple).

Professor Mishal also discusses cloning. Mammalian cloning is based on the technology of somatic cell nuclear transfer. The notion of human cloning was entertained by some scientists but was met with strenuous objections from scientific, ethical, and religious bodies. Some countries have outlawed the practice. While it is possible to clone the genome of the individual, the individual as a whole cannot be cloned. This is due to the crucial influence of the environment on the individual from conception to death. Every Muslim seminar, fatwa council and individual scholar have prohibited human reproductive cloning. Professor Mishal details the juristic opinions and the rationale for the prohibition. Therapeutic cloning uses the same method of somatic cell nuclear transfer. It generates autologous embryonic stem cells from the cloned embryos for the purpose of

tissue replacement. As the obtained stem cell has the same DNA as the original somatic cell from which it was derived, and the stem cells are going to be transplanted into the same individual, there is no risk of immune rejection and immunosuppression therapy is unnecessary. This technique is very promising. Therapeutic cloning is considered permissible by some Muslim jurists in special circumstances. This issue needs further discussions in *fiqh* councils, in view of recent scientific breakthroughs. (For a more detailed discussion of the Islamic perspectives on stem cell research and cloning see reference 2).

I conclude by thanking all the authors for their contributions to this issue. I especially thank members of the Editorial Board, Drs. Aly Mishal, Abul Fadl Mohsin Ebrahim, and Musa Mohd Nordin for their valuable input and guidance. I pray that we can continue working on the project of the Encyclopedia in the following years and complete this great project in our lifetime. I sincerely appreciate the work of Dr. Mishal's staff for copyediting and proofreading of the manuscripts especially Ms. Elham Mohammad Swaid.

I pray that Allah (ﷻ) accept and bless our efforts in His service. May Allah (ﷻ) guide us to the right path and have mercy on us. Amin.

Wassalam

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References:

1. Fadel HE, Misha'l AA, Ebrahim AFM, Nordin MM. Termination of pregnancy (TOP). In: Fadel HE, Misha'l AA, Ebrahim AFM, Nordin MM. Encyclopedia of Islamic Medical Ethics (Part 1). Amman: Jordan Society for Islamic Medical Sciences; 2014:35-52.

2. Fadel HE. Developments in stem cell research and therapeutic cloning: Islamic ethical positions, a review. Bioethics. 2012;26:128-35. http://dx.doi.org/10.1111/j.1467-8519.2010.01840.x

Federation Of Islamic Medical Associations (FIMA) in Brief

- Established at the outset of the 15th Hijrah century, December 1981, in Orlando, Florida, USA, where senior leading medical professionals representing ten Islamic medical organizations, from various parts of the world, convened and laid down the foundation of the Federation.
- Subsequently, in March 1999, FIMA was incorporated in the State of Illinois as a non-profit organization, and a tax-exempt status was acquired. FIMA acquired the special consultative status with the United Nations Economic and Social Council (UN-ECOSOC).
- Since that time, FIMA membership progressively expanded to include 29 full members, 13 associate members, and more than 15 prospective and collaborating organizations from all over the world.
- Most FIMA activities and achievements are based on the endeavors of its member Islamic Medical Associations (IMAs), in constructive mutual cooperation, and harmonious understanding.
- Islamic medical activities of FIMA have a holistic nature. Leadership, mutual cooperation and innovation are prerequisites for the welfare of our communities, our Ummah and humanity at large.
- These activities include, but are not limited to:

1. Cooperation in humanitarian medical relief work, where and when needed in disaster stricken countries, regardless of ethnicity, religion or race. The FIMA Save Vision Program was initiated in early 2005. To date, more than 125,000 eye surgeries were performed by volunteer ophthalmologists and teams from IMAs in several countries in Africa, South and Southeast Asia, where visual impairments are rampant. The program included training of local medical professionals to continue and widen this activity by qualified local talents. The program also included establishment of local eye hospitals or eye sections in existing general hospitals, in deprived communities.

   This activity qualified FIMA for a distinguished award from the American College of Physicians (ACP), designated for outstanding humanitarian medical achievements.

   Over the past five years, two new humanitarian activities were launched: The cleft lip/palate, and the vesico-vaginal fistula projects, both highlighted as significant medical and psychosocial problems in several needy communities.
2. Collaboration with regional and international organizations in areas of preventive medicine and community health education.
3. Scientific, professional and ethical jurisprudence related conferences, seminars and publications.
4. Establishment of the Consortium of Islamic Medical Colleges (CIMCO), to foster cooperation in improvement of curriculum, training, research, administration, and up-bringing of model medical practitioners.
5. Establishment of the Islamic Hospitals Consortium (IHC), to pursue cooperation and coordination among medical professionals and hospital administrators in

areas of experience exchange, benchmarking, improvement of health care delivery, ethical, administrative and operational activities, to meet the most advanced international standards, in the context of Islamic principles.

6. Publication of FIMA Year Books, which address biomedical, scientific, ethical, and other related issues that are needed for medical practitioners, educators as well as Jurists.
7. In 2013, FIMA committee on Bioethics embarked on the project of Encyclopedia of Medical and Health Ethics. In view of the extensive effort needed, this project is expected to span over several years.
8. Medical students' activities, including conferences, seminars, publications, camps, Umrah and Ziarah programs, pioneered by IMA-Saudi Arabia.
9. Collaboration to extend a helping hand to Muslim medical practitioners in underprivileged countries, to work together and organize professional medical societies, to serve their communities.
10. Activities to combat HIV/AIDS and sexually transmitted infections (STIs): FIMA established long standing educational, prophylactic and capacity building activities in many countries, especially in Africa and Asia, which was pioneered by Uganda IMA in the 1980s. Ten years ago, FIMA launched the parallel project [Protection of Our Youth From STIs and AIDS], pioneered in Jordan, with wide spread activities of education, nurturing and preparation of thousands of local youth leaders in around 30 countries in various regions of the world.
11. Activities to combat all forms of addiction. The project is organized and directed by the Green Crescent Society, based in Istanbul-Turkey, with programs conducted in several countries. The theme of FIMA Yearbook in 2014 was on addiction.

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ETHICS IN GENETIC RESEARCH

*Omar Hasan Kasule Sr**

Introduction

Genetic research is *ijtihad* involving reading Allah's signs in humans (*ayat al Allah fi al anfus)*. Its ethically adverse side effects can be avoided if we have a joint reading (*al jam'u baina al qira'atain)* of the book of empirical knowledge (*kitaab al kawn)* and the book of revealed knowledge (*kitaab al wahy)*. This paper explores issues of human dignity, underlying intentions, consent, disclosure, and genetic therapeutic intervention from the theory of purposes of the law (*maqasid al shari'at)*[1], and principles of *fiqh* (*qawa'id al fiqh)*[2].

Human Dignity

Human dignity is a concern in genetic research[3]. A human is a deliberate and best of creation being a duality between body and *ruh*. Allah dignified the human being giving him superior intelligence (*'aql*), a conscience to tell right from wrong (*hidayah)*, limited willpower (*iraadah*) and control over the environment (*tashkir*). Preserving human dignity is one of the fruits of the purpose of *hifdh aldin*. Genetic research if misconceived could lead to loss of human dignity by looking at the human as a collection of genes with their random mutations that determine both biology and behavior. Genetic research could lead to violation of human dignity if it results in discrimination based on genetic make-up[4], commodification of the human corps, especially if commercialized[5]. On the other hand, by showing the common origin of the human genome, genetic research can restore human dignity by de-emphasis of external variations of race, color, ethnicity, tribe, and family lineage.

Intentions

According to the *fiqh* principle of intention (*qa'idat al qasd*) all matters are judged by the underlying intentions (al *umuur bi maqasidiha*) and not the outwardly expressed intentions, Genetic research can have good underlying intentions in the diagnosis, prevention, and treatment of disease, for example genetic susceptibility studies[6] can lead to effective preventive measures. Genetic research may have intentions of financial gain from patenting new diagnostic or treatment processes.

Genetic research may have bad underlying intentions if it is used to (a) provide scientific support for forbidden sexual orientations[7,8] and addictions (b) creating a potential for stereotyping such as genetic research on intellectual disability[9], community genetic survey for anti-social personality[10] or genetic research on intelligence[11].

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Consent

Like all other forms of research, genetic research requires consent of the research subject. The process is more rigorous than other forms of research because more information must be provided to and must be understood by the research subject mainly related to the disclosure of both main and incidental findings. Genetic findings in a research subject disclose genetic details of all members of the family or community and require additional privacy and confidentiality measures. Some forms of findings may upset the research subject or the family and they need advance counseling and consent. Study of community genetic variation requires consent at the level of the community. Study of individual disease risk requires consent by the individual. Genetic therapy requires consent[12].

Privacy, Confidentiality, and Disclosure of Anticipated Findings

Strict privacy and confidentiality must be maintained in genetic research to prevent harm to patients and relatives. Non-disclosure, whatever its benefits, may lead to loss of patients of trust in the researchers[13]. We may need to weigh the harm and benefits of the disclosure according to detailed rules of the principle of injury (*qa'idat al darar)*. Public interest (*al maslahat al 'ammah)* of either disclosure or non-disclosure takes precedence over individual interest (*almaslahat al khaassah)*.
This principle can be applied in the following cases to allow disclosure to relatives: a gene of a preventable or treatable condition is found in a patient who refuses disclosure to relatives who could benefit, a gene of treatable disease is found from the patient after death?[14], and genetic data collected from critically ill patients who die soon after?[15].

Disclosure of Incidental Findings

Issues of disclosure of anticipated findings in research can be resolved in advance by the consent process. Problems arise regarding disclosure of incidental findings. Among questions that arise are: Is it ethical to look for incidental findings? Should we disclose incidental findings to the patient or the family? Should we break the anonymity of genetic research on bio-banked material to disclose an incidental finding that will require medical intervention? [16]. Should we disclose wrongly attributed parentage? Should we disclose an incidental finding of an unsuspected but treatable genetic disease? The genetic researcher must avoid the complications of incidental findings by not looking for them or even noticing them because he is bound by the principle of intention to carry out only what he set out to do. It is therefore not obligatory to look for incidental findings[17]. According to the principle of certainty (*yaqin*) the findings must have a high degree of certainty (validity: analytical and clinical) before we even consider disclosure. According to the principle of injury the disclosure must have benefit (value, clinical utility, clinical relevance, and actionability) and the patient must consent to be told (volition) because he/she is the best protector of self-interest[18,19,20].

References

1. Abu Ishaq Ibrahim Bin Musa al Shatibi (d. 790H). Al Muwafaqaat fi Usuul al Shari'at. Dar al Marifat, Beirut 2008G.
2. Majallat al Ahkaam al Adliiyyat Dar Ibn Hazm Beirut 2004 G / 1424H.
3. Chan DK. The Concept of Human Dignity in the Ethics of Genetic Research. Bioethics. 2015 May;29(4):274-82.
4. Alfonso Farnós I, Hernández Gil A, Rodríguez Velasco M. Update of the work of the ethics research in evaluat-

ing genetic research and its role as an external ethics committee biobank. Rev Derecho Genoma Hum. 2013 Jul-Dec;(39):173-203.
5. Hoeyer K. The role of ethics in commercial genetic research: notes on the notion of commodification. Med Anthropol. Jan-Mar;24(1):45-70. 2005.
6. Ayuso C, Tellería JJ, Tejedor JC, Gracia D. Ethics on genetic research (2). Genetic susceptibility studies. Med Clin (Barc). 2011 Jun 11;137(1):22-6.
7. Schüklenk U, Stein E, Kerin J, Byne W. The ethics of genetic research on sexual orientation. Hastings Cent Rep. 1997 Jul-Aug;27(4):6-13.
8. Murphy TF. Abortion and the ethics of genetic sexual orientation research. Camb Q Healthc Ethics. 1995 Summer;4(3):340-50.
9. Goodey CF. On certainty, reflexivity and the ethics of genetic research into intellectual disability. J Intellect Disabil Res. 2003 Oct;47(Pt 7):548-54
10. Rodriguez MV. The ethics of a genetic screening study for antisocial personality disorder with Mesoamericans: case study in the ethics of mental health research. J Nerv Ment Dis. 2012 Mar;200(3):260-4.
11. Reiss MJ. The ethics of genetic research on intelligence. Bioethics. 2000 Jan;14(1):1-15
12. Dhar HL. Ethics in genetic research. J Assoc Physicians India. 2002 Nov;50:1395-7.
13. Mandava A, Millum J, Berkman BE. When Should Genome Researchers Disclose Misattributed Parentage? Hastings Cent Rep. 2015 Feb 11
14. Taylor HA, Wilfond BS. The ethics of contacting family members of a subject in a genetic research study to return results for an autosomal dominant syndrome Am J Bioeth. 2013;13(10):61..
15. Freeman BD, Kennedy CR, Frankel HL, Clarridge B, Bolcic-Jankovic D, Iverson E, Shehane E, Celious A, Zehnbauer BA, Buchman TG. Ethical considerations in the collection of genetic data from critically ill patients: what do published studies reveal about potential directions for empirical ethics research? Pharmacogenomics J. 2010 Apr;10(2):77-85.
16. Wolf LE, Bouley TA, McCulloch CE. IRB. 2010 Mar-Apr;32(2):7-18. Genetic research with stored biological materials: ethics and practice..
17. Gliwa C, Berkman BE. Do researchers have an obligation to actively look for genetic incidental findings?. Am J Bioeth. 2013;13(2):32-42.
18. Eckstein L, Garrett JR, Berkman BE. A framework for analyzing the ethics of disclosing genetic research . J Law Med Ethics. 2014 Summer;42(2):190-207.
19. Ayuso C, Millan JM, Dal-Re R. Management and return of incidental genomic findings in clinical trials. Pharmacogenomics J. 2015 Feb;15(1):1-5.
20. Wolf SM. Return of individual research results and incidental findings: facing the challenges of translational science. Annu Rev Genomics Hum Genet. 2013;14:557-77.

GENOMICS: CONTEMPORARY SCIENTIFIC, ETHICAL AND ISLAMIC PERSPECTIVES

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Abstract

Significant progress and innovations in genomics have been achieved over the past several decades, particularly after completion of the Human Genome Project (HGP) in April, 2003. Sequencing of the human genome created breakthroughs in understanding, research and clinical applications. Parallel to that, was the progress in information technology which became instrumental in establishing databases, along with marked improvements in sequencing of known and hypothetical genes and proteins, and detailed analysis of various data.
This concise overview of Genomics will present an update of recent information of some aspects of this broad topic, aiming at better understanding of their scientific basis to both scientific-medical professionals, and to concerned scholars in ethical and Islamic jurisprudence. It is hoped the interaction between the two groups will be instrumental in formulating ethical-Islamic guidance to researchers, practitioners and to the public at large.

Keywords: Genomics, Human Genome Project, ethics, Islamic Jurisprudence.

Contents

Introduction

In the nucleus of the human cell, most of the heritable genetic information is stored in the deoxyribonucleic acid (DNA), a linear polymer comprising of the 4 bases: adenine,

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guanine, thymine and cytosine. A typical DNA molecule comprises of 4 distinct nucleotides each composed of a sugar, a nucleoside base, and phosphate groups covalently linked to one another by phosphodiester bonds. The hydrogen bonding that occurs between the complementary base pairs of adenine- guanine and cytosine-thymine results in the double helical structure[1,2].

This sequence of complementarity, in which the sequence of bases on one strand determines the sequence on the other, forms the structural basis for DNA replication, in which the two strands are separated and new DNA is synthesized complementary to each strand. Additionally, it forms the basis for the sequence specific of hybridization, the chemical reaction whereby two complementary strands of DNA bind[2].The sequence of the four nucleotides varies among the DNAs of different organisms. Indeed this is the nuclear basis of genetic diversity[3].

All genetic characteristics are transmitted from parent to progeny. This evolves by unwinding of the two chains, and polymerization of two daughter chains along the separated parental strands.

The nucleotide sequence and the genetic information are conserved during this process, because each nucleotide in the daughter chains is paired specifically with its complement, in the parental or template chains by polymerization.

DNA → RNA → Protein

To translate its genetic information into a protein, a segment of DNA is first transcribed into messenger ribonucleic acid (mRNA). The mRNA contains a sequence of purine and pyrimidine bases that is complementary to the bases of the antisense strand of DNA[3].

Thus each DNA triplet codon is transcribed into a corresponding RNA triplet codon that will be translated into a corresponding amino acid.

The mRNA for each gene is processed extensively within the cell nucleus, including splicing to remove intronic sequences. It then crosses the nuclear membrane and enters the cytoplasm, where it serves as a template for the synthesis of a specific protein.

The flow of information from DNA to RNA to protein is predominantly unidirectional. Unlike RNA and protein, DNA molecule is extremely stable, and resists various environmental adversities, even many years following death of individuals[4].

The Genetic Code

The sequence of the bases in a gene dictates the sequence of amino acids in a specific protein. This collinearity between the DNA molecule and the protein sequence is achieved by means of the genetic code[5].

If one were to print one copy of one strand of the haploid human genome, the information encoded in it would fill a text 170 times the size of Harrison's Textbook[3].

This analogy of the human genome to a large text can be carried out further. The text can be envisioned as being bound into 23 paired volumes of various sizes, each the equivalent of one pair of chromosomes. Individuals would inherit one paternal set of 23 volumes and one maternal set of 23 volumes.

This text can also be envisioned as having been copied over and over again for thousands of generations[5].

When one thinks of this endless knowledge of Allah that is encoded in His creation, one would contemplate on this issue, by remembering some of the comprehensive Qur'anic verses, the following are just examples:

"قُل لَّو كَانَ الْبَحْرُ مِدَادًا لِّكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَن تَنفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا "

"Say: if the ocean were ink (where with to write out) the words of my Lord, sooner would the ocean be exhausted than would the words of my Lord, even if we added another ocean like it …"[6].

"وَلَو أَنَّما في الأَرضِ مِن شَجَرَةٍ أَقلامٌ وَالبَحرُ يَمُدُّهُ مِن بَعدِهِ سَبعَةُ أَبحُرٍ مَّا نَفِدَتْ كَلِمَاتُ اللَّهِ ..."

"And if all the trees on earth were pens and the ocean (were ink), with seven oceans behind it, to add to its (supply), yet would not the words of Allah be exhausted (in the writing)…"[7].

The Human Genome Project

The construction of genetic maps of different organisms and plants started as early as 1913, when elucidation of genetic maps of some flies, bacteria, viruses, mice, corn and wheat were accomplished with mapping the locations of genes.

In the mid 1980s, an international effort started, aiming at producing a comprehensive sequencing of the human genetic complement, the double helix of deoxyribonucleic acid (DNA) which contains all of human genes.

The Human Genome Project (HGP) was launched in 1990, by collaboration and funding from the US Department of Energy's Office of Health and Environment Research (DOE), and the National Institute of Health (NIH). Very soon, this major undertaking culminated into an international scientific research project, that included, USA, UK, Japan, France, Germany, Spain and China[8]. The primary goal was to determine the sequence of chemical base pairs which make up human DNA, and to indentify and map the total genes of the human genome, from both physical and functional standpoints[9]. The HGP is the largest collaborative biological project ever launched[10].

Mapping of the human genome involves sequencing multiple variations of each gene[11]. There are approximately 20,500 genes in human beings[12].

The essentially complete genome was announced in April 2003[13]. In May 2006, another milestone was achieved, when the sequence of the last chromosome was published[14]. The accuracy rate of most samplings exceeded 99.99%[15].

The "genome" of any individual is unique.On each chromosome, the nucleotides are arranged in a way that distinguishes each individual from all others since the creation of humans. This unique arrangement is referred to as: the genetic map[16], and later on the term (genetic print) was used.

The sequencing of the (HGP) provided very significant applications and benefits in the understanding of diseases, including:

- Identification of oncogenes and mutations linked to different forms of cancer[17].
- Advancement in forensic applied sciences.
- Genotyping of specific viruses to understand their pathogenicity and to direct appropriate treatment.
- Biofuels and other energy applications, agriculture, livestock breeding, bioprocessing, risk assessment, evolution, anthropology, and bioarcheology.
- Commercial development of genomics research related to DNA –based personalized products.

The work on interpretation of genome data is still in its initial stages. It is anticipated that detailed knowledge of the human genome will provide new avenues for advances in medicine and biotechnology[9].

Deeper understanding of disease processes at the molecular level may determine new therapeutic procedures and advances that may not have been possible without them[17].

The sequence of the DNA is stored in huge databases accessible to anyone on the inter-

net. Computer programs have been developed to analyze that data, which has significantly facilitated proper analysis and interpretation of the data.

Role of genetics in the practice of medicine

Genomic medicine is beginning to change the way medicine is practiced, particularly in regard to interventions for common diseases[18].

Testing for predisposition to disease has the potential of extraordinary benefits, but, it has the potential for harm because it may open the possibility of various forms of discrimination[18].

Genotypic analysis is standard practice for diseases in which a single gene plays a prominent role, but, its role in complex disease traits, where multiple genes and non genetic factors are implicated, is less established. It, nevertheless, has the potential of reducing the burden of these disorders[18].

Genetic Examination, Counseling and Testing

Initially, the field of medical genetics sought to explain the cause of rare diseases with a single gene Mendelian inheritance pattern, such as cystic fibrosis and Huntington disease. Recent discoveries have led to an exponentially increased understanding of causes of many common diseases, in which disease risk often is determined by the combined effects of genetic and non-genetic factors. This applies to adult onset conditions, such as cancer, diabetes, and cardiovascular disease[19].

The hope of genetic screening and testing for heightened genetic risk will allow specifically tailored (personalized) interventions, such as encouraging lifestyle changes, and ultimately preventing disease.

Targeting appropriate individuals for genetic counseling and testing is key to the successful transfer of genetics research to improvement of health and quality of life.

Genetic counseling depends mainly on the primary care physicans[20], proper family history[21], and patients' classification to average, moderate or high risk categories.

Genetic testing can be done for both diagnostic and predictive purposes such as identification of carriers of disease traits, preimplantation gamete testing, prenatal testing, and newborn screening.

The predictive value of a positive genetic test is limited by variable penetrance and expression of various disease entities[22]. Genetic testing of an affected individual is important to facilitate interpretation of testing in other family members.

The long-term management of patients who have an inherited risk of a certain disease may involve aggressive screening at an earlier age, counseling of lifestyle modifications, initiation of pharmacologic or other interventional preventive measures including prophylactic surgery, and management of related psychosocial issues[23,24].

Genetic examination is usually the initial step for most genetic interventions in humans. Genetic examination can also be undertaken for purposes such as contracts of marriage, employment and insurance.

Jurisprudence on genetic counseling and testing

Ethical, legal and psychosocial impacts of genetic testing must be taken in consideration[25].

The issue of genetic counseling and testing is a new one, not tackled by past Muslim jurists. Several seminars, fatwa councils and individual scholars have addressed this issue over the past several decades[26-30].

In the International Islamic Code for Medical and Health Ethics[31], the Islamic juris-

prudence view was outlined in the following account:

- Hereditary (Genetic) Counseling: The (IOMS) seminar has discussed this subject, and it recommends the following:

A.Family genetic counseling services should be made available on a large scale to families and those who plan to get married. These services should be staffed by qualified specialists. In addition, public awareness should be promoted and people should be educated by every possible means to guarantee benefits to all.

B. Genetic counseling should not be compulsory, and its findings should not result in any compulsory measure.

C. The findings of genetic counseling should be kept completely confidential.

D. Services of genetic counseling should be expanded in medical and health institutions, schools, media, and mosques after sufficient training is given to counselors to expand their knowledge and make them qualified.

E. Since statistics show that the marriage of relatives (as permitted in Islamic Law) may result in a higher rate of transmission of congenital anomalies, the public should be made aware of this so that choices would be informed, particularly in the case of families where some members are afflicted with a hereditary disease.

Diseases for which genetic testing must be compulsory, and those where it is optional:

1.The seminar believes that efforts should be made to spread awareness of genetic disorders and diseases and limit their incidence.

2. The seminar calls for the encouragement of genetic tests before marriage through the promotion of greater awareness in auditory and visual media, symposia and mosques.

3. The seminar urges health authorities to increase the number of human genetics units, in order to make physicians available for genetic counseling, and to extend health services in the field of genetic diagnosis and treatment to all pregnant women in order to improve procreative health.

4.No person should be compelled to have a genetic test.

Pre-symptomatic genetic diagnosis and susceptibility testing

Genetic technologies have been proposed as tools for identification of individuals who may harbour pathological variations in genes for a late-onset disease, if they live long enough, such as Huntington disease, Alzheimer disease, heart disease or breast cancer, among many others.

Susceptibility, or predictive testing, may identify risk of future disorders in certain individuals, but it should be noted that these individuals may never develop the disorders[32-34].

The following potential benefits have been cited by proponents of testing[32].

- Testing can be instrumental in prevention of serious disorders, e.g. familial polyposis coli, breast cancer, medullary thyroid carcinoma, and heart disease. Other serious diseases such as Type 2 diabetes, atherosclerosis can be prevented or their incidence significantly decreased by implementing changes in lifestyle or by environmental modifications.
- Individuals could use testing results to better plan for their own lives, their reproductive choices, and for the wellbeing of other family members who may be also at risk or affected.

For proper planning, concerned individuals should be provided proper information related to the inherited disorder in question: its risk, severity and timing.

Availability of support systems, and cultural/religious values should be taken into consideration[32].

On the other hand, susceptibility genetic testing may result in harms, including psychosocial disorders, breaches of confidentiality that result in stigmatization and discrimination in employment, insurance, school and social relationships[33-36].

Guidelines and recommendations have been adopted by WHO to maximize benefits and minimize harms of susceptibility testing[32]:

- Encourage genetic testing in individuals with pertinent family history of the disease in question, e.g. heart disease, cancer and other common preventable or treatable diseases.
- Testing should be voluntary.
- Population testing: It is unethical to screen for disorders that cannot be treated or prevented.

On individual basis, however, this kind of testing should be available for adults who want it, provided confidentiality is observed. Tested individuals should be properly informed about limitations, uses and possible harms of testing.

In the area of prenatal and preimplantationgenetic diagnosis of inherited diseases (and conditions) in fetuses, embryos, or preembryos, there are genuine ethical and religious implications. What are the pathological conditions that justify termination of pregnancy, or justify selection of the preembryo, in IVF centers? If certain conditions (e.g. schizophrenia, depression, rheumatoid arthritis, certain tumors or autoimmune diseases) are proven to be inherited, and are diagnosed that early,could the affected pre-embryos not be implanted, or the affected embryos / fetuses aborted?

The other extremely sensitive and complex question is related to how to keep the privacy and confidentiality of this genetic information. If this issue is not properly and carefully taken care of, subjects with certain "faulty genes" could be stigmatized and discriminated against in employment, insurance, schools and social relationships.

Some other disadvantages are repeatedly cited by human rights defendants. In view of the high cost of genetic screening and testing programs, and their future medical utilizations, it is conceivable that only privileged individuals and societies could benefit from them, thus reflecting on inequitable access to services.

Genetic Engineering

Genetic engineering technological applications have progressed over the past 4 decades, but are still at early stages. Medications, especially hormones, vaccines and other factors have been successfully produced.

Prior to that, gene transplantation was undertaken from one plant to another.

Experimentation on DNA in plants, microorganisms, animals, then in humans, with modifications (engineering) of the genetic code, represented the basis of many applications, including gene therapy.

The basic technological undertaking is introduction of a functional gene to replace, or supplement, the activities of a resident defective gene[37].

Transgenic animal models have been engineered for medical aims, such as organ transplantation.

These new technologies have been instrumental in studying human illnesses, such as diabetes, obesity, cancer, Parkinson's and many others [37,38].

The applications of this technology are still somewhat uncertain in regards to future

hazards to human beings or to other creatures.
The hazards involved in genetic engineering applications in animals, plants and microorganisms, include the absence of scientific controls to guarantee that no genetic tampering that threatens the safety of animals takes place. Some genetically modified animals have an obscure gene that may be hazardous to human and environmental health.
The hazards involved in genetic engineering applications in human beings include technical difficulties and risks of side effects from genomic manipulations, gene delivery tools, utilization in multigene disorders, and more understanding of genes interactions with environment. Another hazard is the potential damage from gene therapy, which is still in the stage of experimentation, which poses the possibility of death or deformity caused by the viruses used in gene transfer or by error in locating a gene on a chromosome of the patient[38,39].

Gene Therapy

Gene therapy is a term applied to therapeutic procedures in which genes are intentionally introduced into human somatic cells. If the gene is inserted in the germline tissue, it can be passed down to that person's descendants.
The goal of gene therapy is to correct the deficiency by introducing a functional gene to replace the patient's defective gene into the appropriate cells[40,41].
Gene therapy has the potential to cure genetically based diseases. The functional gene must be introduced into a sufficient number of cells, and also be adequately expressed for its product to correct the deficiency.

There are two main approaches:

- *In vivo* therapy: in which genes are delivered directly to target cells in the body.
- *Ex vivo* gene therapy, in which the target cells are genetically modified outside the body and then reimplanted[42].

Gene transfer into human cells is not a new concept. Viral genes, for example, are introduced into human cells when a viral vaccine is administered to protect against disease[43].
Technologies in gene therapy involve isolation (cloning) of certain genes, then manipulating them (engineering), followed by transferring them into human cells.
Transfer of therapeutic genes into human cells is accomplished by inserting them into vectors that transport them into the nuclei of target cells[43-45]. Vectors in gene therapy are viral (from which the viral genes are removed to allow insertion of therapeutic genes), or non-viral (synthetic), by condensing of DNA with lipids, proteins, or coated onto micro projectiles.
Inside these target cells, transferred genes are decoded (expressed) to produce therapeutic proteins, and, by doing so, stable genetic modification takes place in cells, tissues and organs in subjects suffering from genetic disease to restore deficient functions.

Problems and limitations in these technologies include:

1.Gene therapy cannot be used for modification of human germline (in testis, ovary, or zygote), in view of worldwide legal, ethical or religious considerations.
It is not allowed in any country.[43]Gene therapy is solely directed to introducing genes into somatic cells.

2.Much of earlier gene therapy research focused on single gene disorders. It has been applied to diseases, such as sickle cell anemia, where vectors have been designed only to deliver healthy genes. There is no way, sofar, to remove or replace defective genes,

in which case their products may continue to produce disease pathology.

3.Current gene delivery systems are incapable of transferring therapeutic genes to most affected cells.

Because of these limitations, research efforts have concentrated on only a few genetic deficiency disorders, such as: cystic fibrosis, hemophilia, and severe combined immune deficiency, in which the target cells are relatively accessible.

Possible future applications of gene therapy include:

- Improved manipulation of human cells and tissues outside the body will greatly facilitate implantation of genes, and other genetically engineered products.
- Application of this new technology could extend to the broader scope of common diseases, such as diabetes, cardiovascular, respiratory, gastrointestinal and neurological disorders.
- Future, more specific and effective vectors may be developed: customized vectors.
- Application of gene therapy in the area of cell and tissues implantation, together with improvements in harvesting, processing, culturing, and genetic modification: will need major resources, and specialized regional gene therapy centers[46].

Gene therapy, which is still in its infancy, has been recently tried in many clinical entities which have been so far, impossible to cure.

It has to be noted, however, that gene therapy is complicated by technical difficulties and significant risks of side effects,that may be lethal, from genetic manipulations[47,48].

Examples of published trials:

(1) X-linked severe combined immune deficiency (SCID), which is caused by mutations in the gene encoding the cytokine receptor subunit gamma-C[49].

From around 30 infants treated in this manner, 90% exhibited engraftment of fully functional mature T cells. Five children, however, developed T cell acute leukemia.

In older children, trials were not as successful[44,45].

The US Food and Drug Administration (FDA) initially prevented all "active gene therapy trials using retroviral vectors to insert genes into blood stem cells". The probable cause of the serious complications was related to the retroviral vectors[50].

Subsequently, such therapy was allowed on a case-by-case basis, if no other treatment was available.

Clinical trials of gene therapy in other conditions, e.g. chronic granulomatous disease (CGD) were not successful, in view of occurrence of insertional activation of cancer-associated genes.

Severe limitations of gene therapy were encountered and formed important obstacles for wider utilization[51].

More advances in gene transduction protocols, and improved viral vectors are needed.

One new promising approach is mutation-targeted therapies, which are at their early stages[52].

(2) Familial hypercholesterolemia which is known to be drug-resistant. Gene therapy has been used in trials to supply a normal LDL receptor gene, in mice and humans[53,54]. This technology is still at an experimental stage.

(3) Gene therapy for cystic fibrosis (CF) began in 1989 with the identification and cloning of the CF trans-membrane conductance regulator (CFTR) gene, the mutations of which account for the disease. Despite early progress, many significant obstacles were encountered. Research is still underway on developing vectors for the safe delivery of

the normal CFTR gene to the airways of affected subjects[55,56].Other obstacles include overcoming host immune responses.

(4) Beta thalassemia:

This condition is caused by mutations of the beta globin locus. There has been some progress in gene therapy through globin gene expression in murine models of beta thalassemia, but it was difficult to achieve significant progress in humans. The donor globin gene must be inserted into the pluripotent hematopoietic stem cells, which requires further research[57].

(5)Duchenne and Becker muscular dystrophy:

These are progressive myopathic disorders, caused by mutations of the dystrophin gene, and inherited as X-linked recessive traits.

Gene therapy, by transferring a functional gene, raised hopes of cure, but the results have not, sofar, been encouraging, due to factors such as problems of cellular immunity[58,59].

(6) Mitochondrial myopathies

Mitochondrial disorders are caused by mutations of genes related to mitochondrial respiratory chain, with wide range of clinical expressions. There are no curative pharmacologic treatments. Genomic research has been ongoing, with many obstacles, including the presence of multiple genomes with multiple mutations. All genetic approaches are, sofar, not standard therapies[60].

(7) In other serious inherited clinical entities, such as: epidermolysis bullosa[61], rheumatoid arthritis[62], and other entities, trials are underway to replace defective genes.They are still at the experimental level, faced with obstacles, which are, more or less similar to the hurdles of gene therapies of the aforementioned entities.

Gene therapy applications in gynecology and obstetrics are detailed elsewhere in this volume.

Genetic Improvement (Enhancement)

Technologies of genetic improvement have been successfully utilized in plants and animals for several decades. Engineering of transgenic plants and animals has been to replace defective genes undertaken for many objectives, including scientific research, production of medical materials, improved productivity, resistance against infections and herbicides, and environmental stresses[63-65].

The complex issue of genetic improvement in humans, aiming at selection of certain features, not necessarily disease-related, to improve physical or intellectual characteristics such as height, color of skin or eyes, and intelligence of certain individuals or races, using the genomic advances, is considered unethical[66-67]

It is looked upon as reminiscent of "Eugenics", a term used several decades ago, to describe the Nazi racial practices.

To the already prevalent unjust, and unbalanced distribution of wealth and social advantages, humanity could face further injustices in genetic qualities!

Interventions in this area are not directed towards medical necessities or considered needs. They only tackle improvement of certain physical or intellectual features,such as height, color of skin or eyes, and intelligence[67].

With these possible applications, humanity may be endangered by stepping into Eugenics[68]. People, because of their financial privileges, will be capable to use costly scientific breakthroughs, to acquire favorable physical or intellectual status, even before birth! This may widen social differences between classes and nations. Privileged people may add the fruits of science, to their already acquired wealth and social advantages.

There is universal understanding that priorities should be carefully set and addressed. The real needs of the wide social classes must be addressed.
Such real and wider needs should never be sacrificed for the sake of narrow classes. Efficient screening, diagnosis and management of genetic disorders, are extremely costly. Proper selection of significant genetic disease entities that benefit the society at large should be regulated and prioritized.
With the possibilities of genetic harm and induction of new unexpected genetic disorders, that may result from the intervention, and taking in consideration there is no necessity or considered need, it is too early to allow these interventions.
Jurisprudence deliberations of this issue were undertaken within the framework of genetic engineering. There is agreement that genetic engineering technologies could be utilized in disease prophylaxis and cure, but not to interfere in, or manipulate non-pathological human features, such as intelligence, character or appearance, height, eye color or other normal heritable features[69,70].
Sometimes, the distinction between cure and enhancement can be difficult to establish[71,72].All genetic undertakings in this regards must comply with *Shari'ah* rulings and controls.

Ethical, Legal and Social Issues[73]

All these issues were raised in regards to how this increased knowledge of the human genome could be used to discriminate against people. The main concerns are related to employers and health insurance companies,that may discriminate against some individuals with possible health concerns indicated by their gene testing[74].
This issue may also play against minorities and ethnic groups on which population-based research is conducted.
Psychological considerations may pose major problems, when healthy individuals discover they have certain susceptibility genes that may, or may not, cause certain disease entities in future years.

Jurisprudence on the Human Genome Project

In its eleventh seminar in 1998, the IOMS[75] ruled that the Human Genome Project is a *Fardh Kifayah* (community obligation). That is, an obligation for which all people are responsible, but it is met if some people undertake it. This is because it is a useful scientific undertaking that helps in curing patients. The IOMS seminar worked out the legal controls for the protection of human rights in the actual performance of human genome tests.
The Eleventh seminar's recommendations included the following:
The Human Genome Project that aims at understanding the entire genetic blueprint of a human being is part of man's effort to know himself, to explore God's laws of creation, and to apply the verse in the Glorious Quran which says:

"سَنُرِيهِمْ آيَاتِنَا فِي الآفَاقِ وَفِي أَنفُسِهِمْ حَتَّى يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُ عَلَى كُلِّ شَيْءٍ شَهِيدٌ "

"We will show them Our signs in the horizons and in themselves"[76], and similar verses.
Since reading the human genome is a means to identify some hereditary diseases and the potential to be afflicted with them, it is a valuable addition to healthcare, medical studies and to disease prevention and treatment. As such, this pursuit is a community obligation.
The first item of the recommendations, which sets general principles, says:
No research, treatment of a human being, or diagnosis related to the genome of a certain person should be undertaken before a very

strict advance assessment of potential risks and benefits associated with such an undertaking is conducted. In the procedure, relevant rulings of Islamic Law must be observed, and voluntary, informed consent must be obtained in advance from the person concerned. When that person does not have the competence to express such consent, the consent or permission of his guardian should be acquired, while the ultimate interest of the person concerned should be sought. When that person does not have the competence to give his consent, no genome of his should be the subject of research, unless that research results in a direct enhancement of his health and the consent of his guardian is obtained.

Every person's right to decide whether he wants to be informed about the findings or consequences of any genetic test should be respected.

All genetic diagnoses, whether filed or intended to be used in research or for any other purpose, should be totally confidential. It can be revealed only in the cases specified at the third IOMS seminar on April 18,1987[77], which dealt with professional confidentiality.

No person should be subject to any form of discrimination which is based on his genetic characteristics and which aims at, or results in, undermining his basic rights, freedom and dignity.

No research or research application related to the human genome, particularly in the fields of biology, genetics, and medicine, should be beyond the scope of the rulings of Islamic Law and of recognition of the human rights endorsed by Islam. Nor should it reduce the basic freedoms or human dignity of any individual or group.

Islamic countries should enter the field of genetic engineering.

The IOMS should form committees concerned with the ethics of medical practice in every Islamic country, as a step towards establishing an Islamic association of medical ethics related to bio-technology.

Genetic engineering:
Overview of Jurisprudence Guidance

Genetic engineering is a new scientific issue (*nazilah*) that was never addressed by past Muslim jurists.

Jurisprudence foundations pertaining to genetics, genetic engineering and its practical human applications are based on the broad guiding principles of medical practice, as perceived by past and contemporary Muslim jurists, namely:

1.The five purposes of *Shari'ah* (*Maqassid al-Shari'ah*)[78]:

- Preservation of religion (*Hifzul-din*).
- Preservation of life (*hifzul-nafs*).
- Preservation of progeny (*hifzul-nasl*).
- Preservation of intellect (*hifzul-a'ql*).
- Preservation of wealth (*hifzul-mal*).

Any medical intervention must fulfill one or more of these purposes.

2.Jurisprudence of seeking remedy[79]: Muslim jurists consider medicine (teaching, training, research and practice) as a *Fardh Kifayah*, that is an obligation, if not properly met by some members of the Muslim *Ummah*, the whole *Ummah* is considered sinful. Its obligation stems from the *Shari'ah* purposes, especially preservation of life, health and intellect. Muslim jurists expressed variable opinions on seeking remedy that range from obligatory, to permissiveness, advice or dislike.

Most juristic evidence points towards permissibility and advice (recommendation-*Istihbab*), rather than obligation[80].

Jurists, however, consider it obligatory in cases where cure is, or most likely, guaranteed as stated by *ImamAl-Baghawi*[81]:

"If cure is known from therapy, that therapy is a must".
Ibn Taymiyyah states: "Some medical treatments may be obligatory (*wajib*), if they result in saving life, that could not be secured by other means"[82].
Qura'nic verses and prophetic *ahadith* have successively confirmed the concept of life and health preservation and this concept comes second to the preservation of religion in the purposes of the Law (*Maqassid Al-Shari'ah).*

Medical interventions, including genetic undertakings, are some of the means by which medical care providers infringe into human body and tissues. General outlines of Islamic Jurisprudence have been discussed and approved in several seminars, councils and *fatawa*[83-86].
The Council of Islamic *Fiqh* Academy-Muslim World League, in its 15th session discussed and approved benefiting from the science of genetic engineering in disease prophylaxis, cure or minimizing harm, provided harm should not exceed benefits[87].
The Council also approved utilization of genetic engineering in the area of agriculture and livestock breeding, provided all due precautions are undertaken to prevent any harm, even long term, to humans, animals or environment[88].
Here is an overview of these guiding principles:

(1) It is permissible to pursue working in the area of genetic engineering provided the aims are to properly utilize these technologies to explore and understand the ways of Allah (*Sunnan*) in His creation, and to utilize them for human benefits in prevention and therapy of illness, and in other perceived benefits of this science[87].
(2) Introduction of human genetic materials into animals or other organisms, aiming at production of medical preparations.

- **A. Jurists allowed using scientific technologies to introduce human genetic material to certain bacteria, with the aim of producing therapeutic materials needed to prevent or treat human diseases.**
- **B. It is permissible to use scientific technologies to introduce human genetic material into animal fertilized ova (zygotes) with the aim of producing milk that contains medical materials needed for prevention or therapy of human disease.**
- **C. It is not permissible to use scientific technologies for purposes rejected by Islamic *Shari'ah*, such as production of distorted creatures (*maskh*), or to cause change of Allah's creation.**

(3) It is permissible to use genetic engineering technologies to introduce healthy genetic materials into the human body in individuals affected with inherited disorders, with the aim of treatment of these disorders, provided *Shari'ah* controls are observed, and provided these procedures do not cause harm that exceeds their benefit.

In this regards, the Council of Islamic *Fiqh* Academy, in its above mentioned deliberations[87], ruled **"It is not permissible to utilize any of the scientific instruments and means of genetic engineering for evil or hostile aims, and in all what are *Shari'ah*-prohibited matters, including futile, useless or harmful manipulations of human characteristics and personal responsibility, or interfering in his genetic constitution, on pretext of improvement of human progeny"[87].**
(4) Aims of genetic engineering must be lawful, such as curing a disease, disfig-

urement, organ transplantation, and other *Shari'ah* -approved aims.

This precaution was stressed in deliberations of the seminar organized by IOMS on Genetics, Genetic Engineering, Human Genome and Gene Therapy held in Kuwait, October, 1998, in which it is stated[85]:
It is not permissible to undertake any research, treatment or diagnosis related to any individual's genetic constitution, unless a strict evaluation is conducted of all possible harms and benefits expected from these undertakings, with full compliance with *Shari'ah* rulings specific for such issues".
(5) Individuals' right to decide their preferences and to be well informed of any genetic testing and its ramifications, must be respected, with observation of confidentiality.
(6) It is not permissible to expose any individual to any kind of discrimination based on his genetic characteristics, with the aim of compromising his basic rights, freedom and dignity.
(7) All experimenting and practical applications of genetic engineering and therapy should be under strict scientific and *Shari'ah* supervision, conducted by a specific *Shari'ah*-scientific forum composed of jurists, and specialist scholars in this vital area, in an effort to prevent mis-utilization of this science for non-permissible objectives, and to safeguard against possible dangers and precautions that may occur from its abuses.

Jurisprudence on gene therapy

Gene therapy could be conducted on germline cells (sperms, ova, and zygotes) or on somatic cells. We will discuss the jurisprudence on each type.

A. In germline cells:

Introduction of foreign genetic material into genital cells will change their structure, and will initiate effects on future generations and produce mixing of lineages which is not permissible[89]. Germline genetic therapy could be for therapeutic purposes or for enhancement. When done for therapy there is possible injury to the existing gene during the procedures and may cause unexpected harm. Islamic jurisprudence considers the process *haram* if the expected benefit is outweighed by the potential harm.

Further, genetic interventions with the aim of improvement (enhancement) of certain human characteristics are also not permissible in view of same reasoning as in therapeutic gene therapy. The non-permissibility here is more significant in view of lack of necessity or considered needs.

The Council of Islamic *Fiqh* Academy-Muslim World League, in its 15th session, issued a ruling to ban genetic enhancement:

"It is not permissible to utilize any of the instruments and means of the science of genetic engineering to manipulate with human personality, his individual responsibilities, or to interfere in his genetic composition on the pretext of improving human offspring".

B.Somatic cells:

Genetic intervention in somatic cells, whether for therapeutic or improvement (enhancement) purposes, is conducted by introducing a healthy gene to substitute a diseased one in somatic cells of a patient afflicted by a genetic disorder, or by introducing a gene that imparts certain human (non-pathologic) body features. This kind of intervention is limited, in its effects, to the recipient's body without transmission to future generations[90].

The intervention could be looked upon as a form of organ transplantation, or even to

blood transfusion which was allowed by jurists, provided it is properly prepared and administered.

Jurisprudence opinion applies on a case-by-case basis. In cases where geneticists have confidence of success that overweighs the harms of a specific gene therapy, it may be considered permissible; otherwise the treatment should not be provided. It is also not permissible to undergo genetic therapy on the basis of experimentation, unless it is performed within the course of well-designed clinical trial.

In practice, however, this type of therapy is still surrounded with doubts in its efficacy, complications and long term effects in causing disease conditions and cancers that have never been previously known. It is too early to consider it an approved standard therapy.

Genetically modified organisms (GMO) in food

Genetic modification (engineering) technologies have developed over the past several decades. Manipulation of DNA and its insertion into the genomes of micro-organisms, plants and animals have been undertaken for scientific, medical, economic and other purposes. DNA can also be synthesized then inserted into the host organism. Genes may be added, removed (knocked out), exons removed and point mutations added[91]. DNA could be injected directly into the host, or into a cell that is then fused (hybridized) with the host[92].

If the genetic material is derived from another species then added to the host, the resulting organism is termed: transgenic.

If the genetic material is derived from the same species, the resulting organism is termed: cisgenic[93].

If the procedure involves removal of genetic material from the organism, it is termed: knockout organism[94].

Polymerase chain reaction (PCR) can be used to amplify up a gene segment to the desired quantities, which can then be isolated and injected into the host organism[95].

An organism that is generated through genetic engineering is considered a genetically modified organism (GMO).

Bacteria were the first organisms to be genetically modified. Plasmid DNA-containing new genes can be inserted into the bacterial cell, which will then express those genes. Medications and vaccines have been produced by this technology[91].

Plants have been genetically modified for insect protection, herbicide and virus resistance, enhanced nutrition, tolerance to environmental stresses and other purposes[1].

Animals have been modified to be used for research, production of pharmaceutical and agricultural products, e.g. cattle with ability to express proteins in large quantities in their milk. Drugs and medicinal proteins have also been produced[91].

Moreover, other forms of genetic modification and gene control mechanisms were introduced by Epigenetic technology of DNA methylation and histone modifications which affect product phenotype without altering their genetic code[96-98].

Genetic engineering technologies in food production continue to stir disputes among scientists, consumers, advocacy groups and biotechnology companies[99].

There is broad scientific consensus that genetically modified (GM) food products pose no greater risk than conventional food[100-102].

A 2013 review of 1783 papers on GM crops and food published between 2002 and 2012, found no plausible evidence of dangers from genetic engineering to humans or animals[103].

The World Health Organization (WHO), the American Medical Association (AMA), the U.S. National Academy of Sciences, the British Royal Society, and other respected organizations, that have examined this issue,

have concluded that consuming GM derived food materials poses no risks more than conventional food consumption.
There is, however, a view from many scientists and regulators, who support GM food, that there is a continuing need for improved testing technologies and protocols to identify and manage risks better[104].
Advocacy groups still have concerns that potential risks of GMOs (including food products) on health and environment have not yet been adequately investigated.
They believe that truly independent research in GM food is systemically blocked by the GM corporations that actually produce it. These corporations own the GM seeds and reference materials[104].
Independence in research has been studied by a 2011 analysis into conflict of interest.The study found a significant correlation between author affiliation to industry and study outcomes in the scientific work published on health risks of GM products[105.] .Most studies were conducted by the biotechnology companies that commercialize the plants[106].
More scientific effort and investigation is needed to ensure that consumption of GM foods is not likely to provoke any form of health problems[107]:

Other concerns:

(1) Gene flow: Genes from a GM organism may pass to another organism, a process called (out crossing). This may produce species of weeds resistant to herbicides[108].
Gene flow may also affect plants, animals and bacteria
(2) Monopolization, ethical issues and intellectual property (patency): Large corporations can manipulate food crops and create unfair control and dependence, especially on developing countries that have to buy seeds every season, and fall under dependence on giant corporations.
(3) Labeling: The controversy is raging between proponents and opponents of labeling of GM food materials. European countries, Australia, New Zealand, China and India require mandatory GM labeling[109,110].
Some other countries advocate voluntary GM labeling, while in the USA no labeling is required[111].
(4) Objectivity of regulatory bodies: Advocacy groups have questioned the attitudes and integrity of regulatory authorities in various countries as being too close to companies that seek approval of GM products, and even suggested that they may have received bribes from such companies.

Islamic perspectives on (GMO):

The Islamic Organization for Medical Sciences (IOMS) addressed this issue in its 11th seminar held in Kuwait in 1998, under the title: Heredity, Genetic Engineering, the Human Genome and Genetic therapy, and published its recommendations in the International Islamic Code for Medical and Health Ethics in 2005[31,90]:

1. Using genetic engineering for human beings is permissible if it is for purposes of disease prevention or therapy, provided that controls are applied to seek benefit, avoid harm, and prevent any confusion of lineage.
2. As for genetic engineering in the case of plants and animals, it is generally permissible, provided that three points are taken into consideration.

The *first point* is a warning against the possibility of long-term diseases that are harmful to human beings and the environment.
The *second* is that it should be declared whether an animal or plant source is natural or produced through genetic engineering, and the genetically engineered percentage

should be pointed out, so that consumers may know the facts.

The third point is that it is advisable to follow the recommendations and resolutions of the United Sates Food and Drug Administration, the World Health Organization, and the UN Food and Agriculture Organization.

The seminar calls for the establishment of consumer protection and awareness organizations in Islamic countries.

The Islamic *Fiqh* Academy-Muslim World League- discussed this issue in its 15th session, held in Makkah al-Mukarramah on 11th of Rajab 1419 H (31st October 1998), and decided:

- **It is permissible to utilize the science of genetic engineering and its means (technologies) in the area of agriculture and animal growing, provided all precautions are undertaken to prevent any harms on humans, animals or environment, including possible long term harms.**
- **The Academy calls upon the companies and factories that produce food and medical materials to show the ingredients of these materials (labeling) to enable information about their utilization and dealings, with precautions towards harms or *Shari'ah* prohibition.**

In conclusion, although the safety of consumption of genetically modified food products has been, to a great extent, accepted sofar, there are considerable concerns of long term and environmental safety. Moreover the issues of patency and monopolization are worrisome.

The scientific as well as the Islamic jurisprudence scholars are called upon to discuss this issue in more depth, taking in consideration that the last jurisprudence discussions were undertaken in 1998.

References

1. Nussbaum RL, Mclnnes RR, Willard HF. Thompson and Thompson Genetics in Medicine, W.B. Saunders, Philadelphia 2007.
2. Strachan T, Read A. Human Molecular Genetics, 4th edition, Garland Science, New York 2010.
3. Baby BA. Principles of molecular genetics, www.uptodate.com/contents/principles-of-molecular-genetics?topicKey=PC%2F...
4. Stenico M, Nasidze IS, De Benedetto G, et al, Mitochondrial DNA sequence in prehistoric human remains from the Alps. European Journal of Human genetics, 8(9), September 2000. PP 669-977.
5. Murphy A, Chu JH, Xu M, et al. Mapping of numerous disease-associated expression polymorphisms in primary peripheral blood CD4+ lymphocytes. Hum Mol Genet 2010; 19:4745.
6. The Glorious Qur'an, Al-Kahf 18: 109.
7. The Glorious Qur'an, Luqman 31: 27.
8. http://www.genome.gov/11006943
9. Human Genome Project. http://en.wikipedia.org/wiki/Human_Genome_Project
10. Economic Impact of the Human Genome Project – Battelle. Retrieved 1 August 2013.
11. Harmon, Katherine (2010-06-28). Genome Sequencing for the Rest of Us. Scientific American. Retrieved 2010-08-13.
12. http://www.genome.gov/12011238
13. Noble, Ivan (2003-04-14). Human genome finally complete. BBC News. Retrieved 2006-07-22.
14. Guardian Unlimited [UK latest] Human Genome Project finalized. The Guardian (London). Archived from the original on October 12, 2007. Retrieved 2006-07-22.
15. Schmutz (2004). Quality assessment of the human genome sequence. Nature 429:365-368. Bibcode: 2004 Natur. 429..365S. doi:10.1038/nature02390. PMID 15164052.
16. Wollmann, H. and F. Berger (2012): Epigenetic reprogramming during plantreproduction and seed development. Current opinion in Plant biology 15(1): 63-69.
17. Snyder M, Du J, Gerstein M (2012). Personal genome sequencing: current approaches and challenges. Genes Dev 24(5): 423-431. doi:10.1101/gad.1864110. PMID 20194435.
18. DeLisi, Charles (2008). Meetings that changed the world: Santa Fe 1986: Human genome babysteps. Nature 455 (7215): 876. Bibcode: 2008Natur. 455….876D. doi:10.1038/455876a.

19. Scheuner MT, Sieverding P, Shekelle PG. Delivery of genomic medicine for common chronic adult diseases: a systematic review. JAMA 2008, 299-1320.
20. Guttmacher AE, Porteous ME, Mclnerney JD. Educating health-care professionals about genetics and genomics. Nat Rev Genet 2007, 8:151.
21. Berg AO, Baird MA, Botkin JR, et al. national Institutes of Health State-of-the-Science Conference Statement: Family History and Improving Health. Ann Intern Med 2009, 151:872.
22. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998, 62:676.
23. Weil J. Psychosocial Genetic counseling, Oxford University Press, New York 2000.
24. Peters JA, Kenen R, Giusti R, et al. Exploratory study of the feasibility and utility of the colored eco-genetic relationship map (CEGRM) in women at high genetic risk of developing breast cancer. Am J Med Genet A 2004, 130A:258.
25. Calyton EW. Ethical, legal and social implications of genomic medicine. N Engl J Med 2003, 349:562.
26. IOMS, 11th Seminar: Genetics, Genetic Engineering, Human Genome and Gene Therapy: Islamic Perspective-Held in Kuwait, 23-25 Jumada Al-Akhirah 1419H, 13-15 October 1998, pp 621-690.
27. Abul-Bassal A.M. Fiqhi Studies of contemporary medical issues (DirasatFiqhiyyah fi QadayaTibbiyyahMuasserah), Dar Al-Nafais Publishing, Amman-Jordan 2001, vol.2, pp687-731.
28. Al-Bar M.A Counseling about genetic disease: An Islamic perspective. Eastern Mediterranean Health Journal, 1999, vol.5, no. 6, pp 1129-1135.
29. Kanaan M.A, The Fiqhi Medical Encyclopedia, 2010, Al-Nafaes publishing, Beirut-Lebanon, pp 891-896.
30. Al-Lodami, T.M. Human Genes and their Applications: A Fiqhi comparative study. A publication of the International Institute of Islamic Though, 2011, pp 251-296.
31. IOMS: The International Islamic Code for Medical and Health Ethics, 2005, pp 406-408.
32. Fletcher JC, Berg K. Wertz DC, Report of Consultations to WHO. World Health Organization, Human Genetics Programmes. WHO/HGN/ETHO/00.4.2003 http://www.who.int/genomics/publications/en/ethical_issuesin_medgenetics%20report.pdf
33. AkosCsaba and Zoltan Papp. Ethical dimensions of genetic counseling. Clinperinatol 30 (2003) pp 81-93.
34. Macer DRJ. Ethics and prenatal diagnosis. In: Milunsky A, editor. Genetic disorders and the fetus: diagnosis, prevention and treatment. Baltimore. John Hopkins University Press, 1998, p.999-1024
35. Joint policy statement. Ethical Issues in assisted reproduction. Journal SocObstetGynecol Can; January-May 1999
36. .Genomics and world health. The Advisory Committee on Health research, World Health Organization – Geneva 2002.
37. Crystal RG: Transfer of genes to humans: Early lessons and obstacles to success. Science 270-404, 1995.
38. Genetic engineering, http://en.wikipedia.org/wiki/Genetic_engineering
39. Tutois 5, Salaun J, Matter MG, et.al.A homozygous lethal insertion in the mouse:Mamm Genome 1991;1:184.
40. Kohn DB. Update on gene therapy for immunodeficiencies. ClinImmunol 2010; 135:247
41. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. Gene therapy for primary immunodeficiencies. Immunol Allergy Clin North Am 2010; 30:237.
42. Rosenberg SA. Aebersold P. Cornetta K, et al. Gene transfer into humans: immunotherapy of patients with advanced melanoma using tumor infiltrating lymphocytes modified by retroviral gene transduction. N Engl J Med 1990" 323:570-8.
43. Russell, SJ, BMJ, vol. 315, 15 November 1997. PP 1289.
44. Sokolic R, Kesserwan C, Candotti F. Recent advances in gene therapy for severe congenital immunodeficiency diseases. CurrOpinHematol 2008; 8:540.
45. Qasim W, Gaspar HB, Thrasher AJ. Update on clinical gene therapy in childhood. Arch Dis Child 2007; 92:1028.
46. Hawkins RE, Russell SJ, Marcus R, et al. A pilot study of idiotypic vaccination for follicular B-cell lymphoma using a genetic approach. Hum Gene Ther 1997; 8:1287-99.
47. Kohn DB. Update on gene therapy for immunodeficiences. Clinical Immunol 2010; 135:247.
48. Bonilla FA, Stiehm ER, TePas E. Gene therapy for primary immunodeficiency. UpToDate, www.uptodate.com@2015.
49. Noguchi M, Yi H, Rosenblatt HM, et al. Interleukin-2 receptor gamma chain mutation results

in X-linked severe combined immunondeficiency in humans. Cell 1993; 73:147.
50. "The Pink Sheet". Gene therapy trial clinical hold will be topic of FDA, NIH meetings, January 20, 2003; 65:7.
51. Rans TS, England R. The evolution of gene therapy in X-linked severe combined immunodeficiency. Ann Allergy Asthma Immunol 2009; 102:357.
52. Hu H, Gatti RA. New approaches to treatment of primary immunodeficiencies: fixing mutations with chemicals. CurrOpin Allergy ClinImmunol 2008; 8:540.
53. Oka K, Pastore L, Kim IH, et al. long-term stable correction of low-density lipoprotein receptor-deficient mice with a helper-dependent adenoviral vector expressing the very low-density lipoprotein receptor. Circulation 2001; 103:1274.
54. Van Craeyveld E, Jacobs F, Gordts SC, DE Geest B. Gene therapy for familial hypercholesterolemia curr Pharm Des 2011; 17:2575.
55. Prickett M, Jain M. Gene therapy in cystic fibrosis. Transl Res 2013; 161:255.
56. .Griesenbach U, Alton EW. Progress in gene and cell therapy for cystic fibrosis lung disease. Curr Pharm Des 2012; 18:642.
57. Cavazzana-Calvo M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature 2010; 467:318.
58. Emery AE. The muscular dystrophies. Lancet 2002; 359:687.
59. Gussoni E, Blau HM, Kunkel LM. The fate of individual myoblasts after transplantation into muscles of DMD patients. Nat med 1997; 3:970.
60. Adhya S, Mahato B, Jash S, et al. Mitochondrial gene therapy: the tortuous path from bench to bedside. Mitochondrion 2011: 11:839.
61. Titeux M, Pendaries V, Hovnanian A. Gene therapy for recessive dystrophic epidermolysisbullosa. Dermatolclin 2010; 28:361.
62. Evans CH, Robbins PD, Ghivizzani SC, et al. clinical trial to assess the safety, feasibility, and efficacy of transferring a potentially anti-arthritc cytokine gene to human joints with rheumatoid arthritis. Hum Gene Ther 1996; 7:1261.
63. http://en.wikipedia.org/wiki/Genetic_engineering
64. The European Parliament and the Council of the European Union (12 March 2001). Directive on the release of genetically modified organisms (GMOs) Directive 2001/18/EC ANNEX I A. Official Journal of the European Communities. P 17.
65. A decade of EU-funded GMO research (2001-2010). Directorate-General for Research and Innovation. Biotechnologies, Agriculture, Food, European Union. 2010. doi:10.2777/97784.ISBN 978-92-79-16344-9.
66. Genomics and world health. The Advocacy Committee on Health research. World Health Organization- Geneva 2002, pp 162.
67. Green RM. Prenatal autonomy and the obligation not to harm one's child genetically. J Law Med Ethics 1997; 25;5-15.
68. Genomics and world health. The Advisory Committee of Health research, World Health Organization- Geneva 2002. Eugenics, pp 162.
69. Council of Islamic Fiqh Academy-Muslim World League, 15th Session, Makkah, 11 Rajab 1419H, 31st October, 1998.
70. IOMS-Seminar on Genetics, Genetic Engineering, Human Genome and Gene Therapy: Islamic Perspectives. Kuwait, 13-15 October, 1998, P 1043-1052.
And published in the IOMS International Islamic Code for Medical and Health Ethics-2005, pp 398-399.
71. Verlinsky Y, Rchisky S, Schooloraft W, strom C, Kaliev A, Preimplantation diagnosis for Fanconi anemia combined with HLA matching. JAMA 2001, 285:3130-3.
72. Green RM. Prenatal autonomy and the obligation not to harm one's child genetically. J Law Med Ethics 1997, 25:5-15.
73. www.en.wikipedia.org/wiki/Human_Genome_Project
74. Greely, Henry (1992). The Code of Codes: Scientific and Social Issues in the Human Genome Project. Cambridge, Massachusetts: Harvard University Press. PP 264-65. ISBN 0-0674-13646-2.
75. IOMS- 11th Seminar: Heredity, Genetic Engineering, The Human Genome, and Genetic Therapy: An Islamic Perspective. Kuwait, October 13-15, 1998, Pp 273-322.
76. The Glorious Qur'an: Fussilat 41:53
77. IOMS, Third seminar: Islamic view on Certain Medical Practices. 18th April, 1987, pp 21-202.
78. Al-Shatibi, Al-Muwafaqat fi usul al-Shari'ah.
79. Council of Islamic Fiqh Academy-OIC, 7th Session, 1993, publisher: Dar al-Qalam-Damascus, 2nd Edition, 1998, p 147.
80. IbnTaimiyah: Al-Fatawa, 24, P 269.
81. Al-Sharwani, In al-Hawashi, Dar al Fikr, Beirut, part 3, p 183.
82. IbnTaimiyah: Al-Fatwa, 24, P 269.
83. Jordan Society for Islamic Medical Sciences-Contemporary Medical Issues in Light of Islamic Jurisprudence, Al-Dostour Publishing, Amman-Jordan, 2000, pp 268-270.

84. B. Kanaan A.M, The Fiqhi-Medical Encyclopedia 2010, Al-Nafaes Publishing, Beirut-Lebanon, pp 891-896.
85. IOMS, Seminal on Genetics, Genetic Engineering, Human Genome and Gene Therapy: Islamic perspective. Kuwait, October 1998. PP 1045-1050.
86. Council of Islamic Fiqh Academy- Muslim World League, The 15th session, held in Makkah Al-Mukarramah, 11-15 Rajab, 1419 H, 31st October- 4th November, 1994.
87. Council of Islamic Fiqh Academy-Muslim World League, 15th session, Makkah, 31st Oct.-4th Nov. 1994.
88. ibid
89. Council of Islamic Fiqh Academy- Muslim World League, 15th Session, Makkah, 15th Rajab 1419 H, 4th November 1998, P313.
90. IOMS, 11th seminar, Heredity, Genetic Engineering, The Human Genome, the Genetic Therapy: An Islamic Perspective Kuwait, 13-15 October, 1998, PP 1043-1052.
91. http://en.wikipedia.org/wiki/Genetic_engineering
92. The European Parliament and the Council of the European Union (12 March 2001). Directive on the release of genetically modified organisms (GMOs) Directive 2001/18/EC ANNEX I A. Official Journal of the European Communities. P 17.
93. Jacobsen, E. Schouten, H. J (2008). "Cisgenesis, a New Tool for Traditional Plant Breeding, Should be exempted from the Regulation on Genetically Modified Organisms in a step by step Approach". Potato Research 51:75-88. Doi:10.1007/s11540-008-9097-y
94. Capecchi, M.R. (2001). "Gnerating mice with targeted mutations". Nature Medicine 7(10):1086-1090. Doi:10.1038/nm1001-1086.PMID 11590420.
95. Kaufman R I and Nixon B T (1996). "Use of PCR to isolate genes encoding sigma54-dependent activators from diverse bacteria". J Bacteriol 178(13): 3967-3970. PMC 2322662. PMID 8682806.
96. Feng S, Jacobsen SE and Riek W. Epigenetic reprogramming in plant and animal development. Science 330; 622-627. doi: 10.1126/science. 1190614.
97. Bird A. (2007). Perception of Epigentics. Nature 447, 396-398. doi:10.1038/nature05913
98. Rodriguez Lopez, CM, and Wilkison MJ. Epi-fingerprinting and epi-interventions for improved crop production and food quality. Frontiers in Plant Science. Review. 05 June 2015. doi:10.3389/fpls.2015.00397. www.frontiersin.org
99. http://en.wikipedia.org/wiki/Genetically_modified_food_controversies
100. American Association for the Advancement of Science (AAAS), Board of Directors (2012). Legally Mandating GM Food Labels Could Mislead and Falsely Alarm Consumers.
101. A decade of EU-funded GMO research (2001-2010) (PDF). Directorate-General for Research and Innovation. Bioechnologies, Agriculture, Food, European Union. 2010. doi:10.2777/97784.ISBN 978-92-79-16344-9.
102. Ronald, (2011). "Plant Genetics, Sustainable Agriculture and Global Food Security". Genetics 188 (1): 11-20.doi.10.1534/genetics.111.128553.PMC 3120150. PMID 21546547.
103. Nicolia A et al. An overview of the last 10 years of genetically engineered crop safety research. Crit Rev Biotechnol, Early Online: 1-12,2013.
104. http://en.wikipedia.org/wiki/Genetically_modified_food_controversies
105. Johan D, Cunha M, Manaia C et al. "Association of financial or professional conflict of interest to research outcomes on health risk or nutritional assessment studies of genetically modified products". Food Policy 36(2):197-203. Doi:10.1016/j.foodpol.2010.11.016
106. Domingo L: GineBordonaba, (2011). "A literature review on the safety assessment of genetically modified plants". Environment International 37(4): 734-42. Doi:10.1016/j.envint.2011.01.003.PMID 21296423.
107. Magana-Gomez, Javier A, De La Barca, Ana M (2009). "Risk assessment of genetically modified crops for nutrition and health". Nutrition Reviews 67(1):1-16. Doi.10.1111/j.1753-4887.2008.00130.x.PMID 19146501.
108. Conner AJ, Glare TR, Nap JP (January 2003). "The release of genetically modified crops into the environment. Part II. Overview of ecological risk assessment". Plant J. 33 (1): 19-46. Doi:10.1046/j.0960-7412.2002.001607.x. PMID 12943539.
109. Scatasta, S, Wesseler, J, Hobbs, J, (2007). "Differentiating the consumer benefits from labeling of GM food products". Agricultural Economics 37(2-3): 237. Doi:10.1111/j.1574-0862.2007.00269.x.
110. O'Connell E, 64 countries around the world label GE food GMO INSIDE. May 13,2013, accessed November 13,2013.

111. Amy Harmon and Andrew Pollack for the New York Times. 24 May 2012 Battle Brewing Over Labeling of Genetically Modified Food.

ROLE OF GENETICS AND GENOMICS IN THE PRACTICE OF MEDICINE

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Abstract

The traditional practice of medical genetics has been limited to the management of rare disorders that are of genetic etiology. The generation of the complete human genomic reference sequence, the cataloging of genetic variations, and the generation of related datasets increased the understanding of the genetic etiology of common disorders. This incorporation of the generated knowledge in the medical practice of rare and common disorders, known as genomic medicine, mandates the education of the health care providers and the public about the available tools and its importance. Genomic medicine is evolving into personalized medicine in which the genetic and genomic information associated with other biologic information aims at maximizing wellness, through prediction, prevention and institution of early treatment, rather than the customary reactive medical practice. In order to implement personalized medicine as a health care system there is a need to develop national public and regulatory policies and establish acceptable standards.

Keywords: Medical Genetics, Medical Genomics, Genomic Medicine, Personalized Medicine, Predictive Medicine, Preventive Medicine, Pharmacogenetics, Pharmacogenomics, Systems Biology, Systems Medicine.

Introduction

Historically, the contribution of genetics to the practice of medicine has been limited to the management of rare genetic disorders within specialist genetic centers, clinics and academic departments. It is now apparent that integrating genetic and genomic knowledge into health care heralds a major change in the mainstream practice of medicine, including primary care[1,2]. Since the initial publications of the draft sequence of the human genome[3-5], substantial progress has been made in the understanding of the etiology of common complex disorders and traits[6].

The diagnosis and treatment of rare, as well as, common diseases are now better understood due to the available genetic and genomic information that has been provided by the Human Genome Project (HGP) and its derivatives, such as transcriptome (RNA), proteome (proteins), epigenome (programing), metabolome (metabolites) and others[7,8]. In addition, genetic and genomic knowledge

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helps in identifying individuals at risk for certain diseases; thus enabling screening, prediction and prevention. Most primary health-care providers have received insufficient genetic education that allows for accepting this major change in approach. Similarly, the patients have not been informed about the added value of their own genetic and genomic information.

Although this manuscript does not address the professional and public educational need in details, it provides an overview of how genetics and genomics are set to improve health-care.

Traditional genetics and rare disorders

Within the nucleus of every cell in the human body lies a full complement of the genetic material of that individual in the form of DNA. The DNA is organized into 20,000 to 25,000 gene pairs, each of which produces one or more proteins that contribute to the cellular and organ functions and structure. The World Health Organization (WHO) defines genetics as the study of heredity, and genomics as the study of genes and their function and related techniques[9]. While genetics examines the function and composition of a single gene, genomics addresses all genes and their inter-relationship in order to identify their combined influence on growth and development of an organism. The genetic material, as well as, the interaction of all genes contained within the genetic complement determines the characteristics of an individual, including those related to health and disease. Although genetic factors contribute to the development of most diseases, the prediction of whether a person will develop a disease or not, its severity or timing is not dependent on a single genetic variant [10]. This does not apply to the rare single gene (Mendelian) disorders, in which a disease-causing mutation will manifest a specific phenotype. It is now apparent that multifactorial disorders, such as diabetes mellitus, hypertension and autism spectrum disorder, result from interactions between a number of predisposing factors, including genotypes at one or more loci and a variety of environmental exposures that trigger, accelerate or exacerbate the disease process.

Medical genetics is a clinical medical specialty that addresses the diagnosis and treatment of conditions that are caused by alteration in the genetic material. A medical genetics service provides genetic counseling and genetic testing to individuals with or at risk for conditions that have a genetic basis such as chromosomal abnormalities (Down syndrome), single gene disorders (cystic fibrosis and Huntington disease), and various multifactorial birth defects (cleft lip and palate).This counseling and testing usually takes place within the context of the family. Cancer is quite common with a considerable percentage of cases being due to predisposing dominantly inherited germ-line mutations[11,12]. The counseling and testing for the hereditary cancer syndromes have been added to the services provided by medical genetics and currently constitutes a considerable proportion of the practice. To mainstream the practice of medical genetics, the American College of Medical Genetics and Genomics issues practice guidelines in a dynamic website that is updated periodically[13].

Reproductive genetics is one of the traditional medical genetics services and it includes premarital genetic testing, prenatal genetic diagnosis, prenatal screening of maternal serum for biomarkers, and preimplantation genetic diagnosis[14].

Genomic medicine

The concept of genomic medicine embraces influencing or basing the clinical care of a patient on the knowledge of the specific genomic variants the patient has[15]. The potential of the integration of the genetic and genomic knowledge into health care and the

development of genomic medicine has been anticipated since the early stages of the HGP[16], but the pace of the clinical application of genomic knowledge has been slow[17]. However, an increasing number of success stories are being reported in which a genome-based diagnosis is made and consequently influenced treatment and produced a favorable outcome. One example is a case in which an accurate and novel genetic diagnosis was made in a male child with intractable inflammatory bowel disease. This was followed by performing an allogeneic hematopoietic progenitor cell transplant which resulted in both preventing the occurrence of hemophagocytic lymphohistiocytosis and in ameliorating the bowel inflammation[18]. Another example is that of using a genomic approach to identify null mutations in *NT5E*, which encodes CD73 enzyme, in patients with lower-extremity arterial calcification and demonstrating a potential successful treatment that is based on rescuing the metabolic pathway involved in ectopic tissue calcification[19]. Clearly there have been failed endeavors in this regards, which unlike success stories are not usually published. Therefore more robust genomic approaches need to be developed to identify the cause of various genetic diseases[20,21].

Genomic discoveries may be leveraged in the expansive field of pharmacogenomics to improve health-care. One example is the increased risk for serious adverse reaction to carbamazepine (Tegretol) in individuals with *HLA-B*1502* and the elimination of that risk with the proper pretreatment genotyping[22]. Another example is the dramatic response patients with metastatic melanoma and the *BRAF* activating somatic mutation, p.V600E, have to the BRAF kinase inhibitor, vemurafenib[23,24]. Similarly, non-small-cell lung cancer can now be treated with specific agents that are chosen based on genotypes analyzed from a panel of genetic variants [25]. The activation of clopidogrel (Plavix), a widely prescribed antiplatelet drug, is dependent on cytochrome P4502C19 and about 30% of individuals carrying specific *CYP2C19* variants are unable to generate the active form and will thus have a diminished response to drug[26,27]. In addition to the clopidogrel experience, there are now known genomic factors that produce variable responses to widely used cardiovascular drugs such as warfarin, heparin, and statins[28].

Disease prognosis is a prominent contribution of genome medicine to health care. Tumor-gene-expression signature models for a variety of cancers are combined with clinical relevant data to predict progress and outcome [29-31]. A 70-gene signature that discriminates breast cancer patient risk for metastatic recurrence within five years was developed at the Netherland Cancer Institute and gained approval by the USA Food and Drug Administration (FDA) in 2007 [32]. The clinical trials that validated this signature model reported accurate prediction[33,34]. Another example, not pertaining to cancer, relates to a specific B-cell lymphocyte signature that identifies kidney transplant recipients who are tolerant to the transplanted kidney and in whom immunosuppression can be discontinued[35].

Reproductive genetics has benefited from the advances in genomic technology in several aspects. Prenatal genetic diagnosis has moved from traditional metaphase karyotyping to microarray based testing (molecular karyotyping) with shorter turn-around time, as there is no need to culture cells and higher sensitivity in detecting chromosomal deletions or duplications (unbalanced)[36]. However, this genomic methodology brought a concern regarding incidental findings, as well as detecting Copy Number Variants (CNVs) of unknown clinical significance [14].In addition, the microarray based testing does not identify balanced chromosomal rearrangements. The detection of cell-free fetal DNA in the maternal blood in 1997 opened a new field of

non-invasive prenatal diagnosis of various genetic conditions[37]. This not only developed into the utilization of genomic technologies to provide non-invasive prenatal diagnosis for specific chromosomal aneuploidies[38], but also into producing whole genome sequencing of the fetus[39].

The wider application of genomic medicine is contingent on the advances in technology that allows providing low-cost, rapid and clinically available tests that produce both interpretable and applicable results. It is also contingent on the education of the primary care health providers about the role genetics and genomics play in their day-to-day practice.

Personalized medicine

This emerging and evolving field is gaining popularity as it advances the use of genomic medicine and widens its application while shifting the health care from being reactive to being predictive and preventive. It involves integrating and coordinating each person's unique clinico-pathological, genetic, genomic and environmental information to createan optimized and evidence-based care plan at every stage of disease, including prediction and prevention[40,41]. It utilizes the molecular understanding of common diseases to enhance prevention of disease during wellness and begin therapy at the earliest stages of disease, if it occurs. The predictive and preventive role of personalized medicine is discussed in the next section. In addition, the characterization and classification of disease based on molecular determinants have been enhanced by genomic technology and its derivative products[41]. This has been applied to multiple disorders both helping in the diagnosis and promoting the understanding of the etiologic mechanisms, such as in childhood epilepsy[42], autism[43], and multiple cancer syndromes[44-47].

The development of personalized medicine into a comprehensive health care system that maximizes wellness rather than just treating disease have been influenced by the convergence of deeper understanding of the system biology and the digital revolution with its ability to generate and analyze large datasets [48]. Systems biology is the study of biological systems as collections of networks at multiple levels ranging from the molecular level, through cells, tissues, organs, organisms, to the population level. Systems medicine is the application of system biology to human disease with the aim of enhancing wellness[49]. The terms "precision medicine" and "P4 medicine (predictive, preventive, personalized and participatory)" are currently used interchangeably to describe the ultimate proactive health care model that can be extended to a population level [50]. What this concept brought is that there is a wealth of knowledge that has been derived from the genomics information, mostly produced through research projects, and that knowledge must be integrated in the health care system for the provision of personalized medicine for the wider population[51]. The application of this knowledge embodies the concept of systems medicine and systems biology and bridges the gap between the science of medicine and the practice of medicine.

Predictive and preventive medicine

To promote the shift of the provision of health care from being reactive to a preventive focus, predictive strategies that assess health risk should be adopted. The family history is a valuable tool for gathering health risk information reflecting on complex combination of shared genetic, environmental and life style factors[52]. This simple tool remains underutilized mainly due to lack of standard collection methods and guidance for interpretation and use specially by primary health care providers[53]. An important component to predictive medicine is the standard health risk assessment that is based on disease model such as in the Framingham

heart study[54], and in the Gail model for breast cancer risk assessment[55]. Clinical decision support systems, now being mostly computerized, provide the physician and patients with guidance and direction for specific health care plan that is based on patient information and thus optimize the utilization of family history and the health risk assessment in disease risk prediction[56].

The incorporation of genomic information into the predictive aspect of personalized medicine has revolutionized the disease risk assessment tool set. The HGP and the completion of the human genome sequence provided derivative datasets, such as the transcriptome, proteome, epigenome, metabolome and others, all could be incorporated in assessing health risks [16,41]. The value of genomic information lies in its stability throughout life that is throughout the continuum of health and disease and can be quantified during health or even at birth[41]. For example, long QT syndrome (LQTS) is a heterogeneous autosomal dominant disease that is associated with mutations in 12 different susceptibility genes[57,58]. Beta-blockers are effective in prevention of the lethal complications of LQTS in patients with mutations in *KCNQ1* but not for those with mutations in *KCNH2* or *SCN5A*[59]. This emphasizes the importance of genetic testing for the disease risk prediction[41]. Another example emphasizes the importance of the identification of women with *BRCA1* or *BRCA2* mutations, which puts them at risk for developing breast and ovarian cancer[60], with the aim of initiation of screening programs or preventive procedures that minimize this risk and maximize wellness[61]. Other genetic factors, also identified from genomic approaches, play extensive roles in prediction of risk for breast cancer development, as well as choice of therapy or preventive measures[62,63].

Newborn screening for metabolic disorders which started more than 50 years ago and have saved newborns whom would have otherwise died or suffered a life-long disability [64]. It aims at identifying disorders prior to the onset of symptoms and providing the necessary treatment that would prevent them from occurring. The technology started with one blood spot for one disorder (phenylketonuria) with the metabolite measured by a bacterial inhibition test and now it includes a screening panel for detection of 29 core disorders and 25 secondary disorders by tandem mass spectrometry[65,66]. Newer technology that may lower the cost and decrease the time, such as microfluidics are emerging with promising results[67]. The utilization of genomic sequence technology in newborn screening is being explored with the promise that it will expand the disorder list[68,69]. The experience form the history of newborn screening exemplifies the predictive, preventive and personalized nature of genomic medicine and shows that the testing is only a part of health care system [66]. It demonstrated that the posttest counseling and the clinical follow-up are of substantial importance.

Integration of genetics and genomics into clinical practice

The rapid advance in genomic and computational technologies led to an exponential accumulation of genomic data from research platforms and exposed the gaps in the infrastructure needed for its implementation into clinical practice. There is a paucity in the standardization and the translation of specific genomic findings into clinical tests[70]. It is also clear that physicians feel unprepared to deliver genomic information to patients, and in turn patients do not appreciate the value of this information[71].

One barrier to the integration of genomic information into clinical practice is the deficiency in genetics knowledge amongst the average health care provider[72-75]. It is imperative that medical education, especially

Continued Medical Education (CME), is enriched with genetics and genomics knowledge to advance the field[76]. The implementation of clinical decision support systems that provide guidance to the busy physicians improves patient care and constitutes a significant venue for incorporating genetics and genomics data into the practice[77].

International standardization of genetic testing, genomics-based pharmaceutical products and genomic-based research and clinical tools are of paramount importance for the integration of genetics and genomics knowledge into clinical practice. Adoption of these standards at a national level is usually incorporated into public and regulatory policies, albeit with some adaptation to the acceptable cultural norms.

Conclusions

The practice of medical genetics has been restricted to managing rare disorders within specialized clinical settings. Since the completion of the HGP, a revolutionary change in the practice of medical genetics took place through the incorporation of genomic knowledge. The genetic and genomic knowledge are changing the mainstream practice of medicine, including primary care, and this change is forming the basis for genomic medicine. Subsequently, genomic medicine is evolving into personalized medicine where the genetic and genomic knowledge, associated with other biologic determinants will maximize wellness, through prediction, prevention and early treatment. To utilize the conceptual advantage of incorporating the personalized medicine in the health care system, it is necessary to educate the health care providers and the public about the importance of the genetic and genomic knowledge. It is also advisable to encourage the legislative bodies in the different countries to develop public and regulatory policies and to establish acceptable standards to be able to harness the benefits of this system.

References

1. Lango H, Weedon MN (2008) What will whole genome searches for susceptibility genes for common complex disease offer to clinical practice? J Intern Med 263:16-27.
2. Pearson H (2008) Genetic testing for everyone. Nature 453:570-571.
3. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, et al (2001) The sequence of the human genome. Science 291:1304-1351.
4. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, et al (2001) Initial sequencing and analysis of the human genome. Nature 409:860-921.
5. International Human Genome Sequencing Consortium (2004) Finishing the euchromatic sequence of the human genome. Nature 431:931-945.
6. Altshuler D, Daly MJ, Lander ES (2008) Genetic mapping in human disease. Science 322:881-888.
7. Burke W (2004) Genetic testing in primary care. Annu Rev Genomics Hum Genet 5:1-14
8. Samani NJ, Tomaszewski M, Schunkert H (2010) The personal genome--the future of personalised medicine? Lancet (London, England) 375:1497-1498.
9. WHO Human Genetics Programme; WHO Definitions of Genetics and Genomics. http://www.who.int/genomics/geneticsVSgenomics/en/.
10. Hall WD, Mathews R, Morley KI (2010) Being more realistic about the public health impact of genomic medicine. PLoS Med 7.
11. Bonadona V, Sinilnikova OM, Chopin S, Antoniou AC, Mignotte H, Mathevet P, Brémond A, Martin A, BobinJY, Romestaing P, et al (2005) Contribution of BRCA1 and BRCA2 germ-line mutations to the incidence of breast cancer in young women: results from a prospective population-based study in France. Genes Chromosomes Cancer 43:404-413.
12. Thompson D, Seal S, Schutte M, McGuffog L, Barfoot R, Renwick A, Eeles R, Sodha N, Houlston R, Shanley S, et al (2006) A multicenter study of cancer incidence in CHEK21100delC mutation carriers. Cancer Epidemiol Biomarkers Prev 15:2542-2545.
13. ACMG The American College of Medical Genetics and Genomics Practice guidelines. https://www.acmg.net/ACMG/Publications/Practice_Guidelines/ACMG/Publications/Practice_Guidelines.aspx?hkey=b5e361a3-65b1-40ae-bb3e-4254fce9453a.
14. Bianchi DW (2012) From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges. Nat Med 18:1041-1051.

15. Varmus H (2010) Ten years on--the human genome and medicine. N Engl J Med 362:2028-2029.
16. Collins FS (1999) The human genome project and the future of medicine. Ann N Y AcadSci 882:42-55; discussion 56.
17. Green ED, Guyer MS, National Human Genome Research Institute (2011) Charting a course for genomic medicine from base pairs to bedside. Nature 470:204-213.
18. WortheyEA, Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B, SerpeJM, Dasu T, TschannenMR, VeithRL, et al (2011) Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med 13:255-262.
19. St Hilaire C, Ziegler SG, MarkelloTC, Brusco A, Groden C, Gill F, Carlson-Donohoe H, Lederman RJ, Chen MY, Yang D, et al (2011) NT5E mutations and arterial calcifications. N Engl J Med 364:432-442.
20. Jiang YH, Yuen RKC, Jin X, Wang M, Chen N, Wu X, Ju J, Mei J, Shi Y, He M, et al (2013) Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. Am J Hum Genet 93:249-263.
21. Yuen RKC, Thiruvahindrapuram B, Merico D, Walker S, Tammimies K, Hoang N, Chrysler C, Nalpathamkalam T, Pellecchia G, Liu Y, et al (2015) Whole-genome sequencing of quartet families with autism spectrum disorder. Nat Med 21:185-191.
22. Wilke RA, Dolan ME (2011) Genetics and variable drug response. JAMA 306:306-307.
23. Chapman PB, Hauschild A, Robert C, HaanenJB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, et al (2011) Improved survival with vemurafenib in melanoma with BRAFV600E mutation. N Engl J Med 364:2507-2516.
24. Puzanov I, AmaravadiRK, McArthur GA, Flaherty KT, Chapman PB, Sosman JA, Ribas A, Shackleton M, Hwu P, Chmielowski B, et al (2015) Long-term outcome in BRAF(V600E) melanoma patients treated with vemurafenib: Patterns of disease progression and clinical management of limited progression. Eur J Cancer 51:1435-1443.
25. Mirnezami R, Nicholson J, Darzi A (2012) Preparing for precision medicine. N Engl J Med 366:489-491.
26. RodenDM, Shuldiner AR (2010) Responding to the clopidogrel warning by the US food and drug administration: real life is complicated. Circulation 122:445-448.
27. Scott SA, Sangkuhl K, Gardner EE, Stein CM, HulotJS, Johnson JA, RodenDM, Klein TE, Shuldiner AR, Clinical Pharmacogenetics Implementation Consortium, et al (2011) Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. ClinPharmacolTher 90:328-332.
28. RodenDM (2015) Cardiovascular pharmacogenomics: current status and future directions. J Hum Genet.
29. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, SmelandEB, GiltnaneJM, et al (2002) The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 346:1937-1947.
30. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, Gupta S, Moore J, WrobelMJ, Lerner J, et al (2008) Gene expression in fixed tissues and outcome in hepatocellular carcinoma. N Engl J Med 359:1995-2004.
31. Parker JS, Mullins M, CheangMCU, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, et al (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. J ClinOncol 27:1160-1167.
32. van de VijverMJ, He YD, van't Veer LJ, Dai H, Hart AAM, VoskuilDW, Schreiber GJ, PeterseJL, Roberts C, MartonMJ, et al (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999-2009.
33. Buyse M, Loi S, van't Veer L, Viale G, Delorenzi M, Glas AM, d'Assignies MS, Bergh J, Lidereau R, Ellis P, et al (2006) Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. J Natl Cancer Inst 98:1183-1192.
34. Glas AM, Floore A, DelahayeLJMJ, Witteveen AT, PoverRCF, Bakx N, Lahti-Domenici JST, BruinsmaTJ, Warmoes MO, Bernards R, et al (2006) Converting a breast cancer microarray signature into a high-throughput diagnostic test. BMC Genomics 7:278.
35. Newell KA, Asare A, Kirk AD, Gisler TD, Bourcier K, Suthanthiran M, BurlinghamWJ, Marks WH, Sanz I, Lechler RI, et al (2010) Identification of a B cell signature associated with renal transplant tolerance in humans. J Clin Invest 120:1836-1847.
36. Friedman JM (2009) High-resolution array genomic hybridization in prenatal diagnosis. PrenatDiagn 29:20-28
37. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, WainscoatJS (1997) Presence of fetal DNA in maternal plasma and serum. Lancet (London, England) 350:485-487.
38. Lo KK, Boustred C, Chitty LS, Plagnol V (2014) RAPIDR: an analysis package for non-invasive prenatal testing of aneuploidy. Bioinformatics 30:2965-2967.
39. Lo YMD (2013) Non-invasive prenatal testing using massively parallel sequencing of maternal plasma DNA: from molecular karyotyping to fetal whole-genome sequencing. Reprod Biomed Online 27:593-598.

40. Hamburg MA, Collins FS (2010) The path to

personalized medicine. N Engl J Med 363:301-304.
41. Chan IS, Ginsburg GS (2011) Personalized medicine: progress and promise. Annu Rev Genomics Hum Genet 12:217-244.
42. Epi4K Consortium (2012) Epi4K: gene discovery in 4,000 genomes. Epilepsia 53:1457-1467.
43. Betancur C (2011) Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. Brain Res 1380:42-77.
44. Garber K (2005) Human Cancer Genome Project moving forward despite some doubts in community. J Natl Cancer Inst 97:1322-1324.
45. Cancer Genome Atlas Research Network (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455:1061-1068.
46. VerhaakRGW, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, et al (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17:98-110.
47. Cancer Genome Atlas Research Network, Brat DJ, VerhaakRGW, AldapeKD, Yung WKA, Salama SR, Cooper LAD, Rheinbay E, Miller CR, Vitucci M, et al (2015) Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. N Engl J Med 372:2481-2498.
48. Hood L, Flores M (2012) A personal view on systems medicine and the emergence of proactive P4 medicine: predictive, preventive, personalized and participatory. New biotechnology.
49. Hood L, Heath JR, Phelps ME, Lin B (2004) Systems biology and new technologies enable predictive and preventative medicine. Science 306:640-643.
50. KhouryMJ, Gwinn ML, Glasgow RE, Kramer BS (2012) A population approach to precision medicine. Am J Prev Med 42:639-645.
51. Chakravarti A (2011) Genomics is not enough. Science 334:15.
52. Guttmacher AE, Collins FS, Carmona RH (2004) The family history--more important than ever. N Engl J Med 351:2333-2336.
53. Rich EC, Burke W, Heaton CJ, Haga S, Pinsky L, Short MP, Acheson L (2004) Reconsidering the family history in primary care. Journal of general internal medicine 19:273-280.
54. Kannel WB, DawberTR, Kagan A, Revotskie N, Stokes J (1961) Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. Ann Intern Med 55:33-50.
55. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, MulvihillJJ (1989) Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst 81:1879-1886.
56. Osheroff JA, TeichJM, Middleton B, Steen EB, Wright A, Detmer DE (2007) A roadmap for national action on clinical decision support. J Am Med Inform Assoc 14:141-145.
57. Hedley PL, Jørgensen P, Schlamowitz S, Wangari R, Moolman-Smook J, Brink PA, KantersJK, Corfield VA, Christiansen M (2009) The genetic basis of long QT and short QT syndromes: a mutation update. Hum Mutat 30:1486-1511.
58. Tester DJ, Ackerman MJ (2011) Genetic testing for potentially lethal, highly treatable inherited cardiomyopathies/channelopathies in clinical practice. Circulation 123:1021-1037
59. Shimizu W (2008) Clinical impact of genetic studies in lethal inherited cardiac arrhythmias. Circ J 72:1926-1936.
60. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M (2010) Genetic susceptibility to breast cancer. Molecular oncology 4:174-191.
61. Trainer AH, Lewis CR, Tucker K, Meiser B, Friedlander M, Ward RL (2010) The role of BRCA mutation testing in determining breast cancer therapy. Nature reviews. Clinical oncology 7:708-717.
62. Trainer AH, Thompson E, James PA (2011) BRCA and beyond: a genome-first approach to familial breast cancer risk assessment. Discov Med 12:433-443.
63. Mavaddat N, PharoahPDP, Michailidou K, Tyrer J, Brook MN, Bolla MK, Wang Q, Dennis J, Dunning AM, Shah M, et al (2015) Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst 107.
64. Therrell BL, Adams J (2007) Newborn screening in North America. J Inherit Metab Dis 30:447-465.
65. Therrell BL, Buechner C (2008) Newborn screening for all identifiable disorders with tandem mass spectrometry is cost effective: supporting arguments. Ann Acad Med Singapore 37:32-34.
66. McCabe LL, McCabe ERB (2008) Expanded newborn screening: implications for genomic medicine. Annu Rev Med 59:163-175.
67. Millington DS, Sista R, Eckhardt A, Rouse J, Bali D, Goldberg R, Cotten M, Buckley R, Pamula V (2010) Digital microfluidics: a future technology in the newborn screening laboratory? SeminPerinatol 34:163-169.
68. Landau YE, Lichter-Konecki U, Levy HL (2014) Genomics in newborn screening. J Pediatr 164:14-19.
69. Baker MW, Atkins AE, Cordovado SK, Hendrix M, Earley MC, Farrell PM (2015) Improving newborn screening for cystic fibrosis using next-generation sequencing technology: a technical feasibility study. Genet Med .
70. Douglas PS, Ginsburg GS (2008) Clinical genomic testing: getting it right. Journal of cardiovascular translational research 1:17-20.

71. Scheuner MT, Sieverding P, Shekelle PG (2008) Delivery of genomic medicine for common chronic adult diseases: a systematic review. JAMA 299:1320-1334.
72. LaphamEV, Kozma C, Weiss JO, BenkendorfJL, Wilson MA (2000) The gap between practice and genetics education of health professionals: HuGEM survey results. Genet Med 2:226-231.
73. Wilkins-Haug L, Hill LD, Power ML, Holzman GB, Schulkin J (2000) Gynecologists' training, knowledge, and experiences in genetics: a survey. ObstetGynecol 95:421-424.
74. Metcalfe S, Hurworth R, Newstead J, Robins R (2002) Needs assessment study of genetics education for general practitioners in Australia. Genet Med 4:71-77.
75. Bancroft EK (2013) How advances in genomics are changing patient care. NursClin North Am 48:557-569.
76. Frueh FW, Gurwitz D (2004) From pharmacogenetics to personalized medicine: a vital need for educating health professionals and the community. Pharmacogenomics 5:571-579.
77. Kawamoto K, Lobach DF, Willard HF, Ginsburg GS (2009) A national clinical decision support infrastructure to enable the widespread and consistent practice of genomic and personalized medicine. BMC Med Inform DecisMak 9:17.

PHARMACOGENOMICS: A BRIEF OVERVIEW

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Abstract

The Human Genome Project (HGP) has irrevocably changed medicine and medical research. The wealth of information gained from the HGP, in parallel with advances in technology, flowed into various new disciplines, among which is pharmacogenomics. Pharmacogenomics is the scientific field that attempts to search for the genetic basis for inter-individual variability in drug response. Recognition of this inter-individual difference in drug response is an essential step towards optimizing therapy.

While recognising that other factors such as age, health status and concurrent therapy play important roles in variability of drug response, individualizing drug therapy with the use of pharmacogenomics has the potential of providing the means for prescribers to identify patients for whom the drugs will be both effective and safe.

Pharmacogenomics has been successfully applied in personalized medicine and some examples of these in clinical practice will be presented. Some examples will also be presented of studies that have been carried out in the Malaysian setting that could contribute to the database for future application of pharmacogenomics in this region.

Keywords: Pharmacogenomics, Genomics, drug response

Background

The Human Genome Project, which started in the early 1990s and took 13 years to complete, had involved more than 1,100 biologists, computer scientists, analysts and others with various expertises at university laboratories in six different countries, and had resulted in the sequencing of the human genome. A peek at the amazing wealth of information that has come along with this decoding of the genome is described by Ferguson[1] in simple words as below:

"There are three billion letters in the Human Genome.
Written out, the Human Genome would stretch 5,592 miles, (9,000 km).
It would take a typist working eight hours a day half a century to type.
It would fill one million pages; 5,000 books stacked 200 feet high; or two hundred telephone directories.
Read out for 24 hours a day, it would take a century to finish.
The human body has 100 trillion (100 000 000 000 000) cells – each contains a copy of the entire Genome ----."

This kind of simple description serves to spark the imagination of many, as to the complex yet marvellously organised machinery within us. The Human Genome Project was hugely significant to biology. It has influenced biological research ever since.

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At the macro level, it is easy to understand the awe with which we view the vastness and the complete order of this universe, with its estimated 100 billion galaxies and an estimated 200 billion stars in each, but scientists now realise that the human body, in its complexity and amazing design is a universe in itself. In the Glorious Quran[2], it is stated that:
"*We will show them Our signs in the universe and within their own beings, until it will become manifest to them that it is the truth*."
Ibrahim Syed[3], Clinical Professor of Medicine at the University of Louisville, eloquently described a part of this "universe" within us, as follows **"---- the human brain has 100 billion neurons, with untold trillions of connections and patterns of endless wiring sequences. We are unaware of what goes on in our cells as our genome tells our cells to assemble amino acids into proteins to make cell walls, and cell walls to split and divide and human beings are unaware of the constant stream of virtual miracles that keep human beings alive, alert and functioning. How did such an astonishingly complex process begin? How did the billions of atoms in each DNA molecule arrange themselves perfectly for the self-perpetuation or what we call life? How did cells, DNA and chromosomes come about? Some argue that the greatest scientific proof that human beings were designed by a higher Power is this: The process of one genome creating a living, self-perpetuating organism cannot happen over time. It has to be right the first time, and it must entail literally billions of designed elements that must be in place and functioning perfectly, or else the cell cannot exist and reproduce. The self-replicating cell exists only because its inherent intelligent systems- each involving billions of functions- interact perfectly. Otherwise it is dead. The chromosomes and cells are extremely complex and beyond imagination that some scholars argue that they could never have evolved through random processes from nothing, even if given the endless time spans evolutionists require for their theory. Evolutionists are unable to explain, for example, how and why heart tissue, liver tissue, skin and blood are distinctly different and have dramatically different functions despite the fact that, surprisingly, each cell of these different organs contains the same DNA. A liver cell's DNA is identical to a brain cell's DNA. Still the mystery is how each cell knows its identity, function and position in the body."**

This glimpse of the complexity of the DNA, and of the process involved in the perpetuation of life is necessary in order to appreciate the complexity that comes with the new knowledge following the Human Genome Project (HGP). In a transcript[4] of an interview with Dr. Francis Collins, Director of the HGP at the National Institutes of Health, USA, carried out in 2001, about two years before completion of the project, Dr Collins stated that **"For me, as a person who believes in a personal God, the opportunity to uncover something about us that nobody knew before but God knew is really a moment not to be missed. It expands the experience of discovery. It's an opportunity both for scientific exhilaration and actually for worship".** Indeed, in line with this, the Glorious Quran, also invites us to look within ourselves as a way to strengthen faith, and has this to say[5]:
"Do they (that is, humans), not think deeply (in their ownselves) about themselves".

In looking into ourselves, we realise the beauty of His creation and cannot help but be in awe of the intricate physiological miracles that have enabled our body to function. Hence, even when we reflect upon the humble process of how the body removes unwanted water through urination, we cannot also help but be grateful for His blessings upon us.
As we reflect on these verses and realize the intricate design of the human body we will be more at awe when we study one of the sciences that have emerged in a big way following the unravelling of the human code through the HGP, namely, the science of pharmacogenomics.

Pharmacogenomics

The field of pharmacogenetics was introduced more than 40 years ago to emphasize the role of

heredity in person-to-person differences in drug response[6,7]. The focus of pharmacogenetic investigations in the early years was the unusual and extreme drug responses resulting from an effect from single gene. On the other hand, pharmacogenomic studies encompass the sum of all genes, that is, the genome. In general, numerous genes play a role in determining drug response and toxicity, introducing a daunting level of complexity into the search for the right candidate genes. The specificity and high speed associated with newly emerging genomic technologies enable the search for relevant genes and their variants to include the entire genome. These new technologies have essentially spawned this new discipline of pharmaco-genomics, which seeks to identify the variant genes affecting the response to drugs in individual patients.

Since the landmark completion of the HGP, and with the development of newer and more refined technologies, there have been major advances in our understanding of genomics as a whole. This genomic revolution continued within the next important phase, which is, understanding the meaning of all the information in the sequenced DNA; in other words, understanding the function of the proteins in the genomes. This new phase involves a number of new genomics areas, and pharmacogenomics is one of them.

Pharmacogenomics is a discipline which seeks to apply knowledge, technologies and processes, from the field of genomics, to improve the efficacy and safety of drugs. It combines traditional pharmacology with an understanding of variations in the human genome. Two types of variations are common in the human genome, namely, (a) single nucleotide polymorphisms (SNPs), which is a change in a single nucleotide base (adenine, cytosine, guanine and thymine) of the DNA, and (b) structural variation in which changes affect portions of DNA which can consequently alter the structure of the entire chromosome. Structural variation can occur in a number of ways, for example, copy number variation (CNV) in which there is either an increase or a descrease in the amount of DNA, which can be due to deletion (an entire block of DNA is missing), insertion (a block of DNA is added in) or duplication (additional copies of a portion of the DNA).

It is estimated that there are approximately 11 million SNPs in the human population, with an average of one every 1,300 base pairs. An individual's response to a drug is often linked to these common DNA variations. In a similar manner, susceptibility to certain diseases is also influenced by common DNA variations. Currently, much of the research in the field of pharmacogenomics is focused on genes encoding either metabolic enzymes that can alter the activity of a drug or defective structural proteins that result in increased susceptibility to disease.

Pharmacogenomics has the potential to lead to personalized medicine, which is the use of information and data from the patient's genotype, or level of gene expression to select a medication, provide a therapy, or initiate a preventative measure that is particularly suited to that patient at the time of administration. This concept has been high-lighted as "therapy with the right drug at the right dose in the right patient"[8]. Its urgency emerged in a survey of studies on adverse drug effects in hospitalized patients: adverse drug reactions may rank as the fifth leading cause of death in the United States[9]. Thus, as anticipated, pharmacogenomics is slowly playing an integral role in disease assessment, drug discovery and development, and selection of the type of drug, thus potentially improving treatment outcomes. Moreover, it may provide information useful to the selection of dosage regimen for an individual patient. It has been said that pharmacogenomics may be one of the most immediate clinical applications of HGP.

It is a fact that humans share about 99 per cent of their genomes.The remaining one per cent or so, that differs between each of us, explains variation in physical characteristics such as eye color, body height, and in the context of genomics, also to inter-individual variations in susceptibility to diseases and response to drugs. This one per cent may not sound like much, but it does mean that there are still millions of differences between the DNA of two

individuals.Hence, even considering variations due only to single nucleotide polymorphisms (SNPs), it is difficult to work out which variation in the DNA causes a particular disease and which ones have no effect on health.

Practical Applications Of Pharmacogenomics in Clinical Practice

Pharmacogenomics has been applied in personalized medicine in the case of warfarin[6]. It is estimated that the new prescriptions for warfarin every year is between 300,000 and 500,000, and that on a daily basis, between 2 million and 5 million people are taking the drug[10]. The use of warfarin has, however, long been associated with risk of adverse bleeding events. A study showed that over 2,000 bleeding events were reported during a 30-month period, of which more than 80% resulted in hospitalization, disability, life-threatening sequelae and/or death[11,12]. Furthermore, warfarin was ranked in the top ten of all drugs with serious adverse events over a 5 year period (2000 to 2005) with more than 6000 reported cases, and accounts for 3.6% of all drug-induced adverse events and 15.1% of all severe drug-induced adverse events. It is with this background, that FDA, in August 2008, updated the labeling for warfarin, to include genetic testing information, stating that the information can help physicians determine the safest starting dose for their patients. The agency then approved a genetic test that is capable of determining patients' variations in two genes, CYP2C9, the enzyme primarily responsible for warfarin metabolism, and VKORC1 gene, the site of action for warfarin. The goal of the warfarin genetic test is to decrease the time it takes to titrate the dose to an effective level while minimizing the risk of bleeding events.

Warfarin was not the first drug for which pharmacogenomic information was included in prescription drug labeling. Herceptin is a genetically specific drug developed earlier, and indicated for the treatment of a type of breast cancer which is usually aggressive, and with high risk of relapse and even death[13]. This drug is only effective in women with a genetic defect which results in the overproduction of the HER2neu receptor which will be present in excessive numbers on the surface of certain breast cells. The HER2neu receptors promote cellular growth which leads to breast tumors. Herceptin is a monoclonal antibody directed against the HER2neu receptor and therefore only helps women who have an increased number of copies of the relevant HER2neu gene, while in all other women, this highly specific drug is much less effective. Herceptin can therefore be used only in conjunction with a genetic test which measures HER2neu overexpression. A positive result predicts response to the drug whereas a negative result redirects therapy elsewhere.

Genetic testing can also dramatically reduce the number of people who are affected by adverse drug effects. Abacavir is a drug that is used with other antiretrovirals in the treatment of human immune-deficiency virus (HIV) infection[14]. It is highly effective for the mentioned indication but approximately five to eight per cent of patients are adversely affected, resulting in rash, fatigue and diarrhea, suggesting that the affected patients are hypersensitive to this medication. In 2002, two groups identified a particular gene variant, called HLA-B*5701, as being the key factor in hypersensitivity to abacavir[15]. Individuals with this allele were found to be more likely to have a hypersensitivity reaction to it. The HLA-B*5701 allele occurs at a frequency of around five per cent in European populations, one per cent in Asian populations and less than one per cent in the African populations. Clinical trials later indicated that screening patients for HLA-B*5701 before treatment has dramatically reduced the number of side effects experienced from abacavir use. In individuals found to have the HLA-B*5701 allele, abacavir is avoided, and alternative HIV treatments are given. The test is now a routine part of clinical practice in certain parts of the world[15,16].

There are now numerous other examples of how the power of genetics, molecular biology, chemistry, and other advanced tools can be harnessed to transform information about molecular mechanisms and targets into therapies directed against diseases, thereby creating methods for preventative care, diagnostics, and

ultimately personalized medicine. Today, the USA Food and Drug Administration (FDA) requires pharmacogenomics information to appear on the labels of hundreds of medications that are currently on the market, whereas before the Human Genome Project, only four drugs carried such a label.

Studies in a Malaysian population

Presently, genomic technologies are being extensively used to identify variants for a specific disease, as a possible target for drug therapy. This is done by comparing the genetic makeup of a large number of people with that specific disease with those who are not afflicted with the disease. This allows the identification of genetic variants that are more common in people with a particular disease as compared to people without it. Hence, if a particular genetic variant is present in 85 per cent of patients with the disease and in only 15 per cent of the healthy population, this is an indicator that this variant is likely associated with risk of that disease. Searching for variants in a single gene that may be associated with a particular disease will not be as complicated as trying to identify variants in many different genes that might be involved in a complex disease such as obesity or metabolic syndrome. In order for this type of comparison to be effective, large sample size is needed, usually in the tens of thousands, to find the variants with subtle effects on disease risk. This approach was used in our laboratory, to study non-alcoholic fatty liver disease (NAFLD), which is the commonest cause of chronic liver disease in many countries and has emerged as an important global health problem, in parallel with increased incidence of obesity. The reported prevalence of NAFLD in the general population is as high as 35%. NAFLD comprises a wide spectrum of derangements, ranging from simple steatosis (presence of fat in the liver) to non-alcoholic steatohepatitis (NASH – presence of fat in the liver, with inflammation and/or fibrosis) and cirrhosis. Simple steatosis is, in general, a benign condition whereas NASH can potentially progress to serious liver complications, including liver cancer. Genetic factors have been shown to play a significant role in the pathogenesis of NAFLD and may be responsible for the different phenotypes among individuals, including predisposition to severe liver disease. Genomic variability in the case of NAFLD may present in various forms such as single nucleotide polymorphisms (SNPs), or structural alterations (deletion, duplication and inversion), however, the commonest is the SNP. Copy number variation (CNV) is another type of mutation that could serve as a genetic marker for the spectrum of NAFLD. The general aim of the NAFLD studies was to investigate the association between SNPs of candidate genes with susceptibility to NAFLD and with severity of the underlying liver disease[17-27]. This study further aimed to identify genomic amplifications and deletions (CNVs) in the spectrum of NAFLD. One hundred and forty-four biopsy-proven NAFLD patients and 198 controls without NAFLD were genotyped for polymorphisms of various candidate genes. Whole-genome array comparative genomic hybridization (aCGH) method was used to detect CNVs in a total of 40 patients with NASH and 40 other age, gender, and ethnicity-matched controls. Polymorphisms of the genes *PNPLA3* and *LEPR* render susceptibility to NAFLD (OR 2.23, 95% CI 1.60-3.11, $P < 0.0001$ and OR 1.64, 95% CI 1.18-2.28, $P = 0.003$, for *PNPLA3* and *LEPR* respectively) while SNPs of the *AGTR1*gene render protection against the condition (OR 0.40, 95% CI 0.20-0.81, $P = 0.01$). The G allele of both the *PNPLA3* rs738409 and the *AGTR1*rs3772622 were associated with increased fibrosis score ($P < 0.05$). For *LEPR* rs1137100, the G allele was associated with lower fibrosis score ($P < 0.05$). Analysis of gene-gene interaction revealed a strong interaction between the *AGTR1* and the *PNPLA3* genes (empirical $P = 0.007$), and the *LEPR* and the *PNPLA3* genes (empirical $P = 0.001$). A total of 73 CNVs were identified in patients with NASH. It can be hypothesised that these CNVs may be implicated in mechanisms that lead to progression to NASH. Of particular note was a 2.63MB deletion on Chromosome X at position 60,000bp to 2,698,000bp. This region is very gene rich and includes *ZBED1* (Zinc-finger DNA replication binding factor) and *CRLF2* (Cytokine receptor). Overall, the study

showed associations of SNPs in *PNPLA3*, *AGTR1* and *LEPR* genes across the NAFLD spectrum. The copy number findings are novel and could serve as potential genetic markers for the identification of the potentially progressive form of NAFLD.

Similar study design has been employed in association studies with regard to drug response. Accumulating evidence strongly suggests that genetic polymorphisms in drug metabolizing enzymes, transporters, receptors, and other drug targets are linked to inter-individual differences in the efficacy and toxicity of many medications and have been studied in many laboratories worldwide.We have carried out several studies on the role of genetic variants in response to drugs that are used in epilepsy patients28-38. Epilepsy is a common neurological disorder affecting over 50 million people worldwide. While seizures can be effectively controlled with anti-epileptic drugs (AEDs) in most, about 20-30 percent of the epilepsy patients do not adequately respond thereby affecting these patients' health and quality of life. The general objective of this set of studies is to determine whether polymorphisms in the transporter and target genes can explain the biological mechanism underlying the response to AEDs, with a potentially significant impact on clinical practice. Genotyping was carried out using Sequenom Mass ARRAY PCR on numerous relevant single nucleotide polymorphisms (SNPs) of drug transporter genes, including ABCC2, ABCB1 and its regulator PXR, and of drug targets, including SCN1A, SCN2A and SCN3A in more than 1100 patients and 1500 controls. Results showed that ABCB1 rs3789243 C>T, C1236T, G2677T/A, rs6949448 C>T, and C3435T polymorphisms and their haplotypes, PXR G7635A polymorphism and interaction between these genes do not contribute to response to carbamazepine (CBZ) or sodium valproate (VPA) monotherapy. However, significant association was observed between ABCC2 rs2273697-rs3740066 GT haplotype with drug responsiveness in the Malaysian Chinese. Data analysis of 39 polymorphisms of the SCN1A, SCN2A and SCN3A genes in Malaysian and Hong Kong epilepsy cohorts showed no significant association with AED responsiveness. The conclusion from these studies suggests that unlike the ABCC2 rs2273697-rs3740066 GT haplotype in the Malaysian Chinese, the common polymorphisms in ABCB1, PXR, SCN1A, SCN2A, and SCN3A do not play significant roles in influencing response to AEDs.

Moving on to another clinical condition, namely schizophrenia, we carried out a study[39] in schizophrenia patients who are on antipsychotic drugs. Many antipsychotic drugs can cause weight gain as an adverse effect. The aim of the study was to determine whether a polymorphism in the α-2A adrenergic receptor is associated with changes in weight and body mass index (BMI) of the patients following switching of antipsychotics which would normally cause increase in weight, to either of two drugs, namely, aripiprazole or ziprasidone. Both of these latter drugs have been reported to have less tendency to cause weight gain. Results showed an association between polymorphism in the α-2A adrenergic receptor and reductions in mean BMI, especially in those receiving ziprasidone. In yet another study, many female patients with Major Depressive Disorder (MDD), who were treated with Selective Serotonin Reuptake Inhibitor (SSRI), were found[40] to have sexual dysfunction. This study showed that this side effect was associated with Serotonin 2A-1438 G/A single nucleotide polymorphism.

Efforts are continuing to discover and characterize gene polymorphisms for different diseases. These are being carried out in different subpopulations within the Malaysian population, with the hope of discovering new diagnostic tests for different medical conditions and for devising better strategies for pharmacotherapy.

Ethical concerns

The sequencing of the human genome and developments in the study of genes has brought with it numerous ethical concerns. It has raised serious questions about the limits to privacy and genetic discrimination that may be used, for example by employers, with regard to diagnosis of diseases at early age or susceptibility of individuals to a given disease or even to adverse drug effects which may come about as a result of

the hereditary genetic make-up. This may perhaps lead to denial of jobs or to the denial of insurance to those with certain genetic predispositions. Concerns have also been raised with regard to the use of such techniques for gene therapy or for customizing personal genetic traits in babies such as physical appearance or level of intelligence.

Conclusions

The human body is so complex and so perfect in its design. Following the Human Genome Project, the sequencing of the three billion base pairs which constitute the human genome has led to major advances in our understanding of genomics and related fields, among which is pharmacogenomics. Pharmacogenomics is a science that attempts to look at the genome of an individual to identify genetic factors that influence their response to a drug. It can potentially lead to personalised medicine if and when genetic tests become available to predict how a certain patient will respond to the drug, what dose is to be given, and which drug is least likely to cause side effects in that particular patient. Pharmacogenomics has been successfully applied in clinical practice, and some pharmacogenomics studies are being carried out in Malaysia with the hope that data from these studies can be utilised to fill in the missing gap to make personalised medicine a reality for this region.

References

1.'The Human Genome: Poems on the Book of Life', the project for which Gillian K Ferguson. http://www.thehumangenome.co.uk/THE_HUMAN_GENOME/Home.html gabriel@thehumangenome.co.uk

2. Glorious Quran, Surah Al-Sajdah (Fusilat) 41:53.

3. The Human Genome Project. Ibrahim B. Syed, Clinical Professor of Medicine, University of Louisville School of Medicine, Louisville, KY 40292 and President, Islamic Research Foundation International, Inc, 7102 W. Shefford Lane, Louisville, KY 40242-6462 , E-Mail: IRFI@INAME.COM ,Website: http://WWW.IRFI.ORG, http://www.irfi.org/articles/articles_1_50/human_genome_project.htm

4. TRANSCRIPT: Bob Abernethy's interview with Dr. Francis Collins, Director of the Human Genome Project at the National Institutes of Health. Religion and Ethics News Weekly. June 16, 2000. http://www.pbs.org/wnet/religionandethics/2000/06/16/transcript-bob-abernethys-interview-with-dr-francis-collins-director-of-the-human-genome-project-at-the-national-institutes-of-health/15204/

5. Glorious Qur'an, Surat Al-Rum, 30:08.

6. Kalow W. Pharmacogenetics: heredity and the response to drugs. Philadelphia: WB Saunders, 1962.

7. Motulsky AG. Drug reactions, enzymes and biochemical genetics. J Am Med Assn 1957;165:835-837.

8. Mancinelli L, Cronin M and Sadee W. Pharmacogenomics: The promise of Personalised Medicine. AAPS PharmSci. 2000; 2 (1): article 4.

9. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients. JAMA. 1998;279:1200-1205.

10. Lesko LJ. Regulatory Perspective on warfarin re-labelling with genetic information. 9th National Conference on Anticoagulant therapy. 2007.

11. Evan. Annals of Pharmacotherapy 2005;38:1181-1188.

12. Wadelius. The Pharmacogenomics Journal 2005;5:262-270.

13. David A Flockhart et al. ACMG Policy Statement: Pharmacogenetic testing of CYP2C9 and VKORC1 alleles for warfarin. Genetics in Medicine (2008) 10, 139–150; doi:10.1097/GIM.0b013e318163c35f.

14. Plosker GL1, Keam SJ. Trastuzumab: a review of its use in the management of HER2-positive metastatic and early-stage breast cancer. Drugs. 2006;66(4):449-75.

15. Hetherington S, Hughes AR, Mosteller M, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. Lancet. 2002 Mar 30;359(9312):1121-2.

16. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. Lancet. 2002 Mar 2;359(9308):727-32.

17. ShamsulMohd Zain, Zahurin Mohamed, Munir Pirmohamed, Hwa Li Tan, Mohammed Abdullah Alshawsh, SanjivMahadeva, Wah-Kheong Chan, Nik Raihan Nik Mustapha, Rosmawati Mohamed. Copy number variation in exportin-4 (XPO4) gene and its association with histological severity of non-alcoholic fatty liver disease.Sci Rep. 2015 Aug 21;5:13306. doi: 10.1038/srep13306.

18. ShamsulMohd Zain, ZahurinMohamed, Muhammad YazidJalaludin, Fatin Fauzia, Anahita Hamidia, Nur Lisa Zaharan. Comprehensive evaluation of the neuropeptide-y gene variants in risk of obesity: A case-control study and meta-analysis. Comprehensive evaluation of the neuropeptide-Y gene variants in the risk of obesity: a case-control study and meta- analysis. PHARMACOGENETICS AND GENOMICS AUGUST 2015. DOI: 10.1097/FPC.0000000000000164.

19. ShamsulMohd Zain, Rosmawati Mohamed, David N. Cooper, RozaimiRazali, Sanjay Rampal, SanjivMahadeva, Wah- Kheong Chan, Arif Anwar, NurulShielawati Mohamed Rosli, AnisShafinaMahfudzPhaik-LengCheah Roma Choudhury Basu,9 and Zahurin Mohamed1,2.

Genome-Wide Analysis of Copy Number Variation Identifies Candidate Gene Loci Associated with the Progression of Non-Alcoholic Fatty Liver Disease. PLOS ONE 9(4):E95604 APRIL 2014 Northeast Ohio Medical University, United States of America. DOI: 10.1371/journal.pone.0095604

20. Zain SM, Rosmawati M, Cooper D, Razali R, Rampal S, Mahadeva S, Chan WK, Anwar A, Nurul SMR, Anis SF, Cheah PL, Basu RC, Tan HL, Mohamed Z. Genome-wide analysis of copy number variation identifies candidate gene loci associated with the progression of non- alcoholic fatty liver disease. PLOS ONE 2014;9 (4):e95604 , PLOS ONE 9(4):E95604 APRIL 2014. DOI: 10.1371/journal.pone.0095604

21. Shamsul Mohd Zain, Zahurin Mohamed, Rosmawati Mohamed. A common variant in the glucokinase regulatory gene rs780094 and risk of non-alcoholic fatty liver disease: A meta-analysis. JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY 30(1) AUGUST 2014, DOI: 10.1111/jgh.12714

22. ShamsulMohd Zain,*, Zahurin Mohamed,*, SanjivMahadeva, Phaik-LengCheah, Sanjay Rampal, Kin-Fah Chin, Roma Choudhury Basu,** Hwa-Li Tan*, and Rosmawati Mohamed (2013). Impact of leptin receptor gene variants on risk of non-alcoholic fatty liver disease and its interaction with PNPLA3 variant. Journal of Gastroenterology and Hepatology 2013;28(5):873-9. doi: 10.1111/jgh.12104.

23. ShamsulMohd Zain, Zahurin Mohamed, SanjivMahadeva, Sanjay Rampal, Roma Choudhury Basu, PhaikLengCheah, Agus Salim, Rosmawati Mohamed. Susceptibility and gene interaction study of the angiotensin II type 1 receptor (AGTR1) gene polymorphisms with non-alcoholic fatty liver disease in a multi-ethnic population. Plos One 2013; 8(3); 1-8. March 2013 | Volume 8 | Issue 3 | e58538(Tier 1; Impact Factor = 5.07)

24. Hwa Li Tan, ShamsulMohd Zain, Rosmawati Mohamed, Sanjay Rampal, Kin-Fah Chin, Roma Choudhury Basu, PhaikLengCheah, SanjivMahadeva, Zahurin Mohamed. Association of glucokinase regulatory gene polymorphisms with risk and severity of non-alcoholic fatty liver disease: an interaction study with adiponutrin gene. JOURNAL OF GASTROENTEROLOGY 49(6) JUNE 2013. DOI: 10.1007/s00535-013-0850-x

25. Zain SM, Mohamed Z, Mahadeva S, Cheah PL, Rampal S, Chin KF, Basu RC, Tan HL, Mohamed R. The impact of LEPR variants on risk of non-alcoholic fatty liver disease and its interaction with PNPLA3 variant. J GastroenterolHepatol ;28(5):873-9.

26. Zain SM, Mohamed Z, Mahadeva S, Rampal S, Basu RC, Cheah PL, Salim A, Mohamed R. Susceptibility and Gene Interaction Study of the Angiotensin II Type 1 Receptor (AGTR1) Gene Polymorphisms with Non-Alcoholic Fatty Liver Disease in a Multi-Ethnic Population. PLOS ONE 8(3):E58538 MARCH 2013. DOI: 10.1371/journal.pone.0058538

27. ShamsulMohd Zain, Rosmawati Mohamed, SanjivMahadeva,CheahPhaikLeng,SanjayRampal,Roma Choudhury Basu, Zahurin Mohamed. A multi-ethnic study of a PNPLA3 gene variant and its association with disease severity in non-alcoholic fatty liver disease. HUMAN GENETICS 131(7):1145-52 JANUARY 2012. DOI: 10.1007/s00439-012- 1141-y

28. HidayatiMohdShaari, Batoul Sadat Haerian, Larry Baum, JunjiSaruwatari, Hui Jan Tan, MohdHanipRafia, Azman Ali Raymond, Patrick Kwan, TakateruIshitsu, Kazuko Nakagawa,KhengSeang Lim, Zahurin Mohamed. ABCC2 rs2273697 and rs3740066 polymorphisms and resistance to anti- epileptic drugs in Asia Pacific epilepsy cohorts. PHARMACOGENOMICS 15(4):459-66 MARCH 2014. DOI: 10.2217/pgs.13.239

29. Larry Baum, Batoul Sadat Haerian , Ho Keung Ng, Virginia C. N. Wong, Ping Wing Ng, Colin H. T. Lui, Ngai Chuen Sin, Chunbo Zhang, Brian Tomlinson, Gary Wing Kin Wong, Hui Jan Tan, Azman Ali Raymond, Zahurin Mohamed, Patrick Kwan. Case control association study of polymorphisms in the voltage- gated sodium channel genes SCN1A, SCN2A, SCN3A, SCN1B, and SCN2B and epilepsy. HUMAN GENETICS 133(5):651-659 MAY 2014. DOI: 10.1007/s00439-013-1405-1

30. SoobithaSubenthiran, Noor Rain Abdullah, Prem Kumar Muniandy, Joyce Pauline Joseph, Kee Chee Cheong, Zakiah Ismail and Zahurin Mohamed. G2677T polymorphism can predict treatment outcome of Malaysians with complex partial seizures being treated with Carbamazepine. GENETICS AND MOLECULAR RESEARCH: GMR 12(4):5937-5944 NOVEMBER 2013. DOI: 10.4238/2013.November.26.3

31. SoobithaSubenthiran, Noor Rain Abdullah, Joyce Pauline Joseph, Prem Kumar Muniandy, Mok Boon Teck, Kee Chee Cheong, Zakiah Ismail and Zahurin Mohamed (2013). Linkage Disequilibrium between Polymorphisms of ABCB1 and ABCC2 to Predict the Treatment Outcome of Malaysians with Complex Partial Seizures on Treatment with Carbamazepine Mono- Therapy at the Kuala Lumpur Hospital. PLOS ONE 8(5):E64827 MAY 2013 Kaohsiung Chang Gung Memorial Hospital, Taiwan DOI: 10.1371/journal.pone.0064827

32. Batoul Sadat Haerian, Larry Baum, Patrick Kwan, Hui Jun Tan, Azman Ali Paymond, Zahurin Mohamed. SCN1A, SCN2A and SCN3A gene polymorphisms and responsiveness to antiepileptic drugs: a multicenter cohort study and meta-analysis. PHARMACOGENOMICS 14(10):1153-66 JULY 2013. DOI: 10.2217/pgs.13.104

33. Batoul Sadat Haerian, Larry Baum, Hui Jan Tan, Patrick Kwan, Azman Ali Raymond, JunjiSaruwatari, Kazuko Nakagawa, Zahurin Mohamed. 2012. SCN1A IVS5N+5 polymorphism and response to sodium valproate: a multicenter study. PHARMACOGENOMICS 13(13):1477-85 OCTOBER 2012. DOI: 10.2217/pgs.12.127

34. BS Haerian, H Roslan, AA Raymond, CT Tan, KS Lim, SZ Zulkifli, EHM Mohamed, HJ Tan and Z Mohamed. Association of ABCB1 gene polymorphisms and their haplotypes with response to antiepileptic drugs: A systematic review and meta-analysis.

PHARMACOGENOMICS 12(5):713-25 MARCH 2011. DOI: 10.2217/pgs.10.212
35. BS Haerian, H Roslan, AA Raymond, CT Tan, KS Lim, SZ Zulkifli, EHM Mohamed, HJ Tan and Z Mohamed. Lack of association of ABCB1 and PXR polymorphisms with response to treatment in epilepsy. SEIZURE 20(5):387-94 FEBRUARY 2011. DOI: 10.1016/j.seizure.2011.01.008
36. BS Haerian, H Roslan, AA Raymond, CT Tan, KS Lim, SZ Zulkifli, EHM Mohamed, HJ Tan and Z Mohamed. Association between ABCB1 polymorphism and response to sodium valproate treatment in Malaysian epilepsy patients. EPILEPTIC DISORDERS: INTERNATIONAL EPILEPSY JOURNAL WITH VIDEOTAPE 13(1):65-75 MARCH 2011.Impact Factor: 0.90 DOI: 10.1684/epd.2011.0419
37. BS Haerian, KS Lim, EHM Mohamed, HJ Tan, CT Tan, AA Raymond, CP Wong, SW Wong, and Z Mohamed. Lack of association of ABCB1 haplotypes on five loci with response to treatment in epilepsy. SEIZURE 20(7):546-53 APRIL 2011. DOI: 10.1016/j.seizure.2011.04.003
38. BS Haerian, H Roslan, AA Raymond, CT Tan, KS Lim, SZ Zulkifli, EHM Mohamed, HJ Tan and Z Mohamed. 2010. ABCB1 C3435T polymorphism and the risk of resistance to antiepileptic drugs in epilepsy: A systematic review and meta-analysis. SEIZURE 19(6):339-46 JULY 2010 , DOI: 10.1016/j.seizure.2010.05.004
39. SitiNorsyuhadaRoffeei, Gavin P. Reynolds, NorZuraida Zainal, Mas Ayu Said, Ahmad Hatim3, Syarinaz Ahmad Aida &Zahurin Mohamed. 2014. Association of ADRA2A and MTHFR gene polymorphisms with weight loss following antipsychotic switching to aripiprazole or ziprasidone. Hum. PsychopharmacolClinExp 29: 38- 45
40. RuzianaMasiran, Hatta Sidi, Zahurin Mohamed, Nur Elia Mohd. Nazree, Nik Ruzyanei Nik Jaafar, MarhaniMidin, Srijit Das, Suriati Mohamed Saini. Female Sexual Dysfunction in Patients with Major Depressive Disorder (MDD) Treated with Selective Serotonin Reuptake Inhibitor (SSRI) and Its Association with Serotonin 2A-1438 G/A Single Nucleotide Polymorphisms. JOURNAL OF SEXUAL MEDICINE 11(4) FEBRUARY 2014 Impact Factor: 3.15 DOI: 10.1111/jsm.12452.

EPIGENETICS IN HEALTH AND DISEASE

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Abstract

Epigenetics or epigenetic control was noted along with understanding of modern genetics but it lagged behind it because of the lack of the tools to understand its exact mechanisms. Recent advances in the genetic studies and tools made it now possible to try to understand how multiple genes are turned on and off.

Multiple mechanisms are proposed to explain this epigenetic control. DNA methylation levels affect the structure of the DNA double strands making them more or less accessible to the transcription process to RNA. Methylation and other types of chemical modifications affect also the histone structure which is responsible for the tight folding of the DNA strands in the nucleus. Small pieces of RNA (of different sizes), which are non-coding, have been recently discovered in cells and are thought to be capable of turning genes on and off. The aim of this line of studies is first to explain normal development and disease causation or pathogenesis, especially for complex multifactorial traits. The other aim is to discover new "drugs or interventions" to modify disease processes such as autoimmune diseases and cancers. However, epigenetic like genetic studies raise many ethical questions that need to be addressed.

Keywords: Epigenetics, DNA methylation, epigenetic control, pathogenesis,

Introduction

The term of epigenetics was first coined by CH Waddengton in 1942[1], and it focused on explaining the control mechanisms for the genetic material. The number of publications in this field is exploding and it is stirring great interest among many kinds of scientists in different fields such as medical doctors, pharmacists, biologists and researchers.

Figure 1 shows the number of articles published so far by a recent Pubmed search. Conducting this type of research is demanding for resources, but promising for future understanding of the pathogenesis of many diseases and possible treatments and cures.

This presentation reviews and discusses the roles of epigenetic factors on the normal and abnormal cell development and behavior, mostly by selecting and quoting key ideas and concepts on each topic especially from the abstracts and conclusion sections from the listed references.

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Genetics

The mechanisms that underlay how genetics work are probably better understood than the mechanisms of epigenetics. The specific composition of the DNA molecule and the sequence of its nucleotide bases dictates the structure of the needed proteins at the end, by first the transcription process of DNA into mRNA then translation to polypeptide chains.
In each human cell (with few exceptions) there is 10^9 single nucleotide bases in 2 sets of chromosomes. The term genome refers to either the complete gene complement (in the sense "geneotype"), or the total DNA amount per haploid chromosome set as thought from the initial use of the term[2].
The DNA double helix is very long and needs to be tightly coiled to fit in the nucleus of the cell, while the genes that need to be transcribed in each cell have to be unfolded to be accessible for the transcription enzymes. There are mechanisms that regulate where to start or end the transcription including stop codons, but this does not explain all the phenomena we observe[3]. In the case of cell division by meiosis or mitosis, DNA has to condense into the visible chromosomes, align itself in the middle of the cell then separate into the opposite poles equally. Structure of the chromatins help in performing these steps of the DNA folding, unfolding, condensation and replication[4,5].
Dynamic structural properties of chromatin play an essential role in defining cell identity and function. Transcription factors and chromatin modifiers establish and maintain cell states through alteration of DNA accessibility and histone modifications[4].
Diseases are the result of the interplay between genetic factors and environmental ones, with varying roles of each of them in each disease. Some diseases are mainly environmental, while in others genetic factors play the major role. Single gene diseases are called Mendelian traits and are organized by the Online Mendelian Inheritance in Man (OMIM) database in the US National Library of Medicine-National Institute of Health, website, part of the main website of the "National Center for Biological information NCBI". OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. The official site www.omim.org and the reference link for the site is http://www.ncbi.nlm.nih.gov/omim/
Even for single gene diseases, there is variation in disease severity or presence due to levels of gene expressions variation or phenomena of gene penetrance variance and the degree of environmental role in the disease.

Epigenetics

Epigenetics deals with the control of genetics and the needed fraction of the almost 25000 total genes becomes activated in each cell type. Authors, like Horsburgh, defined epigenetics stating:
"It is the study of mitotically or meiotically heritable phenotypes that occur as a result of modifications to DNA, thereby regulating gene expression independently of changes in base sequence due to manipulation of the chromatin structure. These modifications occur through a variety of mechanisms, such as DNA methylation, post-translational histone modifications, and non-coding RNAs, and can cause transcriptional suppression or activation depending on the location within the gene. Environmental stimuli, such as diet and exercise, are thought to be able to regulate these mechanisms, with inflammation as a probable contributory factor. However, research into these areas is still in its infancy"[6].

The authors noted that epigenetics is consistent with a theory proposed in 1809 as Lamark's theory of heritability of acquired characteristics. Lamark suggested that **"traits acquired during a lifetime can be passed on to future generations. This theory was generally abandoned in biology and replaced by the classical Mendelian laws of inheritance, namely, the law of segregation, the law of independent assortment, and the law of dominance. Indeed, it was widely accepted that the only way for traits to be passed on through generations was through the inheritance of genes and that the environment could not influence them. Lamark's theory, that environment plays a role in inherited phenotype, is now being accepted by the scientific community[6].**

"DNA consists of nucleotides: a deoxyribose molecule bound to a phosphate group on one side, creating the backbone of DNA, and bound to one of four nitrogenous bases on the opposite side. The double-ringed purine bases adenine (A) and guanine (G) pair with the single-ringed pyrimidine bases thymine (T) and cytosine (C) (A with T, G with C) (figure 2). Nucleosomes, which consist of ~147 base pairs of double helix structured DNA wrapped around an octamer of histone proteins, are the packaging units of DNA that form chromatin fibers, and when condensed further, form chromosomes (Figure 3).

Post-translational modifications to histones are key moderators of gene activity.

Acetylation and methylation are the best characterized of these modifications but ubiquitation, phosphorylation, sumoylation, ADP-ribosylation and citrullination also occur.

Acetylation of lysine (K) residues within the N-terminal tail of the histone proteins is associated with gene activation by neutralizing the positive charge of lysine, thus decreasing attraction between histones and DNA. Additionally, the attachment of an acetyl group, via histone acetyltransferase (HAT), can act as an attachment site for other proteins that are able to recruit chromatin remodeling complexes. Consequently, chromatin is less tightly bound which allows transcription factor binding, thus resulting in gene activation and protein formation. In contrast, methylation of histones, catalyzed by histone methyl-transferase (HMT), can correlate with either transcription or repression, depending upon the locus of modification. For example, tri-methylation of lysine residue 4 of histone 3 (H3K4me3) causes gene transcription, whereas tri-methylation of lysine 9 or 27 (H3K9me3/H3K27me3) results in gene silencing.

Non-coding RNAs (ncRNA), RNA molecules that are not translated into a protein, can be classified into many subgroups, including, but not limited to, micro RNAs (miRNA), involved in post-transcriptional gene silencing; piwi-interacting RNAs (piRNA), which direct DNA methylation at transposable elements; and long non-coding RNAs (lncRNA), which direct epigenetic machinery such as chromatin remodeling complexes"[6].

In prokaryotes, DNA sequence information is maximally used, whereas in higher organisms there is a large amount of DNA which is apparently functionless. However, this concept is rapidly changing in light of recent discoveries of small nuclear RNAs. Prokaryotes are devoid of Cytosine phosphate Guanine (CpG) islands. Even in lower eukaryotes there are no defined CpG islands. In cold blooded animals primitive type of CpG islands are observed. The higher vertebrates (warm blooded animals) have well defined CpG islands. CpG islands get methylated and demethylated. Methylation of CpG islands is the founding phenomenon of epigenetic operations. In the heavily methylated eukaryotic genome, CpG islands in promoter regions have been kept free of methylation which is an interesting mechanism of gene expression regulation[7].

Development and Embryogenesis

According to Naumova et at:

"Epigenetic modifications are crucial for maintaining and faithfully transmitting the identity of each cell type during cell division. During mammalian germ cell development, the acquisition of the ability to form a totipotent zygote is associated with extensive epigenetic reprogramming that affects all major developmental processes, including genomic imprinting, X-inactivation, retroelement (a gene segment from the proviral origin like HIV) silencing and gene expression"[8].

Tollefsbol addressed the issue of epigenetics of aging:

"Recent advances have merged the broad fields of epigenetics and aging. For instance, global hypomethylation of the genome and regional hypermethylation of specific genes occur during the aging of cells. Age-related diseases such as cancer, Alzheimer's disease, autoimmunity, and osteoarthritis may be associated with epigenetic alterations and or changes in the epigenetic machinery. These have also been reported in premature aging diseases such as progeria. While epigenetics impacts many different biological processes other than aging and aging involves numerous mechanisms other than epigenetics, it is clear that these two processes are linked and it seems likely that epigenetics will be proven to have a major role in aging, not only in the aging of cells, but also in organismal aging"[9].

A recent review by Coppede discussed how **"epigenetic control plays a role in the normal or abnormal development as in the example of Down syndrome (DS) where incidence increases with advancing maternal age. This review has shown the role of folate supplementation or deficiency in this disease caused by the faulty segregation of the two number 21 chromosomes at the time of meiosis in the ovum"[10].**

Coppede's large-cohort study revealed that lack of maternal folic acid supplementation at peri-conception resulted in increased risk for a DS birth due to errors occurring at maternal meiosis in the aging, oocyte, and it was shown that the methylation status of chromosome 21 peri-centromeric region could favor recombination errors during meiosis leading to its malsegregation.

In this regard, two recent case – controlled studies revealed association of maternal polymorphisms or haplotypes of the *DNMT3B* gene, coding for an enzyme required for the regulation of DNA methylation at centromeric and peri-centromeric regions of human chromosomes, with risk of having a birth with DS. Furthermore, congenital heart defects (CHD) are found in almost a half of DS births, and increasing evidence points to a possible contribution of lack of folic acid supplementation at peri-conception, maternal polymorphisms of folate pathway genes, and resulting epigenetic modifications of several genes, at the basis of their occurrence[10].

Epigenetics and Diseases

Epigenetics is considered to play roles in the pathogenesis of wide varieties of disease entities and an explosion of results are coming out from recent research about this association. Different modes of epigenetic modification and candidate genes affected by these factors are proposed for different diseases in populations. In the following sections, examples of the reports of the associations will be listed.

Diabetes Mellitus, Hypertension, Obesity and the Metabolic Syndrome

Smith, in his review, stated:

"Historical epidemiologic observations have led to further study of the association between the intrauterine environment and subsequent disease[11].

Most notably, the Dutch famine of 1944–1945 led to the epidemiologic observation that children born to mothers who were in the

early stages of pregnancy during the famine were at significantly increased risk of cardiometabolic disorders in adulthood. A relationship between low birth weight and increased risk for type 2 diabetes (T2D) in a British cohort was also observed, leading to the proposal of the "thrifty phenotype" hypothesis, which posited that malnutrition during pregnancy results in structural and functional changes in the developing fetus[11]. Since the proposal of the thrifty phenotype hypothesis in 1992, numerous studies have replicated the finding that low birth weight and other pregnancy complications confer increased risk for adult chronic disease in the offspring, including hypertension, T2D, and cardiovascular disease. There is strong evidence both on epidemiological human research and in experimental animal models for the relationship between maternal and early postnatal diet, maternal obesity, insulin resistance and metabolic syndrome, pregnancy complications and epigenetic programming and subsequent risk of obesity, diabetes and metabolic syndrome in the offspring. It is clear that many of these factors overlap and may mutually confound these relationships[12].

Multiple genes (two mitochondrial genes, *ATPASE6, CYTB, lipogenic genes, Srebf1, Fasn, Pparg1 and Pparg2 and IGF2) and epigenetic factors like microRNA's* are found. Animal models of intrauterine exposures and pregnancy complications and the resulting changes in the offspring, including structural and epigenetic, have been particularly valuable for studying the developmental origins of chronic diseases from a developmental programming perspective.

In particular, rodent models of maternal obesity and insulin resistance, nutrition intervention, intrauterine growth restriction (IUGR), and early postnatal growth provide a rich source of evidence for the developmental programming of metabolic syndrome and other metabolic disorders[13].

As for hypertension, Zang stated that

"The epithelial Na+ channel (ENaC) consists of α, β, γ subunits. Its expression and function are regulated by aldosterone at multiple levels including transcription. ENaC plays a key role in Na+ homeostasis and control of blood pressure. Mutations in ENaC subunit genes result in hypertension or hypotension, depending on the natureof the mutations. Studies suggest that a gene of Dot1a-Af9 complex represses αENaC by directly binding and regulating targeted histone H3 K79 hypermethylation at the specific subregions of αENaC promoter as an example for epigenetic control over hypertension"[14].

It was noted by Yang that:

"Heart failure (HF) is a complex patho-physiological syndrome that arises from a primary defect in the ability of the heart to take in and / or eject sufficient blood. Genetic mutations associated with familial dilated cardiomyopathy, hypertrophic cardiomyo-pathy, and arrhythmogenic right ventricular cardiomyopathy can contribute to the various pathologies of HF. Therefore, genetic screening could be an approach for guiding individualized therapies and surveillance.

In addition, epigenetic regulation occurs via key mechanisms, including ATP-dependent chromatin remodeling, DNA methylation, histone modifica-tion, and RNA-based mechanisms. MicroRNA is also a hot field in HF research"[15].

Malignancy

There is a wealth of research about the epigenetic control and its role and participation in the malignant phenotype.

Research can be found either in single articles and even books or volumes about the

association between many types of cancers and certain epigenetic modifications.

Yi, et. al. discussed the association between epigenetic control and many different cancers of the lungs, colon, blood, breast and prostate[16]. Morales et al found links between a certain rare brain tumor (diffuse intrinsic pontine glioma) and the histone modifications giving rise to possibilities for the treatment by studies about this tumor[17]. References like the ones edited by Sarkar[18] and Minarovits[19] are examples of comprehensive overviews illustrating the complexities of the regulation and deregulation of genes in the development and progression of human malignancies through epigenetics.

According to Sarker, cancers of the colon, prostate, breast, brain and many other types are associated with epigenetic changes[18].

It is well known that genetic aberrations, especially those inherited through parents (somatic genetic alterations), contribute to the development of less than 10 % of all cancers, yet epigenetic alterations in genes are responsible for the development and progression of the vast majority of cancers.

Among many alterations in the expression of genes, epigenetic regulation of genes, especially through selective methylation and acetylation, appears to play an important role in the development and progression of human cancers.

Understanding the role of epigenetics in the regulation of genes, especially through deregulated expression of microRNAs (miRNAs), will allow scientists to devise targeted therapeutic strategies for re-expression of the lost genes or down-regulating the genes that are over-expressed in order to eradicate cancer.

It is hoped that targeting epigenetics will not only target cancer cells, but it will also target the whole tumor micro-environment in the entire host aiming for the objective of complete eradication of cancer[18].

Psychological, Behavioral And Neurological Disease

There is a wealth of literature addressing behavioral and brain epigenetics, representing a novel frontier in neurobiological and psychiatric research. A significant statement by Petronis et al elustrates this concept:

"One of the primary objectives of behavioral epigenetics is to understand the molecular basis of various brain functions (e.g., memory, cognition, homeostasis, and adaptation to new environments). Of particular interest is the putative role of epigenetic dysfunction in brain pathology and mental illness. Epigenetic mechanisms -often more efficiently than genetic ones-are able to integrate a number of apparently unrelated clinical, epidemio-logical, and molecular data into a new theoretical framework. It is important to note that epigenetic changes that are partially both inherited and acquired can be the primary disease causes, rather than just one of numerous secondary or further downstream epiphenomena. Another pertinent question is how exposure to a wide scope of environmental factors, such as toxins, drugs of abuse, infection, nutrition, and stress can affect epigenetic regulation in the brain that ultimately translate into alterations in behavior. Epigenetic studies will provide new insights into the interface between the environment and the genome, and the mechanisms by which exposures at key points in development may mediate long-term effects on behavior"[20].

Another statement by Sassone-Corse, et al shed additional dimensions:

"On a daily basis, neurons convert a variety of external stimuli into rapid or long-lasting

changes in gene expression. A variety of studies have centered on the molecular mechanisms implicated in epigenetic control and how these may operate in concert. It will be critical to unravel how specificity is achieved. Importantly, specific modifications seem to mediate both developmental processes and adult brain functions, such as synaptic plasticity and memory. Many aspects of the research in neurosciences and endocrinology during the upcoming decade will be dominated by the deciphering of epigenetic control. The physiological implications of epigenetic regulation of neuronal functions were highlighted. Also highlighted is the increasing understanding of the molecular mechanisms that operate within neurons to translate epigenetic control into long-lasting neuronal responses"[21].

Autoimmune and Allergic Diseases

Wu, et. al., and other workers, reviewed the possible epigenetic contribution to a disease like Systemic lupus erythematosus (SLE), an autoimmune disease involving multiple organs in which anti-nuclear antibodies are present.

"The pathogenesis of SLE has been intensively studied but remains far from clear. B and T lymphocyte abnormalities, dysregulation of apoptosis, defects in the clearance of apoptotic materials, and various genetic and epigenetic factors are implicated in the development of SLE. The latest research findings point to the association between abnormal epigenetic regulation and SLE, which has attracted considerable interest worldwide. Research is done to investigate the relationship between aberrant epigenetic regulation and SLE. These include studies of DNA methylation, histone modifications and microRNAs in patients with SLE, as possible mechanisms of immune dysfunction caused by epigenetic changes. A better understanding of the roles of aberrant epigenetic regulation in the initiation and development of SLE is hoped to provide an insight into therapeutic options in SLE[22].

Yi et al addressed this concept:

"Recently significant effort has been invested in uncovering genetic and epigenetic factors, which may increase the risk of Inflammatory Bowel Disease (IBD), but progress has been slow, and few IBD-specific factors have been detected so far. It has been known for decades that DNA methylation is the most well studied epigenetic modification, and analysis of DNA methylation is leading to a new generation of cancer biomarkers.

It was suggested that DNA methylation should be studied in depth to understand the molecular pathways of IBD pathogenesis"[16].

Allergic tendency or atopy is another example of research for epigenetic control over phenotypes. Evidence for this type of phenomena has been provided by a study from Li et al on the transgenerational link of smoking and asthma. It was found that there was an increased risk (odds ratio 2.1) of an unexposed child developing asthma if the grandmother smoked during the mother's pregnancy[23]. They hypothesize that tobacco products alter the DNA methylation patterns in fetal oocytes and the changes in immune function and detoxification can be passed on to subsequent generations, increasing the risk for asthma. While interesting, much work is needed to verify this concept[24].

Modifications and treatments for epigenetic abnormalities

The value and aim of any scientific or medical research, after knowing the pathogenesis or causes, is to design or come up with treatment or cure for the disease. The initial possible advice for the Mendelian traits is avoidance of the disease by minimizing consanguinity, or early diagnosis by genetic

screening. Changing the genes of a human is unavailable or difficult to achieve because of the need to change in the makeup in every cell genes (bone marrow transplantation nowadays is rare exception because of the ease to handle blood cells). Research is being done to be able to produce drugs that affect the steps involved in the epigenetic modifications.

A study by Hui and Ye on the lysine methyltransferase which catalyzes methyltion of histone and non-histone proteins, showed that **"it plays a crucial role in diverse biological processes and has emerged as a promising target of treatment for various human diseases, including cancer, inflammation, and psychiatric disorders. However, inhibiting lysine methyltransferases selectively has presented many challenges to medicinal chemists. During the past decade, lysine methyltransferase inhibitors covering many different structural classes have been designed and developed. That article discussed the current state of lysine methyltransferase inhibitors and discuss future directions and opportunities for lysine methyltransferase inhibitor discovery"** [25].

Histone deacetylases are enzymes that modify chromatin structure and contribute to aberrant gene expression in cancer. Research over the past decade has led to the development of histone deacetylase inhibitors as anticancer agents. In addition to their effect on chromatin and epigenetic mechanisms, Histone Deacetylases (HDAC) inhibitors also modify the acetylation state of a large number of cellular proteins involved in oncogenic processes, resulting in antitumor effects [26]. While all these drugs are promising, this research is still in its infancy and far from being used in clinical trials[27].

Ethical, Moral
And Legal Considerations

Epigenetics, like genetics, raises a lot of issues and considerations from the ethical, moral and legal aspects, although there are some clear differences between them. Epigenetics is still in its infancy as a science and there are not as many opinions or laws about them in the developed world. Epigenetics falls under the guidance of how to perform medical research in general, as developed by the World Health Organization (WHO) and the Helsinki Declaration. Respect for the human life and the ethical considerations of patient's information, privacy, governance of medical research by peer review approval before a study, and informed consent should be observed. Equality in health services and non-discrimination against the handicapped also apply to treatment, medical insurance and employment should be used at both the stage of research or clinical practice of epigenetic results [28].

Usually these aspects are observed and regulated by the national laws and practices, and in the developed world courts have clear guidelines for them. One potential difference from genetics, is that epigenetics are more variable in its markers than the genes and are dose dependent when affected by environmental exposures leading to confusion in cases of litigation[28].

The general principles of human and life respect, equality and non-discrimination are consistent with the Islamic principles of the *Shari`ah* and Laws and I hope that Islamic countries apply and adopt them.

Conclusions

Epigenetics is a new and promising area of science and medicine trying to explain what is not yet understood in areas of health and disease. Epigenetics will work only if there is proper genetic machinery. Only few mechanisms for epigenetic control have been discovered so far that indicate that they are involved in many diseases including malignancies, non-communicable and autoimmune diseases. Till now no therapeutic treatment resulted from this research but few of them are being investigated. Ethical, moral and legal considerations have to be established and followed in this new arena of medicine.

References

1.Penny, D., *Epigenetics, Darwin, and Lamarck*. Genome Biol Evol, 2015. **7**(6): p. 1758-60.

2. Gregory, T.R., *The evolution of the genome*. 2005, Amsterdam ; London: Elsevier Academic.

3. Nakamoto, T., *Evolution and the universality of the mechanism of initiation of protein synthesis*. Gene, 2009. **432**(1-2): p. 1-6.

4.Shchuka, V.M., et al., *Chromatin Dynamics in Lineage Commitment and Cellular Reprogramming*. Genes (Basel), 2015. **6**(3): p. 641-61.

5. Melters, D.P., et al., *Chromatin Dynamics in Vivo: A Game of Musical Chairs*. Genes (Basel), 2015. **6**(3): p. 751-76.

6. Horsburgh, S., et al., *Exercise and inflammation-related epigenetic modifications: focus on DNA methylation*. Exerc Immunol Rev, 2015. **21**: p. 26-41.

7. Kundu, T.K., *Epigenetics : development and disease*. Subcellular biochemistry,. 2013, Dordrecht ; New York: Springer. xxvi, 689 p.

8. Naumova, A.K. and C.M.T. Greenwood, *Epigenetics and complex traits*. xxii, 341 pages.

9. Tollefsbol, T.O., *Epigenetics of aging*. 2010, London: Springer. Introduction "Epigenics and the aging process" pp 2-3

10. Coppede, F., *The genetics of folate metabolism and maternal risk of birth of a child with Down syndrome and associated congenital heart defects*. Front Genet, 2015. **6**: p. 223.

11. Smith, C.J. and K.K. Ryckman, *Epigenetic and developmental influences on the risk of obesity, diabetes, and metabolic syndrome*. Diabetes Metab Syndr Obes, 2015. **8**: p. 295-302.

12. Hales, C.N. and D.J. Barker, Type2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. diabetologia, 1992.

13. Seki, Y., et al., *Minireview: Epigenetic programming of diabetes and obesity: animal models*. Endocrinology, 2012. **153**(3): p. 1031-8.

14. Zhang, W., *Epigenetics of epithelial Na(+) channel-dependent sodium uptake and blood pressure regulation*. World J Nephrol, 2015. **4**(3): p. 363-6.

15. Yang, J., W.W. Xu, and S.J. Hu, *Heart failure: advanced development in genetics and epigenetics*. Biomed Res Int, 2015. **2015**: p. 352734.

16. Yi, J.M. and T.O. Kim, *Epigenetic alterations in inflammatory bowel disease and cancer*. Intest Res, 2015. **13**(2): p. 112-21.

17. Morales La Madrid, A., R. Hashizume, and M.W. Kieran, *Future Clinical Trials in DIPG: Bringing Epigenetics to the Clinic*. Front Oncol, 2015. **5**: p. 148.

18. Sarkar, F.H.e., Book Editor, "*Epigenetics and cancer"*. Springer Science + Business Media Dordrecht 2013. Sarkar FH, Prevace p v.

19. Minarovits, J. and H.H. Niller, *Patho-epigenetics of disease*. 2012, New York: Springer. xiii, 465 p.

20. Petronis, A. and J. Mill, Book Editors "*Brain, behavior and epigenetics"*. 2011, Heidelberg: Springer. Preface pp viii-ix.

21. Sassone-Corsi, P. and Y. Christen, *Epigenetics, brain, and behavior*. Research and perspectives in neurosciences,. 2012, Heidelberg: Springer. Forward of the volume pp v-vi.

22. Wu, H., et al., *The real culprit in systemic lupus erythematosus: abnormal epigenetic regulation*. Int J Mol Sci, 2015. **16**(5): p. 11013-33.

23. Li, Y.F., et al., *Maternal and grandmaternal smoking patterns are associated with early childhood asthma*. Chest, 2005. **127**(4): p. 1232-41.

24. Pawankar, R., S.T. Holgate, and L.J. Rosenwasser, *Allergy frontiers*. 2008, Tokyo ; London: Springer.

25. Hui, C. and T. Ye, *Synthesis of lysine methyltransferase inhibitors*. Front Chem, 2015. **3**: p. 44.

26. Gasser, S.M. and E. Li, *Epigenetics and disease: pharmaceutical opportunities*. Progress in drug research. 2011, Basel ; London: Springer. x, 270 p.

27. Ruiz-Hernandez, A., et al., *Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence*. Clin Epigenetics, 2015. **7**(1): p. 55.

28. Randy L. Jirtle, Frederick L. Tyson Book Editors, "*Environmental epigenomics in health and disease*". Springer-Verlag Berlin Heidelberg 2013. Chapter 14 "Legal and Ethical Implications of Epigenetics". Mark A. Rothstein, page 292.

Figure 1. Number of articles about epigenetics published yearly accessed on August 16-2015 url link http://www.ncbi.nlm.nih.gov/pubmed/?term=epigenetics (The number for year 2015 is an estimate by the same trend)

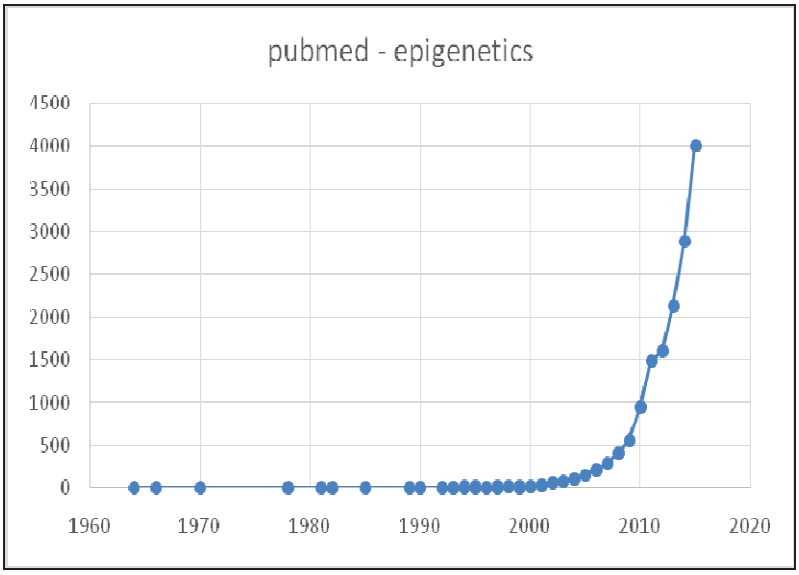


Figure 2 Structure of the DNA.
From https://upload.wikimedia.org/wikipedia/commons/e/e4/DNA_chemical_structure.svg

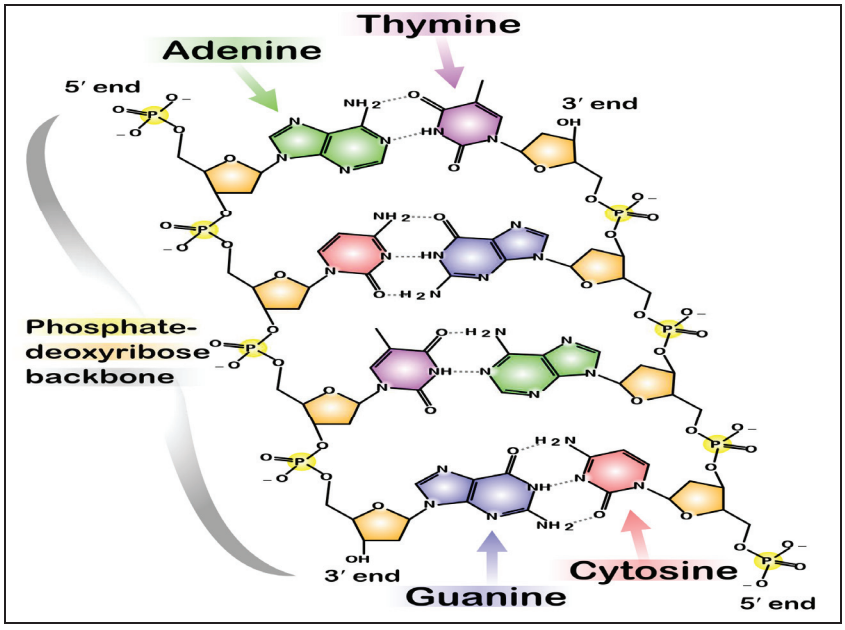
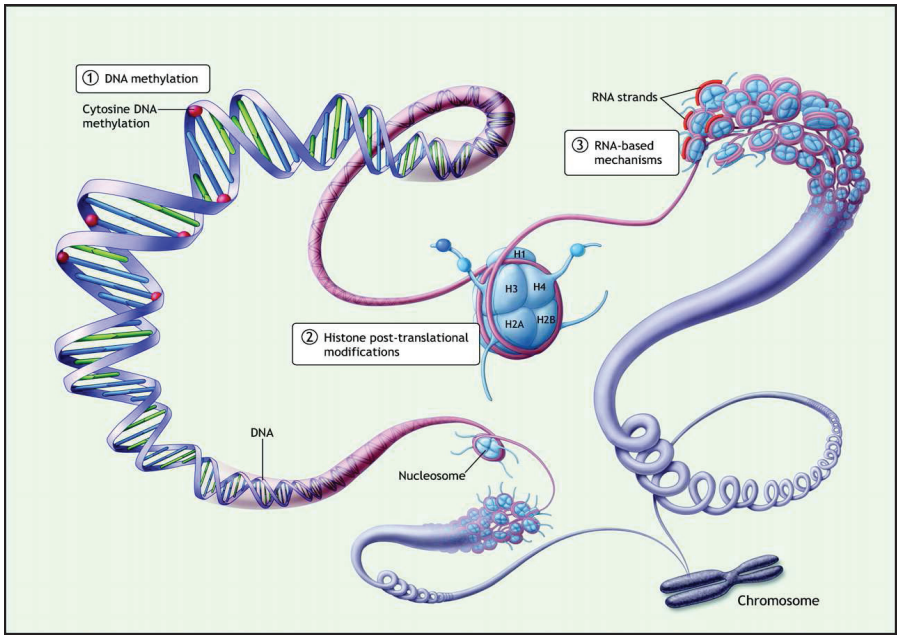


Figure 3:
Structure of the DNA strands into nucleosomes around histones, to be condensed into chromosomes.
From http://circres.ahajournals.org/content/102/8/873.



GENETIC FINGERPRINTING: IMPLICATIONS ON ISLAMIC LAW ON PATERNITY

Aly A. Misha'l *

Abstract

DNA fingerprinting is one of the applications of the Human Genome Project. This new technology is currently utilized, with unprecedented precision, for paternity establishment and negation.
Muslim scientists and jurists started discussions on the relevance of using this technology within the framework of the classical Islamic rulings pertaining to paternity issues.
This paper addresses the new innovations on DNA structure, and genetic diversity responsible for a wide range of physical characteristics, as a prelude to the study of its implications on ethical, legal issues and the Islamic *Shair`ah* established methods of establishing and negating paternity. Specifically addressed is the extent to which DNA testing may be utilized within the hierarchy devised by Muslim jurists.
Contemporary Jurisprudence outcomes, especially of the two Islamic *Fiqh* Academies, and the combined scientists-jurists seminars held by the Islamic Organizations of Medical Sciences (IOMS), together with individual new publications, were the main sources for this paper.

Keywords: DNA fingerprinting, DNA testing, Human Genome Project, paternity testing, Islamic Jurisprudence, paternity negation.

Introduction

Genetic fingerprinting is also referred to in the literature as DNA typing, DNA profiling and genotyping. The DNA (deoxyribonucleic acid) molecule varies in different organisms, and this forms the basis of genetic diversity[1]. The Human Genome Project (HGP) has opened unprecedented avenues to understand the unique details of the human DNA and to identify and map the sequences of all genetic and translated protein arrangements which constitute the distinguished DNA structure of each individual in a way that distinguishes him/her from all other individuals in the world, since human creation, except his / her own identical twin. These genetic variations have been extensively studied to understand their roles in disease associations and drug responses[2]. Variation of the DNA sequence contributes to the inter-individual variability for a wide range of physical characteristics[3]. There are three categories of genetic variation[3]:

- DNA sequence variation.
- Structural variation.
- Epigenetic variation.

DNA sequence variation has been extensively studied, with single nucleotide polymorphisms (SNPs) standing as the most common form of DNA variation[3]. The unique DNA molecular sequences entail unique sequence of genetic information, which is the same in every cell of the organism, being a skin cell, a hair root or cells from the gums on a tooth brush.

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The specific DNA sequences at a particular gene (or locus) are termed alleles[3]. An individual's genotype refers to the combination of multiple of loci joined together in 23 pairs of chromosomes.
If an individual has 2 copies of the same allele at a particular locus on each of the two chromosomes, that individual is homozygous for this locus (or gene)[3].
If, on the other hand, the 2 alleles differ, that individual is heterozygous for this gene.
The term (haplotype) refers to the combination of alleles in a region of DNA sequence[3].
DNA features retain their distinctiveness, prolonged stability and durability throughout the life of the organism, and continue after its death[4,5].

Structural genetic variation affects large segments of DNA (i.e. >1000 bases) or a whole chromosome, commonly affecting the function of multiple genes and having pronounced phenotypic effects. This class includes copy number variation (CNV), and chromosomal translocations, inversions and rearrangements.

Epigenetic variation refers to modifications of DNA, by methylation or histone modulation. Such variation does not directly alter the DNA sequence but can have significant effects on protein expression.

There are multiple technologies for DNA fingerprinting, the choice of which depends on their specific applications in[3,6,7]:

- Medical diagnosis.
- Criminal and forensic science.
- Identity verification.
- Parental testing.
- Food production and quality.

And many others.

The role of genetic testing in modern clinical medicine is termed (Personalized medicine)[3]. Its main utilities are in areas of:

- Genetic counseling and testing.
- Prenatal screening, including free fetal DNA in maternal blood.
- Prenatal testing.
- Pre-implantation genetic diagnosis (PGD).
- Newborn screening.
- Carrier testing, and others.

Genetic fingerprinting in paternity issues in Western countries has been utilized for more than a decade in legal disputes and paternity verification, to impose or revoke parental obligations, paternal disputes and verification of fidelity of spouses[8,9,10].
Some countries have developed national DNA databases for several purposes including paternity issues and identity verification.

Ethical and legal concerns

With continuously expanding utilizations of the DNA fingerprinting technologies, there have been concerns related to sample contamination, faulty preparation and issues of interpretations.
Initially, Restriction Fragment Length Polymorphism (RFLP) was utilized. This technology requires large quantities of high-quality DNA, which may not be guaranteed with degraded, scanty samples taken from postmortem sources.
The use of RFLP technology therefore subsided with development of the Polymerase Chain Reaction (PCR) technology which amplifies the desired fragment of DNA many times, creating thousands of copies. Moreover, this technology works even with partially degraded DNA[11].
Other ethical concerns stem for the increasing commercialization and lack of governmental regulations and supervision, to guard against technical and human errors.
Many paternity testing laboratories provide services through mail-order operations, by phone or on the internet [9,10]. Experts also warn

of lack of guidance and supervision to preclude abuses, especially against minorities and disadvantaged groups[10-12].
Another significant concern is the lack of safeguards to preclude misuses of such sensitive information to reveal possibilities of latent genetic illnesses or future health conditions these tests can reveal[10-13].
More than any other modern scientific discovery, DNA fingerprinting raises crucial issues about balancing the use of technology to help society, against an individual's right to privacy.
These concerns prompted the establishment of a research group, within the Human Genome Project, that focuses on ethical, legal and social issues. The group found it necessary to develop a statement on the proper conduct of genetic research[10-14,15].
The above multiple considerations related to ethics, data retrieval, accuracy, interpretation and data storage remain to be further explored.

Lineage in Islamic *Shari`ah*

As a necessary prelude to the discussions on Islamic law pertaining to paternity, the *Shari`ah* principles of human lineage will be briefly outlined.
Two basic principles are established in Islamic *Shari`ah*:

A) **Establishment of Paternity:**

Shari`ah* always looks forward to establishment and confirmation of lineage, even with the least means, methods and evidence[16]. Lineage is one of the five aims (*Maqassid*) of *Shari`ah[17].
Ibn Qudamah states[18]:
"Lineage is established upon the testimony (*shahadah*) of only one woman, on the birth of a child, and is confirmed merely by the claim".

This principle of Islam's utmost care of lineage, is manifested by severe disappointment and strict warnings to parents when they proceed to deny the established paternity or maternity of their children, and to exonerate themselves from them.
The same *Shari`ah* ruling also applies to those who link to themselves the parentage of children who, in fact, are not from their lineage. In this regards, the Prophet (ﷺ) says: "Any woman who introduces (a child) on a people (family) who is not really so, she is devoid of anything (that pleases) Allah, and Allah will never grant her admission to Paradise (*al-Jannah*), and any man who denies (disclaims) his son looking at him (in the eye), Allah will not reveal Himself to him on the Day of Judgment, and Allah will expose (disgrace) him towards the first and last people"[19].

The Prophet (ﷺ) also says: "Anyone who linked himself (to the paternity) of a father, knowing he is not his father, paradise (*Jannah*) is forbidden (*haram*) on him"[20].
Islam connects the person whose lineage is unknown, to a father that claims him by merely declaring that, taking into consideration the son is most likely the son of that father.

All these are clear evidences of the utmost care of *Shari`ah* towards progeny and lineage in the context of Divine wisdom to fulfill significant aims in stability of family and society.

On the other hand, *Shari`ah* is extremely strict on the issue of negation of lineage, and established to that the strongest measures, and the mutual oath of condemnation (*li`an*) is the principle means in this regards[21].

Establishment of paternity within the context of *Shari`ah*:

Islamic law provides a comprehensive legal structure that governs various aspects of

Muslim's life. In no other area has *Shari`ah* been as detailed and specific as it is in the area of family law[16]. Rules pertaining to marriage, divorce, inheritance and family matters are rooted in the Qur'an and the *Sunnah* of the Prophet (ﷺ).

Within this Islamic legal structure, paternity has always been linked with licit sexual relationship, either through marriage, or, at certain earlier times, ownership of a slave woman[16].
In the case of marriage, children born within wedlock are automatically attached to the *bona fide* husband who also becomes the *bona fide* father[16].
Based on the Qur'an and *Sunnah* of the Prophet (ﷺ) the formal kinship system in Islam is built on patrilineal pedigree[16].

Establishment of paternity, in classic Islamic family structure, is based on a primary principal method[10,16], namely: *al-firash* (the shared marital bed), which is the licit sexual relationship through marriage, and through ownership of a slave woman, when this was a recognized system in the early history of Islam.

The Prophet (ﷺ) is reported to have said. "The child belongs to *al- firash*, and the adulterer receives the stone"[22].
"الولد للفراش وللعاهر الحجر"
Ibn al-Qayyim stated: "And for establishment of lineage by *al-firash*, this is the consensus of the *Ummah*"[23].
In cases where fulfillment of this primary principle method is absent, or difficult to achieve, four other methods could be resorted to, in a hierarchical manner[10,16]:
1. Admission of paternity by the father (*iqrar*). Another term used is (*istilhaq*), when the father accepts attachment of a child to himself as a son. The man who makes *iqrar* must meet certain conditions, and the person for whom *iqrar* is made must be of unknown or disputed paternity, such situations were prevalent in *Jahiliyyah* (era of ignorance , before the advent of Islam) and the transition period to Islam.
2. Evidence (*bayyinah*), usually by testimony of qualified witnesses, that a child is the son/daughter of a father, based on his (their) knowledge the parents were married, and hence the child is legitimate.
3.Resemeblance of physical features between a child and a father, as judged by known experts in examination and comparing of physical features (*qiyafah*), which is resorted to in the absence of *al-firash*, or witnesses, in situations with un-established paternity, or when there is dispute between 2 or more men who claim paternity. It is not unanimously approved by jurists:
4. Lot-casting (*qur'ah*): Its admission is questionable among many jurists, and was not approved by *al-jamhoor*. Some jurists resorted to it in the post-*Jahiliyyah* period, in situations of disputes where it is extremely difficult for any one claim to outweigh other claims (such as disagreement among witnesses, or between experts in *qiyafah*), especially when a woman was married to more than one man (polyandry).

B) Negation of Paternity

Negation of paternity under Islamic law is only conducted by *li`an*, the mutual oath of condemnation, as clearly established in the Qur'an[24]:

"وَالَّذِينَ يَرْمُونَ أَزْوَاجَهُمْ وَلَمْ يَكُن لَّهُمْ شُهَدَاء إِلاَّ أَنفُسُهُمْ فَشَهَادَةُ أَحَدِهِمْ أَرْبَعُ شَهَادَاتٍ بِاللَّهِ إِنَّهُ لَمِنَ الصَّادِقِينَ، وَالْخَامِسَةُ أَنَّ لَعْنَتَ اللَّهِ عَلَيْهِ إِن كَانَ مِنَ الْكَاذِبِينَ، وَيَدْرَؤُا عَنْهَا الْعَذَابَ أَنْ تَشْهَدَ أَرْبَعَ شَهَادَاتٍ بِاللَّهِ إِنَّهُ لَمِنَ الْكَاذِبِينَ، وَالْخَامِسَةَ أَنَّ غَضَبَ اللَّهِ عَلَيْهَا إِن كَانَ مِنَ الصَّادِقِينَ"

"And those who accuse their wives [of adultery] and have no witnesses except themselves - then the witnesstes of one of them [shall be] four testimonies [swearing] by Allah that indeed, he is of the truthful. And the

fifth [oath will be] that the curse of Allah be upon him if he should be among the liars. But it will prevent punishment from her if she gives four testimonies [bear witness] by Allah that indeed, he is of the liars. And the fifth [oath will be] that the wrath of Allah be upon her if he was of the truthful".
These methods reveal how *Shari`ah* established paternity with the least available evidence, while negation of paternity was established with very strict methods.
There are also several supportive prophetic traditions. In one of them, narrated by *Ibn Umar* (RA): "A man conducted *li`an* against his wife at the time of the Prophet (ﷺ), and denied her son. The Prophet (ﷺ) separated them, and annexed the son to the woman"[25].

The husband can negate the child who is born within marriage, when he is confident his wife has committed adultery, with his inability to produce the four witnesses[10].

Li`an* should be initiated upon the husband's request to the judge or ruler. Followed by that of the wife. There are Islamic legal conditions for the validity of *li`an, and there are specific statements to make it valid.

The jurists stipulated that the statement uttered by the disputing spouses during the exchange of oaths of condemnation must follow the manner and order specified in the *li`an* verses[24,27].

Three conditions for the validity of *li`an* are needed: Immediacy, absence of prior acknowledgment, and life of the infant (must be living).
The jurists have disagreement on timing of paternity negation: during pregnancy, after childbirth, etc.
Jurists are in agreement that denial of paternity is not valid if it is preceded by prior acknowledgement and acceptance of paternity of the same child.

Li`an is initiated to resolve a complicated marital dispute involving children whose paternity is cast in doubt, in a manner that allows both parties to save face and preserve their dignity.
This resolution of dispute, however, is not meant to establish guilt or to verify the veracity of the claims[10]. *(*Although the parties save face and preserve dignity in this life, one of them will suffer in the Hereafter*)*.

Li`an, therefore, cannot remove the ambiguity that results from the unverified claims of the disputing spouses, which are bound to remain at that level of uncertainty.
In the context of the new innovation of the Human Genome Project and discovery of DNA printing, it became possible this new technology can now be used to resolve the question of paternity, almost beyond doubt. It is, therefore, being debated by Muslim jurists whether DNA testing could replace the traditional method of *li`an*[10].

This issue was discussed by Islamic seminars, conferences, *Fatwa* councils, and Muslim jurists, over the past two decades, following innovations of DNA testing.

The Islamic Organization of Medical Sciences (IOMS), held its 11th seminar in 1998, on genetics, human genome project, genetic engineering and gene therapy[28]. The issue of DNA printing and its use in confirming and negating paternity was discussed in detail. One of the main topics in these deliberations was the extent to which DNA testing may impact the continued validity of *li`an*. The majority of participating jurists, ruled that DNA testing cannot replace *li`an*, but it was given the weight to replace *qiyafah*, and to assume its position in the hierarchy devised by the classical Muslim jurists, in both the establishment and negation of paternity[10,29]
The majority of jurists adopted the opinion that *li`an* is the only legal method for paternity

negation. Without *li`an*, paternity cannot be denied. In certain exceptional cases, however, denial of paternity can occur without *li`an*. The jurists gave some examples that include[30]:

- If a wife gives birth immediately after marriage, or less than six months after marriage.
- If the husband is less than ten years old.
- Or if the husband is impotent.

The tenacity of Islamic law and its procedures in the modern times, including the issue of *li`an*, is ascribed as much to the religious dimension of Islamic law as to the decision-making process in areas of interface between Islamic law and modern science and technology[10]. The underlying argument in giving priority to *li`an* over DNA testing is both textual and rational[10].

The textual component pertains to the authentic and clear sources in the Qur'an and prophetic tradition.

The rational component, on the other hand, refers to the Divine wisdom and *Shari`ah* aims that emphasize the need to avoid grave social and psychological consequences that often accompany and follow such disputes, and to safeguard people's privacy and confidentiality, even if this means the disputing claims will remain forever unverified[10]. There are much to relegate to the consciences of disputants.

The majority of participants in the two IOMS seminars held in 1998[28], as well as the rulings of the International Islamic *Fiqh* Academy held in Bahrain in 1998[31], favored the admission of DNA testing in paternity disputes as the modern equivalent of *qiyafah*, and refrained from acknowledging it as a substitute for *li`an*.

In the 11th IOMS seminar in 1998, the following overview and recommendations on the issue of genetic printing were adopted[28]:

The seminar has discussed the question of the genetic imprint, which is the elaborate genetic structure that establishes the identity of a particular individual. In practice, the genetic imprint is an almost error-proof method of determining biological parentage and confirming identity, particularly in the field of forensic medicine, and is equal to other items of strong evidence that are admitted by most jurists in cases other than those of applying prescribed punishments (*hudud*). It is a great contemporary development in the area of tracing similarities, on which the majority of Islamic jurisprudence (*fiqh*) schools rely in determining a contested parentage. A genetic imprint test, however, should be made at several laboratories.

On May 3rd-4th, 2000, IOMS held a special session, in response to a recommendation from the 11th seminar in 1998, to address the validity of a genetic imprint in confirming parentage. This special session issued the following recommendations[32]:

Having looked into those rulings, the comments of jurists, the explanation of the genetic imprint made by scientists from the Genetics Center, and the detailed discussion and debate on the subject….the participants have arrived at the following:

1. **Every human has his own, unique genetic pattern in every cell in his body, which is not shared by any other individual throughout the world. This pattern is known as the genetic imprint. In practice, the genetic imprint is an almost error-proof method of determining biological parentage and confirming identity, particularly in the field of forensic medicine, and is equal to other items of strong evidence that are admitted by most jurists in cases other than those of applying**

prescribed punishments. It is a great contemporary development in the area of tracing similarities, which the majority of Islamic jurisprudence (*fiqh*) schools rely on in determining a contested parentage. Therefore, the seminar decides that, with all the more reason, the genetic imprint evidence should be admitted in all the cases in which tracing similarities (*qiyafah*) is applied.

2. **The discussion session rules that the genetic imprint should be resorted to in cases where, in the absence of other evidence or with both (or all) sides having equal evidence, more than one man claims the fatherhood of a child whose lineage has not been established.**

3. **A person who claims to be the parent of a child, whose lineage is unknown, is entitled to the parentage of that child when his claim satisfies the conditions set by Islamic law. Consequently, such a person has no right to withdraw his claim, and any denial of one of that person's other children of the kinship of the claimed child, is not admitted, and the genetic imprint evidence is not applicable in such a case.**

4. **The admission of some siblings that a child of unknown lineage is their sibling is not binding to the other siblings, the lineage cannot be confirmed, the consequences of the admission are limited to the inherited share of the siblings making the admission, and the genetic imprint evidence is not admitted in such a case.**

5. **In the discussions of this subject, varying points of view were made and the debate went on for a long time on whether a woman's claim of parentage of the child to a particular father is admissible in the case of a child of unknown lineage. It was therefore decided to allow more time for the consideration and investigation of this question.**

6. **The genetic imprint is not accepted as evidence of a "conjugal bed", since the proof of marriage has to follow the procedures set in Islamic law.**

7. **The participants believe that the following controls should be operative when a genetic imprint test is to be made:**

   a. **A test should be made only after permission from the concerned authority is obtained.**
   b. **The test should be made in at least two different accredited laboratories, with proper precaution taken to guarantee that none of the laboratories involved has access to the findings of the other (s).**
   c. **Government-owned laboratories are preferable, but if such laboratories are not available, the test can be made at government-supervised laboratories. Whatever the case is, one condition that has to be met is that the applicable local and international conditions and controls are met.**
   d. **Another condition is that the staff of these laboratories must be qualified and trustworthy, and none of them should be on**

terms of kinship, friendship, enmity or common interest with any of the claimants, nor should have a record of a dishonorable offence or an act of dishonesty, or common interest with any of the claimants.

It is pertinent here to enlist the comprehensive rulings of the Council of The Islamic *Fiqh* Academy of the Muslim World League, in its 16th session, held in *Makkah Al-Mukarramah* on 21-26 *Shawwal*, 1422 H, (January 2002)[33], that states:

The results of genetic printing are almost conclusive in confirming or negating paternity of children to their parents, and in confirming the specimen (blood, semen, saliva ….), that may be located in the scene of accident, to its real owner, being much stronger than expert physical resemblance (*qiyafah*). Error in genetic printing is not existent in it as a technology, but may exist due to human error or factors of contamination and other factors (not related to the technology), and depending on the above, the Council decided:

First:
There is no *Shari`ah* objection to depend on the genetic print in criminal investigations, and to consider it a confirmation evidence in criminal acts that do not include *Shari`ah* punishment (*Hudud* or *Qissas*), in view of the principle (avoid *hudud* by suspicions), in order to fulfill society's justice and safety, and to end up in punishing the criminal and aquitting the aquisited (accused), which is an important aim (*maqssid*) of *Shari`ah*.

Second:
Utilization of genetic print in the area of lineage should be surrounded by utmost attention, precaution and confidentiality, therefore the *Shari`ah* statements and principles should have precedence on genetic print.

Third:
It is not permissible to depend on genetic print to negate paternity, and it should not be given precedence over the mutual oath of condemnation (*Li`an*).

Fourth:
It is not permissible to use the genetic print, with the intention to confirm the validity of *Shari`ah* established lineage (paternity) by *al-firash*. The official authorities should prevent that, and should impose punishments, since this prohibition is instrumental in protection of peoples' honor and lineage.

Fifth:
It is permissible to depend on the genetic print in the area of lineage (paternity) confirmation, in the following situations:

A. Cases of conflict on an individual with unknown lineage, in all types of conflict mentioned by jurists.
These cases include conflicts stemming from lack of evidence (e.g. conflicting testimonies), or balance, or from taking part in suspected sexual relations (*watu al shobhah*) and other similar situations.

B. Situations in which there is suspicion in identification of newborns in hospitals, child care centers or similar situations. This also includes suspicions of mixing in In-vitro fertilization (IVF) centers.

C. Situations of loss and mixing of children in wars, disasters or accidents, where it becomes

impossible for their families to identify them.

This includes identification of unidentified corpses, or confirming identities of prisoners of war or lost individuals.

Sixth:

It is not permissible to sell or grant the human genomes of races, peoples or individuals for any purpose, in view of the harms (*mafasid*) that result from this sale or grant.

Seventh:

The Council recommends the following:

A. The State should prevent conduction of the genetic print, except upon a judicial demand. This testing should be limited to laboratories that belong to special official authorities. Laboratories of the private sector, that aim at profit, should be prevented from conducting this testing, in view of great dangers if conducted that way.

B. Formation of a (special genetic imprint committee) in each state, with participation of jurists, physicians and administrators, with responsibility to supervise the outcomes of genetic print and authenticate its results.

C. A meticulous mechanism should be adopted to prevent fraud, cheating, contamination and other human errors, in genetic print laboratories, to insure quality and correctness of results and prevent doubt.

Recent Jurist Opinion

The issues of paternity establishment and negation in the wake of recent breakthroughs of the Human Genome Project and DNA fingerprinting, have been comprehensively addressed in several new publications by *Ayman Shabana*, a leading researcher of the Islamic Medical and Scientific Ethics Project at Georgetown University's School of Foreign Service in Qatar.

His three published essays[10,26,34], are most informative and elaborative on the very significant issues of family structure in Islamic teachings, classical Islamic law and contemporary Islamic jurisprudence and various arguments related to paternity establishment and negation.

According to *Shabana*[34], contemporary jurists are classified into those who adopt a limited incorporation of DNA analysis in paternity verification, as long as it does not conflict with the established methods of *Shari`ah*, namely *li`an*, and those who adopt a comprehensive incorporation of DNA analysis. The latter group emphasizes the need to utilize new DNA technology as a definitive way to establish and negate paternity.

Their views will be discussed in some detail in the following segments.

Other contemporary jurists have individually addressed the issue of DNA fingerprinting, specifically in negation of paternity[34].

Sa'ad el-Din Hilali, professor of comparative *fiqh* at al-Azhar University in Egypt[35] calls for integration of DNA analysis in paternity verification, calling the technology as a type of strong circumstantial evidence that denotes definitiveness.

A similar stance was taken by *Muhammad Uthman*[36], from the same institution.

Although this line of thinking does not explicitly call for replacement of the legal method of al-*firash* by the DNA testing, the argument is clear that DNA analysis can be a method for true identification of the "true" *firash*.

One of other strong promoters of this line of thinking is *Abdul Rashid M. Bin Qasim,* who wrote a comprehensive essay on this issue. The following is a pertinent segment[37]:
"*Al-Bayyinah* (evidence) in Qur'an and *Sunnah* is not limited to the testimony only. It includes all what is capable of exposing and proving the truth". He quoted a statement of *ibn al-Qayyim*:

" فالبينة اسم لكل ما يبين الحق ويظهره، ومن خصّها بالشاهدين أو الأربعة .....الخ، لم يوفّ مسماها حقه ..."

"*Al-Bayyinah* (evidence) is a term for all what clarifies the truth and exposes it. And anyone who limits it by the two or four witnesses …etc, fails to provide the truth of this term".

He also quoted the prophetic *Hadith*:

" البينة على المدعي"، والمراد به: أن عليه ما يصحح دعواه ليُحكم له، والشاهدان من البينة، ولا ريب أن غيرها من أنواع البينة قد يكون أقوى منها.... الخ"

"Al-*Bayyinah* is the responsibility of the claimant, which means it is incumbent on the claimant to support and authenticate his claim in order to secure ruling to his favor. The two witnesses (or more) are one type of *al-Bayyinah*. Undoubtedly, other types of *Bayyinah* may be stronger (than the witnesses) …etc.
Bin Qasim included several other pieces of evidence from the Qur'an and *Sunnah* to support his view, and concluded that DNA fingerprinting could be depended upon to negate paternity, and could nullify the husband's claim to deny paternity, because in that situation, his claim is against intellect and fact. In such situations, the judges should consider DNA testing prior to *li`an*, since *li`an* is conditioned on absence of witnesses. So, if one of the couple has this evidence (*Bayyinah*) to support his/her view, there will be no reason for *li`an*. Using this technology fulfils the objectives of *Shari`ah* in the context of the proper preservation of lineage.

The validity of DNA fingerprinting in these cases is a strong *Bayyinah* (evidence) that is considered a strong witness.

Concluding remarks

Contemporary innovations in DNA sciences have opened unprecedented avenues in applications of human genetics, including utilization in paternity establishment and negation. Across the world, concerns over ethical and legal issues stirred continued debates. In the Islamic world, scholarly deliberations among jurists and specialists in medical sciences took place over the past two decades. As in other new achievements of medical sciences, Muslim jurists confirmed the *Shari`ah* principles of safeguarding family structure, lineage, psychosocial integrity and people's privacy.

Genetic fingerprinting, in the majority jurists opinion, is considered as corroborative evidence used within the framework of the established *Shari`ah* methods, even though this undertaking may not definitively verify or negate the disputed claims.
Some contemporary Muslim jurists, however, argue that DNA fingerprinting could be considered as the required testimony (*Shahadah* / *Bayyinah*) that negates the need for *li`an* in classical Islamic law. In view of earlier precedents in Islamic legal tradition, and of the differences among contemporary jurists, more Juristic deliberations and *ijtihad* are needed to explain *Shari`ah* standpoints, in the wake of DNA technological discoveries.

References

1.Principle of molecular genetics, www.uptodate.com/contents/principles-of-molecular-genetics?topicKey=PC%2F
2. Abinaya E, Narange P and Bhardwaj A. FROG-Finger Printing Genomic Variation Ontology. PLOS ONE/DOI:10.1371/journal.pone.0134693, August 2015.

3. Raby BA, Slavotinek A and Tirnauer J. Basic principles of genetic disease. www.uptodate.com , © 2015 UpToDate
4. Genetic fingerprinting explained. Department of Genetics- University of Leicester. http://www2.le.ac.uk/departments/genetics/jeffreys/explained
5."DNA fingerprinting" *Encyclopedia Britanica*, Encylcolpedia Britancia Online Inc, Web. 21 September, 2015.
<http://www.britanica.com/science/DNA-fingerprinting>
6. Read MM, (ed). 2005. Trends in: DNA Fingerprinting Research. New York, USA: Nova Science Publications, Inc.
7. Heras J, Dominguez C, Mata E, et al. A Survey of Tools for Analyzing DNA Fingerprints. Brief Bioinform. 2015.doi:10.1093/bib/bbv016
8. Blustein J. 2005. "Ethical Issues in DNA- based Paternity Testing". In Genetic Ties and the family; The Impact of Paternity Testing on Parents and Children, pp. 34-49. Baltimore, Md.: John Hopkins University.
9. Nelkin D. 2005. "Paternity Palaver in the Media. Selling Indentify Tests". In Genetic Ties and the Family: The Impact of Paternity Testing on Parents and Children. Pp 3-17. Baltimore, Md.:John Hopkins University.
10. Shabana A. Paternity Between Law and Biology: The Reconstruction of the Islamic Law of Paternity in the Wake of DNA Testing. Zygon, vol. 47, No.1, March 2012, pp. 214-239. www.zygonjournal.org
11."DNA fingerprinting" *Encyclopedia Britanica*, 2015.
<http://www.britanica.com/science/DNA-fingerprinting>
12. Selman-Ayetey, J. 2009. "DNA Profiling". In *Encyclopedia of Race and Crime*. Vol. 1, 207-09. Thousand Oaks, Calif: Sage Publications Inc.
13. Williams R and Johnson P. 2005. "Inclusiveness, Effectiveness and Intrusiveness: Issues in the Developing Uses of DNA profiling in support of criminal Investigations". Journal of Law, Medicine and Ethics 33: 545-58.
14. Connell, S. 2005. "Bioethics: ELSI". In *Encyclopedia of Life Sciences*. Vol.3, 179-82, Chichester, UK, Wiley, 20 vols.
15. Kelavkar, U. 2005. "Human Genome Project". In *Encyclopedia of Life Sciences*. Vol. 9, 283-93. Chichester, UK. Wiley, 20 vols.
16. Al-Lodami, TM. Al-Jeenat al-basharyyah wa tatbiqatuha: Dirasah Fiqhiyyah Muqarinah, 2011, A publication of The International Institute of Islamic Thought. PP 95-117.
17. Al-Shatibi (d.790/1388), al-Muafaqat fi ash-Shari`ah, 1975, vol.2:8-11, P 10.
18. Ibn Qudamah Al-Maqdisi, Al-Mughni, vol. 6, p 46.
19. Sunan Abi Dawod, volume 2, No. 2263.
20. Sahih Al-Bukhari, No. 4326, Sahih Muslim No. 63.
21. Ref. #16, pp 98-101.
22. Sahih Al-Bukhari, kitab al-Boyui, no. 2053.
23. Ibn al- Qayyim al-Jouziyyah in Zad ul Maad, Cairo, Dar al Taqwa, 1999. Volume 4, p 204.
24. Glorious Qura'n, Surat al Nour: Chapter 24: 6-9
25. Ibn al-Athir, Usd al-Ghabah fi Ma'rifat al-Sahabah, Beirut-Lebanon: Dar al-kutub al-Ilmiyyah, 5:380.
26. Shabana A. Negation of Paternity in Islamic Law between *Li`an* and DNA fingerprinting. Islamic Law and Society 20-3 (2013), 157-201. Doi:10.1163/15685195-0008A0001.
27. Ibn Qudamah: al-Mughni, 11/79.
28. IOMS, 11th Seminar, Ru'yah Islamiyyah: Genetics, Genetic Engineering, the Human Genome Project and Gene Therapy. Kuwait, 23-25 Jumada Al-Akhirah, 1419 H, 13-15 October, 1998. PP 323-529.
29. Ibid, PP 463-499.
30. Ibn Qudamah, Al-Mughni, 11: 167.
31. The International Islamic *Fiqh* Academy. The Organization of Islamic Cooperation (OIC), Jeddah, Saudi Arabia. www.fiqhacademy.org.sa , see: Majallet Majma' al-Fiqh al-Islamia 11.3 (1998): 533-43.
32. IOMS, The International Islamic Code for Medical and Health Ethics. December 11-14, 2004, held in Cairo- Egypt. Published in Kuwait, 2005, ed: Ahmad R. El-Gendy. PP 402-405. http://www.islamset.org
33. Council of The Islamic *Fiqh* Academy- Muslim World League, 16th session, Makkah Al-Mukarramah, 21-26 Shawwal, 1422H, (5-10 January,2002).
34. Shabana A. Islamic Law of Paternity Between Claassical Legal Texts and Modern contexts: From Physiognomy to DNA Analysis. Journal of Islamic Studies 25:1(2014). PP 1-32, doi:10.1093/jis/ett057
35. Hilali SM, al-Basma al-wirathiyyah, Kuwait: Majlis al-Nashr al-Ilmi, 2001, pp 240-1.
36. Uthman, MR, al-Madda al-wirathiyyh al-jinum (Cairo: Maktabat Wahba,2009, pp 325-339).
37. Bin Qasim, AM, Genetic fingerprinting, (Al-Bassmah al-Wirathiyyah), June 16,2004, Islam Today: islamtoday.net, http://www.islamtoday.net/bohooth/services/printart-86-3866.htm

CONSANGUINEOUS MARRIAGE

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Abstract

Consanguineous marriage such as marriage between cousins carries a stigma in Western countries and is often associated with Islam. However, consanguineous marriage is actually part of shared human history, continues to be preferred in many parts of the world, and is not advocated by Islam. There are genetic consequences for offsprings, particularly if a recessive disease is known to run in the community, but for certain families this risk is outweighed by social benefits of consanguineous unions. Modern genetic/genomic techniques with proper counseling can minimize and even eliminate specific recessive diseases that recur in a given community.

Keywords: Consanguineous, endogamous, homozygous, recessive, Islam, heterozygous, genetic counseling.

Definitions

"Consanguineous" refers to a union between two individuals who have a recent common ancestor, as opposed to between two random individuals from the human population at large. The term "inbreed" is sometimes used synonymously. "Endogamous" refers to a union between two individuals from the same tribe or clan. Humans are diploid organisms, meaning that autosomal genes are present in pairs, one copy (allele) coming from each parent. Consanguineous and endogamous marriages, as opposed to outbreed unions, increase the percentage of autosomal gene pair in which both alleles are the same in the offspring. When both alleles in an autosomal gene pair are the same, the genotype at that locus is "homozygous." When the two alleles at a given autosomal gene locus are different, the genotype is "heterozygous" for such locus. Autosomal recessive disease occurs when both alleles of a given autosomal gene pair have a detrimental recessive mutation, with either both alleles of the gene pair having the same identical mutation (homozygous recessive disease) or each allele of the gene pair having a different mutation (compound heterozygous recessive disease).

Autosomal recessive disease is classically fully penetrant, i.e, biallelic mutations in a given individual results in the disease phenotype. However, there can be exceptions with reduced penetrance, for example, certain biallelic mutations can cause cystic fibrosis in some individuals but not in others[1].

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In addition, autosomal recessive disease classically has minimal variable expressivity, i.e., it presents with the same degree of severity in all individuals with the biallelic mutations. However, there can be exceptions where some individuals have a less severe or later-onset phenotype. For example, recessive mutations in the gene *CYP1B1* typically cause severe newborn glaucoma, but can sometimes cause a later onset juvenile or even adult-onset glaucoma[2].

In clinical genetics, consanguineous marriage is defined as a union between individuals related as second cousin or closer, which results in their offspring having a co-efficient of inbreeding (F) of >=0.0156[3]. Second cousin and first cousin marriages are the most common forms of consanguineous marriage worldwide. Unions between biological first-degree relatives (e.g, siblings), considered incest, are socially reprehensible to most individuals across cultures, are generally illegal, and are not well-studied. They were common in ancient times among some ruling classes such as in Pharonic Egypt[4].

Background
And Contemporary Attitudes

Our human ancestors successfully migrated out of Africa some 40,000–70,000 years ago in relatively small breeding populations, perhaps in groups no more than 1000 individuals[5].

Thus in the past, consanguineous/endo-gamous marriage was commonplace and a necessity.

As humans settled in different environments throughout different parts of the world, local societies developed with different forms of social class stratification. Mate choice was often encouraged within one's social class, which continued to promote consanguinity and endogamy, particularly in geographic isolates, villages, and tribal groups.

With population growth, urbanization, international trade, and technological advancements, the human population has since globalized. Consanguineous marriage is still practiced in several regions of the world, but there have been major attitudinal shifts in Western populations and some Asian countries[3].

In Western societies prior to the mid-nineteenth century, first-cousin marriages were common in Europe particularly among elite ruling families, while in the United States, it was restricted, particularly among poorer immigrant populations[6]. In the mid-1800's in the United States, anecdotal observations that physical or mental disabilities could occur in some offspring of some first-cousin marriages prompted vocal critics to argue that the practice was unhealthy. This was before the era of medical genetics as a specialty and was largely based on anecdotal observations rather than scientific study. For example, an often cited example of the potential detrimental effects of inbreeding was the increased incidence of hemophilia in European royal families of 18th and 19th centuries. However, it is to be noted that this increased incidence would have occurred even if those families were not consanguineous because hemophilia is an X-linked rather than an autosomal recessive disease[7]. In any case, the ensuing debate, met with little or no opposition, led to many American states passing legislation prohibiting first-cousin marriage. Currently more than half the states in the United States ban the practice or stipulate special requirements before it can be allowed[6]. Given our current knowledge that there are greater genetic risks to offspring in other situations for which there are no prohibitions, this legal restriction of first-cousin marriage is somewhat illogical. As discussed below under “Genetic Consequences,” the risk for a congenital malformation from a first-cousin marriage, in general is in absolute terms only 1-3% over that of the general population[8]. In contrast,

for a given individual with recognized autosomal dominant disease, such as the fatal neurodegenerative disease Huntington's disease, the affected individual has 50% risk of passing the disease to his/her offspring when the spouse is not affected and there are no legal restrictions regarding marriage for such an individual.

In the mid-1800's a similar debate regarding consanguineous marriage as a potentially unhealthy practice also took place in Great Britain and Ireland. First-cousin marriages among the local population thereafter declined, although no legislation prohibiting first-cousin marriage was passed, and to date, there are no laws banning the practice in Europe. However, in some countries in Asia, legislation has been passed banning first-cousin marriage-these include China, Taiwan, both Koreas, and the Philippines[6]. Currently, there continues to be stigma and taboo regarding first-cousin marriage among the general population in Western and certain Asian countries, particularly in the United States[8].

In contrast to these developments in Western and certain Asian countries over the last 150 years, in many cultures in Africa, Asia, and the Middle East, first-cousin marriages continue to be preferred[3]. There are various reasons for this continued practice. Economically, the family benefits as assets and family property stay within the family. Socially, because the in-laws are known beforehand, pre-nuptial understandings and agreements are simplified. There tends to be better harmony between the spouse and his/her new in-laws in consanguineous unions[9], and divorce rates have been reported as lower[10]. In addition, the fact that the practice is traditional, is itself a reason for its continued preference in certain cultures.

Consanguineous Marriage and Islam

Consanguineous marriage is common in the Middle East, particularly in Muslim communities. Prior estimates have varied between 25% in Beirut to 60% in Saudi Arabia, reaching 90% or more among some Bedouin communities in Kuwait and Saudi Arabia[3,11]. Currently the average consanguinity rate in Saudi Arabia seems to be 56% although this can be higher in certain regions[12,13].

Because consanguineous marriage is often preferred in many Muslim countries, it is often assumed that Islam recommends the practice. This is not necessarily the case. There is nothing in the Qur'an advocating consanguineous marriage, while there is reportedly a Hadith that discourages it.

"Marry outside the family, lest the offspring be thin and weak." *[Ibn Hajar, Talkhis al-Habir]*"[14].

In addition, the second Caliph, *Omar ibn al-Khattab*, was said to have advised the *Bani Assayib* tribe to intermarry with other tribes and avoid cousin marriage.

"Marry from far away tribes, otherwise you will be weak and unhealthy"[15].

Clearly, consanguineous/ endogamous marriage is a pre-Islamic tradition. However, the practice may have been encouraged by Qur'anic rules of inheritance[16]. Daughters inherit half of the amount received by sons and the wife inherits a determinate share from her husband[17]. According to Islamic law, a dower (*mahr*) is specified as part of the marriage contract, and these goods are transferred to the bride at the time of marriage[18,19]. Thus a woman's share of her family wealth would be retained within the family or tribe by marriage to her paternal cousin.

Also, although there is the aforementioned Hadith reportedly discouraging consanguineous marriage[14], there are examples of consanguineous marriage in the early history of Islam. For example, *Fatima*, the daughter of the Prophet ﷺ , married *Ali (RAA)*, the Prophet's ward and first cousin[16].

While the Glorious Qur'an does not recomm-

end first-cousin marriage, it does not explicitly prohibit the practice. On the other hand, the Glorious Qur'an does specifically prohibit certain intra-familial marriages:

"Prohibited to you (in marriage) are: Your mothers, daughters, sisters; father's sisters, mother's sisters; brother's daughters, sister's daughters; foster-mothers (who gave you suck), foster-sisters; your wives' mothers; your step-daughters under your guardianship, born of your wives to whom you have gone in,- no prohibition if you have not gone in, (those who have been) wives of your sons proceeding from your loins; and two sisters in wedlock at one and the same time, except for what is past; for Allah is Oft-forgiving, Most Merciful"[20].

While the prohibition on uncle–niece marriage is observed in Middle Eastern Arab communities[21], in different regions of India, uncle–niece marriages have been reported in Muslim communities[22], possibly reflecting pre-Islamic marital customs among converts from the Hindu or Buddhist faiths.

Genetic Consequences

When both parents of a child share a recent common ancestor following a consanguineous marriage, each parent will inherit an identical copy of certain chromosomal regions (haplotypes).

Some of these haplotypes shared by both parents will be received by their offspring as a double copy in a homozygous, or more precisely autozygous state.

If there is a deleterious recessive gene mutation on a parental shared haplotype, the homozygous child will inherit two copies of the recessive disease gene (i.e., have biallelic homozygous mutations) and thus be affected by the disease. The closer the biological relationship between the parents, the greater is the homozygosity of the offspring, and thus the greater the probability their offsprings will inherit a detrimental recessive gene mutation. First cousins are predicted to share 12.5% (1/8) of their genes. Thus, on average, their progeny will be homozygous at 6.25% (1/16) of gene loci.

Because all individuals carry different recessive gene mutations in the heterozygous state, the fact that consanguineous marriage results in increased homozygosity in offspring means an increased probability for expressing a recessive disease in offspring[23]. However, overall, first-cousin marriages do not carry a high risk of congenital disease, particularly if there is no history for significant disease in the family, and it is only a minority of consanguineous unions that are at high risk for such disease. In general, first-cousin marriage increases the risk of significant birth defects in offspring only 1-3% over that of the general population (from 0.8-2.1% to 2.5-4.5%)[8]. In absolute terms this is approximately double the risk, but in relative terms it is only a 1-3% increased risk. The risk for consanguineous marriage is higher when a recessive disease is known to run in a particular community or family, when there is concurrent endogamy, and/or when families have large numbers of children. A recessive founder or *de novo* mutation can quickly rise to a high frequency in a tribal sub-community that practices endogamy, due to population stratification[24]. In this way, an otherwise rare genetic condition can become relatively common from a single founder mutation in particular subgroup in a country, such as congenital glaucoma (caused by recessive mutations in the gene *CYP1B1*) in Saudi Arabia[2,23]. Moreover, a high birth rate in the setting of consanguinity/endogamy further increases the probability that new recessive mutations inherited by both parents from a recent common ancestor will come together in the homozygous state in at least one of the several offspring and thus cause disease. This combination of consanguinity, endogamy, and preference for a large number of

children, as seen in several Arab countries, clearly increases the incidence of homozygous recessive disease mutations in offspring and thus the number of children affected by rare autosomal recessive disorders[23,25], sometimes to alarmingly high levels, and sometimes including diseases that are not seen elsewhere in the world[23,25]. When both parents are carriers for a given autosomal recessive disease with full penetrance, the chance of an affected offspring is 25%, the chance of a carrier (heterozygous) offspring is 50%, and the chance of a non-carrier offspring is 25%. If carrier parents have one or two children, the likelihood is none will be affected by the disease. However, if the carrier parents have 10 children, the likelihood is that more than one will be affected by the disease and several will be carriers.

Offspring of consanguineous unions may also be at increased risk for additional disorders other than single gene autosomal recessive ones, such as disorders of multifactorial or complex inheritance[26]. These include congenital abnormalities/ syndromes, hypertension, high blood pressure, and adult-onset glaucoma. However, there are few studies evaluating the effect of consanguinity on multifactorial diseases of childhood and adulthood and the studies to date are not conclusive as to whether consanguinity increases the risk for multifactorial disease, as it is difficult to separate out confounding socioeconomic, environmental, and subpopulation-related factors[8].

Furthermore, the increased prevalence of Down's syndrome observed in the Middle East, relative to the West could be related to an autosomal recessive gene predisposing to non-disjunction in certain families[26].

Similarly, it is difficult to separate a predisposeing role of consanguinity in this increased prevalence from the other confounding regional factors, such as advanced maternal age, high birth rate, and a preference not to terminate pregnancy based on results of prenatal genetic testing[27].

On the other hand, it has been suggested that consanguinity can "weed out" deleterious gene mutations, resulting in overall more healthy offspring other than the occurrence of autosomal recessive diseases in the minority of cases. However, again this is difficult to prove as it is difficult to separate out socioeconomic, environmental, and subpopulation-related factors[8].

What can be done about Homozygous Recessive Disease?

When a homozygous recessive disease is known to run in a particular family or group that wants to continue to practice consanguinity/endogamy, genetic testing and counseling to identify carriers (heterozygotes) is recommended. Marriage unions between carriers and non-carriers or between two non-carriers should be encouraged, and unions between two carriers should be strongly discouraged. Done in an organized fashion, this strategy can dramatically reduce the incidence of a recessive disease that is associated with a specific population, as was achieved with the reduction of Tay-Sachs disease in Jewish populations[28].

Genetic testing and counseling to identify carriers has been implemented by the government of Saudi Arabia for two common hereditary hemoglobinopathies in the region, thalassemia and sickle cell disease. On February 21, 2004 it became mandatory for potential Saudi couples to undergo carrier testing for thalassemia and sickle cell disease before they could be issued a marriage certificate[29]. Those found to be at high risk for having affected offspring have the right to marry regardless of the results of the testing, but they must undergo counseling before they are issued a marriage certificate. In the first two years of the program, it was estimated

that nearly 90% of potential Saudi couples who were found to be high risk couples as a result of the testing (both were carriers for the recessive gene) proceeded with marriage regardless of their high risk of having affected offsprings[29].

However, with improved counseling and awareness, over the next five years, the frequency of voluntary marriage cancellation has increased five-fold[30]. During the same period, the incidence of β-thalassemia has dramatically decreased[30]. Nevertheless, there is some discussion about legislation to ban marriage of couples who are both carriers for the recessive gene.

When a recessive disease occurs in a particular family or group that wants to continue to practice consanguinity/endogamy but the disease gene is not known, modern genomic techniques that take advantage of consanguineous family structure can often identify the underlying disease gene which in turn can facilitate genetic counseling and treatment research[31].

Homozygosity mapping, a genomic technique that tracks patterns of homozygosity that result from the bi-parental inheritance of a shared ancestral haplotype, has been very successful in helping to uncover new recessive disease mutations in affected consanguineous families, sometimes even from a single affected patient[31]. The clinician plays a key role in such situations as careful phenotyping is essential. For example this led to the gene discovery in the syndrome of ectopia lentis, spontaneous filtering blebs, and craniofacial dysmorphism[32].

Homozygosity-analysis guided exome sequencing in a Saudi woman from a consanguineous family who was clinically recognized to have this rare but distinct phenotype, uncovered the mutated gene, *ASPH,* which was then confirmed to be mutated in other similarly affected individuals from Lebanon[32].

Summary and Recommendations

Consanguineous marriage is part of our shared human heritage and continues to be preferred in many parts of Asia, Africa, and the Middle East. Over the last 150 years the practice has become stigmatized in Western and some Asian countries through anecdotes and legislation rather than on the basis of scientific study.

Consanguineous marriage increases homozygosity in offspring and thus the risk for expressing recessive gene mutations (homozygous recessive disease). In general, the overall risk of significant congenital disease from first-cousin marriage is not much higher than the risk from a non-consanguineous marriage (1-3% greater). However, if a recessive disease is known to run in a family or community, the risk is greater. Also, the coupling of consanguinity with endogamy and a preference for a large number of children, as is often seen in Arab Muslim communities, can increase the number of offspring with an autosomal recessive disease that is rare or virtually absent in the rest of the world to unacceptably high levels.

In many families/communities without a history for significant recessive disease, there are cultural, social, and economic reasons for consanguineous marriage that outweigh a theoretical increased 1-3% probability of a rare disease. Islam does not advocate first-cousin marriages but does not prohibit it.

When a recessive disease associated with an identified gene is known to run in a particular family / community and consanguineous marriage is desired, genetic testing/ counseling can reduce the burden of the disease and even effectively eliminate it from the family/ community. If the recessive disease gene is not known, modern genomic techniques that take advantage of consanguineous family structure, can allow discovery of the gene, which in turn can lead

to better treatments as well as enable genetic testing/counseling that can reduce the burden of the disease and even effectively eliminate it from the family/community.

References

1. Thauvin-Robinet C, Munck A, Huet F, Genin E, Bellis G, Gautier E, et al. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. J Med Genet. 2009 Nov;46(11):752-8.
2. Khan AO. Genetics of primary glaucoma. Curr Opin Ophthalmol. 2011 Sep;22(5):347-55.
3. Bittles A. Consanguinity and its relevance to clinical genetics. Clin Genet. 2001 Aug;60(2):89-98.
4. Middleton, R. Brother–sister and father–daughter marriage in Ancient Egypt. Am Sociol Rev. 1962 27:603–11.
5. Liu H, Prugnolle F, Manica A, Balloux F. A geographically explicit genetic model of worldwide human-settlement history. Am J Hum Genet. 2006 Aug;79(2):230-7.
6. Paul DB, Spencer HG. "It's ok, we're not cousins by blood": the cousin marriage controversy in historical perspective. PLoS Biol. 2008 Dec 23;6(12):2627-30.
7. Rogaev EI, Grigorenko AP, Faskhutdinova G, Kittler EL, Moliaka YK. Genotype analysis identifies the cause of the "royal disease". Science. 2009 Nov 6;326(5954):817.
8. Bennett RL, Motulsky AG, Bittles A, Hudgins L, Uhrich S, Lochner Doyle D, et al. Genetic counseling and screening of consanguineous couples and their offspring: recommendations of the National Society of Genetic Counselors. J Genet Couns. 2002 Apr 11;2,:97-119.
9. Hamamy H, Bittles AH. Genetic clinics in arab communities: meeting individual, family and community needs. Public Health Genomics. 2009;12(1):30-40.
10. Saadat M. Association between consanguinity and survival of marriages. Egypt J Med Hum Genet. 2015;16:67-70.
11. Warsy AS, Al-Jaser MH, Albdass A, Al-Daihan S, Alanazi M. Is consanguinity prevalence decreasing in Saudis?: A study in two generations. Afr Health Sci. 2014 Jun;14(2):314-21.
12. El-Mouzan MI, Al-Salloum AA, Al-Herbish AS, Qurachi MM, Al-Omar AA. Regional variations in the prevalence of consanguinity in Saudi Arabia. Saudi Med J. 2007 Dec;28(12):1881-4.
13. El Mouzan MI, Al Salloum AA, Al Herbish AS, Qurachi MM, Al Omar AA. Consanguinity and major genetic disorders in Saudi children: a community-based cross-sectional study. Ann Saudi Med. 2008 May-Jun;28(3):169-73.
14. Hussain R. Community perceptions of reasons for preference for consanguineous marriages in Pakistan. J Biosoc Sci. 1999 Oct;31(4):449-61.
15. Albar MA. Counselling about genetic disease: an Islamic perspective. East Mediterr Health J. 1999 Nov;5(6):1129-33.
16. Bittles AH, Hamamy HA. Ch. 4: Genetic disorders among Arab populations. In Teebi AS (ed.). Endogamy and consanguineous marriage in Arab populations. Springer-Verlag 2010; Berlin. pp. 85-108.
17. The Glorious Qur'an Surah 4 (An-Nisa), Verses 7,11,12.
18. Khuri, FI. Parallel cousin marriage reconsidered: a Middle Eastern practice that nullifies the effects of marriage on the intensity of family relationships. Man. 1970 5;597–618.
19. Tucker, JE. Marriage and family in Nablus, 1720–1856: toward a history of Arab marriage. J Fam Hist. 1988 13;165–79.
20. The Glorious Qur'an Surah 4 (An-Nisa), Verse 23.
21. Teebi AS, Marafie MJ. Uncle-niece/aunt-nephew marriages are not existing in Muslim Arabs. Am J Med Genet. 1988 Aug;30(4):981, 983.
22. Bittles AH, Hussain R. An analysis of consanguineous marriage in the Muslim population of India at regional and state levels. Ann Hum Biol. 2000 Mar-Apr;27(2):163-71.
23. Khan AO. Ocular genetic disease in the Middle East. Curr Opin Ophthalmol. 2013 Sep;24(5):369-78.
24. Bittles AH. A community genetics perspective on consanguineous marriage. Community Genet. 2008;11(6):324-30.
25. Al-Gazali L, Hamamy H, Al-Arrayad S. Genetic disorders in the Arab world. BMJ. 2006 Oct 21;333(7573):831-4.
26. Fadel HE. Strategies to decrease the incidence of genetic disorders in Arab countries. JIMA. 2008;40:98-103.
27 Afifi HH1, Abdel Azeem AA, El-Bassyouni HT, Gheith ME, Rizk A, Bateman JB. Distinct ocular expression in infants and children with Down syndrome in Cairo, Egypt: myopia and heart disease. JAMA Ophthalmol. 2013 Aug;131(8):1057-66.
28. Kaplan F. Tay-Sachs disease carrier screening: a model for prevention of genetic disease. Genet Test. 1998;2(4):271-92.
29. Alhamdan NA, Almazrou YY, Alswaidi FM, Choudhry AJ. Premarital screening for thalassemia and sickle cell disease in Saudi Arabia. Genet Med. 2007 Jun;9(6):372-7.
30. Memish ZA, Saeedi MY. Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and beta-thalassemia in Saudi Arabia. Ann Saudi Med. 2011 May-Jun;31(3):229-35.

31. Alkuraya FS. Impact of New Genomic Tools on the Practice of Clinical Genetics in Consanguineous Populations: The Saudi Experience. Clin Genet. 2013 Mar 1.
32. Patel N, Khan AO, Mansour A, Mohamed JY, Al-Assiri A, Haddad R, et al. Mutations in ASPH cause facial dysmorphism, lens dislocation, anterior-segment abnormalities, and spontaneous filtering blebs, or Traboulsi syndrome. Am J Hum Genet. 2014 May 1;94(5):755-9.

PREMARITAL GENETIC TESTING FOR HEREDITARY DISEASE IN LIGHT OF ISLAMIC MEDICAL JURISPRUDENCE

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Abstract

Premarital genetic testing (PGT) for prospective couples includes tests for genetic, infectious and blood transmitted diseases to prevent any risk of transmitting these diseases to their children. It is deemed important in view of the increasing number of children affected with genetic or blood transmitted diseases. In Arab countries, for example, in United Arab Emirates (UAE), Saudi Arabia and Bahrain, their Ministries of Health have made it mandatory for all prospective married couples to undergo this screening process before getting married. This is being enforced precisely because of the high prevalence of hereditary diseases, like sickle cell disease and Thalassemia which may be associated with the high prevalence of consanguineous marriages in such countries. Whilst there are obvious benefits of PGT, Muslim scholars are divided on the issue of the enforcement of this test upon the population. This paper addresses their concerns and an attempt is made to justify mandatory PGT in light of Islamic Medical Jurisprudence.

Keywords: Premarital genital testing, genetic diseases, consanguineous marriages, Islamic jurisprudence.

Introduction

Generally, diseases that affect humans are categorized as acquired or hereditary. An acquired disease is not present at birth (congenital), but develops later on in life, for example, tuberculosis, etc. Hereditary diseases, on the other hand, are diseases that are present in the genetic makeup of a person and may to be passed on from generation to generation[1]. Premarital genetic testing (PGT) is a test carried out on prospective couples who intend to get married. The objective is to test them in order to ascertain whether they are carriers of genetic diseases which could be transmitted to their offspring[2].

It is important to note that many parents are unaware that they are carriers of certain genetic disorders in view of the fact that a carrier of a genetic disorder does not carry any morbidity nor does the disorder manifest in one's physical constitution (normal phenotype). Hence they only become aware that they are carriers of a particular disorder after their children are born with that specific genetic disorder.

For example, in the United Arab Emirates, Saudi Arabia and Bahrain there is a high prevalence of hereditary diseases like sickle cell anemia and Thalassemia which resulted from consanguineous marriages.

This reality prompted the governments in these countries to make it mandatory for all prospective couples to undergo PGT[3].

Merits of Premarital Genetic Screening

In a study conducted in Bahrain[4] where consanguineous marriages were prevalent, it was found that as a result of mandatory premarital genetic counseling and screening, the following positive results were achieved:

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- Increase of 'separation before engagement' in 'arranged' marriages.
- Decline of 50% in the incidence of sickle cell disease in neonates.
- Decline in the rate of cousin marriages from 45.5% in the previous generation to 39.4% in the present generation.
- Increased awareness of the negative effects of consanguineous marriages.

Shaykh `Abd al-Rashid Qasim[5] concedes that PGT will enable prospective marriage couples who were unaware of the fact that they were both carriers of the same genetic disease to be better informed. They would have the option to either call off the marriage or to take precautionary measures not to have children if they nevertheless wish to get married to each other.

Concerns

In the context of mandatory PGT, however, several concerns may be raised and some of them are hereunder discussed:

(a) Violation of autonomy

Autonomy is defined as a principle of medical ethics which necessitates one to respect the rights of others so that they could freely make their own choices and decisions[6]. Thus the imposition of mandatory PGT by the State compromises the autonomy of the populace and may be considered un-ethical.

(b) Falsification of medical certificates

According to Albar[7](1998) when the Arab countries were contemplating making PGT mandatory in the latter part of the 1900s for their citizens, it was feared that the cost of such a test would be too exorbitant and hence most governments would not be able to fund such a test for their subjects. Moreover, citizens residing in the less affluent Arab countries, like for example, Egypt, Morocco and Syria, would decline the test or evade it on the grounds of poverty. Furthermore, it was argued that if PGT were to be imposed as a precondition for the solemnization of marriage, then that would inadvertently open the doors for trafficking in false medical certificates. However, today, PGT is a gratis routine mandatory procedure for the populace in the affluent Middle Eastern countries.

(c) Breach of confidentiality

The results of PGT ought to be confidential in the sense that a physician or other health professionals are expected to hold secret all information related to the clientele, unless the clientele gives consent permitting disclosure (thefreedictionary.com). Hence breach of confidentiality insofar as the results of PGT is concerned will undermine their (especially women) chances to have further marriage proposals[8] if they are tested positive as carriers of genetic disorders.

(d) Limitations of PGT

It is important to note that due to the cost factor, mandatory PGT is limited to two or three genetic diseases common amongst the local population. However, having undergone PGT, the prospective couples, who find out that they are non-carriers of any of the genetic diseases for which they had been tested for, may have the false notion that their offspring would be immune from other forms of genetic disorders[9]. In order to circumvent this, the State which imposes mandatory PGT safeguards itself by making the prospective couples sign an indemnity form clearly stipulating the genetic disease/s they had been tested for so as to indemnify the State from litigation.

Dissenting Views

There are some Muslim scholars who oppose mandatory PGT and they substantiate their objections on the basis of the following arguments[10]:

Firstly, they state that the conditions for contracting *nikah* (marriage) have clearly been defined in both the Glorious Qur'an and in the *Sunnah*. For example, Muslim women are not allowed to marry non-Muslims[11], Muslim men are not allowed to marry two sisters at one and the same time and likewise Muslims in general are cautioned against marrying those who are related to them through blood relationship[12]. Hence they point out that PGT cannot be made a condition for marriage since *Sayyiduna* Muhammad (SAAS) said: "Every condition that

does not exist in the Book of Allah (*Qur'an*) is invalid[13]".

Secondly, while one of the primary objectives of marriage is procreation, women who have passed the age of child-bearing may also wish to marry for the sake of companionship and not for procreation. It would therefore be meaningless to impose PGT on such prospective elderly couples[14].

Thirdly, undergoing PGT does not in any way guarantee that the offspring of the prospective couples would be totally safeguarded from genetic disorders. Hence, they are of the view that prospective couples ought to supplicate for pious and healthy offspring just like *Sayyiduna* Zakariyya did: *"Then Zakariyya prayed unto His Lord and said: "My Lord! Bestow upon me Your bounty, a goodly offspring. Lo! You are the Hearer of prayer"*[15]. In other word, the prospective couples must place their *tawakkul* (trust) in Allah (SWT) and acknowledge the power of *du`a'* (sincere supplication).

Fourthly, while they do concede that the Muslim ruler has the prerogative to enforce certain matters as mandatory, they are of the view that it should be restricted to matters which involve the interest of wider public. They do not accept PGT to be in the interest of public welfare since its negative consequences far outweigh its benefits[16]. Thus in the context of mandatory PGT, they contend that an individual's autonomy should not be compromised and hence prospective couples should be encouraged to undergo PGT and to be educated about its benefits, but at the same time they should be allowed to exercise their autonomy either to undergo the test or not.

Endorsement of Mandatory PGT

Islamic Medical Jurisprudence is in essence the science which deals with bioethical issues in the field of healthcare and medicine[17]. Thus in light of Islamic Medical Jurisprudence, mandatory PGT can be substantiated on the basis of the following considerations:

(a) Textual Evidence

The Qur'anic *ayah*: "*Obey Allah and obey His messenger and those in authority amongst you*"[18] justifies Muslims to uphold the State's imposition of mandatory PGT in the interest of a pressing social need, namely, to safeguard its population from inheriting genetic disorders.

Moreover, the Qur'anic *ayah*: "*Do not make your hands contribute to your own destruction*"[19] may be cited to justify prospective Muslim parents taking the initiative to avoid producing offspring who would be suffering from genetic disorders. Caring for children with genetic disorders would place them in financial difficulties which would have adverse effects on their mental sanity.

(b) *Maqsid al-Shari`ah*[20]

One of the five objectives of Islamic Law is *hifz al-nasl* (protection of progeny). Hence taking steps to ensure that future generations are free from genetic disorders is in conformity with this objective.

(c) Justifications for the compromizing of autonomy on the basis of *al-Qawa'id al-Fiqhiyyah*[21] (Legal Maxims).

One of the Legal Maxims is *akhaff al-dararayn* (lesser of two evils). In the context of this paper, the two evils are (i) mandatory PGT and (ii) the prevalence of birth of children with genetic disorders. It would therefore be a lesser evil to compromise autonomy for the sake of a pressing social need, i.e. to prevent the births of children with genetic disorders.

Another principle of Islamic Jurisprudence is *sadd al-dhara`i* which implies blocking the means to an expected end (evil) which is likely to materialize if the end towards it is not obstructed. In other words, therefore, it would be permissible to block autonomy in the context of PGT to an expected end of safeguarding the progeny from inheriting genetic disorders.

(d) Genetic counseling

Genetic counseling for the prospective couples who are at risk of passing the genetic disorder to their unborn offspring should be encouraged in view of the fact that such counseling conforms to the following *hadith*: "*Al-Din nasihah*"[22] (Religion is sincere advice). They should be advised on contraception and if they wish to have children, it is important that they are made aware of that in light of Islamic Medical Jurisprudence, it is unacceptable for them to resort to sterilization, to solicit third party

intervention in the form of artificial insemination with donor sperm, egg donation and surrogate motherhood and adoption[23]. Likewise, they cannot legally adopt children, but they could foster a child, but that would entail observance of the rules of *`awrah* (privacy) within their homes. In other words, when the female who is foster-parented attains puberty, she would not be able to freely come out in the presence of her foster father without observing the rules of seclusion of her privacy. In the same way, the foster mother will have to observe the rules of seclusion in front of her foster son. A viable option for them would be to resort to pre-implantation diagnosis (PGD), if they so wish to have a child who would be free from hereditary disease[24].

Reflection on the above two viewpoints

It is important to note that under dissenting views, the first objection raised implies that PGT is a (condition) for marriage, which is not necessarily the case. PGT is only required prior to marriage, and the decision to go ahead with the marriage is left entirely to the prospective couple intending to be married.

Under endorsement of mandatory PGT, it should also be noted that undergoing PGT before marriage can effectively avert the procreation of children who could be susceptible to inheriting chronic and disabling genetic disorders thus reducing the severe psychosocial problems on the family and the economic burdens on both the family and the State. Hence, imposing PGT upon all prospective marriage couples would markedly outweigh the costs of testing.

Moreover, under (d) above, genetic counseling, it should equally be noted that in the area of reassurance and counseling of carriers, it is important to educate the prospective couples that[25]:

- Being a carrier is not a disease, and not shameful.
- It may be associated with some health advantages for survival: e.g., established Malaria survival in carriers of sickle cell trait noted in Africa.
- The advantage of knowing one is a carrier enables him/her to better plan for a healthy family and avoid genetic diseases.
- Any carrier can safely marry a non-carrier.

Conclusions

In countries where certain genetic disorders are prevalent, it would be perfectly in order, in light of Islamic Medical Jurisprudence, for the State to impose mandatory PGT upon its populace who intend to get married for that would be in fulfilment of a pressing social need, namely, to safeguard them from bearing children with genetic diseases. However, confidentiality ought to be guarded at all times. Thus, it is important that the populace receive genetic counseling both prior to and after PGT[26]. However, if a couple who are both carriers of a particular genetic disorder were to still wish to take the risk and be married to each other, they should not be discouraged to do so, bearing in mind the power of *du`a'* (supplication). They should thus be counseled about contraception and if they do want to have children they should be advised as to what is acceptable within the ambit of Islamic Medical Jurisprudence *vis-à-vis* their right to procreate[27].

References

1. Oman Hereditary Blood Disorder Association. 20015. Premarital Genetic Screening. Available from: http://www.omancares.org/en/premaritalScreening.php.
2. Eastern Biotech and Life Sciences. 2014. Pre Marital Genetic Screening. Available from: http://www.easternbiotech.com/resource_Pre%20marital%20Screening.php.
3. Rahman, MM et al. PremaRITAL Health Screning – A Review and Update. *JAFMC* Bangladesh. Vol 10, No 1 (June) 2014, p. 105.
4. Al-Arrayed, S.S. Review of the spectrum of genetic disease in *Bahrain Eastern Mediterranean Health journal*, Vol. 5, No. 6.
5. Qasim, `Abd al-Rashid. Mandatory Genetic Medical Examinations. Available from: http://en.Islamtoday.netstarshow-403-3348.htm.
6. Farlex. The Free Dictionary. Available from: http://medical-dictionary.thefreedictionary.com/autonomy.
7. Albar, Muhammed Ali. April – June 1998. Genetic Counselling and Genetic Issues: An Islamic Perspective. The Fountain on Life, Knowledge and Belief. Available from:

http://www.fountainmagazine.com/Issue/detail/Genetic-Counselling-and-Genetic-Disseases- An-Islamic-Perspective.
8. Qasim, `Abd al-Rashid. Mandatory Genetic Medical Examinations. Available from: http://en.Islamtoday.netstarshow-403-3348.htm.
9. Ibid.
10. Ibid.
11. The Glorious Qur'an. *Al-Baqarah*, 2:221.
12. The Glorious Qur'an. *Al-Nisa*', 4:22-23.
13. Khan, Muhammad Muhsin. The Translation of the Meanings of *Sahih al-Bukhari*. 1997. The Book of Sales (Bargains). Vol. 3, Hadith no. 2168, p. 212.
14. Qasim, `Abd al-Rashid. Mandatory Genetic Medical Examinations. Available from: http://en.Islamtoday.netstarshow-403-3348.htm.
15. The Glorious Qur'an *Al `Imran*, 3:38
16. Qasim, `Abd al-Rashid. Mandatory Genetic Medical Examinations. Available from: http://en.Islamtoday.netstarshow-403-3348.htm.
17. Ebrahim, Abul Fadl Mohsin. 2008. *An Introduction to Islamic Medical Jurisprudence*. Durban. Islamic Medical Association of South Africa p. 3.
18. The Glorious Qur'an. *Al-Nisa,* 4:59.
19. The Glorious Qur'an. *Al-Baqarah*, 2:195.
20. *An Introduction to Islamic Medical Jurisprudence*, op. cit., p. 3.
21. Ibid, p. 15.
22. Al-Nasayburi, Muslim ibn al-Hajjaj.N.D. *Sahih Muslim*. Cairo. Dar al-Sha`b. Vol. 1, p. 238.
23. Albar, Mohammed A. Ethical considerations in the prevention and management of genetic disorders with special emphasis on religious considerations. *Saudi Med. J* 2002; Vol. 23 (6).
24. Haji Ahmad, Norhayati. Islamic Views on the Science of Procreation. *Eubios Journal of Asian and International Bioethics* 13 (2003), 59-60).
25. Al-Bar MA and Chemsi-Pasha H. contemporary Bioethics- Islamic Perspectives-Springer open. PP 191-192. Doi 10.1007/978-3-319-18428-9-1.
26. El-Hazmi, Mohsen A.F. Genetical Aspects of Research and Medical Services in Islamic Countries. Available from: http://www.researchgate.net/profile/Mohsen_ElHazmi/publication/221917595_Genethical_Aspects_of_Research_and_Medical_Services_in_Islamic_Countries/links/0046352b0a2a7275c0000000.pdf.
27. Al-Aqeel, I Aida. Ethical guidelines in genetics and genomics. An Islamic perspective. *Saudi Med J* 2005; Vol. 26 (12).

PRENATAL DIAGNOSIS: A MODEL FOR GENETIC COUNSELING AND TESTING

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Abstract

Over the past 4 decades, there have been revolutionary changes in the approach to prenatal diagnosis of fetal congenital defects during pregnancy. Prenatal diagnosis evolved from assessment of risk based on maternal age alone to the advent of serum screening and ultimately combined testing utilizing serum markers and ultrasound nuchal translucency (NT) to detailed evaluation of fetal anatomy and invasive diagnostic procedures involving sampling of intrauterine amniotic fluid, placental villi or fetal blood. The result is improved detection rates of Down's Syndrome from approximately 30% to greater than 90% over the past 20 years. The recent introduction of non-invasive prenatal testing (NIPT) via cell-free fetal DNA analysis has created exciting opportunities to expand and improve genetic testing options with a high sensitivity (>99%) coupled with a low false positive rate.

A pendulum has swung back and forth, between which test to start with, as new technologies have been developed. The novelty and complexity of these technologies, combined with the commercial interest to implement these tests rapidly into clinical care, have created challenges for physicians and patients. Overall, prenatal diagnosis has moved along two parallel paths (imaging and tissue diagnoses) that sometimes converge. Often, clinicians are experts in one diagnostic modality or the other and there are a very limited number who are experts in both. As a result, there is often a huge variability in approach to screening and diagnosis depending on by whom and where a patient is seen.

Keywords: prenatal screening, DS, plasma free fetal DNA, Mendelian disorders, genetic counseling.

Introduction

From the moment of conception to the time of delivery, the growing baby (fetus) goes through several stages of development before he or she is ready to be born. Fortunately, the majority of babies are born healthy; however, 2-3% of infants are born with congenital abnormalities or defects, some of which are major ones that endangers their health. Fetuses with abnormal number of chromosomes (aneuploidy) account for 6–11% of all stillbirths and neonatal deaths.

Fetal congenital defects might be hereditary due to chromosomal or genetic disorders, or spontaneous non-hereditary due to developmental defects, genetic mutation, or exposure to teratogens.

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Parents are always anxious about their fetus and eager to know if it has any problem before it is born. Certain fetal conditions can be treated before the baby is born, while others may need special treatment immediately after delivery. Without knowledge gained by prenatal diagnosis, there could be an untoward outcome for the fetus or the mother or both. Prenatal diagnosis provides prospective parents with information that enables them to make decisions about the health care for their infant.

Prenatal diagnosis is a new field in which doctors, Maternal-Fetal Medicine specialists (perinatologists) and geneticists, determine whether a fetus has a problem or congenital defects. Prenatal diagnosis involves genetic counseling to prospective parents, performing screening tests on mothers to assess their risks for having a child with congenital abnormalities, and diagnostic tests to confirm or disprove the results of maternal screening tests. Prenatal diagnosis is now a part of routine prenatal care. Ideally, every pregnant woman is to be offered screening for hereditary disorders and chromosomal aneuploidy. The aim is to detect birth defects such as neural tube defects, Down's syndrome, other chromosome abnormalities, and hereditary genetic disorders.

In the past decades, numerous markers and strategies for screening and diagnosing congenital fetal defects and chromosomal aneuploidies have been developed. The recent advances in technology have created exciting opportunities to expand and improve genetic testing options that are available to women during pregnancy. The novelty and complexity of these technologies, combined with the commercial interest to implement these tests rapidly into clinical care, have created challenges for physicians and patients. Obstetric providers are challenged continuously with the evaluation of the potential benefits and harms of new screening and diagnostic procedures or technologies for their patients (mother and fetus). The pregnant women are challended to understand the intricacies of these tests and to make the appropriate decision based on the results of these tests.

The purpose of this article is to present and evaluate the available screening and diagnostic strategies offered in clinical practice and to help prospective parents to better understand the pros and cons of each method.

Chromosomes and Genetic Disorders

Few areas in science and medicine have advanced at the pace we are experiencing as the fields of genetics and genomics. The Human Genome Project, at the beginning of the 21st century, have provided a complete sequence of our genome that now serves as the foundation to our understandings of gene regulation and genetic disorders. This knowledge of genetic principles are now not restricted to any one medical specialty but in fact have permeated all areas of medicine as virtually any disease is the result of the combined action of genes and environment.

The human genome consists of large numbers of deoxyribonucleic acid (**DNA**) molecules that contain within their structure the genetic information needed to specify all aspects of embryogenesis, development, growth, metabolism, and reproduction, essentially all aspects of what makes a human being a functional organism. Every nucleated cell in the body carries its own copy of the human genome, which contains approximately 20,000 to 25,000 protein coding genes. The genes are the functional units of genetic information encoded in the DNA of the genome. These genes are organized into a number of rod-shaped organelles in the nucleus of each cell, called chromosomes.

The composition of genes in the human genome, as well as the determinants of their expression, is specified in the DNA sequence of the 46 human chromosomes in the nucleus

plus the mitochondrial chromosome. Each human nuclear chromosome consists of a single continuous double helix DNA and the nuclear genome consists, therefore, of 46 linear DNA molecules totaling more than 6 billion nucleotide pairs. The DNA helices are packaged with several classes of specialized proteins (histone and non-histone) to make **chromatin**. In the non-dividing cell, chromatin is distributed throughout the nucleus, and as the cell divides, its genome condenses to appear as microscopically visible chromosomes.

Each species has characteristic chromosome compliment (**karyotype**) in terms of the number, morphology, and contents of the chromosomes that make up its genome. The genes are localized in linear order along the chromosomes and each gene has a precise position or **locus**. The study of the chromosomes, their structure and inheritance is called **cytogenetics**[1]. With the exception of cells that develop into gametes (the **germline**); all cells that contribute to one's body are called **somatic cells**. The genome contained within the nucleus of human somatic cells consists of 46 chromosomes arranged in 23 pairs. Of those 23 pairs, 22 pairs are alike in males and females and are called **autosomes**. The remaining pair is the two different types of **sex chromosomes**: an X and Y chromosome in males and two X chromosomes in females. Members of paired chromosomes (referred to as **homologous**) carry matching genetic information, same genes in the same order. At any specific locus, the genetic information might be identical or may vary slightly in sequence; these different forms of a gene are called **alleles**. One member of each pair of chromosomes is inherited from the father and the other from the mother[2].

Mitochondrial chromosome is a small but important part of the human genome. Human cells can have hundreds to thousands of mitochondria, each containing a number of copies of small circular mitochondrial chromosome. The mitochondrial DNA molecule is only 16 kb in length. It is just a tiny fraction of the length of even the smallest nuclear chromosome. It encodes only 37 genes and the products of these genes function in the mitochondria. The genes of the mitochondrial chromosome exhibit exclusively maternal inheritance.

Among congenital anomalies caused wholly or partially by genetic factors, three main types are recognized: chromosome disorders, single-gene disorders, and multifactorial disorders[3].

Chromosome disorders are due to the excess or deficiency of the genes located on entire chromosomes or chromosome segments. As a group, chromosome disorders are common, affecting approximately 7 per 1000 live born infants and accounting for approximately half of all spontaneous abortions occurring in the first trimester of pregnancy.

Single-gene defects are caused by pathogenic mutations in individual genes. The mutation may be present on both chromosomes of a pair (homozygous) or only on one chromosome of a pair (heterozygous). This leads to a critical error in the genetic information carried out by these individual genes with a classic Mendelian inheritance patterns in families (autosomal recessive, autosomal dominant, or X-linked). Most of single-gene defects disorders are rare, with a frequency that varies between 1 in 500 to 1 in 1000 individuals. However, single-gene disorders are responsible for a significant proportion of disease and death. Overall, the incidence of serious single-gene disorders in pediatric population is estimated to be approximately 1 per 300 live born infants.

Multifactorial disorders with complex inheritance contribute to the majority of diseases in which there is a genetic contribution. Multifactorial disorders include congenital heart defects, cleft lip and palate,

and Hirschsprung disease, as well as many common disorders of adult life, such as diabetes, coronary heart disease, and Alzheimer disease. There appears to be no single error in the genetic information in many of these conditions. Rather, the disease is the result of the combined impact of variant forms of many different genes; each variant may cause, protect from, or predispose to a serious defect, often in concert with or triggered by environmental factors. Estimates of the impacts of multifactorial disease range from 5% in the pediatric population to more than 60% in the adult population[4].

Understanding the genetic origin, prevalence, and inheritance mechanism of these congenital anomalies are crucial for proper prenatal screening and counseling of pregnant women, as well as for determining their proper diagnosis.

Genetic counseling

The initial step in providing prenatal diagnosis for every prospective parents is receiving genetic counseling. Family history plays a critical role in assessing the risk of inherited medical conditions and single gene disorders. A common tool used is the family history questionnaire or checklist. Positive responses on the questionnaire are usually followed up by the healthcare provider to obtain more detail, including the relationship of the affected family member(s) to either parent, exact diagnosis, age of onset, severity of disease, and if there is evidence of consanguinity (relation by blood or by common ancestor).

Another family history assessment tool, commonly used by genetics professionals, is the family pedigree. The pedigree may assist in determining the mode of inheritance of a specific condition and identify the members at increased risk of being carriers or of developing the condition. This will help the healthcare provider or geneticist in estimating the probability that the fetus might be affected and in offering the particular test (s) to diagnose the condition.

Single-gene (Mendelian inheritance) conditions are inherited as either autosomal dominant, recessive or sex linked patterns. Certain conditions are more common in specific ethnic groups, although it is essential to note that there are no disorders found uniquely in a certain ethnic or racial group and that many families may not have an obvious predominant ethnicity. If carrier testing is to be done on the basis of ethnicity, it is reasonable to offer this to the pregnant woman first and then test the father only if the mother is positive. If testing is being considered on the basis of an affected relative, the test is offered to the family member of the affected individual first. Table 1 includes recommendations for genetic testing based solely on ethnic identity.

Apart from genetic counseling based on single gene disorders or family history the most common risk assessment is that for aneuploidies, specifically for Down's Syndrome (DS). Aneuploidies,DS and other trisomies are primarily the result of meiotic nondisjunction, which increases with maternal age. Fetuses with aneuploidy may have major anatomic malformations that often are discovered during an ultrasound examination. An abnormality involving a major organ, or the finding of two or more minor structural abnormalities in the same fetus indicates an increased risk of fetal aneuploidy. Although advanced maternal age (> 35 years) is by itself a risk for fetal aneuploidy, genetic counseling and screening for aneuploidy is offered to all women who seek prenatal care regardless of their age. Women should be counseled regarding the differences between screening and diagnostic tests. Genetic counseling also should include information about the detection and false-positive rates of the screening test to

be used. Information about the limitations as well as the risks and benefits of any diagnostic procedure that might follow the screening tests should be available to patients so that they can make informed decisions. In couples in which the male partner is 45 years or older, counseling should also address the increased risk of new onset autosomal dominant disorders (such as neurofibromatosis or Marfan syndrome) that are associated with increased paternal age. Some patients may benefit from a more extensive counseling with genetics professional or a Maternal-Fetal Medicine specialist, especially if there is a family history of a chromosome abnormality, genetic disorder, or congenital malformation.
It is very important that the patient be informed during genetic counseling that screening test results only represent numerical information regarding her risk of having a fetus with aneuploidy. Communicating and interpreting the numerical risk assessment is crucial to enable women and their partners to balance the consequences of having a child with the particular disorder against the risk of an invasive diagnostic test that she might consider. It is often useful to contrast this risk with the general population risk and their age-related risk before screening.

Prenatal screening

In the 1970s, prenatal screening was simple and included carrier screening for some Mendelian disorders and for those who are at increased risk for chromosomal aneuploidy because they will be 35 years or older at the time of delivery. Since then, there has been an explosion of testing possibilities and a simultaneous significant increase in the number of births to women 35 years of age and older. These now constitute almost 15% of all pregnant women[6]. Today, there are dramatic differences in approach, including acceptance and tolerance of genetic risk and an increase of the available pan-ethnic screening for dozens of genetic Mendelian disorders[5]. Much of the change in attitude has been due to improvements in the ability to accurately detect genetic health status of the fetus.

The main indications for prenatal screening are maternal risk for non-disjunctional aneuploidy because of advanced maternal age and for neural tube defects (NTDs). Although Down's syndrome (DS) represents only a small proportion of the serious genetic disorders observed worldwide (Box 1), it continues to be the forefront indication. Other indications include a previous affected offspring, patients with a balanced structural rearrangement of parental chromosomes, or structural fetal anatomical abnormality noted on ultrasound examination[6]. There are now many strategies available to screen for chromosomal abnormalities. These incorporate maternal age and a variety of first-trimester and second-trimester ultrasonography and biochemical markers that include nuchal translucency (NT) measurement and pregnancy associated plasma protein-A, human chorionic gonadotropin (hCG), maternal serum α-fetoprotein (MSAFP), estriol, and inhibin-A levels[5]. The choice of a screening test depends on many factors, including gestational age, number of fetuses, test sensitivity and limitations, desire for early test results, and reproductive options. The goal is offering screening tests with high detection rates and low false-positive rates.
Ideally, patients seen early in pregnancy can be offered first-trimester aneuploidy screening, or integrated or sequential aneuploidy screening that combines first-trimester and second-trimester testing.
The options for women who are first seen during the second trimester are limited to quadruple (or "quad") screening and ultrasound examination (Table 2).
Ultrasound (US) evaluation of fetal anatomy in the second trimester is another strategy for prenatal screening. US markers for fetal chromosome anomalies are observed in 3% to

5% of pregnancies[7]. Some major structural defects are frequently associated with chromosomal anomalies such as omphalocele and heart defects. Other US anatomical findings called "soft markers", such as choroid plexus cyst, echogenic focus of left ventricle of the heart, single umbilical artery, pyelectasis (dilated renal pelvis), and echogenic bowel have been associated with increased risk for chromosomal aneuploidy[8].

Second trimestr US evaluation of fetal anatomy is also the main screening and diagnostic tool for fetal NTDs, such as spina bifida, and abdominal wall defects, such as omphalocele and gastroschisis. Patients with elevated MSAFP levels should undergo detailed US evaluation by a Maternal-Fetal Medicine specialist to rule out the existence of fetal neural tube defects, abdominal wall defects, or central nervous system (CNS) structural abnormalities.

The single, most important advance in the prenatal diagnosis of DS in the past 2 to 3 decades has been the understanding that the US visualization of fetal nuchal translucency (NT) was a very powerful marker of DS. US measurement of fetal NT in the first trimester is a powerful marker of DS, aneuploidy, and congenital heart defects[9].
However, pregnancies with increased NT measurements and normal karyotypes represent a distinct risk group. More than 100 genetic conditions have been found in such cases—the most common of which are cardiac anomalies and Noonan syndrome[10].
Therefore, when NT measurements greater than 3-3.5 mm is noted on first trimester US examination a fetal echocardiogram in the second trimester to search for cardiac anomalies should be recommended.

Recently, other first trimester US markers have been suggested, including hypoplasia of nasal bone, tricuspid valve regurge, and abnormal cardiac axis of fetal heart, and are increasingly incorporated in prenatal screening for fetal aneuploidy.

Prenatal Diagnostic Procedures

Prenatal diagnostic tests or procedures are the next step offered for pregnant women with positive screening tests or structural anatomical abnormalities noted on US examination. Diagnostic procedures often involve direct assessment of fetal tissue. This has been possible via US guided aspiration of placental chorionic villi or amniotic fluid or occasionally fetal blood. Other tissues such as skin, muscle, and liver, have been obtained occasionally for those diagnoses that cannot be accomplished by sampling of amniotic fluid, chorionic villi or fetal blood.
As in prenatal screening testing, prenatal diagnostic procedures require proper counseling for patients about the aimed benefits, possible risks, and limitations. Patients should also be informed beforehand that normal karyotype results after the diagnostic procedure does not eliminate submicroscopic chromosomal aberrations and other non-chromosomal developmental abnormalities.
Patients should be also informed about their options, including termination of pregnancy (TOP) if the diagnostic procedure confirmed serious fetal congenital defects or chromosomal aneuploidies. Personal and religious perspectives need to be considered and respected as part of the patient's autonomy. For the Islamic perspective of the termination of pregnancy, specifically for a fetal malformation, the reader is referred to a comprehensive review published in FIMA yearbook 2013[11].

Genetic Amniocentesis: Invasive procedures for diagnosis of fetal genetic disorders have been available since techniques for culturing and karyotyping of amniotic fluid fibroblasts

were developed in the late 1960s to early 1970s[12]. It has been the mainstay for 40 years, however a major disadvantage of amniocentesis is that results are usually not available until late in the second trimester, generally at 17 to 20 weeks of gestation, by which time the pregnancy is very visible, the mother has felt the baby moving, the bonding process has been established and accelerated, and consideration of TOP is more ethically, emotionally and physically burdensome than earlier in gestation[13]. Improvements in US and increasing expertise in US-guided procedures enabled physicians in the 1980s to move to the first trimester, introducing chorionic villous sampling (CVS) which can be done at 12 weeks and then early amniocentesis (EA) which can be done between 10 and 14 weeks of gestation. However, EA has been almost completely abandoned because it has almost the same risk of fetal loss and in addition was associated with amniotic fluid leakage that led to talipes equinovarus[14,15].

Occasionally, an abnormally elevated amniotic fluid alphafeto protein-AFP (AF AFP) is noted when there is no obvious cause, such as US-visualized neural tube defect (NTD). This situation indicates the need to test for acetylcholinesterase (AChE) which, if positive, suggests the presence of a small non-visualized NTD. The combination of an abnormally elevated AF AFP and a negative AChE is associated in most cases with fetal malformations or with fetal death[16].

The safety and complications of genetic amniocentesis were the subject of many investigations[17]. As with all prenatal procedures, there is always a constellation of factors that render definitive assessment of the risks and complications of amniocentesis difficult. There is a well-understood background rate of fetal loss and there is a dramatic correlation between maternal age and the risk of spontaneous loss. A randomized trial of amniocentesis versus no amniocentesis in low-risk patients found nearly a 1% increase in pregnancy loss following amniocentesis. More recent studies have suggested a much lower complication rate, with the general consensus being that, in experienced hands, fetal loss after amniocentesis is approximately 1 in 350 to 400[18]. Trauma to the fetus during amniocentesis has been reported; including central nervous system injury.[19] However, fetal injury caused by the amniocentesis needle was never common and should now be very rare with US-guided procedures. Maternal complications, such as sepsis and death are, also very rare[20,21].

Chorionic Villous Sampling: Since the introduction of genetic amniocentesis, there has been a constant desire to move prenatal diagnoses as early in gestation as possible[6]. In the mid-1980s, the combination of increasingly sophisticated US imaging and laboratory cytogenetic advances made first-trimester sampling of chorionic villi possible.

With the rare exception of those patients whose primary risk is for a NTD, virtually any patient seen in the first trimester, who would be considered a candidate for amniocentesis, is also a candidate for CVS[6]. CVS has the advantage of earlier diagnosis, allowing earlier intervention when chosen, and increased privacy in patients' reproductive choices. With developments in screening, principally the first trimester, combined protocol of free β-hCG, PAPP-A, and NT, most DS pregnancies can now be identified in the first trimester. Therefore, it is not reasonable to identify high-risk patients in the first trimester and then let them wait a month for an amniocentesis when a CVS could provide the answer much sooner.

CVS is now routinely performed at about 12 weeks. In general, placental location determines whether the approach will be trans-cervical (TC) or trans-abdominal (TA). For most cases, this decision is straightforward. If the placenta is low-lying, posterior, or previa, a TC approach is appropriate. If the placenta is higher or lateral, if the uterus is retroverted, or

if there are fibroids, TC CVS becomes more technically challenging. However, the placental "position" can often be maneuvered by judicious manipulation of bladder volume (by its overfilling).On the other hand, if the placenta is anterior or fundal, a TA approach is usually indicated[22].

Safety of chorionic villus sampling was always a concern since inception. In the early 1990s, it was suggested that CVS might be associated with specific fetal malformations, particularly limb reduction defects (LRDs)[20,23]. Based on published data, it is clear that there is no increased risk for LRDs or any other birth defect when CVS is performed at greater than 70 days of gestation. There is a minimal risk between 8 and 9 weeks, and about a 1% risk of LRD, if the procedure is performed between 28 and 42 days after fertilization (6–8 weeks LMP)[22]. Recent data suggest that CVS is in fact safer in experienced hands than mid trimester amniocentesis[21]. A meta-analysis has shown that the loss rates from amniocentesis and CVS were equivalent and that the incidence of late-term complications is actually lower in the CVS group than in those who had amniocentesis[21,24].

Post diagnostic procedure counseling

This is an important step in assisting patients to clearly understand the meaning of the results, and the expected short and long term effects on the fetus / newborn infant. Counseling should include the availability of both medical and surgical antenatal treatment for some of the diagnosed abnormalities. There are specialized fetal therapy centers for in-utero intervention for some fetal structural defects, such as NTDs, posterior urethral valve obstruction, diaphragmatic hernia, or for twin-twin transfusion syndrome. Consultation with neonatal and pediatric surgery specialists are often needed to coordinate post-delivery management.

Non-Invasive Prenatal Screening with Cell-Free Fetal DNA

For decades the ultimate goal of prenatal screening was to develop a method whereby one can obtain fetal cells from a maternal blood sample and thus avoids the need and risk of an invasive, diagnostic procedure for aneuploidy. Non-invasive prenatal testing (NIPT) for aneuploidy using cell-free DNA in maternal plasma is revolutionizing prenatal screening and diagnosis. Traditional fetal aneuploidy screening tests, based on ultrasonography and maternal biochemistry, have a detection rate of 50–95% at a 5% false-positive rate[25]. The discovery of cell-free fetal DNA in maternal plasma[26], and the invention of massively parallel sequencing (MPS)[27] techniques have made NIPT for DS a clinical reality[27,28]. NIPT has been proved to be highly accurate in detecting fetal trisomy 21, with sensitivity and specificity both > 99% and a non-reportable (failure) rate of 0–4.9%[29]. Although initially less successful, the detection rates for trisomy 18 and 13 were reported to be 100% and 91.7%, respectively[30,31]. The detection rate for sex-chromosome abnormalities has been reported to be 96.2%, with a false-positive rate of 0.3%[32].
However, it is important to recognize that, despite the reported high sensitivities approaching 99% for DS, 99% sensitivity is not a 99% positive predictive value[33]. Of those cases "at risk," diagnostic procedures such as amniocentesis or chorionic villus sampling (CVS) must then be offered for confirmation. It is also well-appreciated that although statistical dogma of sensitivity and specificity do not vary with prevalence, the predictive values do.[23] Thus, although in a 40 year old woman with a positive DS screening using cffDNA, the likelihood of actually having a fetus with DS may be 70% or greater, in

younger women the likelihood is much lower. For example, with a 99% sensitivity and a 99% specificity (1% false positives), the positive predictive value in a 26 year old woman can be as low as 11%. That would be comparable to a 3-mm NT measurement on US. Therefore it should be understood that both the NT measurements and NIPT results are indicators of the odds and not a definitive answer for the presence of the chromosomal aneuploidy. These results ought to be looked at as screening tests and followed by a detailed ultrasound examination to identify ultrasound markers of fetal aneuploidy or anatomical malformations. This needs to be followed by a diagnostic test such as amniocentesis or CVS to obtain chromosomal karyotyping or molecular microarray so as to have a definitive diagnosis.

Summary

There have been tremendous advances in the ability to screen for the "odds" of having a genetic disorder (both Mendelian and chromosomal). At the same time, diagnostic capabilities have increased as genetics research moves into more accurate cytogenetic and molecular techniques for both chromosomal and Mendelian disorders. With safe ultrasound guided procedures to obtain fetal samples, karyotype and microarray analyses-to reach a definitive diagnosis-can be offered to pregnant women at risk for chromosomal aneuploidy and genetic disorders regardless of age.

Ultimately, as sequencing techniques replace other laboratory methods, the only question will be whether these tests are performed on villi, amniotic fluid cells, or maternal blood. Recently NIPT has been added to the armamentarium. It is a sensitive tool in prenatal screening and diagnosis, provided adequate genetic counseling regarding its strengths, limitations, and interpretation of its results is provided.

Box 1. Worldwide birth defects in 2006

Congenital heart defects	1,040,835
Neural tube defects	323,904
Hemoglobin disorders	307,897
Down's syndrome	217,293
Glucose-6-phosphate dehydrogenase deficiency	177,032
Data from Global report on birth defects: the hidden toll of dying and disabled children White Plains (NY): March of Dimes Birth Defects Foundation; 2006.(34)	

Table 1. Recommended prenatal genetic carrier screening tests based on ethnic background(35)

Ethnic Background	Screening Tests to Offer	Additional screening Tests to make available
Caucasian	Cystic fibrosis	
African descent (African, African-American, African-Caribbean)	Sickle hemoglobinapathies, ß-thalassemia, α-thalassemia	Cystic fibrosis

Ethnic Background	Screening Tests to Offer	Additional screening Tests to make available
Ashkenazi Jewish (European Jewish)	Tay-Sachs disease, familial Dysautonomia, cystic fibrosis, Canavan disease	Mucolipidosis IV, Niemann-Pick disease type A, Fanconi anemia group C, Bloom syndrome, Gaucher disease
Southeast Asian	ß-thalassemia α-thalassemia (if microcytic anemia)	Cystic fibrosis
French Canadian and Cajun	Tay-Sachs disease	Cystic fibrosis
Mediterranean	ß-thalassemia	Cystic fibrosis

Table 2. Down's syndrome screening protocols over the past 50 years (36)

Method Positive rate (%)	Components	Time Frame	Sensitivity	(%)FPR* False
Maternal age	Birthday	1960s to present	35	15
Low MSAFP + age	AFP	1980s	50	~5
Double	AFP/HCG	1990s	55	~5
Triple	AFP/HCG/estriol	1990s	60	~5
Quad.	AFP/HCG/estriol/inhibin	1990s/2000s	65	~5
NT	US measurements	1990s to present	60	~5
Combined	Free B HCG/PAPP-A/NT	2000s to present	85	~5
Sequential	Combined + quad	2000s to present	85-90	~5
Free fetal DNA	Sequencing/targeted	Since 2011	98	0.2-1

References

1. Frazer KA: Decoding the human genome, genome res 2012; 22: 1599-1601.
2. DeiningerP; Alu elements: know the SINES, Genome Biol2011; 12: 236.
3. Ferro WG, Guttmacher AE, Collins FS: Genomic medicine: an updated primer, N Engl J Med 2012; 2001-11.
4. Ginsburg G, Willard HF, editors: Genomics and personalized medicine (vol 1 & 2), ed 2, New York, 2012, Elsevier.
5. Srinivasan BS, Evans EA, Flannick J, et al. A universal carrier test for the long tail of Mendelian disease. Reprod Biomed Online 2010; 537–51.
6. Evans MI, Hallahan TW, Krantz D, et al. Meta-analysis of first trimester Down syndrome screening studies: free beta-human chorionic gonadotropin significantly outperforms intact human chorionic gonadotropin in a multimarker protocol. Am J Obstet Gynecol 2007; 198–205.
7. Benacerraf BR, Nadel A, Bromley B. Identification of second trimester fetuses with autosomal trisomy by use of a sonographic scoring index. Radiology 1994; 135–40.
8. Lau TK, Evans MI. Second trimester sonographic soft markers: what can we learn from the experience of first trimester nuchal translucency screening? Ultrasound Obstet Gynecol 2008; 123–5.

9. Wright D, Syngelaki A, Bradbury D, et al. First trimester screening for Trisomies 21, 18, and13 by ultrasound and biochemical testing. Fetal Diagn Ther 2014; 118–26.
10. Souka AP, Von Kaisenberg CS, Hyett JA, et al. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol 2005; 1005–21.
11. Fadel HE, Mishal A, Ebrahim AM, Nordin MM. Termination of pregnancy (TOP) (Abortion). In Fadel HE, Mishal A, Ebrahim AM, Nordin MM, eds. FIMA Yearbook 2013, Encyclopedia of Islamic Medical Ethics Part 1. Amman, Jordan; Jordan Society for Islamic Medical Sciences; 2014. p. 35-52 http://fimaweb.net/documents/FIMA%20Year%20Book%202013.pdf. Last accessed December 1,2015
12. Jacobson JB, Barter RH. Intrauterine diagnosis and management of genetic defects. Am J Obstet Gynecol 1967; 795–801.
13. Fletcher JC, Evans MI. Maternal bonding in early fetal ultrasound examinations. N Engl J Med 1983; 392–3.
14. Hanson FW, Happ RL, Tennant FR, et al. Ultrasonography-guided early amniocentesis in singleton pregnancies. Am J Obstet Gynecol 1990; 1376–81.
15. Wilson RD. Early amniocentesis: a clinical review. Prenat Diagn 1995; 1259–73.
16. Drugan A, Sokol RJ, Syner FN, et al. Clinical implications of amniotic fluid AFP in twin pregnancies. J Reprod Med 1989; 977–81.
17. Mujezinovic F, Alfirevic Z. Procedure-related complications of amniocentesis and chorionic villus sampling: a systematic review. Obstet Gynecol 2007; 687–94.
18. Eddleman KA, Malone FD, Sullivan L, et al. Pregnancy loss rates after midtrimester amniocentesis. Obstet Gynecol 2006; 1067–72.
19. Squier M, Chamberlain P, Zaiwalla Z, et al. Five cases of brain injury following amniocentesis in mid-term pregnancy. Dev Med Child Neurol 2000; 554–60.
20. Akolekar R, Beta J, Picciarelli G, et al. Procedure related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015; 16–26.
21. Wulff R, Tabor A. Risk of fetal loss from invasive testing. Presented at the meeting of Fetal Medicine Foundation 2013.
22. Evans MI, Rozner G, Yaron Y, et al. CVS in Evans MI. In: Johnson MP, Yaron Y, editors. Prenatal diagnosis: genetics, reproductive risks, testing, and management. New York: McGraw Hill Publishing Co; 2006. p. 433–42.
23. Silverman NS, Sullivan MW, Jungkind DL, et al. Incidence of bacteremia associated with chorionic villus sampling. Obstet Gynecol 1994; 1021–4.
24. Mujezinovic F, Alfirevic Z. Procedure-related complications of amniocentesis and chorionic villus sampling: a systematic review. Obstet Gynecol 2007; 687–94.
25. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004; 45–67.
26. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. Lancet 1997; 485–7.
27. Schuster SC. Next-generation sequencing transforms today's biology. Nat Methods 2008; 16–18.
28. Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, Foo CH, Xie B, Tsui NB, Lun FM, Zee BC, Lau TK, Cantor CR, Lo YM. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci U S A 2008; 20458–63.
29. Benn P, Cuckle H, Pergament E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. Ultrasound Obstet Gynecol 2013; 15–33.
30. Dan S, Wang W, Ren J, Li Y, Hu H, Xu Z, Lau TK, Xie J, Zhao W, Huang H, Xie J, Sun L, Zhang X, Wang W, Liao S, Qiang R, Cao J, Zhang Q, Zhou Y, Zhu H, Zhong M, Guo Y, Lin L, Gao Z, Yao H, Zhang H, Zhao L, Jiang F, Chen F, Jiang H, Li S, Li Y, Wang J, Wang J, Duan T, Su Y, Zhang X. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11,105 pregnancies with mixed risk factors. Prenat Diagn 2012; 1225–32.
31. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW, Nelson SF, Canick JA. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med 2012; 296–305.
32. Mazloom AR, Dzakula Z, Oeth P, Wang H, Jensen T, Tynan J, McCullough R, Saldivar JS, Ehrich M, van den Boom D, Bombard AT, Maeder M, McLennan G, Meschino W, Palomaki GE, Canick JA, Deciu C. Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma. Prenat Diagn 2013; 591–7.

33. Chitty LS, Bianchi DW. Noninvasive prenatal testing: the paradigm is shifting rapidly. Prenat Diagn 2013; 511–3.
34. http://www.marchofdimes.org/materials/global-report-on-birth-defects-the-hidden-toll-of-dying-and-disabled-children-full-report, White Plains, NY, 2006.
35. http://www.acog.org/Resources-And-Publications/Guidelines-for-Perinatal-Care. Preconception and Antepartum Care, page 121.
36. Evans MI, Andriole S, Evans SM. Genetics: update on prenatal screening and diagnosis. Obstet Gynecol Clin North Am. 2015 ;42 :193-208.

PREIMPLANTATION GENETIC SCREENING AND DIAGNOSIS

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Abstract

Preimplantation genetic screening and diagnosis (PGS, PGD) is a contemporary measure to reduce the incidence of congenital malformations, which account for 20-30% of perinatal deaths, in addition to significant morbidity and poor quality of life.

PGD is performed in assisted reproductive centers as part of *in-vitro* fertilization (IVF) procedures, when one or both parents is genetically screened, prior to conception, and found to be a carrier of a certain genetic or chromosomal disorder. Implantation of only the unaffected embryos is undertaken into the wife's womb.

High costs and low success rates are the major drawbacks, in addition to lack of proper standardization, guidelines and regulation of the clinical and technological aspects in most countries.

This article summarizes the techniques used for embryo biopsy, and addresses medical, ethical, legal and religious considerations.

Keywords: Pre-implantation genetic diagnosis, embryo biopsy, congenital abnormalities, *in-vitro* fertilization.

Introduction

Preimplantation genetic screening and diagnosis (PGS, PGD) is an increasingly utilized contemporary undertaking to identify embryos with genetic defects so as to avoid implanting them into the mother's womb.

It is performed in IVF centers, when one or both parents is found to be a carrier of a certain genetic disorder, or a chromosomal abnormality, or when the woman is at an increased risk for karyotypic abnormalities because of advanced reproductive age[1,2]. Many serious genetic diseases could thus be avoided, including beta thalassemia, sickle cell disease, spinal muscular dystrophy, Tay Sach's disease, and others.

Moreover, since X-linked recessive disorders such as hemophilia A and B are known to be passed on to male children, it is possible, through PGD, to discard all of the male embryos and only implant female embryos.

PGD detects "at risk" cytogenetic and Mendelian disorders, but cannot detect fetal structural anomalies or any other unforeseen genetic disorder.

PGD has an advantage over the conventional prenatal diagnosis, that is avoidance of the need for pregnancy termination in case an abnormality is found [3,4].

PGD, at this stage, does not involve manipulation of genes in embryos. Possible future use for gene therapy (Germ-line gene therapy) needs further research. In preimplantation genetic screening or diagnosis, embryo biopsy can be done at different stages of embryo development during IVF procedures by one of the following techniques:

A. Polar body biopsy (PB): is usually performed 8-14 hours after Intracyto-plasmic Sperm Injection (ICSI). The perivitelline space of the fertilized ovum is entered either by mechanical or laser dissection. PB biopsy analysis provides important information about aneuploidies. This technique has drawbacks; limited diagnosis of genetic and chromosomal abnormalities and the high incidence of post meiotic chromosome abnormalities.

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B. Cleavage stage biopsy: this is typically performed on day 3 of in vitro development by extracting one blastomere. Extracting two blastomeres from an embryo with at least 4 blastomeres was shown to have detrimental effect on embryo and should be avoided. The major drawback of blastomere biopsy is the risk of mosaicism which might be responsible for false positive or negative results[5].
C. Blastocyst stage biopsy: it consists of removing 5-10 trophectoderm cells on day 5 or day 6 of embryo development. Blastocyst biopsy provides more DNA templates than day 3 biopsy, which should improve the sensitivity and specificity of PGD and is associated with lower rates of mosaicism, and increased chance of live birth rate[6].

The use of PGD is increasing in particular among women of advanced age; and it has been suggested that PGD will become a standard procedure for women undergoing IVF.

Although observational studies comparing IVF with and without PGD have shown that the use of PGD is associated with higher implantation rates for transferred embryos but not with an increase in the rate of ongoing pregnancy per initiated cycle or per follicular aspiration[7].

PGD procedure starts by fixing the blastomere and analysis using fluorescence in situ hybridization (FISH). Nuclei then are analyzed for the different chromosomes according to the probe used.

The genetic material obtained is tested for either single gene mutation using molecular biology techniques, Polymerase Chain Reaction (PCR, PCR-multiplier) or for chromosomal translocation and aneuploidy, using cytogenetic techniques such as FISH or Comprehensive Chromosomal Screening (CCS)[8,9]. The latter is the emerging new cytogenetic technique that consists of identifying the whole chromosomal compliments (24 chromosomes)[10]. CCS can be accomplished by microarray technology such as Comparative Genomic Hybridization (CGH) or Single Nucleotide Polymerase (SNP) or through PCR[11-13].

Embryos are classified as:

- Normal: if two copies of each chromosome are present and the sex chromosomes are either XX or XY.
- Abnormal: if a different chromosomal constitution is found.
- Undetermined: if no determination of chromosomal constitution could be made either due to absence of nuclei after fixation of the blastomere or if the biopsy was not performed because the embryo contained fewer than four blastomeres.

Indications for Preimplantation Genetic Diagnosis:

- Patients with a hereditary genetic disorder such as cystic fibrosis with a high risk for transmission to the fetus.
- In cases where one or both biological parents is a carrier of an abnormal chromosome.
- Gender selection.
- Compatible HLA typing for a savior sibling: fertilized zygotes are tested for genetically compatible human leucocyte antigen; HLA antigen.Only zygotes that are compatible with an existing child with, for example combined immune deficiency disorder, are implanted[14,15].
- Indentifying hereditary cancers with variable penetrance, eg. BRCA 1, 2 status and late onset genetic diseases such as Huntington's disease.
- In infertility treatment, particularly in women delaying child bearing to older age (>35 years) as aneuploidy is increased, and in women with repeated IVF failure and recurrent spontaneous miscarriages.

Examples of single gene disorders indicating PGD[16]:

- B-Thalassemie
- Cystic fibrosis
- Huntington's disease.
- Fragile X chromosome.

- Myotonic dystrophy
- Spinal muscle atrophy
- Hemophilia-A
- Duchene's muscular dystrophy.
- Neurofibromatosis.

Cytogenetic techniques used in PGD and PGS:

1. Fluorescence in situ hybridization-FISH.
2. Array comparative genomic hybridization -ACGH.
3. Single nucleotide polymerase array, SNP micro array.
4. Relative quantitative polymerase chain reaction (qPCR).

1. Fluorescence in Situ Hybridization (FISH):

This cytogenetic technique uses fluorescent probes that bind to only those parts of the chromosome with high degree of sequence complimentarity.
It is used to detect and localize the presence or absence of a specific DNA sequence on chromosome.
Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosome. It is indicated for aneuploidy screening and chromosomal translocation.

2. Polymerase chain reaction (PCR):

This technique is used to amplify a single copy or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
PCR is used in PGD for an x-linked disorder. This allowed the determination of embryo sex and the transfer of unaffected females.
The number of diseases currently diagnosed via PGD-PCR is approximately 200 and includes some form of inherited cancer such as retinoblastoma and breast cancer susceptibility gene BRCA-2 and in HLA matching[17-19].

PGD for Chromosomal Translocation

There are two types of chromosomal translocations; Robertsonian and reciprocal. If the translocations are balanced, these individuals will be phenotypically normal. But these individuals carry the risk of producing zygotes in which the translocation is unbalanced. These will result in infertility, spontaneous abortion and / or delivery of babies with mental retardation, developmental delay or structural malformations[20]. These reproductive risks, can be significantly reduced by transfer of only embryos which are chromosomally normal or with balanced translocations following PGD. The technique usually used is FISH-PGD. A CGH and SNP microarray using trophectoderm biopsy are now used for PGD in couples carrying balanced translocation[21,22].

Embryo-screening in IVF cycles

In IVF cycles, 40-60% of human embryos are abnormal and this number increases to 80% in women 40 years or older. These abnormalities result in low implantation rates of embryos transferred in IVF cycles[23].
The risk of chromosomally abnormal blastocysts is 2-6% in women aged 26-37 years, but rises to 33% at the age of 42 and rises to 53% at the age of 44. Embryo's potential to implant depends mainly on its chromosomal status.
Assessment of the embryos' chromosomal status can be done by a technique called Comprehensive Chromosomal Screening (CCS), where all 24 chromosomes obtained from trophectoderm biopsy were assessed. Implantation and pregnancy rates were reported to be higher in cases where embryos are screened before transfer using array comprehensive genomic hybridization aCGH.Using this technique, the implantation rate was 72.2% but on embryos selected on morphologic criteria alone, the rate was 46.5%[6].

Nowadays, new IVF outcome data showed clearly that implantation rates were significantly higher in groups using frozen single blastocyst transfer following CCS (65.1%), than with either frozen single blastocyst transfer (52%), or day-5 fresh single embryo transfer (49.2%) based on morphology alone. Therefore, chromosomal aneuploidy screening represents a promising way to reach the full objective of routine single embryo transfer (eSET)[24].

Counseling of couples undergoing PGD

In 2007, the American Society of Reproductive Medicine (ASRM) published recommendations regarding PGD counseling which should include information relating to the following[25]:

1. Couples should be informed about risks of IVF technologies.
2. Couples should be able to not choose IVF and PGD.
3. Couples should know the risk associated with embryo biopsy.
4. Carriers of X-linked disorders should be informed about the disorder and quality of life for an affected child.
5. Couples should know the technical limitations and pitfalls of PGD including the risk of misdiagnosis and the need for subsequent confirmatory prenatal diagnosis tests via CVS or amniocentesis.
6. The possibility that no embryos may be transferred if all are affected.
7. The disposition of embryos that are not transferred due to inconclusive results.

Recommendations

Given the advent of new cytogenetic techniques the practice of prenatal diagnosis has seen major advances in the reproductive science. Although amniocentesis and CVS remain the main techniques of traditional prenatal testing, improvements in pre implantation genetic diagnosis have revolutionized the world of genetic diagnosis.

The following are recommendations which need to be respected as outlined by the European Society of Human Reproduction and Embryology (ESHRE) and Society of Obstetricians and Gynecologists of Canada (SOGC):

1. Before preimplantation genetic diagnosis is performed, genetic counseling must be provided by a certified genetic counselor to ensure that patients fully understand the risk of having an affected child, the impact of the disease on the affected child and the benefits and limitations of all available options for pre implantation diagnosis.
2. Couples should be informed that pre implantation genetic diagnosis can reduce the risk of conceiving a child with a genetic abnormality carried by one or both parents if that abnormality can be identified with tests performed on a single cell or multiple trophechtoderm cells.
3. Trophechtoderm biopsy has no impact on embryo development as compared to blastomere biopsy and should be the method of choice in embryo biopsy.

Summary

Pre implantation genetic diagnosis for single-gene disorders and chromosomal translocations is an alternative to prenatal diagnosis for detection of genetic disorders in couples at risk of transmitting genetic conditions to their offspring. Ideally, detection should be performed by multiple PCR genetic analysis on trophectoderm cells.

Ethical considerations

PGD provides the facility to avoid the implantation of defective embryos, thus giving the couple, who are at risk of having an affected child with chromosomal and genetic defects, the chance to avoid implantation of defective embryo and avoiding the need for the prenatal diagnosis and abortion.

In various countries with various ethical or religious denominations, there are different views on PGD and selection of embryos, on that basis, for implantation in the mother's womb.

In certain countries PGD is regulated by law or by professional recommendations, with three different attitudes:

(1) Those who consider the pre-embryo as a group of cells with no consideration of interest or rights, and can be discarded in case it carries genes for serious diseases[26].

(2) Those who view the pre-embryo as the early stage of a human being, and thereby prohibit performance of PGD and subsequent steps of selective implantation in the mother's womb.

(3) A third view considers the pre-embryo as a potential human being, that should be handled with dignity and respect, but still can be biopsied and discarded if found to be defective.

Other related considerations:

- PGD to avoid genetic/chromosomal disorders should not be confused with "eugenics" where embryo selection aims at achieving desirable (non pathological) features, or improvements of human race.
- The PGD procedures' safety and efficiency should be clearly explained, with its limitations, especially related possible lack of accuracy, and the possibility of unknown genetic anomalies not discovered by the tests provided.
- This counseling should be documented by a fully informed consent.

Ethical-Islamic Perspectives

PGD was discussed by Professor Fadel[27], and a combined seminar organized by the Jordan Society for Islamic Medical Sciences, with participation of scholars in Islamic jurisprudence and medical specialists. The outcome of deliberations was published in September 2000, in a book titled: Contemporary Medical Issues in Light of Islamic Jurisprudence. The following is a summary of these outcomes[28]:

A- It is permissible to conduct scientific research on human sperms and ova, prior to fertilization, to diagnose heritable disorders, provided proper consents are obtained from concerned subjects, and provided that sperms and ova are not used in any impermissible fertilization. Obtaining sperms and ova should be undertaken by means approved by *Shari`ah*. Such research should be supervised by expert and trustworthy committees in ethics and science.

This permissiveness includes surplus ova in *in-vitro* fertilization centers, and ovaries removed surgically for approved therapeutic purposes.

B- Human ova fertilized in IVF laboratories (outside the womb): It is permissible to conduct scientific experimentation on fertilized ova in case of necessity, such as identification of heritable diseases carried by these ova, aiming at diagnosis, prevention or treatment of these diseases.

Examples include availability of family history of certain hereditary diseases in one or both parents that may appear in their offspring.

Medical practitioners are permitted not to implant affected ova into the mother's womb, if it was proven to produce an affected fetus with the heritable disease.

This issue was more recently discussed by the Academy of the Islamic International *Fiqh* Council- Islamic Cooperation Organization, in its 21st session held in Riyadh, Saudi Arabia, on November 18-22, 2013[29]. Among several juristic rulings that addressed the (human genome, gene therapy, genetic engineering, genetic counseling

and prophylactic genetic screening), the Academy issued the following ruling to implanting the fertilized ovum in the mother's womb:

It is permissible to undertake genetic diagnosis following fertilization outside the womb (test tube babies), provided all necessary procedures are implemented to guarantee non-mixing and safety of samples (ova).

Using PGD in IVF centers for the purpose of fetal sex pre-selection was discussed by individual jurists, seminars and *Fiqh* councils. It has been unanimously approved in families with known sex-related inherited diseases. In other situations, jurists' opinion is divided between rejection and approval for "considered needs" on a case by case basis [see a comprehensive review published in FIMA Yearbook-2013][30].

References

1. Abul Fadl M. Ebrahim, Introduction to Islamic Medical Jurisprudence. Published by the Islamic Medical Association of South Africa, July 2008, First Printers, Durban, South Africa, PP 120-123.
2. Center for Genetics and Society. 2004, Preimplantation Genetic Diagnosis (PGD) and Screening. Available: http://www.genetics-and-society.org/technologies/other/pgd.html [20 July 2007].
3. Shulman LP. Preimplantation genetic diagnosis. Up-to-date, April 2005, www.uptodate.com
4. Verlinsky Y, Cohen J, Munne S, et al. Over a decade of experience with preimplantation genetic diagnosis: a multicenter report. Fertile steril 2004, 82(2), 292-294.
5. Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T , wright G- et al correlation between an observational study in two centers involving 956 screened blastocysts . Human repord . 2014,29; 1173-81.
6. SchoolCraft WB , Fragouli E , Stevens J , Munne S , et al. Clinical application and comprehensive chromosomal screening at the blastocyst stage. Fertel Steril 2010; 94: 1700-6
7. Gianaroli L, Magli MC , Ferraretti AP, Munnes. Pre implementation diagnosis for aneupliodes in patients undergoing inVitro fertilization with poor prognosis FertalSteril 1999,72, 837-44
8. Masten broek S, Twisk M, Van Echten-Arends at al. In Vetro fertilization with pre implementation genetic screening N Eng J Med 2007, 357;9-17
9. Capalbo A, Wright G , Ettiott T , Ubaldi FM Rienzi L, Nagy ZP et al. FISH re analysis of inner cell mass and trophectoderm samples of previously array – CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage human reprod, 2013,28: 2298-307.
10. Scott RT Jr. Upham KM, Forman EJ, HongK H, scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in Virto fertilization implantation and delivery rates, a randomized control trial. Fertil Steril 2013; 100: 697-703.
11. Forman EJ, Tao X, Ferry KM, Tylor D, Treff NR, Scott RJ. Jr. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Human Reprod. 2012; 27: 1217-22.
12. Treff NR, Su J, Tao X, Levy B, Scott RT. Accurate single cell 24 chromosomes aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays. Fertil Steril 2010;94: 2017-21.
13. Keltz MD, Vega M, Sirota I, Lederman M, Moshier El, Gonzales E, et al. Preimplantation genetic screening (PGS) with comparative genomic hybridization (CGH) following day 3 single cell blastomere biopsy markedly improves IVF outcome while lowering multiple prepancies and miscarriages J Assist Reprod Genet 2013; 30: 1333-9
14. Dickens BM, preimplantation genetic diagnosis and savior siblings'. Int J Gynecol Obstet 2005; 88: 91-6.
15. Whittaker Am. Reproduction opportunists in the new global sex trade. PGD and non medical sex election. Reprod Biomed online 2011; 23: 609-17.
16. Dahdouh E, Balayla J, Audibert F. SOGC, technical update No. 323 May 2015. J Obstet Gynecol Can. 2015; 37(5) 451-463.
17. Offit K, Kohut K, Clagett B, et. Al. cancer genetic testing and assisted reproduction. J Clin Oncology 2006;24 :4775-82.
18. Girarder A, Hamamah S, Anhory T, Dechaud H, Sarda P, Hedon B, et. al. First preimplantation genetic diagnosis of hereditary retinoblastome using informative microsatellite marker. Mol Hum Reprod. 2003; 9: 111-6.
19. Verlinsky Y, Rechitsky S, Scholcraft W, Strom C, Kuliev A. Preimplantation diagnosis for Fanconi anemia combined with HLA marching JAMA. 2001; 285: 3130-3.
20. Chang EM, Han JE, Ewak IP, Lee WS, Yoon TK, Shim SH. Primplantation genetic diagnosis for couples with a Robertsonian translocation. Practical information for genetic counseling, J Assist Reprod Genet. 2012; 29: 67-75.

21. Tan YQ, Tan K, Zhang Sp, Gong F, Cheng DH, Xlong B. et al. Single nucleotide polymorphism microarray-based preimplantation genetic diagnosis is likely to improve the clinical outcome for translocation carriers. Hum Reprod 2013; 28: 2581-92.
22. Fiorentino F, Spizzichinoa L, Bono S, Biricik A, Kokkali G, Rienzi L, et al. PGD for reciprocal and Robertsonian translocation using array comparative genomic hybridization. Human Reprod. 2011; 26: 1925-35.
23. Munne S, preimplantation genetic diagnosis for aneuploidy and translocations using array comparative genomic hybridization. Curr Genomics 2012; 13: 463-70.
24. Schoolcraft WB, Katz-J MG. Comprehensive Chromosome Screening of trophectoderm with vitrofication facilitates elective single embryo transfer for infertile women with advanced maternal age. Fertil Steril 2013; 100: 615-9.
25. Practice committee of the society for assisted reproductive technology; Practice Committee of American Society of Reproductive Medicine. Preimplantation genetic testing, a Practice Committee opinion. Fertil Steril 2007; 881: 1497-504.
26. Robertson JA. Ethical issues in new uses of preimplantation genetic diagnosis-extending PGD. The ethical debate.Hum.Reprod 2003;18,465-471.
27. Fadel, H.E. Preimplantation Genetic Diagnosis : Rationale and Ethics, an Islamic Perspective. J Islam Med Assn, 2007; 39: 150-7
28. Contemporary Medical Issues in Light of Islamic Jurisprudence. Jordan Society of Islamic Medical Sciences, volume 2, September 2000, pp 208-230 and 269.
29. Academy of the Islamic International *Fiqh* Council, Islamic Cooperation Organization, 21st session, held in Riyadh, Saudi Arabia, November 18-22, 2013.
30. Encyclopedia of Islamic Medical Ethics-I, FIMA Yearbook-2013, pp. 53-63. Website: http://fimaweb.net/cms/index.php/publications/fima-yearbook

ETHICAL AND LEGAL IMPLICATIONS OF MITOCHONDRIAL DONATION

(This article is a modified part of MSc degree theses submitted by the author in 2014)

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Abstract

The mitochondrial DNA has distinctive features with high mutations, that may cause significant and serious medical syndromes, the management of which is currently mainly supportive and symptomatic. Recent advances in Mitochondrial replacement therapy (MRT) have added new hopes of preventive measures.
Mitochondrial donation is a new form of IVF that was approved by the UK parliament in February 2015.The aim is to remove mitochondrial disease caused by pathogenic maternal mitochondria to prevent a disorder and its transmission. This is done at the level of fertilization. The technology uses the egg of a donor with healthy mitochondria.
This article will address the scientific advances in MRT, and explore its legal and ethical repercussions.

Keywords: Mitochondrial genetics, mitochondrial disorders, mitochondrial donation, egg donation, reproduction, ethics, Islam.

Introduction

As of October 29th 2015[1], treatments relating to mitochondrial replacement therapy (MRT) can now take place in the UK. There was much debate about this new form of IVF and it was reflected in the UK parliament on February 2015 when the vote took place[2]. This is the first time that a human egg can legally be altered for therapeutic purposes. This article explores the scientific nature of the two proposed methods and thereby concludes on their ethical applicability, specifically in the Muslim world.
A case study into Sharon Bernardi[3], highlights the extent to which mitochondrial disease can affect its carriers. Sharon had 7 pregnancies, most were miscarriages, and those that survived, did so until the age of two years. Sharon's fourth child, Edward, was the exception, he lived until the age of 21 years. At the beginning, it was difficult to diagnose why Sharon had miscarriages. There was a strong genetic link but where? It was not found in the nuclear genome itself, but in the DNA of the mitochondria. By the time Sharon was delivering Edward, doctors were prepared, as they knew the cause. Sufferers of mitochondrial disease express it in differing ways. However, they will usually have three or more organs affected and that is a key sign[4].

Mitochondria are organelles found in the cell cytoplasm. They produce energy required for normal cellular function of organelles and thereby the cell.

The kinds of diseases suffered by mitochondrial defect carriers are dependent on the pathogenic mitochondrial load. The diseases relating to defective (mtDNA) vary. Discrete and sometimes serious clinical syndromes have been described. The following are examples[5]:

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- Kearns-Sayre syndrome (KSS): Pigmentary retinopathy, cerebellar ataxia, heart block. Additional features may include deafness, myopathy, diabetes, dementia and others.
- Chronic progressive external ophthalmoloplegia, bilateral ptosis, and proximal myopathy.
- Encephalopathy with lactic acidosis and stroke-like episodes (MELAS).
- Myoclonic epilepsy with ragged-red fibers (MERRF).
- Neurogenic weakness with ataxia and retinitis pigmentosa (NARP).
- Leigh syndrome (LS): Relapsing encephalopathy, cerebellar and brain stem signs.

Considerable clinical variability exists, and many individuals do not fit neatly in one particular category.
Moreover, a high incidence of mid and late pregnancy fetal loss is also a common feature of mitochondrial disorders.
Mitochondrial disorders are more common than was previously thought. In the literature, more than 12 clinical syndromes of mitochondrial disease have been reported. Epidemiological studies reveal the prevalence of all mitochondrial disease at around 11.5 in 100,000.
Mitochondrial dysfunction should be considered in the differential diagnosis of any progressive multisystem disorder.

The discussion in this article will start with the science of mitochondria, MRT and the potential ethical dilemmas of its proposed methods. Then, various scholarly opinions taken regarding MRT will be addressed. The UK and US law will be briefly described followed by conclusions, high lighting potentially the most favorable method of MRT in the Muslim world.

The Science

There are about 1000-2000 mitochondria in the cytoplasm of each cell depending on the cell's function[6]. Their primary role is respiration, which is vital for the survival of cells. The release of adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) is a defining feature of a mitochondrion's function. The mitochondrion also has its own DNA, which spans 37 genes. This mitochondrial DNA (mtDNA) is separate from the nuclear DNA (nDNA).
Mitochondrial defects occur in most of us naturally through the process of aging[7].This is through the release of free radicals due to the specific reactions taking place in the mitochondria. This causes a slow natural deterioration in a person. On the other hand, mitochondrial defects can also be caused by mutations in mtDNA, or in nDNA. Both these defects lead to disease entities. The mitochondrial defect caused by mtDNA is of interest in the techniques proposed for MRT.
mtDNA is circular, and spans 16.6 kilobase pairs with almost no introns. There are no checking features in mtDNA replication, so errors are likely, and thereby mutations in mtDNA[8]. Thirteen of the 37 genes found in mtDNA are related to OXPHOS. The rest of the genes encode for translation of organelle via transfer RNAs (22 genes) and ribosomal RNA (2 genes).
The mitochondrial function is dependent on the type of cell it is in. Nuclear-encoded genes and mitochondrial genes instruct function for the mitochondria.

Factors relating to disease transmission in mitochondria

A given cell can have all normal mitochondria. On the other hand, a person's cells can have all abnormal mitochondria. These are both homoplasmic respectively. The individual with homoplasmy of defective mitochondria will die soon after birth. There are also those who possess both normal and mutant forms of mitochondria. This is known as heteroplasmy. In this circumstance, the healthy mitochondria make up in function for the mutant mitochondria. However, if the mutant load reaches a certain threshold, there may not be enough healthy mitochondria to make up for them in function.
The way in which mtDNA is inherited is entirely different from the Mendelian mode of inheritance of nDNA. This difference is vital in

understanding the ethical dilemma and its proposed solution. When fertiliza-tion occurs, pronuclei with nDNA of egg and sperm come into contact, and the chromosomes form a spindle.

A recombination of DNA from both mother and father occurs. This is the nature of meiosis. Alternatively, the mitochondria are replicated onto daughter cells post meiosis straight from the maternal egg cell. The sperm cell has no contribution as it loses its mitochondria during the fertilization process. There is no recombination that occurs. Hence, the mitochondria are only inherited maternally.

There is an interesting phenomenon which occurs during the fertilization process. It is known as the mitochondria bottleneck[9]. A woman carries eggs, and each egg has its own DNA. A woman with mitochondrial defect will have eggs that reflect this. The higher her number of defective mitochondria, more of her eggs will reflect this.

As fertilization occurs, the mitochondria from only the mother are replicated onto daughter cells. These cells are then divided further in order to form a zygote. The dividing cells will also be dividing mitochondria. So, as this occurs, the small number of mitochondria that the initial ooplasm contained has now been amplified in number in the resulting offspring. Thereby, worsening the prognosis of the resultant child and increasing the mutation load of carriers. If the mutation load reaches a certain threshold, multi-system decline occurs because normal functioning cells cannot compensate for the loss caused into this heteroplasmy[10].

Current methods of treament

There is no cure for mitochondrial disease. The current treatment lies in managing the disease to make life as comfortable as possible, for the possessor, by avoiding an energy crisis[11].

It is possible to use pre-implantation genetic diagnosis (PGD) or go through prenatal diagnosis (PND), but the aim of both is to discard an embryo or abort a fetus in which the disease mutation is suspiciously high. PGD could perhaps be useful in finding those eggs with low levels of mutated heteroplasmy. PND can be used to confirm this. Neither can be considered an effective method of eradicating mitochondrial defects. These techniques aid in acquiring only the most heteroplasmic entity of normal mitochondria, but this is no solution for people like Sharon Bernardi with badly affected eggs.

Proposed techniques for MRT

There are two novel forms of IVF for mitochondrial donation; pronuclear transfer (PNT) and maternal spindle transfer (MST). Both of these techniques are for those couples where the mother has pathogenic mitochondrial defects exclusively due to mtDNA. These techniques will have no impact on a person presenting with mitochondrial defects from mutation in their nDNA.

It is important to understand the differences between these two techniques because these differences form the basis of the ethical solution proposed. Prior to either form of MRT, the affected couple will need an egg donor with healthy mitochondria to donate her egg(s) for this form of therapy.

In PNT, an egg cell from intended mother is fertilized with the intended father's sperm. At the same time, the donor egg is also fertilized with intended father's sperm. The two pronuclei from the donor-father combination are removed and discarded leaving behind an enucleated cell with healthy mitochondria. The two pronuclei formed in the intended mother-father are taken out of the egg cell, with defective mitochondria, and placed into the enucleated cell. This new cell formed from the egg donor and intended parents is now placed in a medium to grow until it is ready for implantation.

The research done by Akitsugu Sato and his team in 2005[12] found that the offspring of mice resulting from PNT were free from symptoms of the parent mice. This means that PNT can enable offspring entirely free from mitochondrial defect. Lindsay Craven and colleagues[13] showed that the resulting zygote can progress

on to a blastocyst following PNT. This is indicative of potential successful pregnancy.

In MST, the transfer occurs at the oocyte level, as opposed to the zygote level as is the case in PNT. The maternal spindle is removed from the donor with healthy mitochondria. Then the maternal spindle, which is at metaphase II, is removed from the intended mother's egg and placed in the enucleated donor egg. Fertilization is then initiated with the use of intended father's sperm. Once this occurs, the egg forms daughter cells and this zygote is prepped for implantation into the uterus of the intended mother.

The difference between the two forms of MRT is simple. Figures 1 and 2 below highlight the difference and the ethical point of contention. This difference makes one technique more favourable than the other. In PNT, fertilization has occurred between father and donor egg. The transfer of pronuclei is after fertilization. This is destroying a zygote.

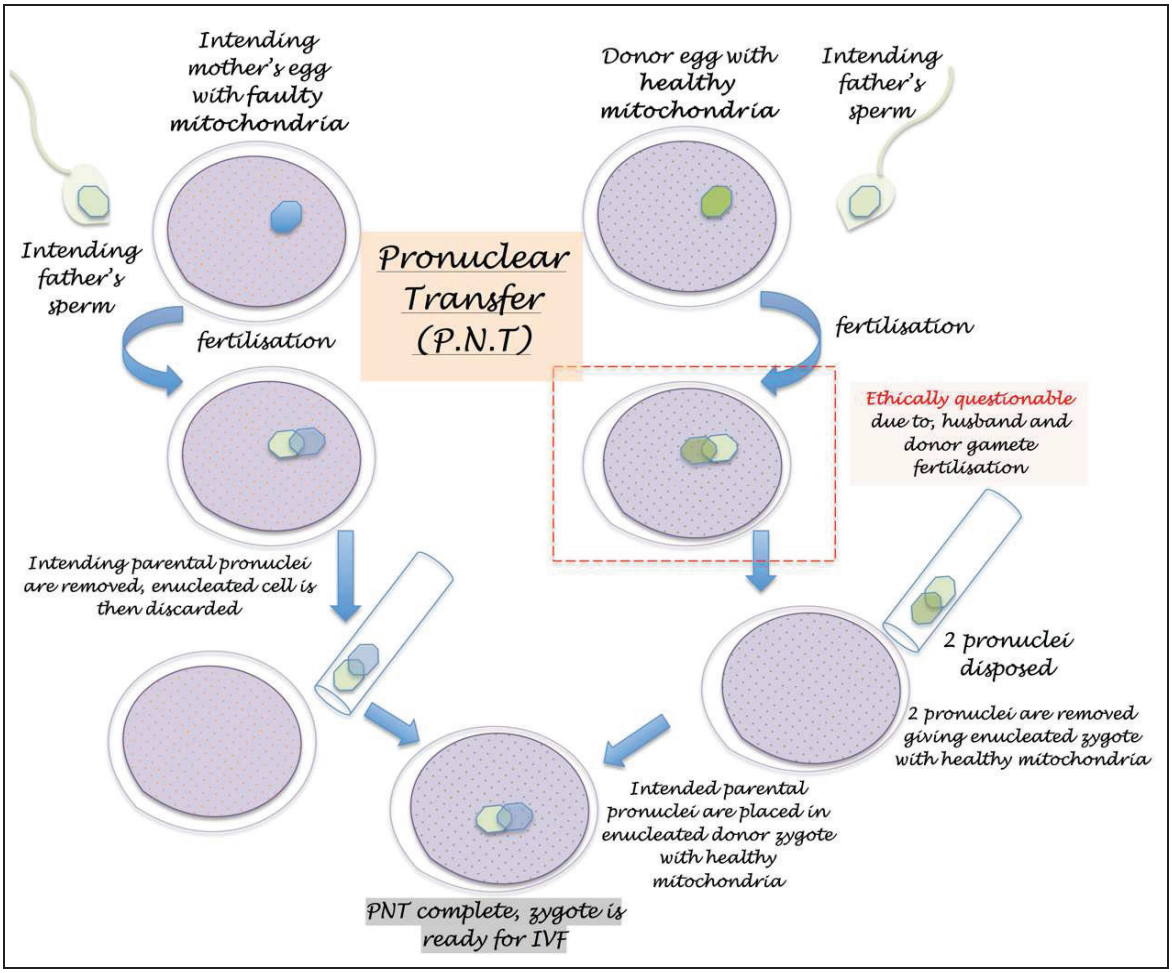

Figure 1: Author's method of explaining the ethical qualm in PNT.

In MST maternal spindle at metaphase II is transferred before fertilization.

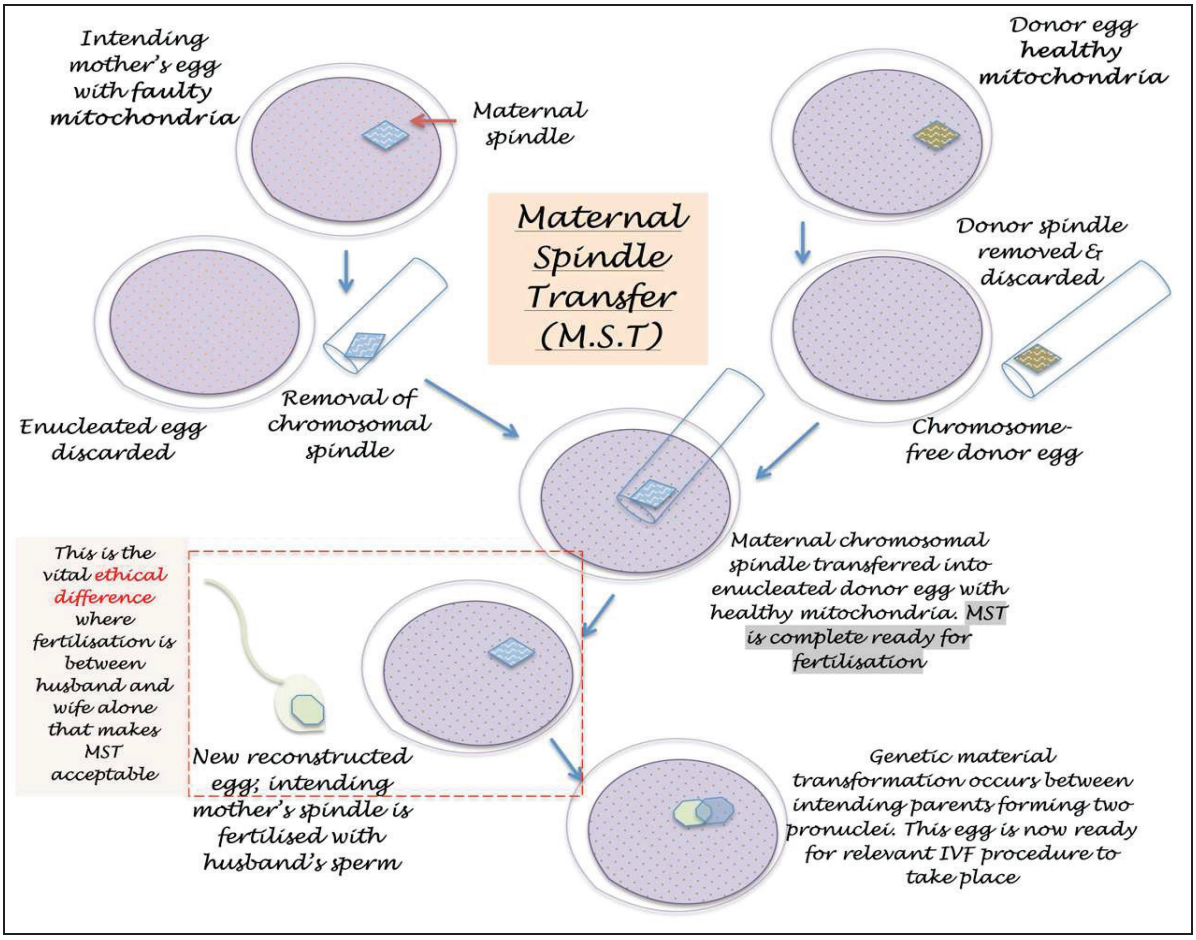

Figure 2: Author's method of explaining the ethical difference found in MST compared to PNT.

There have been alternative methods to these two techniques, like ooplasmic transfer[14] where healthy donor ooplasm was injected into unhealthy ooplasm to rejuvenate it, but due to the lack of success, its use has been discontinued. There were however, a couple of successful deliveries and the children are completely healthy[15].

Egg donor

An aspect of MRT that bears heavily ethically is the use of donor eggs. In PNT, the donor egg is also being fertilized, despite absence of donor-father nDNA in offspring. This can be a cause for concern ethically to those parents who only wish for the married couple's egg/sperm to fertilize. On the other hand, in MST, the egg donor is being used for her enucleated egg prior to fertilization. This kind of egg usage is similar to tissue transplant as no fertilization has occurred between father-donor.

Germline changes

The vertical, uniparental transmission of donor mtDNA is inevitable. Any girl born will be transmitting donor mtDNA to generations to come. On the other hand, there will be no transmission of donor mtDNA if a baby boy is born.

Ethical concerns

Physicians have a consensus that faulty mtDNA defects cause array of harrowing disorders for the sufferer, and quality of life may be extremely poor. In order to protect a patient's welfare and interest, it is important to look at all related potential therapies. Hence, MRT techniques are being explored along

with consideration of the ethical issues of their implementation.

Experimental

There is a risk that the procedures of MRT are not safe as they have been in use for only a short time, and complications may develop later. The UK government is urging scientists to prove their safety and efficacy before trying it on the public[16].If any problems arise, then, like the case of ooplasmic transfer, the therapy shall be stopped. This will be easier to do when the affected child is a boy, because boys do not cause germline effects. Due to this, it will be important to ensure only boys are being born, until MRT is honed.

Identity

An issue, which often surfaces, is that the baby will not know its identity. This is because of its tri-genetic makeup. nDNA comes directly from the intended parental unit but mtDNA comes from the donor woman. The genes relating to us as humans, and our traits, all come from nDNA. mtDNA is like a battery in a car[17,18]. Its only contribution is in releasing fuel. Hence, this angle of discussion is a gross misnomer. The child should be considered his/her genetic parents' baby. The donor woman should have no contribution in the child's life.

Germline modification

mtDNA can only be replicated onto daughter cells. It is unlike the process of fertilization whereby egg and sperm nDNA recombines (meiosis) to form daughter cells. mtDNA is inherited as nDNA, however, mtDNA is inherited only through the maternal line. Therefore, daughters will carry this donor mtDNA for generations to come but sons will not. Hence, it is hoped that when MRT is implemented, it will initially be only for boys. Once it is confirmed there are no negative side-effects, then girls can also be born through MRT. The aim is to have disease-free individuals whilst putting as few people at risk as possible.

Counseling

MRT is still at an experimental level, so counseling is vital. The intended parents need to understand the risks involved and be psychologically sound to take them on. There may be dire side effects in the resultant offspring due to MRT, so they need to be prepared for this possibility. However, because these parents have had much trouble conceiving normal healthy children, they will, most likely, be all set for the newest resort.

It is important to have regular check-ups and follow-ups with parents and their children born as a result of MRT. This would provide a platform for counseling to the parents, as well as a way in which efficacy of MRT can be analysed. Dr Cohen and his team in 1997[14] conducted ooplasmic transfer on 25 women. This had limited results and the main drawback to the report was the lack of follow-ups of the successful deliveries. Although ooplasmic transfer proved unsuccessful overall, something good could have come out of it if regular follow-ups were enforced as part of the therapy.

A question related to germline modification is that would MRT be entering a slippery slope to further gene manipulations[19]? MRT aims to retain the integrity of the nuclear genome itself, hence it is hoped the sanctity of the individual born is maintained.

Rights of the egg donor

The egg donor woman has her own rights. She is to be given the opportunity of informed consent under all circumstances. In general, if a woman knew her egg was simply used for its healthy ooplasm, she may be more inclined to donate her egg. This is because there would be no repercussions of the offspring being her child.

It is important to inform the egg donor that her egg could be used for either PNT or MST. Therefore, she should be aware that potentially her zygote or egg will be destroyed. She could have a contention with the destruction of her zygote as there is potentiality of life.

Egg retrieval is a painful procedure and hence the egg donor needs to be made aware of this.

Who can be eligible for MRT?

A woman with proven mitochondrial defect of high mutant load is most eligible. This is likely to be an intending mother who has had a couple of failed attempts already, or had children with mitochondrial disease. The mitochondrial disease needs to be localised to the mitochondria, not to the mitochondrial genes found in nDNA. The woman would need to be of a child-bearing age. She should otherwise be healthy and mentally stable. A good support network of family and friends will act as an asset through-out this process.

Cost to public

A person affected with mitochondrial disease will have a low quality of life. They will have a constant need for medical intervention and they will be highly dependent. Despite all of that, they will never be healthy. This will inevitably be a high cost to maintain for public health services, as well as the family. On the other hand, if MRT is conducted successfully, this will be a one-off cost directly reducing the cost for not only the offspring, but potentially generations to come.

The law

In January 2012, the Human Fertilization and Embryology Authority (HFEA) was asked to ascertain public opinion on new IVF techniques including those for prevention of mitochondrial disease[20].The results of which can also be found on the HFEA website. The themes discussed were based on germline modifications, identity issues, treatment of mitochondrial egg donor, regulation of MRT and views on the law.

The results showed that educating a person on the procedure makes them more open to MRT. There were workshops given to ensure people had a good understanding of the techniques proposed. People's reservations lay in 'playing God', creating 'designer babies' and entering the 'slippery slope'. "Patient focus groups" on the other hand, are involved with those in contact with or affected by mitochondrial disease. They were all in support of MRT. This comes as no surprise.
When alternatives of MRT were discussed such as PGD/PND, people were not too keen on them either. This is because there was a potentiality of discarding a zygote. Hence, there was a group of people who were against PNT form of MRT and not MST.

UK law

As of 29th October 2015, extensive regulatory information is outlined in the law on mitochondrial donation. It covers the techniques used, the scope of the egg donor, and licensing of this form of IVF in institutions.
The HFEA now allows for an egg/embryo of mixed genetic origin to be placed inside a woman for specifically MST and PNT. The components must all be of human origin.

The second section in the HFEA Act relates to 'parenthood' of the child. This part states that an egg donor for her mitochondria will not be considered a mother of the child.

United Nations Educational Scientific Cultural Organization (UNESCO) has a constitutional commitment to 'the democratic principles of dignity, equality and mutual respect of men'. The constitution covers respect for human dignity. This should be reflected in molecular terms including the genome.

In 2009, Dr Hwan Woo-suk was in violation of this basic right[21]. His quest for new medical technologies led him to fabricating fabulous results as well as many other offences against his colleagues. He was tried and prosecuted for his offences.

US law

The Food and Drug Administration (FDA) is the governing body pertaining to experimental techniques. The FDA limited Jacques Cohen's research into ooplasmic transfer[22,23]. Ooplasmic transfer involves the transfer of egg cytoplasm from a healthy egg donor to rejuvenate eggs of intended mothers. MRT, is a complete transfer of ooplasm so it is a more refined version of ooplasmic transfer. Of the 30 babies conceived through ooplasmic trans-

fer, only 15 are thought to be normal. Two have Turner's syndrome. This information is hazy because follow-ups were not made rigorously. The issues raised varied from entering a possible slippery slope with a rouse for curing mitochondrial disease, to potential interaction of donor ooplasm and maternal nDNA.
The FDA is strict in its regulations relating to MRT due to lack of safe results. The UK is hopeful its proposed MRT techniques are effective.

Islamic perspectives

MRT is a new form of preventive medicine aimed at removal and replacement of disordered mitochondria at the fertilization level. So far, the issue of MRT has never been discussed by any *Fiqh* council or at any combined Islamic jurist-scientist forums.
Individual opinions, however, have been issued by notable Muslim jurists and scholars, in forms of personal communications.
Dr. Mohammad Ali Albar, Chairman of Medical Ethics Center in Saudi Arabia, stated that "donating an un-nucleated ovum from a healthy donor to save an embryo from a serious genetic disorder is commendable in view of necessity. The number of genes in mitochondria is so small (around one in a thousand of the total number of genes). Moreover, it may be acceptable, within the Muslim family structure, to have more than one mother, such as a milk mother. MRT has been approved in UK, and if outcomes are safe, then jurisprudence scholars could provide a constructive opinion to deal with this issue". Dr. Tariq Ramadan, from the Research Center for Islamic Legislation and Ethics in Qatar, responded that MRT is acceptable if the ovum donor is another wife of the husband, provided this marriage is based on sound *Shari'ah* grounds. It is not acceptable for a marriage to take place for the sake of the wife's ova. Marriages are meant to be a life-time union.
The issue of PNT as compared to MST is worthy of analysis and deliberations. Unlike PNT, in MST no fertilization occurs between the intended father's sperms and the donor's ovum, which is devoid of its DNA (nucleus totally discarded). The donated ovum could be considered as donated tissue. On this basis both Dr. Tariq Ramadan and Professor Abul Fadl M. Ebrahim (Professor Emeritus-School of Religion, Philosophy and Classics-University of Kwazulu-Natal-South Africa), approved of the MST technique.
Jurists and scientists need to conduct dialogue, seminars and deliberations to reach at sound standpoints in accordance with *Shari`ah*.

Concluding remarks

Mitochondrial disorders and mitochondrial replacement therapy have been progressively studied, aiming to minimize the harrowing medical disorders, and to prevent their propagation from affected women to their offspring at the fertilization level.

The proposed MRT techniques are surrounded by safety, legal and ethical concerns. In UK, concerned authorities had urged scientists to prove MRT safety and efficacy prior to trying on the public. Subsequently, in February 2015, mitochondrial donation was approved by the UK parliament, when a panel of expert scientists reported no evidence to suggest that MRT was unsafe.

Ethical concerns are still debated. In the Muslim world, no systematic discussions were undertaken by *fiqh* councils, or by combined jurist-medical scholars. Only few individual opinions have been reported in the form of "personal communications". Muslim jurists and medical scientists are called upon to study MRT in depth in pursuit of approved *Shari`ah* guidelines.

References

1. Human Fertilization and Embryology Authority S and ID. Mitochondrial donation [Internet]. [cited 2016 Mar 6]. Available from: http://www.hfea.gov.uk/9933.html
2. UK Parliament. Commons Debate [Internet]. 2015 Feb [cited 2015 Oct 27]. Available from: http://www.publications.parliament.uk/pa/cm201415/cmhansrd/cm150203/debtext/150203-0002.htm

3. The woman who lost all seven children. BBC [Internet]. 2012 Sep 20 [cited 2014 Jan 27]; Available from: http://www.bbc.co.uk/news/magazine-19648992
4. Possible Symptom's [Internet]. The United Mitochondrial Disease Foundation; 1996. Available from: http://www.umdf.org/site/pp.aspx?c=8qKOJ0MvF7LUG&b=7934631
5. Chinnery, PF. Mitochondrial disorders overview. GeneReviews. www.ncbi.nlm.nih.gov/books/NBK1224 (Accessed on March 29,2012)
6. Robin ED, Wong R. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. J Cell Physiol. 1988 Sep;136(3):507–13.
7. Beckman KB, Ames BN. The Free Radical Theory of Aging Matures. Physiol Rev. 1998 Apr 1;78(2):547–81.
8. www.mitomap.org. Morbid Map of the Human mtDNA [Internet]. 2013 [cited 2014 Sep 2]. Available from: http://www.mitomap.org/pub/MITOMAP/MitomapFigures/mitomapgenome.pdf
9. Wai T, Teoli D, Shoubridge EA. The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. Nat Genet. 2008 Nov 23;40(12):1484–8.
10. Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nat Rev Genet. 2005 May;6(5):389–402.
11. Bruce H. Cohen. Mitochondrial Cytopathies: A primer [Internet]. Cleveland Clinic Foundation; 2000 [cited 2014 Oct 2]. Available from: http://www.umdf.org/atf/cf/%7B858ACD34-ECC3-472A-8794-39B92E103561%7D/mitochondrial_cytopathies_APrimer.pdf
12. Sato A, Kono T, Nakada K, Ishikawa K, Inoue S-I, Yonekawa H, et al. Gene therapy for progeny of mito-mice carrying pathogenic mtDNA by nuclear transplantation. Proc Natl Acad Sci U S A. 2005 Nov 15;102(46):16765–70.
13. Craven L, Tuppen HA, Greggains GD, Harbottle SJ, Murphy JL, Cree LM, et al. Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. Nature. 2010 Apr 14;465(7294):82–5.
14. Cohen J, Scott R, Schimmel T, Levron J, Willadsen S. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. Lancet. 1997 Jul 19;350(9072):186–7.
15. Moses Gold. Michigan Girl, 13, Ends Up With Three Biological Parents | Religion [Internet]. Before It's News | Alternative News | UFO | Beyond Science | True News| Prophecy News | People Powered News. 2013 [cited 2014 Jan 28]. Available from: http://beforeitsnews.com/religion/2013/12/michigan-girl-13-ends-up-with-three-biological-parents-2461010.html
16. Review of scientific methods to avoid mitochondrial disease 2014 [Internet]. [cited 2016 Mar 7]. Available from: http://www.hfea.gov.uk/8807.html
17. Collins N. Three parent babies "incompatible with human dignity." Telegraph.co.uk [Internet]. 15:27 [cited 2014 Jan 27]; Available from: http://www.telegraph.co.uk/science/science-news/10356387/Three-parent-babies-incompatible-with-human-dignity.html
18. Helen Weathers. When surrogacy turns sour: Miscarriages, money wrangles and fall-outs. Mail Online [Internet]. UK; 2014 Jan 15 [cited 2014 Jan 29]; Available from: http://www.dailymail.co.uk/femail/article-2540167/When-surrogacy-turns-sour-Louise-expecting-baby-thatll-bring-joy-desperate-childless-couple-five-failed-pregnancies-wrangles-money-bitter-fallings-out.html
19. Nick Collins. Babies with three parents: Britain gives green light to radical IVF plan - Independent.ie [Internet]. 2013 [cited 2015 Oct 28]. Available from: http://www.independent.ie/life/family/mothers-babies/babies-with-three-parents-britain-gives-green-light-to-radical-ivf-plan-29379540.html
20. Human Fertilisation and Embryology Authority S and ID. Mitochondria public consultation 2012 - HFEA [Internet]. www.hfea.gov.uk. 2012 [cited 2014 Jan 29]. Available from: http://www.hfea.gov.uk/6896.html
21. Profile: Hwang Woo-suk. BBC [Internet]. Korea; 2009 Oct 26 [cited 2014 Jan 20]; Available from: http://news.bbc.co.uk/2/hi/asia-pacific/4554704.stm
22. TeachIslam.com. Halal (Lawful & Haram (Unlawful) [Internet]. Teach Islam. 2010 [cited 2014 Oct 2]. Available from: http://www.teachislam.com/content/view/5143/693/
23. Barritt J, Willadsen S, Brenner C, Cohen J. Cytoplasmic transfer in assisted reproduction. Hum Reprod Update. 2001 Aug;7(4):428–35.

MOLECULAR GENETICS OF CANCER: A REVIEW

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Abstract

Cancer is an abnormal growth of cells, presenting as a tumor. It is believed to arise from a single cell with altered genetic material, which undergoes multiple divisions producing a clone of cells with same abnormality. These genetic defects are identifiable in all subsequent cells, which arise from this initial cell, also referred to as the cancer stem cell. Recent advances have provided a detailed view of the mechanisms involved in the development and progression of cancer. A thorough understanding of the molecular mechanisms at play in malignant cells provides us the tools necessary to interrupt these aberrant genetic pathways and successfully treat this dreaded disease. This review provides a brief overview of the molecular abnormalities in the aberrant genes causing cancer. The history of evolution of knowledge in the field, and technologies involved are described. Therapeutic interventions currently based on molecular targets are tabulated. Ethical issues raised by this knowledge are discussed.

Keywords: Cancer, Genetics, Molecular Genetics, Genes.

Introduction

There is general consensus that cancer results from somatic mutations, which allow cells with abnormal genes to have competitive advantage over normal cells[1]. They not only proliferate but also invade neighboring tissue and spread to distant sites eventually leading to mortality. Advances in molecular biology and genetics have allowed scientists to study the mechanisms at the level of DNA, RNA, protein transcription, and post transcriptional activity in the malignant cell. These insights have opened new vistas for developing targeted drugs specifically neutralizing the effects of aberrant genes. These new treatments are more efficacious and less toxic than conventional treatments. Every area of cancer research and clinical practice has been affected by the evolving knowledge in this field.

Molecular level abnormalities are being targeted not only for therapy but also for screening and prevention. However, this knowledge also raises ethical issues about knowing the future, its implications are not only medical, but emotional and financial. Society is debating and learning how to handle this information in an ethical and socially responsible way. This article briefly provides an overview of the current state of knowledge in the field.

History

In 1855 Rudolph Virchow, a German physician published his famous paper "Omnis cellula e cellula" meaning "Every cell arises from another cell"[2]. This set the stage for study of cell as the fundamental unit of body

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for understanding its physiology and pathology. The source of all cells in the human body is the embryonic cell. A little over a decade later, an Austrian monk Gregor Mendel applied scientific principles to study the enigma of heredity – like father like son observation. In the garden of a monastery he demonstrated that specific traits like color of flowers was passed from one generation to the next in a mathematically predictable fashion[3]. The mysterious processes of inheritance were governed by a set of rather simple laws. Mendel recognized that traits were carried by discrete factors later called genes. Mendel's work was not appreciated until the turn of the 20th century. The actual substance in the cell, which carried these discrete factors, was unknown. Friedrich Miescher had isolated a substance from the nucleus, which he called nuclein[4]. He determined that it was made up of Hydrogen, Oxygen, Nitrogen, and Phosphorus. The Phosphorus and Nitrogen were in a unique ratio. He published his findings in 1871. The significance of Miescher's discovery only recognized when Oswald Avery in 1944 showed that DNA contained in the nucleus was the carrier of these hereditary factors now called genes[5]. This set the stage for Watson and Crick's famous paper proposing a model of the molecular structure of DNA.[6]Rapid advances subsequently have not only allowed analysis of the DNA but its manipulations. Today, scientists are not only able to study DNA for understanding the molecular mechanisms of life, but they have also developed interventions ranging from production of human insulin by inserting human genes in algae to developing drugs for cancer targeting the specific molecular abnormality.

Molecular Alterations in Carcinogenesis

In the normal process of cell division, the DNA is passed on to daughter cells without alteration. There are many steps involved in the cell cycle leading to cell division, and there are several major regulatory mechanisms that a healthy cell employs to ensure its genome remains stable throughout this process. In the DNA synthesis phase of the cell cycle (S-phase), DNA polymerase replicates the genetic code, proofreading for errors in replication. Errors that occur through the process of division are corrected. A cell that has damaged DNA is not allowed to progress to the next phase of the cycle, but it is channeled to the programmed cell death (apoptosis) pathway. These mechanisms ensure that daughter cells receive identical chromosomes creating two perfect clones.

An unstable genome arises when these regulatory mechanisms malfunction. When a cell with altered genomic material survives, it could lead to the development of cancer. This altered genome could involve overexpression of a gene involved in growth or suppression of a gene involved in apoptosis, which can lead on to a path of pre-cancerous growth. Subsequent additional genetic abnormalities at the molecular level may confer not only a growth advantage but also an ability to metastasize leading to clinically evident metastatic disease.

When there are mutations in hereditary genes such as those coding for DNA repair proteins, an individual will be more predisposed to cancer. These proteins, like the very important tumor suppressor p53, usually recognize damage in the DNA. When they are not fully functional, cancerous DNA can slip through the cracks more easily. Also, certain inherited mutations in genes BRCA1 and BRCA2 carry a 75% risk of breast and ovarian cancer. Overall, 3-10% of all cancer is due inherited abnormal genes.

Genetic alterations can result from environmental carcinogens that damage DNA, such as the chemical benzopyrene in tobacco smoke and charred coal. Other environmental carcinogens that can alter the genome direct-

ing it towards malignancy include radiation, either ionizing or UV. Ionizing radiation physically damages DNA altering the chromosomes. If this alteration involves a gene coding for growth, it could lead to development of a malignant clone. Even non-ionizing radiation such as UV light from the sun or tanning beds can physically alter the DNA of skin cells leading to the development of cancer.

Certain individuals are born with inherited abnormal genes, which do not by themselves lead to cancer unless additional environmental factors come into play. Individuals with such inherited abnormal genes, in the presence of social factors such as poor diet, obesity and smoking play a role in bringing to surface genes that cause cancer which otherwise would remain benign.

Certain infectious agents are also a known cause of cancer, with 18% of cancer deaths worldwide are attributed to them. These agents are primarily viruses known as oncoviruses. These include human papillomavirus, which causes cervical carcinoma, and the Epstein-Barr virus, which causes B-cell lymphoproliferative disease and nasopharyngeal carcinoma.

Hormones that naturally induce cell proliferation are often linked to cancer. For example, a correlation has been established between testosterone levels and occurrence of prostate cancer. Obesity is related to 30-35% of cancer deaths for many reasons, one of them being higher levels of growth hormones such as estrogens.

MMR, BER, NER

There are three primary repair mechanisms a cell employs when it encounters DNA damage. These include, in the order from small to large DNA lesions: mismatch repair (MMR), base excision repair (BER), and nucleotide excision repair (NER). Each step involves a group of proteins that are designed to recognize and repair specific types of DNA damage. Genes involved in these repair proteins, when damaged, would allow altered DNA to remain in the cell, which would have normally been fixed. Ultimately, a malignant cell can result from malfunctioning DNA repair proteins.

DNA mismatch repair (MMR) is aimed at correcting wrongly incorporated nucleotides in DNA replication, and repairing small DNA damage sites. An example of an MMR target would be a mismatch of bases, such as an incorrect guanine-thymine pair when the DNA polymerase should have instead placed a cytosine to match its complement guanine. In this case, three "Mut" proteins nick the daughter strand, make a loop, and excise the mutated DNA. DNA polymerase and ligase then correct the error. The proteins are named "Mut" because upon discovery, they were inactivated in bacteria and resulted in hypermutable strains.

Microsatellite instability is implicated in mutations of the "Mut" proteins and is present in most human cancers. Cancers with high microsatellite instability (MSI-H) carry a better prognosis. An example is colon cancer where MIS-H confers a 15% better prognosis compared to it's absence.[7] Microsatellite instability is also a marker suggesting the possibility of hereditary nonpolyposis colorectal cancer also known as Lynch syndrome[8].

Base excision repair (BER) comes into play when the base itself is chemically altered but does not alter the helical shape of the DNA. These alterations can occur naturally and include oxidization, alkylation, or deamination. BER involves DNA glycosylases that recognize problem sites and apurinic/apyrimidic endonucleases that cleave the altered bases[9]. Once the damage is removed, the DNA can be replaced by one of two repair processes known as short patch and long patch. Both

involve proteins normally found in the DNA replication process. BER malfunctioning has been linked to cancer. Mutations in polymerase β (involved in the long-patch repair) has been found in 30% of human cancers[10]. Mutations in a specific BER glycosylase can increase susceptibility to colon cancer[11].

The cell employs nucleotide excision repair (NER) when a large portion of the DNA is damaged enough to alter the shape. This mechanism is complicated, involving nine different proteins specifically aimed at recognition of lesions, adducts or structures that disrupt the DNA double helix, removal of a short oligonucleotide containing the offending lesion, synthesis of a repair patch copying the opposite undamaged strand, and ligation, to restore the DNA to its original form[12]. One of the most common ways bulky lesions are created in DNA is through UV-light exposure. The high-energy electromagnetic waves can cause two thiamine bases at different locations in the helix to link and form a dimer. In patients with xeroderma pigmentosum, NER is dysfunctional and unable to repair these large DNA lesions. Patients with this genetic disorder display multiple basal cell carcinomas (basaliomas) and other skin malignancies such as malignant melanoma and squamous cell carcinoma.

Epigenetic alterations favoring malignant transformation

Epigenetics involves modifications of gene expression rather than alterations of the genetic code itself. Changes in post-transcriptional mRNA can lead to a variety of phenotypic changes in the cell, which may facilitate development of cancer. The common epigenetic mechanisms that favor biological processes fundamental to the genesis of cancer include altered DNA maturation and histone modification. Epigentic mechanisms can also confer the ability for the malignant cells to self-renew, block differentiation, evade apoptosis, and develop potential for tissue invasion, all hallmarks of malignancy[13].

DNA methylation is a way of inactivating genes. Tumor cells are often found to have hypermethylation of tumor suppressor genes such as cell cycle regulators APC, p16, and BRCA1 a DNA repair gene. This hypermethylation inactivates the suppressor genes allowing unopposed activity of promoter genes leading to development of cancer.

The length of the DNA requires it to be condensed so it can fit in the nucleus. It is condensed with histones into structures called nucleosomes. Histones can be modified via methylation, similar to DNA methylation and acetylation. Alterations in the histones can affect the function of DNA, which is another mechanism that can facilitate development of cancer.

MicroRNA (miRNA) regulates about 60% of the transcriptional activity of protein-encoding genes[14]. MicroRNAs are responsible for down-regulating oncogene activity to protect against cancer. When transcription of these miRNAs is silenced by methylation, the oncogenes they usually suppress are expressed.

Human genome and the cancer genome

A genome is a complete set of genes of an organism, which is necessary for its formation and development. In humans, it consists of 3 billion base pairs of DNA and is present in every cell with a nucleus. Since cancer is a disease caused by mutations in the genes, understanding both the normal human genome and the cancer genome is necessary for understanding the molecular basis of cancer. Understanding the differences between the human genome and the cancer can guide the path to development of cancer therapies.

The proof that mutation of normal genes could cause cancer led scientists to the realization that a sequence of all human chromosomes would be the foundation for understanding cancer[15]. The human genome project was the world's largest collaborative biological project[16]. In a span of 13 years beginning in 1990, the human genome was sequenced, revealing 3.3 billion base pairs. It was a collaborative endeavor of the U.S. National Academy of Science, National Institute of Health, and the Department of Energy. This provided the blueprint for further work to analyze and compare a cancer genome to that of a healthy human one. With the genome sequenced, identification of mutations linked to different forms of cancer was made possible.

Rapid advances in technology have allowed complete DNA sequencing of a large variety of cancer cells[17]. The somatic mutations that are seen in this so-called cancer genome include substitutions of one base by another; insertions or deletions of small or large segments of DNA; rearrangements, in which DNA has been broken and then rejoined to a DNA segment from elsewhere in the genome; copy number increases from the two copies present in the normal diploid genome, sometimes to several hundred copies (known as gene amplification); and copy number reductions that may result in complete absence of a DNA sequence[17]. Additionally, the cancer genome may contain exogenous DNA such as that from human papilloma virus (associated with cervical cancer), epigenetic changes, and alterations to the mitochondrial genome. Currently, there are 350 cancer genes catalogued, with many more infrequently mutated genes playing a role as well[18].

The Cancer Genome Atlas Map

The cancer genome, which is the entire genome for cancer cells, was a natural sequel to the human genome project to unravel the mechanisms of malignancy and find avenues for therapeutic interventions. In 2004, about 291 abnormal genes were identified in the cancer genome. This accounted for about 1% of the total genome. 90% of these genes were somatic mutations, 20% showed germ line mutations, and 10% involving both.

The Cancer Genome Atlas (TCGA) was a project started in 2006 by the National Cancer Institute and the National Human Genome Research Institute, both parts of the National Institutes of Health, with a goal of mapping and highlighting the key changes occurring at the molecular level in brain and ovarian cancers. A secondary goal of the project was to build a genomic database with a compilation of cancer causing mutant genetic sequences.

As the project progressed, the initial focus was expanded to encompass several different types of cancers. The TCGA first began studying glioblastoma, the most common primary form of brain cancer. 6 years into the study, the TCGA researchers discovered 5 specific mutated genes, which were also linked to other cancers such as breast cancer and neurofibromatosis. Glioblastoma was also noted to have an incorrect translation of the enzyme responsible for telomere production on chromosomes. These findings have provided potential loci for targeted therapy in tumor cells. Four of the most common mutant oncogenes in glioblastoma are epidermal growth factor receptor (EGFR) and platelet derived growth factor(PDGFR), and deletion mutations in the DNA of tumor suppressor genes PTEN and p53. These genes are regulatory genes, which affect many pathways in the progression to cancer cells. Knowing the genome of glioblastoma cells can provide the necessary information to develop and use inhibitors in the pathways of cancer progression[19].Adenocarcinoma, the leading cause of

cancer death in the world, was found to have 18 significantly altered somatic genes, caused by intercellular alternative RNA splicing[20]. The analysis of proteins and growth factors made by genes and the subsequent antibodies to affected tissues in adenocarcinoma has led to the advancement of targeted therapies for adenocarcinoma. The Cancer Genome Atlas also documented 279 head and neck squamous cell carcinomas (HNSCCs) containing somatic genetic mutations[21].

The future goals of TCGA include using genomics to devise a more effective diagnosis of cancer and using information about a patient's genetic mutations to provide tailored treatment unique to the specific cancer of the patient.

A majority of the mutations studied were a result of chromosomal translocations.[22] Before the human genome project, the tyrosine kinase gene was identified as a high frequency mutation site in cancer genes. After the human genome project, 90 different tyrosine kinases were identified from the genome[23]. This discovery was one of several that contributed to the understanding and development of targeted treatments for cancer.

The hallmark of identified cancer genome is the alterations of the nucleotide base pairs in chromosomal segments[24]. Mutations in the cancer genome are common. Mutated genes in cancer cells encode for many proteins that are involved in the cell cycle and DNA regulation, leading to abnormal growth. Identifying the differences in base pairs between a cancer genome and a normal human genome could identify specific loci that are responsible for causing malignancy[25].

As of 2014, TCGA has completed whole-genome sequencing on 1,000 tumor samples, which has led to more than 2,700 publications in research journals. Aside from great progress in identifying subtypes of gastric, prostate, and head and neck cancers, the effort has also yielded potential drug targets for bladder cancer and others[26].

The research being conducted by TCGA has increased our understanding of the genetic drivers of cancer exponentially. Many of the common pathways and several specific mutations occurring in malignant disorders have been described. Louis M. Staudt, Director of the Center for Cancer Genomics explains that, "TCGA has led to an appreciation of cancer pathways that weren't even considered 5 or 10 years ago"[20]. This growing database of genetic information will continue to play a large role in understanding the molecular genetics of cancer and provide targets for developing better and safer drugs.

Methodology for Studying Molecular Genetics

The study of the molecular genetics of cancer requires the use of various techniques that can provide information about the genetic material and proteins associated with malignancy. Lab techniques used in identifying cancer have revolutionized cancer diagnosis and treatment.

Sanger Sequencing

Genome sequencing before the 1970s was merely a concept. In 1975, Frederick Sanger discovered a fundamental key to understanding the makeup of genes: the sequence of nucleotides in conjunction with chemically altered nucleobases, leading to a chain termination method[27]. The double stranded DNA is initially heated to form two single stranded pieces of DNA. These strands are added into a reaction vessel containing a primer and a DNA polymerase. This DNA is then placed into 4 reaction vessels, each containing a radio labeled version of the four-nucleotide

bases. As the reaction proceeds, every time a radio labeled nucleotide attaches to the template DNA strand, the replication of that strand would be terminated. Gel electrophoresis can then separate out the DNA chains by size and the overlaying of the DNA can provide the sequence of DNA. One of the shortcomings of the Sanger sequencing method in cancer diagnostics is that it is unable to detect mutations when there are mixtures of normal and cancerous tissues in a heterogeneous solution[28].

PCR

Kary Mullis first discovered the polymerase chain reaction technique, or PCR, an enormously useful technique in the study of DNA. It is widely used to multiply small quantities of DNA into larger amounts.

When a small quantity of DNA is available, it can be heated, which breaks up the double stranded DNA. The single stranded DNA is then attached to an initiator primer sequence, a sequence that functions as the starting point for replication. If DNA polymerase and base nucleotides are presented in the solution, which then is cooled to allow replication of the single stranded DNA to two exactly similar double stranded DNA's. This process can be repeated by alternative heating and cooling the solution to continuously double the DNA content with each heating and cooling cycle. The exponential increase in DNA allows for ample quantities of DNA for various analyses.

cDNA

Complimentary DNA, or cDNA, is a simple way to document coding sequences of genes in cells. cDNA is formed by the reverse transcription of mRNA in the cell. cDNA is preferred over mRNA sequences because mRNA is regularly degraded in the cell and is difficult to isolate. Once the mRNA of choice is identified, the enzyme reverse transcriptase in the presence of deoxy-nucleotide bases builds the complementary DNA strand to the mRNA sequence. A DNA polymerase through standard replication then replicates this single stranded DNA. The double stranded cDNA can be transcribed to produce mRNA, which can synthesize essential proteins and identify mutations in the transcription and translation mechanisms in the cell.

A compilation of an organism's reverse transcribed mRNA makes a cDNA library. A cDNA library compiles the mRNA sequences, which together make up an individual's transcriptosome. cDNA library can allow the insertion of specific mutant transcription factors, oncogenes, and tumor suppressors into model organisms and observe phenotypic changes within the organism compared to the phenotypic picture before insertion. This analysis is utilized to identify transcripts such as alternative splicing variants, fusion genes, and regulatory RNAs[29].

Whole-Exome Analysis

The human genome is a vast, with parts of the genome that code for proteins and other portions that do not code for proteins. Study of the coding portions, the Exons a small part of the entire genome is easier and faster. Once the mRNA is processed from the DNA within the nucleus of a cell, a spliceosome, a ribonucleoprotein, removes the non-coding parts of the mRNA known as introns[30]. What remains are the exons. The processed transcript is then translated outside of the nucleus. About 180,000 exons exist in the human genome, making up 3% of the genome. These 180,000 exons are arranged in about 22,000 genes. About 85% of diseases caused by genetic variances contain mutations in the exons[31]. Therefore, sequencing all the exons in a genome, known as the exome, is thought to be an efficient method of analyzing a patient's DNA to discover the genetic cause of

diseases or disabilities. Exome sequencing allows for the detection of variations in the protein-coding region of any gene to be identified, rather than the sequencing of individual genes of interest.

The advent of whole-exome sequencing has catalyzed the use of a specialized technique known as mRNA profiling, which compiles the transcriptosome in one or more cells. Transcriptosome mapping in specific cancer cell groups has shown genetic variability compared to the mRNA produced by the wild- type gene. These cancer-causing genes can be grouped as either tumor suppressor or oncogene. In head and neck squamous cell carcinomas, NOTCH1 was initially thought to have been an oncogene, but whole-exome analysis of the carcinoma found the NOTCH1 gene's translation stopped early in nearly 40% of all cases, predicting NOTCH1 may be a tumor suppressor gene rather than an oncogene in the cancer [32].

Mutations that cause disease mostly occur in exons. Exome and whole genome sequencing has led to discovery of DNA variations outside of exons that can alter gene activity and protein production and potentially lead to cancer.

Next-Gen Sequencing – Whole Genome Sequencing

Next Generation Sequencing (NGS) is widely considered to be one of the most revolutionary technological innovations in molecular genetics in the last century. In NGS, a relatively large sample of DNA is broken down into 100-150 base pair (bp) segments to be analyzed. These DNA samples have adapters attached to them in order for them to be attached to the surface of the slide and then through bridge amplification, each 100-150 bp segment is copied repeatedly. These double stranded bridges then become single stranded bridges by breaking the hydrogen bonds between the two strands. The slide is flooded with nucleotides and DNA polymerase. These nucleotides are fluorescently labeled, with a particular color corresponding to a particular DNA base. A terminator is included so that only one base is added at a time. Every time a base is added, a particular color is emitted, indicating the order of bases at one particular location on the slide. An accumulation of many color emissions at a particular location illuminates the order of the DNA bases on that particular 100-150 bp(base pairs) strand of DNA. By identifying the overlaps in the DNA sequences and placing the strands on top of each other in terms of overlaps, the entire genome can thus be pieced together[33].

Prior to NGS, the only genes that were sequenced fully were genomic loci, which were mutated. NSG is significantly cheaper, faster, requires a small sample and is more accurate. This has led to rapid advances in identifying oncogenes and tumor suppressor genes. Additionally, mutations in epigenetic regulators and RNA splicing mechanisms were found through the systematic Whole Genome sequencing using NGS[34].

Next generation sequencing provides the entire sequence of an individual's genome, which can then be compared to a reference genome to determine rates of mutation at specific oncogene and tumor suppressor gene loci. The discovery of independent mutations within these genes can lead to specific targeted gene therapies. As more evidence emerges on common driver-mutations found across a number of cancer types, newly developed molecularly targeted therapies will provide a means to improve cancer treatment outcomes across a number of cancers with common biological/molecular mechanistic pathways.

Genetic Abnormalities in Cancer
Numerical Abnormalities

Cancer is a disease that is caused by accumulation of multiple mutations in the somatic cells. Various factors can cause these muta-

tions. The various genetic abnormalities noted in the cancer cells include abnormal number of chromosomes, abnormal structures of chromosomes. These chromosomal abnormalities create mutations or abnormal genes. In a typical human cell there are 23 pairs of chromosomes. Numerical abnormalities in the chromosomes include aneuploidy i.e. unequal number of chromosomes, hypodiploidy i.e.decrease in the number of chromosomes, or hyperdiploidy i.e. increase in the number of chromosomes.

Structural Abnormalities

The second type of chromosomal abnormalities is structural, which include deletions, translocations, inversions, rearrangements, and amplifications.
A deletion involves the removal of a chromosomal segment from the set. A terminal segment of the chromosome can be deleted, i.e. terminal deletion, or a segment from the non-terminal part of the chromosome can be deleted which would require 2 breaks and a fusion of the remaining part of the chromosomes, which is known as interstitial deletion. This process of deletion and fusion can activate an oncogene or inactivate a tumor suppressor gene[35].

Translocation is a genetic change that results from one piece of chromosome breaking off and attaching to a different, nonhomologous, chromosome or to a new site on the same chromosome. Translocation is most commonly seen in lymphomas, sarcomas, and leukemias. The most well-known being chronic myelogenous leukemia (CML). In CML the long arm of chromosome 22 is attached to chromosome 9 resulting in the oncogene BCR-ABL that is discussed in another section in detail. Multiple advances have enabled scientists to screen for translocation through a special karyotyping technique called multi-color fluorescence *in situ* hybridization (FISH). When translocation is present, FISH is able to detect the translocation due to its sensitive genome-wide overview on the patient's chromosomes.
In inversions a segment of a chromosomes gets detached and re-inserted in the opposite direction. This leads to an aberrant expression in the genome, either activating an oncogene or de-activating a tumor suppressor gene. The oncogene can become activated by being exposed for transcription after an inversion. The tumor suppressor gene could be interrupted by an inversion leading to its inactivation. An example would be an inversion seen at band 11q23 which juxtaposed the Mixed-Lineage Leukemia gene (MLL) to the Clathrin Assembly Lymphoid Myeloid Leukemia (CALM) gene which leads to acute myeloid leukemia (AML) of infants.

Amplifications can lead to increased production of RNA found in a cell thus leading to an increase in the amount of protein production. If the excess production involves proteins promoting cell cycle pathway, it could lead to uncontrolled growth of cells manifesting as cancer. Amplifications can happen anywhere in the genome but the most alarming amplifications would be the amplifications of oncogenes. These amplifications are usually detected through DNA-based techniques, molecular cytogenetic techniques, and comparative genomic hybridization. An example would be the epidermal growth factor receptor 2 (HER2) amplicon which is one of the oncogenes for breast cancer. About 20-30% of patients with breast cancer have this amplification[36,37]. Multiple other rearrangement of chromosomes can occur leading to malfunction of genes.

Most tumors have concurrent multiple abnormalities. Higher grade tumors have more abnormalities than lower grade tumors. Cancer cells can, not only have abnormalities in the chromosome but they can have abnormalities at the molecular level also. These molecular abnormalities involve oncogenes, tu-

mor suppressor genes, fusions genes, mutations in metabolic and enzymatic pathways, which can lead to malignant proliferation of cells.

Oncogenes

Oncogenes are genes that are capable of transforming regular cells into tumor cells. They are a mutated form of normal genes called proto-oncogenes. Proto-oncogenes have three functions: promoting cell growth, regulating differentiation, and minimizing apoptosis. These functions all take part in the growth and development of the body, and are necessary for embryogenesis. When a proto-oncogene is mutated, it has the potential to become cancerous[38].

Proto-oncogenes have the potential of becoming oncogenes due to outside factors such as mitogens and carcinogens. Carcinogens damage DNA and can increase the chance of error during replication. These outside factors can also make the process of repairing damaged genes ineffective, and they can also overpower the effects of tumor suppressor genes. Carcinogens can be found in both natural and synthetic substances. Examples can include tobacco smoke, radiation, and asbestos[39].

There are several mechanisms that can convert proto-oncogenes into oncogenes. The three mechanisms that cause a proto-oncogene to convert into an oncogene are mutations in the DNA, gene amplification, and chromosomal rearrangement. The mutation of the DNA of the gene itself or in a regulatory region of the gene, such as the promoter, can cause the overproduction of proteins, potentially leading to a malignant outcome. Gene amplification and increased mRNA stability prolongs the existence of mRNA in the gene, which can cause a normal protein to be over-expressed. Translocations of genes to nearby regulatory elements such as promoters can induce the over-expression of proteins[38].

An example of a common oncogene is the *Src* oncogene, which is present in sarcoma, a tumor found in mesenchymal cells. The oncogene was initially found in a chicken retrovirus, and functions as an oncogene because it causes the overproduction of a certain protein.[15] Just like a car needs a brake pedal; a normally functioning cell has a mechanism to regulate the production of proteins. An oncogene has no "brake" mechanism, making it continuously active, just as a car would continuously accelerate. The *Ras* oncogene is also a common oncogene, and it codes for guanosine triphosphate hydrolase enzymes (GTPase) in cells[40]. This oncogene is found in thyroid tumors and certain types of leukemia. Another example of an oncogene is the *Myc* oncogene, which codes for transcription factors that induce cell proliferation. The *Myc* oncogene is overexpressed due to the translocation of chromosomes 8 and 14. This translocation commonly leads to Burkitt's lymphoma[41]. RTK oncogenes are similar to *Src* oncogenes in that they code for tyrosine kinases. In this case, the RTK oncogene codes for receptor tyrosine kinases (hence the name RTK) which are located along the cell surface. This oncogene can turn on receptors permanently even in the absence of signals, which can cause cancer. An example of a receptor tyrosine kinase is vascular endothelial growth factor (VEGF).[42]

A well-known example of an oncogene is the BCR-Abl fusion gene, which is associated with chronic myelogenous leukemia.[43] The BCR-Abl gene, which is located on the Philadelphia chromosome, is derived from the translocation that occurs between chromosomes 9 and 22. It was known as the first gene and oncogene known to induce malignant growths. Because of the translocation, the gene is separated from the controlling gene and transferred to another gene, which

causes the continuous and unregulated production of tyrosine, leading to excess cell growth[44].

Tumor Suppressor Gene

A tumor suppressor gene is involved in repairing DNA mistakes, controlling the rate of cell division based on the need, and regulating the process of apoptosis, or programed cell death. The tumor suppressor gene was first described from a study of retinoblastoma, a form of childhood eye tumor. In order to become susceptible to retinoblastoma a child must not only have inherited a single dominant trait but two additional mutations which both result in the loss of the retinoblastoma protein tumor suppressor gene(Rb)[45]. Another notable tumor suppressor gene is the p53 gene which when mutated or inactivated is linked to leukemias, lymphomas, sarcomas, brain tumors, and carcinomas of the breast, colon, and lung. When there is a mutation or error in a tumor suppressor gene it loses its function. This occurs because the inactivation of the tumor suppressor gene eliminates the regulatory protein, which if especially combined with an activation of an oncogene, could lead to the development of cancer.

Fusion Gene

A fusion gene is created by joining two different genes together through structural abnormalities in the chromosome. 20% of morbidities from cancer in humans are caused by gene fusion abnormalities[46]. These fusion genes lead to cancer through multiple pathways. The abnormal pathways include overexpression, suppression, changing the location of a protein, and removing the regulatory domains of specific genes. Recent data suggest that fusion genes are commonly seen in epithelial cancers. Advancement in technology has allowed scientists to disrupt the effects of malfunctions created by fusion genes for therapeutic benefits.

Passenger and Driver Mutations

The mutations involved in the cancer can also be classified as passenger and driver mutations. Driver mutations are mutations which confer growth advantage to the cancer cell and are required for maintenance and survival of the cancer cell[47]. As the cancer has multiple mutations some of them are nonfunctional but persist in the cancer cell and these are called “passenger mutations”. These mutations sometimes could be of value in research and in diagnosis as they are frequently present with the driver mutations.

Mutations in Metabolic Enzymatic Pathways

Mutations in the metabolic enzymatic pathways can provide the cancer cells with a favorable environment to proliferate. In the normal cellular metabolic pathway glucose is converted to pyruvate, which then enters the Krebs Cycle in mitochondria to generate adenosine triphosphate (ATP). Cells under hypoxic conditions convert pyruvate to lactic acid. This is an inefficient system for generating energy. However, cancer cells are able to use this effectively as they are frequently proliferating under hypoxic conditions because they are separated from the underlying stroma and blood vessels because of their rapid growth[48]. This increase in non-aerobic glycolysis creates acidic microenvironment which is toxic to the normal cells but mutations in the malignant cells allow them to adapt and resist the acid induced cell toxicity[49].

In the process of cell division and metabolism abnormalities can occur. There are inbuilt mechanisms in the cell machinery to check and repair these abnormalities or destroy the cells, which cannot be repaired. When these checkpoint processes become

deranged or defective, the potential for developing cancer increases or favorable environment for creation of a malignant clone occurs.

Exciting recent developments in manipulating the checkpoint mechanisms to control cancer have resulted in approval of drugs, which work through this pathway. One example is the newly approved drug nivolumab (Opdivo) which is a human immunoglobulinG4 monoclonal antibody that specifically targets programmed death-1 (PD-1) receptor[50].

Metastasis

Cancer cells not only have the potential to grow and invade locally but also to spread to other parts of the body, a process known as metastasis. Any kind of cancer can become metastatic, and most commonly spreads to the bone, liver, and lung. Approximately 90% of deaths caused by cancer are from metastasis[51]. Although metastasis can happen with any type of cancer there are genes which suppress the metastatic process.

These genes which have been studied include nonmetastatic gene 23 (NM23), and Kangai 1 (KAI1). The deactivation of these metastatic suppressor genes allows the tumor to spread in the body.

Angiogenesis

Angiogenesis, which is the creation of new blood vessels is a pivotal step in the growth and spread of cancer.

The process of angiogenesis itself involves a complex multi-step cascade, which is regulated through different angiogenic factors.

The key growth factors necessary for angiogenesis are the vascular endothelial growth factor (VEGF) and the hepatocyte growth factor (HGF).

Mutations leading to overproduction of these growth factors or increasing the sensitivity of the receptors to these growth factors assists the malignant cells to grow and metastasize.

Apoptosis

Apoptosis, the process of a programed cell death is activated when there is unrepairable damage or the cell is no longer necessary for the body's functioning. The process of apoptosis is genetically controlled. P53, a tumor suppressor gene, when downregulated decreases the functioning of the apoptotic process. A cell which has undergone mutations, which are deleterious would normally be channeled to the apoptotic pathways to rid the body of this cell. When the apoptotic pathway is nonfunctional as in the suppression of p53, this cell would proliferate and manifest as cancer. Disruption in the balance between pro-apoptotic and anti-apoptotic proteins can facilitate development of malignancy. An example of the genes involved in pro-apoptotic and anti-apoptotic mechanism is the Bcl-2 family. Abnormalities in Bcl-2 genes are commonly seen in B-cell lymphoma. Downregulation of these genes allows a lymphocyte to survive much longer than its usual lifespan while the body continues to produce new lymphocytes leading to a marked increase in the number of lymphocytes. This is one of the mechanisms leading to the development of chronic lymphatic leukemia[52].

Drug Resistance

Tumors, like humans, are capable of adapting for survival, as therapies directed against cancer interrupt the growth and metastatic process they develop mechanisms to resists the drugs targeting them. The resistance to chemotherapy can be either intrinsic or acquired. Intrinsic resistance to chemotherapy implies that the tumor contains resistance-mediating factors. Acquired resistance involves mutations that are developed during treatments. Several studies have shown that

the primary mechanism of resistance involves over expression of the P-glycoprotein and the multidrug resistance-associated protein (MRP). These two proteins play a key role in the molecular pumps, which eliminate the drugs from the cell so that the drug is unable to reach its target. Unfortunately targeting these two proteins with specific drugs is toxic to the body because the liver, kidneys, and gastrointestinal tract use the same mechanism to eliminate toxins from the cell.

Cancer genome and
New taxonomy of Tumors

Malignant tumors traditionally have been classified based on the site of origin, like breast cancer. They have also been classified based on the morphology like adenocarcinomas or glandular cancers.
The classification provided the basis for staging, understanding biological behavior and planning treatment. However it has been recognized that tumors arising from same organs behave in different ways. Genomic analysis now has clarified the reasons for this heterogeneity. The classic example is lung cancer, where a tumor with a mutation in EGFR leads to a different natural history. It has a different cause (these generally occur in nonsmokers), and responds to anti-EGFR tablets while the other lung cancers do not[53]. Similar information has changed our understanding of several other cancers. This knowledge of molecular abnormalities in the cancer is redefining the way we classify tumors providing a more rational understanding of their biology, prognosis, and appropriate treatment options. This will eventually lead to a new classification and nomenclature of all malignant tumors.

Molecular Genetics of Selected Tumors

The classification and characterization of this large collection of diseases called cancer is essential for understanding and treating them. While all cancers share certain commonalities, gaining insight on what makes each cancer unique has vastly expanded the number of strategies we currently have to combat them. Clinicians have classified cancers based on site of origin, histopathology and biological behavior.

In the last few decades, with the advent of many advanced molecular techniques, researchers have been able to characterize these different diseases with a much higher level of detail and precision. Modern technology has allowed researchers and physicians to understand and target specific molecular abnormalities in cancer with greater success and less toxicity. Such approaches have also allowed scientists to design drugs targeting those abnormalities. The sheer number of publications detailing the molecular pathways observed in cancer is a testament to the promise and proven results of this basic science approach to fighting cancer.

CML

One of the first cancers to be understood on the molecular level is Chronic myeloid leukemia(CML). This manifests as uncontrolled proliferation of granulocytic series of white blood cells. In 1845 John Hughes Bennet first described this disease. It almost always meant a death sentence. It was believed then that the underlying pathology was due to, “the presence of purulent matter in the blood.”[54]

The first breakthrough in the disease did not occur until over a century later when Peter Nowell and David Hungerford noticed a small, aberrant chromosome in patients with CML[55]. This structure, later deemed the Philadelphia chromosome (Ph^1), is a result of a translocation between chromosomes 9 and 22[56]. As a consequence of this molecular rearrangement of genetic material, an abnormal fusion gene is created called Break point

cluster region/Ableson murine leukemia (BCR/ABL). This is an oncogene, which continuously produces a tyrosine kinase leading to uninhibited growth and production of white cells. The discovery of the Ph[1] has given researchers a very specific understanding of this disease and a specific target for therapeutic intervention. However, most other cancers have not yielded such specific clues yet.

Breast Cancer

Breast cancers are a much more heterogeneous collection of tumors than CML. In contrast to CML, common solid tumors of the breast, colon, brain or pancreas have an average of 33 to 66 somatic mutations[57]. In his book biography of cancer, Siddhartha Mukherjee writes of a forty-three year old woman whose breast cancer had 127 mutated genes. In view of such genetic diversity, it is hard to come up with a simple, all-encompassing description of the genome of a breast tumor. Scientists have identified a subset of oncogenes and tumor suppressor genes believed to play a large role in many cases of solid tumors

A clinically relevant mutation seen in 20-30% of breast cancers is the amplification of the human epidermal growth factor receptor 2 gene (HER2)[36,37]. HER2 is a transmembrane tyrosine kinase which increases cell growth, proliferation, angiogenesis, motility, and resistance to apoptosis. Scientists have been able to target the overexpression of HER2 by monoclonal antibodies, which has improved outcomes of patients with this subset of this disease. The gene coding for the estrogen receptor (ER) also seems to play a significant role in as many of two-thirds of breast cancers. When activated by estrogen, or growth factors, ER activates transcription by oncogene c-MYC and cyclin D1[58]. Fortunately, this also has been exploited as a therapeutic target by developing drugs that are anti-estrogenic.

While only 5-10% of breast cancers are hereditary, they have been the subject of extensive research[59]. The two most well characterized genes responsible hereditary breast cancers are breast cancer susceptibility gene 1 and 2 (BRCA1 and BRCA2). These genes are found in 80% of familial breast cancers. They are tumor suppressor genes that normally function to repair of DNA breaks[60,61]. The presence of these genes not only increases the lifetime risk of breast cancer, but it also occurs at a younger age[62].

Colorectal Cancer (CRC)

Colorectal neoplasms are inherited in 10-25% of cases.[63] One of the inherited mutations leading to colorectal cancer is the familial adenomatous polyposis syndrome. These mutations have been studied in detail and have helped elucidate some of the defining features of this type of cancer. Familial adenomatous polyposis (FAP) is one of these inherited diseases. FAP is a disease in which thousands of polyps begin to develop in the colon during the individuals in their 20's[63]. These polyps, if not treated early, will develop into CRC in 100% of patients[64]. The cause of FAP is the mutation in the tumor suppressor gene adenomatous polyposis coli (APC). The APC gene normally functions to regulate proliferation, differentiation, apoptosis, and migration. Acquired mutation of this gene subsequently have been implicated in approximately 85% of sporadic CRC[65,66].

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, is the most common inherited colon cancer syndrome. It is caused by mutations in no fewer than five mismatch repair genes. When mutated, they allow DNA replication to continue in spite of errors leading to malignant clones. HNPCC compromises around 5% of colorectal cancers.

Many other mutations occur in colorectal cancer. A common mutation seen in most

human cancer is that of the tumor suppressor gene p53. This gene is a transcription factor that can arrest cellular growth and lead to apoptosis when triggered by cell stress.[67] K-RAS is another cell proliferation controlling protein that has been noted to be defective in many CRC patients. It is mutated in one third of all human cancers.[40,68] While there are many other genetic abnormalities seen in CRC, the mutations described here encompass a large number of cases, and can provide targets for therapeutics.

Targets for Therapeutic Interventions

The chart below is a selected group of targeted drugs developed from the understanding of the molecular genetics of specific neoplastic diseases. These drugs have already been approved based on their efficacy. There are many other drugs currently in trials, or in advanced stages of development holding great promise for the future.

Molecular Target	Generic Name (Trade Name)	FDA Approved Use(s)
EGFR	Afatinib (Gilotrif) -oral	•Non-small cell lung cancer
	Erlotinib (Tarceva) -oral	•Non-small cell lung cancer •Advanced pancreatic cancer
	Gefitinib (Iressa) -oral	•Non-small cell lung cancer
HER2	Trastuzumab (Herceptin) -IV	•Breast cancer •Gastric adenocarcinoma
	Lepatinib (Tykerb) -oral	•Breast cancer
VEGF	Bevacizumab(Avastin) -IV	•Colorectal cancer •Non-squamous non-small cell lung cancer •Breast cancer •Glioblastoma •Renal cell carcinoma
Multi-Kinase	Axitinib (Inlyta) -oral	•Renal cell carcinoma
	Sorafenib (Nexavar) -oral	•Hepatocellular carcinoma •Renal cell carcinoma
	Sunitinib (Sutent) -oral	•Gastrointestinal stromal tumors •Renal cell carcinoma •Pancreatic neuroendocrine tumors

Molecular Target	Generic Name (Trade Name)	FDA Approved Use(s)
BCR/ABL	Imatinib (Gleevec) -oral	•Chronic myeloid leukemia •Acute lymphoblastic leukemia •Dermatofibrosarcoma protuberans •Gastrointestinal stromal tumors
	Nilotinib (Tasigna) -oral	•Chronic myeloid leukemia
	Bosutinib (Bosulif) -oral	•Chronic myeloid leukemia
	Dasatinib (Sprycel) -oral	•Chronic myeloid leukemia •Acute lymphoblastic leukemia
ALK	Crizotinib (Xalkori) -oral	•Non-small cell lung cancer
	Ceritinib (Zykadia) -oral	•Non-small cell lung cancer
BRAF	Dabrafenib (Tafinlar) -oral	•Melanoma
PI3K5	Idelalisib (Zydelig) -oral	•Chronic lymphocytic leukemia •Follicular lymphoma •Small lymphocytic lymphoma
PD1	Nivolumab (Opdivo) -oral	•Melanoma •Non-small cell lung cancer •Renal cell carcinoma
JAK2	Ruxolitinib (Jakafi) -oral	•Myelofibrosis •Polycythemia vera
CD20	Rituximab (Rituxan) -IV	•Non-Hodgkin's lymphoma

Prevention

Advances in understanding of the molecular genetics of cancer will allow us to predict the probability of disease in healthy people who can be screened. Interventions could be developed before the disease manifests. This would not only apply to cancer but more common disease like diabetes and hypertension, which have a multifactorial etiology combining a genetic predisposition with lifestyle[69].

Future prospects

The future of cancer management appears very bright with a deeper understanding of the molecular mechanisms at work leading to

this illness. This knowledge has already yielded many applications in practice today from diagnostics to therapeutics. Current work in progress suggests that a majority of cancers will become easily manageable in the near future.

Ethics

Cancer in majority of the cases is a result of sporadic mutations in somatic cells, while only a fraction of them are inherited genetically. Currently tests are available for identifying healthy people who are at risk for developing certain cancers from inherited or familial factors. This knowledge, while providing advance information to allow preventive measures, also creates stress, anxiety and social stigma[70]. Additionally, such knowledge, if made available to others could be exploited for denying employment and insurance. Laws have been enacted to protect such individuals. However, the potential for misuse of this information exists. Predicating the future may have its benefits but society has to learn how to handle such information in an ethically and socially responsible way.

Islamic Perspectives

"*Iqra*," meaning to read, was the first word revealed in the Glorious Qur'an[71]. The command to read has been interpreted by scholars as an obligation to seek knowledge. In one of the early revelations, the Glorious Qur'an states:

"...وَقُل رَّبِّ زِدْنِي عِلْمًا"

"Oh my Lord, advance me in knowledge"[72].

The Glorious Qur'an is referring to all knowledge, not simply spiritual knowledge as clarified in the following *hadith*: A desert dweller once asked the Prophet (ﷺ), "Oh Messenger of Allah, should we seek medical treatment?" The Prophet (ﷺ) replied, "Seek medical treatment, for Allah has not sent a disease without sending a cure for it. Those who have the knowledge of the cure know it and those who are ignorant of it do not"[73]. There are nearly 750 verses in the Glorious Qur'an directing and encouraging mankind to ponder and fully utilize the wonderful creations of the Almighty. Thus, it is clear that seeking knowledge, including medical knowledge, is a part of the faith. The ultimate goal of medical knowledge and research is to save lives. The Glorious Qur'an emphasizes the value of saving a life, stating:

"...أَنَّهُ مَن قَتَلَ نَفْسًا بِغَيْرِ نَفْسٍ أَوْ فَسَادٍ فِي الْأَرْضِ فَكَأَنَّمَا قَتَلَ النَّاسَ جَمِيعًا وَمَنْ أَحْيَاهَا فَكَأَنَّمَا أَحْيَا النَّاسَ جَمِيعًا..."

"...whosoever killeth a human being for other than manslaughter or corruption in the earth, it shall be as if he had killed all mankind, and whoso saveth the life of one, it shall be as if he had saved the life of all mankind..."[74].

This was revealed when Adam's son Cain killed his younger brother Abel. Humanity today is about 7.2 billion people. Saving a life is the greatest reward one can seek in this world. The pursuit of knowledge to discover and administer treatments to save people from deadly diseases is an act of worship of the highest order.

References

1. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med*. 2004;10(8):789-799. doi:10.1038/nm1087.
2. Magner LN. *A History of the Life Sciences, Revised and Expanded*. CRC Press; 2002. https://books.google.com/books/about/A_History_of_the_Life_Sciences_Revised_a.html?id=YKJ6gVYbrGwC&pgis=1. Accessed January 31, 2016.
3. Haq MM. Medical genetics and the Human Genome Project: a historical review. *Tex Med*. 1993;89(3):68-73. http://www.ncbi.nlm.nih.gov/pubmed/8451744. Accessed January 31, 2016.
4. Dahm R. Friedrich Miescher and the discovery of DNA. *Dev Biol*. 2005;278(2):274-288. doi:10.1016/j.ydbio.2004.11.028.
5. Cobb M. Oswald Avery, DNA, and the

transformation of biology. *Curr Biol*. 2014;24(2):R55-R60. doi:10.1016/j.cub.2013.11.060.

6. WATSON JD, CRICK FH. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature*. 1953;171(4356):737-738. http://www.ncbi.nlm.nih.gov/pubmed/13054692. Accessed November 26, 2014.
7. Popat S. Systematic Review of Microsatellite Instability and Colorectal Cancer Prognosis. *J Clin Oncol*. 2004;23(3):609-618. doi:10.1200/JCO.2005.01.086.
8. Buecher B, Cacheux W, Rouleau E, Dieumegard B, Mitry E, Lièvre A. Role of microsatellite instability in the management of colorectal cancers. *Dig Liver Dis*. 2013;45(6):441-449. doi:10.1016/j.dld.2012.10.006.
9. Liu Y, Prasad R, Beard WA, et al. Coordination of steps in single-nucleotide base excision repair mediated by apurinic/apyrimidinic endonuclease 1 and DNA polymerase beta. *J Biol Chem*. 2007;282(18):13532-13541. doi:10.1074/jbc.M611295200.
10. Starcevic D, Dalal S, Sweasy JB. Is there a link between DNA polymerase beta and cancer? *Cell Cycle*. 2004;3(8):998-1001. http://www.ncbi.nlm.nih.gov/pubmed/15280658. Accessed January 28, 2016.
11. Farrington SM, Tenesa A, Barnetson R, et al. Germline susceptibility to colorectal cancer due to base-excision repair gene defects. *Am J Hum Genet*. 2005;77(1):112-119. doi:10.1086/431213.
12. Spivak G. Nucleotide excision repair in humans. *DNA Repair (Amst)*. 2015;36:13-18. doi:10.1016/j.dnarep.2015.09.003.
13. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell*. 2012;150(1):12-27. doi:10.1016/j.cell.2012.06.013.
14. Friedman RC, Farh KK-H, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009;19(1):92-105. doi:10.1101/gr.082701.108.
15. Wheeler DA, Wang L. From human genome to cancer genome: The first decade. *Genome Res*. 2013;23(7):1054-1062. doi:10.1101/gr.157602.113.
16. Economic Impact of the Human Genome Project – Battelle. http://www.battelle.org/docs/default-document-library/economic_impact_of_the_human_genome_project.pdf. Accessed January 28, 2016.
17. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. *Nature*. 2009;458(7239):719-724. doi:10.1038/nature07943.
18. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. *Nature*. 2007;446(7132):153-158. doi:10.1038/nature05610.
19. Mischel PS, Cloughesy TF. Targeted molecular therapy of GBM. *Brain Pathol*. 2003;13(1):52-61. http://www.ncbi.nlm.nih.gov/pubmed/12580545. Accessed January 28, 2016.
20. The Cancer Genome Atlas (TCGA): The next stage - TCGA. http://cancergenome.nih.gov/newsevents/newsannouncements/TCGA_The_Next_Stage. Accessed January 28, 2016.
21. Lawrence MS, Sougnez C, Lichtenstein L, et al. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*. 2015;517(7536):576-582. doi:10.1038/nature14129.
22. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer*. 2004;4(3):177-183. doi:10.1038/nrc1299.
23. Robinson DR, Wu YM, Lin SF. The protein tyrosine kinase family of the human genome. *Oncogene*. 2000;19(49):5548-5557. doi:10.1038/sj.onc.1203957.
24. Loeb KR, Loeb LA. Significance of multiple mutations in cancer. *Carcinogenesis*. 2000;21(3):379-385. http://www.ncbi.nlm.nih.gov/pubmed/10688858. Accessed January 28, 2016.
25. Pleasance ED, Cheetham RK, Stephens PJ, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. *Nature*. 2010;463(7278):191-196. doi:10.1038/nature08658.
26. The Cancer Genome Atlas Research Network. Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature*. 2014;507(7492):315-322. doi:10.1038/nature12965.
27. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*. 1977;74(12):5463-5467. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=431765&tool=pmcentrez&rendertype=abstract. Accessed July 10, 2014.
28. Javle M, Rashid A, Churi C, et al. Molecular characterization of gallbladder cancer using

somatic mutation profiling. *Hum Pathol*. 2014;45(4):701-708. doi:10.1016/j.humpath.2013.11.001.

29. Yang W, Ying D, Lau Y-L. In-depth cDNA library sequencing provides quantitative gene expression profiling in cancer biomarker discovery. *Genomics Proteomics Bioinformatics*. 2009;7(1-2):1-12. doi:S1672-0229(08)60028-5.
30. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Processing of Eukaryotic mRNA. 2000. http://www.ncbi.nlm.nih.gov/books/NBK21563/. Accessed January 28, 2016.
31. Marian AJ. Recent developments in cardiovascular genetics and genomics. *Circ Res*. 2014;115(7):e11-e17. doi:10.1161/CIRCRESAHA.114.305054.
32. Agrawal N, Frederick MJ, Pickering CR, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science*. 2011;333(6046):1154-1157. doi:10.1126/science.1206923.
33. Shendure J, Ji H. Next-generation DNA sequencing. *Nat Biotechnol*. 2008;26(10):1135-1145. doi:10.1038/nbt1486.
34. Watson IR, Takahashi K, Futreal PA, Chin L. Emerging patterns of somatic mutations in cancer. *Nat Rev Genet*. 2013;14(10):703-718. doi:10.1038/nrg3539.
35. Dong JT. Chromosomal deletions and tumor suppressor genes in prostate cancer. *Cancer Metastasis Rev*. 2001;20(3-4):173-193. http://www.ncbi.nlm.nih.gov/pubmed/12085961. Accessed January 28, 2016.
36. Berns EMJJ, Foekens JA, van Staveren IL, et al. Oncogene amplification and prognosis in breast cancer: Relationship with systemic treatment. *Gene*. 1995;159(1):11-18. doi:10.1016/0378-1119(94)00534-Y.
37. Ross JS, Fletcher J a, Bloom KJ, et al. Targeted therapy in breast cancer: the HER-2/neu gene and protein. *Mol Cell Proteomics*. 2004;3(4):379-398. doi:10.1074/mcp.R400001-MCP200.
38. Chial H. Proto-oncogenes to Oncogenes to Cancer. 2008. http://www.nature.com/scitable/topicpage/proto-oncogenes-to-oncogenes-to-cancer-883. Accessed January 28, 2016.
39. Ames BN, Gold LS. Paracelsus to parascience: the environmental cancer distraction. *Mutat Res*. 2000;447(1):3-13.

http://www.ncbi.nlm.nih.gov/pubmed/10686303. Accessed January 28, 2016.

40. Bos JL. ras oncogenes in human cancer: a review. *Cancer Res*. 1989;49(17):4682-4689. http://www.ncbi.nlm.nih.gov/pubmed/2547513. Accessed May 11, 2015.
41. Nesbit CE, Tersak JM, Prochownik E V. MYC oncogenes and human neoplastic disease. *Oncogene*. 1999;18(19):3004-3016. doi:10.1038/sj.onc.1202746.
42. Zwick E, Bange J, Ullrich A. Receptor tyrosine kinase signalling as a target for cancer intervention strategies. *Endocr Relat Cancer*. 2001;8(3):161-173. http://www.ncbi.nlm.nih.gov/pubmed/11566607. Accessed December 12, 2015.
43. Bhise SB, Nalawade AD, Wadhawa H. Role of protein tyrosine kinase inhibitors in cancer therapeutics. *Indian J Biochem Biophys*. 2004;41(6):273-280. http://www.ncbi.nlm.nih.gov/pubmed/22900354. Accessed January 28, 2016.
44. Kurzrock R, Kantarjian HM, Druker BJ, Talpaz M. Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. *Ann Intern Med*. 2003;138(10):819-830. http://www.ncbi.nlm.nih.gov/pubmed/12755554. Accessed January 10, 2016.
45. Cooper GM. Tumor Suppressor Genes. 2000. http://www.ncbi.nlm.nih.gov/books/NBK9894/. Accessed January 29, 2016.
46. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. *Nat Rev Cancer*. 2007;7(4):233-245. doi:10.1038/nrc2091.
47. Pon JR, Marra MA. Driver and passenger mutations in cancer. *Annu Rev Pathol*. 2015;10:25-50. doi:10.1146/annurev-pathol-012414-040312.
48. Gillies RJ, Gatenby RA. Hypoxia and adaptive landscapes in the evolution of carcinogenesis. *Cancer Metastasis Rev*. 2007;26(2):311-317. doi:10.1007/s10555-007-9065-z.
49. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer*. 2004;4(11):891-899. doi:10.1038/nrc1478.
50. Sundar R, Cho B-C, Brahmer JR, Soo RA. Nivolumab in NSCLC: latest evidence and clinical potential. *Ther Adv Med Oncol*. 2015;7(2):85-96. doi:10.1177/1758834014567470.
51. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100(1):57-70. http://www.ncbi.nlm.nih.gov/pubmed/10647931. Accessed July 10, 2014.

52. Gaidano G, Foà R, Dalla-Favera R. Molecular pathogenesis of chronic lymphocytic leukemia. *J Clin Invest*. 2012;122(10):3432-3438. doi:10.1172/JCI64101.
53. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304(5676):1497-1500. doi:10.1126/science.1099314.
54. BENNETT JH. Case of Hypertrophy of the Spleen and Liver in Which Death Took Place from Suppuration of the Blood. 1845;64. http://www.researchgate.net/publication/37374084_Case_of_Hypertrophy_of_the_Spleen_and_Liver_in_Which_Death_Took_Place_from_Suppuration_of_the_Blood. Accessed December 6, 2015.
55. Nowell PC, Hungerford DA. National Academy of Sciences. *Science*. 1960;132(3438):1488-1501. doi:10.1126/science.132.3438.1488.
56. ROWLEY JD. A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining. *Nature*. 1973;243(5405):290-293. doi:10.1038/243290a0.
57. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127):1546-1558. doi:10.1126/science.1235122.
58. Roy PG, Thompson AM. Cyclin D1 and breast cancer. *Breast*. 2006;15(6):718-727. doi:10.1016/j.breast.2006.02.005.
59. Margolin S, Lindblom A. Familial breast cancer, underlying genes, and clinical implications: a review. *Crit Rev Oncog*. 2006;12(1-2):75-113. http://www.ncbi.nlm.nih.gov/pubmed/17078207. Accessed December 29, 2015.
60. Rosen EM, Fan S, Pestell RG, Goldberg ID. BRCA1 gene in breast cancer. *J Cell Physiol*. 2003;196(1):19-41. doi:10.1002/jcp.10257.
61. Gudmundsdottir K, Ashworth A. The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. *Oncogene*. 2006;25(43):5864-5874. doi:10.1038/sj.onc.1209874.
62. Peto J, Collins N, Barfoot R, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. *J Natl Cancer Inst*. 1999;91(11):943-949. http://www.ncbi.nlm.nih.gov/pubmed/10359546. Accessed December 29, 2015.
63. Bogaert J, Prenen H. Molecular genetics of colorectal cancer. *Ann Gastroenterol Q Publ Hell Soc Gastroenterol*. 2014;27(1):9-14. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3959535&tool=pmcentrez&rendertype=abstract. Accessed January 17, 2016.
64. Bisgaard ML, Fenger K, Bülow S, Niebuhr E, Mohr J. Familial adenomatous polyposis (FAP): frequency, penetrance, and mutation rate. *Hum Mutat*. 1994;3(2):121-125. doi:10.1002/humu.1380030206.
65. Näthke IS. The adenomatous polyposis coli protein: the Achilles heel of the gut epithelium. *Annu Rev Cell Dev Biol*. 2004;20:337-366. doi:10.1146/annurev.cellbio.20.012103.094541.
66. van Es JH, Giles RH, Clevers HC. The many faces of the tumor suppressor gene APC. *Exp Cell Res*. 2001;264(1):126-134. doi:10.1006/excr.2000.5142.
67. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature*. 2000;408(6810):307-310. doi:10.1038/35042675.
68. Andreyev HJ, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the "RASCAL II" study. *Br J Cancer*. 2001;85(5):692-696. doi:10.1054/bjoc.2001.1964.
69. William WN, Heymach J V, Kim ES, Lippman SM. Molecular targets for cancer chemoprevention. *Nat Rev Drug Discov*. 2009;8(3):213-225. doi:10.1038/nrd2663.
70. Clayton EW. Ethical, legal, and social implications of genomic medicine. *N Engl J Med*. 2003;349(6):562-569. doi:10.1056/NEJMra012577.
71. Glorious Qur'an: 96:1.
72. Glorious Qur'an: 20:114.
73. Musnad Ahmad: 18456.
74. Glorious Qur'an: 5: 32.

USING GENETIC ENGINEERING TO TREAT CANCER

*Roslan Harun**

Abstract

Cancer development and progression are driven by genetic and epigenetic aberrations in multiple oncogenes and tumor suppressor genes (TSGs). Recent large scale genome sequencing efforts have identified increasing numbers of candidate oncogenes and TSGs with novel driver mutations that can be potential therapeutic targets. Recent advances in genetic engineering technologies have made it possible to modify specific DNA sequences and proteins in cancer cells and explore the roles of the genes or proteins in cancer development, progression and therapeutic response. In this review, several novel approaches to treat cancer using genetic engineering methods will be discussed.

Keywords: cancer, genetic engineering, genome editing, immunotherapy.

Introduction

Cancer is characterized by multiple genetic and epigenetic alterations in oncogenes and tumor suppressor genes. Several large-scale cancer genome sequencing efforts have identified increasing numbers of genetic alterations present in human tumors. Amongst a background of passenger mutations, which are presumed not to directly affect the tumorigenic process, driver mutations directly promote the transformation of normal cells to cancer cells through mutational activation of oncogenes and/or inactivation of tumor suppressor genes. Oncogenes are typically activated via gain-of-function mutations, whereas tumor suppressor genes are usually inactivated via loss-of-function mutations.There is an increasing body of evidence to suggest that the immune system also acts as a significant barrier to tumor initiation and progression. Evasion of immune destruction has been recognized as one of the two emerging hallmarks of cancer. In recent years, immunotherapy has been used to manipulate patient's own immune system to kill cancer cells.

Surgery, chemotherapy and radiation therapy have been the cornerstones of cancer treatment for many years. Specific biologic therapies targeting specific molecular changes, seen primarily in cancer cells, have also emerged as standard treatments for certain types of cancer for the last decade. Genetic engineering technologies have made it possible to modify specific DNA sequences in the genomes of cells to explore the role of genes implicated in cancer development, progression and therapeutic response.

Bispecific Antibodies

Monoclonal antibodies (mAbs)have become an important class of protein-based drugs for the treatment of cancer and other diseases. More than 20 mAbs have been approved by the USA Federal Drug Administration (FDA) for the treatment of cancers and other diseases.

Several approaches have been used to enhance the efficacy of mAbs to target tumor antigens including direct conjugation with various effector compounds, such as toxins and cytotoxic drugs.

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Another novel strategy to increase anticancer antibody potency is using dual-targeting antibodies referred to as bispecific antibodies (biAbs).Variable domains of the desired mAb scan be genetically engineered into a single bispecific antibody. Anticancer response can be increased by targeting both cellular and humoral immune responses. Bispecific antibodies activate immune effector cells by targeting the CD3 complex, costimulatory CD28 molecule on T-cells or Fcγ receptors (FcγRs) on accessory cells, and can bind simultaneously to tumor-associated antigens (TAAs) on tumor cells. Several clinical trials have been conducted with chemically linked or genetically engineered biAbs targeting TCRs or FcγRs and TAA, either alone or in combination with IFNγ, GM-CSF or G-CSF [1,2]. A variety of immune effector cells, such as CTLs, NK cells, neutrophils, and macrophages, can be activated and redirected using biAbs. For T-cells, cytotoxicity is redirected to tumor targets, bypassing MHC restrictions by targeting CD3 on T-cells. Cellular immunotherapy is administered after debulking chemotherapy, surgery and/or radiation, highlighting the importance of lymphodepletion, decreased tumor burden and/or regulatory T-cell depletion.

Oncolytic Viruses as Cancer Vaccines

Oncolytic viruses (OVs) are tumor-selective agents with multi-mechanistic antitumor activities. They have the ability to selectively infect and replicate in cancer cells and associated endothelial cells. Cytokines, particularly interferons and tumor necrosis factors, and complement systems play important roles in dictating the viral tropism. The viruses kill the infected cancer cells via direct oncolysis causing apoptosis, necrosis, pyroptosis and autophagic cell death. Oncolytic viruses also have anti-angiogenesis and anti-vasculature properties which promote apoptosis and necrosis of uninfected cancer cells. In addition, OVs activate innate and tumor-specific immune cells and help eliminate the uninfected cancer cells in primary and metastatic nodules. Genetic engineering of OVs further potentiates the viruses as cancer vaccines. Oncolytic viruses armed with GM-CSF (such as T-VEC and Pexa-Vec) or other immunostimulatory genes, induce potent anti-tumor immunity in both animal models and human patients.

Tumor cells and associated stromal cells express a wide variety of tumor associated antigens (TAAs) including mutated proteins, fusion proteins, developmental proteins or tissue-specific proteins. The TAAs are targets for cancer immunotherapy, either through active immunization or adoptive transfer of activated immune cells. Cancer cell death induced by OVs is mostly immunogenic. Oncolytic viruses, delivered either intratumorally or systemically, will replicate in tumors and stromal cells causing induction of immunogenic cell death (ICD). Then ecrotic cells present signals on the cell surface and release danger signals such as HMGB1, HSP90/70, ATP and PAMPs. Apoptotic bodies are engulfed by antigen presenting cells (APCs) and TAAs are processed and presented along with MHC complex and costimulatory molecules. The danger signals activate and mature dendritic cells (DCs), and TAAs are cross-presented to naive T cells.

Oncolytic viruses have been explored as therapeutic cancer vaccines for few decades. Genetic engineering has been used to make OVs better cancer vaccines that produce potent oncolysis and antitumor immunity. For example, Toda et al. have shown that a genetically engineered oncolytic HSV G207 induced specific anti-tumor immunity in CT26 colon cancer model[3]. Genetic modifications of OVs aim to reduce suppression of immune responses by the OVs with deletion of viral immune evasion genes, and to enhance antitumor immune responses by inserting immune-enhancing transgenes into the OV vectors. Toll-like receptors (TLRs) play key roles in modulating the innate immunity. These receptors recognize pathogen-associated molecular pattern (PAMPs) and damage-associated molecular pattern (DAMPs) molecules and trigger the activation and maturation of DCs. For example, TLR9 recognizes unmethylated CpG motifs in

the viral dsDNA; thus OVs enriched with CpG sequences are believed to be stronger immunogens. Raykov et al. demonstrated that rat lung hepatoma cells infected with CpG enriched parvovirus had a significant reduction in metastatic rate compared with controls[4].

Other important proteins that are induced and released during cellular stress and necrosis are heat shock proteins (HSPs). Due to their abilities to act as molecular chaperones, HSPs can bind potential antigens and deliver them to a variety of APCs. Oncolytic adenoviruses can be modified to overexpress several HSPs such as HSP70, HSP90 and HSF1. The HSPs can function as oncolytic cancer vaccines and induce an MHC associated tumor antigen-specific CD8+ T cell response in syngeneic melanoma, colorectal and prostate cancer models in immunocompetent mice[5].

An HSP70-overexpressing oncolytic adenovirus has also been tested in a phase I clinical trial[6].
Oncolytic viruses can be engineered to express cytokines, chemokines or costimulatory molecules to enhance immune response and antitumor immunity.

Talimogenelaherparavec (T-VEC) is a good example of an engineered oncolytic virus that can be potentially used as an oncolytic vaccine. Liu et al.showed that mutations of several herpes simplex virus (HSV) genes CP34.5 and ICP47 of a potent JS1 strain enhancedtumor cell killing and replication of HSV mutants in tumor, and improved the immune stimulating properties of the virus[7].

In addition the human GM-CSF-encoding gene was inserted into the JS1/34.5-/47- construct to provide viruses with maximum immune stimulating properties. When OV is armed with GM-CSF gene, its antitumor immunity and cytotoxicity are further enhanced. GM-CSF mediates antitumor effects by recruiting NK cells and by induction of tumor antigen-specific cytotoxic T cells through the action of APCs.

Engineering Immune Cells To Treat Cancers

More recently, evasion of immune destruction has been recognized as one of the two emerging hallmarks of cancer[8]. This capability allows cancer cells to evade immunological destruction, particularly by T and B lymphocytes, macrophages and natural killer cells. In recent years, there is an increasing body of evidence to suggest that the immune system acts as a significant barrier to tumor initiation and progression. Previous studies have shown that tumors grew more frequently and rapidly in the immunodeficient mice compared to immunocompe-tent controls, in particular deficiencies in the function of CD8+ cytotoxic T lymphocytes, CD4+ TH1 helper T cells or natural killer (NK) cells[9]. This indicates that both innate and adaptive immune systems contribute significantly to immune surveillance and tumor eradication.
Immunotherapy has been used to manipulate patient's own immune system to kill cancer cells. This adoptive immunotherapy, using viral antigen-specific T cells, is a well-recognized procedure and has been proven to be effective in the treatment of transplant-associated viral infections and viral related malignancies. In a study in 1988, tumor infiltrating lymphocytes were isolated from 20 patients with melanoma, expanded in vitro and subsequently transferred back to the same individuals. This method did mediate metastatic melanoma regression[10].The tumor infiltrating lymphocytes (TILs) were expanded *ex vivo* and re-infused back into the patients. Unfortunately the results were modest and not durable. Furthermore, the generation of TILs requires good manufacturing procedures-compliant facilities and laborious *ex vivo* expansion procedures.
As an alternative, T cells can be genetically engineered, by using gene transfer vector, to express a T cell receptor (TCR) or a chimeric antigen receptor (CAR) specific for antigens expressed by the tumor. TCRs and CARs enable the T cells to recognize specific tumor-associated antigens on tumor cells. These engineered T cells are then expanded in the laboratory and then infused back into the patient. With guidance from their engineered receptors, the T

cells are able to recognize and kill cancer cells that harbor the antigen on their surfaces.Treatments using these engineered immune cells have shown remarkable outcomes in patients with advanced cancer in several small clinical trials.

TCR engineered T cells

In this approach, T cells are transduced with transgenes encoding for α and ß TCR chains specific for a given tumor associated antigen. Most of the earlier clinical trials have adopted TCRs targeting HLA restricted tumor-associated antigens, such as gp100 and Melan-A/MART-1, or cancer germline antigens such as NY- ESO-1 and MAGE3. TCR-directed T cells recognize tumor-associated antigen-derived peptides after being presented by HLA molecules. Unfortunately, some of these antigens, in particular the former antigens, are also expressed in healthy cells, which leads to "off-tumor-on-target" toxicity[11]. The side effects associated with TCR transfer approach are either due to cross-recognition of non-tumoral targets, generation of unexpected antigen specificities or autoimmune reactions. The toxicity is much less when germline antigens are used as targets.

More efforts have been focused to identify TCRs that recognize cancer-specific mutated antigens, particularly in the cancer-driving genes. Most recently, Tran et al. demonstrated that a CD4+ T helper 1 cell response against a mutated antigen could be harnessed to mediate regression of a metastatic cholangiocarcinoma[12]. Using a whole-exomic-sequencing-based approach, they demonstrated that the tumor-infiltrating lymphocytes (TIL) contained CD4+ T helper 1 cells that recognized a mutation in ERBB2 interacting protein (ERBB2IP) expressed by the cancer. After adoptive transfer of TIL containing about 25% mutation-specific polyfunctional T(H)1 cells, the patient achieved a decrease in the metastatic lesions with prolonged stabilization of disease.

Chimeric Antigen Receptors (CAR)

Unfortunately, tumors often evade T-cell immune surveillance by down regulating HLA or molecules involved in antigen processing and presentation. The advantage of the recombinant CARs over the TCRs is that CARs recognize cell surface molecules independently of HLA expression.CARs are constructed by fusing the extracellular antibody-derived single chain variable fragment (scFV), capable of recognizing TAA, with one or more TCR-derived signaling domains. This chimeric receptor provides T cell specificity and simultaneously enhances responsiveness of transduced T cells. Interaction of scFV with a given TAA induces T cell activation and consequent tumor killing. The promise of this approach has been recently highlighted by the success of CAR T cells specific for the CD19 antigen.

In an early proof-of-principle clinical trial involving patients with chronic lymphocytic leukemia (CLL), chimeric antigen receptor–modified T cells that target CD19 produced a durable complete remission in a small number of patients[13]. Relapsed acute lymphoblastic leukemia (ALL) is a considerable therapeutic challenge, particularly in patients who do not have a complete remission or have a relapse after stem-cell transplantation. Brentjens et al. used engineered CD19-targeted T cells (CTL019 cells) to treat refractory B-cell ALL and they found profound responses in a small number of children and adults[14].In their study, an anti-CD19 single-chain Fv domain was coupled to intracellular T-cell signaling domains of the T-cell receptor, thereby redirecting cytotoxic CD8+ T lymphocytes to cells expressing this antigen. The CTL019 (or CART19) T cells express a chimeric antigen receptor in which the T-cell activation signal is provided by the CD3-zeta domain, and the co-stimulatory signal is provided by the CD137 (4-1BB) domain.

In a more recent study by Maude et al., treatment of relapsed and refractory ALL with CTL019 T cells was associated with a high remission rate and durable remissions up to 24 months, even among patients whom stem-cell transplantation had failed[15]. Thirty children and adults with relapsed or refractory ALL were infused with autologous T cells transduced with a CD19-directed chimeric antigen receptor (CTL019) lentiviral vector and they were monitored for a response, toxic effects, and the expansion and

persistence of circulating CTL019 T cells. All the patients had cytokine-release syndrome which was effectively treated with the anti–interleukin-6 receptor antibody tocilizumab. Severe cytokine-release syndrome (27%) was associated with a higher disease burden.

Genome Editing

Genomic instability and mutability provide cancer cells with genetic alterations that drive tumor development and progression. Therefore, experimental approaches to manipulate specific DNA sequences in the genome of normal and cancer cells are critical for modeling the disease as well as systematically studying the many genes involved in cancer initiation, progression and therapeutic response.

Targeted genome editing is a novel approach that can efficiently modify any sequence of interest in living cells including cancer cells. This technology depends on the use of engineered nuclease, a sequence-specific DNA-binding domain fused to a nuclease that cleaves DNA in a non-sequence-specific manner. These nucleases are capable to induce double-strand breaks into specific DNA sites, which are then repaired by mechanisms that can be exploited to create sequence alterations at the cleavage site. Gene-editing nucleases offer the potential to directly assess the impacts of gene disruption or alterations of specific sequence variants on gene function in somatic cell-based models of disease. In addition, targeted nucleases also offer the potential to both gene-correction or gene-disruption strategies for the treatment of a wide range of genetic diseases.

Zinc Finger Nucleases

Majority of the studies using targeted genome editing have been performed using zinc finger nucleases (ZFNs)[16]. ZFNs can be used to introduce genomic alterations including point mutations, deletions, insertions and translocation. ZFNs can also be used for therapeutic purposes such as disruption of the expression of HIV co-receptor CCR5 gene by a specific ZFN for treatment of AIDS. The high efficiencies of alterations observed have already inspired efforts to use ZFNs as a potential therapeutic approach for genetic-based diseases.

Transcription activator-like effector nucleases (TALENs)

Transcription activator-like effector nucleases (TALENs) have rapidly emerged as an alternative to ZFNs for genome editing and introducing targeted double-strand breaks. These engineered nucleases consist of a non-specific FokI nuclease domain fused to a customizable DNA-binding domain which is composed of highly conserved repeats derived from naturally occurring transcription activator-like effectors (TALEs) encoded by *Xanthomonas* proteobacteria. TALENs have a very high success rate for high-throughput genome editing, thus can be used to target essentially any DNA sequence of interest in human cells[17].

CRISPR-Cas9 System

The prokaryotic type II clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system has rapidly revolutionized the landscape of genetic engineering. CRISPR-Cas9 system allows researchers to alter the genomes of a large variety of organisms with relative ease. This highly versatile system, which is derived from a prokaryotic adaptive immune system, comprises of two biological components: the RNA-guided DNA endonuclease Cas9 and a chimeric single guide RNA (sgRNA). The sgRNA molecule contains both a CRISPR RNA (crRNA) component and a trans-activating crRNA (tracrRNA) component. sgRNA binds to Cas9 and directs it to a genomic sequence of interest via base pairing to the target sequence[18].

Large-scale cancer genome sequencing efforts have produced an expanding catalogue of the genetic alterations present in human tumors[19]. Driver mutations promote the transformation of normal cells to cancer cells through mutational activation of oncogenes and/or inactivation of tumor suppressor genes. In contrast, passenger mutations are presumed not to directly affect the tumorigenic process. In addition to simplifying the study of oncogenes and tumor suppressor genes, the CRISPR-Cas9 system also allows for

rapid discrimination between driver and passenger mutations. Matano et al. used the CRISPR-Cas9 system, which acts as a molecular "scissor" that can cut and paste any piece of DNA, in his studies.They recently demonstrated the utility of this system to systematically engineer both loss-of-functions (LOF) in tumor suppressor genes and gain-of-functions (GOF)in oncogenes mutations in untransformed human intestinal organoids in order to model human colorectal cancer (CRC)[20]. The ability of the CRISPR-Cas9 system to modify multiple target mutations offers the opportunity to investigate combinatorial vulnerabilities in cancer cells and synthetic lethal interactions.

Conclusions

Surgery, chemotherapy, radiation therapy and more recently specific biologic therapies have been the cornerstones of cancer treatment. However, the prognosis for many cancers, particularly when at the advanced stages, is still poor. Evasion of immune destruction and genomic instability has been recognized as the two emerging hallmarks of cancer. Novel genetic engineering technologies allow modification of the immune system and correction of the cancer genome mutations. They offer the opportunity to develop effective novel strategies to treat this deadly disease.

References

1. Liu Y, Cheung LH, Marks JW, Rosenblum MG. Recombinant single-chain antibody fusion construct targeting human melanoma cells and containing tumor necrosis factor. Int J Cancer. 2004; 108(4):549–557.
2. Huang TH, Morrison SL. A trimeric anti-HER2/neuScFv and tumor necrosis factor-αfusion protein induces HER2/neu signaling and facilitates repair of injured epithelia. J PharmacolExpTher. 2006; 316(3):983–991.
3. Toda M, Rabkin SD, Kojima H, Martuza RL. Herpes simplex virus as an in situ cancer vaccine for the induction of specific anti-tumor immunity. Hum Gene Ther 1999, 10:385–393.
4. Raykov Z, Grekova S, Leuchs B, Aprahamian M, Rommelaere J: Arming parvoviruses with CpG motifs to improve their oncosuppressive capacity. Int J Cancer 2008, 122:2880–2884.
5. Huang XF, Ren W, Rollins L, Pittman P, Shah M, Shen L, Gu Q, Strube R, Hu F, Chen SY: A broadly applicable, personalized heat shock protein-mediated oncolytic tumor vaccine. Cancer Res 2003, 63:7321–7329.
6. Li JL, Liu HL, Zhang XR, Xu JP, Hu WK, Liang M, Chen SY, Hu F, Chu DT:
   A phase I trial of intratumoral administration of recombinant oncolytic adenovirus overexpressing HSP70 in advanced solid tumor patients. Gene Ther 2009, 16:376–382.
7. Liu BL, Robinson M, Han ZQ, Branston RH, English C, Reay P, McGrath Y, Thomas SK, Thornton M, Bullock P, et al: ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumor properties. Gene Ther 2003, 10:292–303.
8. Hanahan, D and Weinberg, RA. Hallmarks of cancer: The next generation. Cell 144 (2011), 646-674.
9. Teng MWL, Swann JB, Koebel CM, Schreiber RD, Smyth MJ. Immune-mediated dormancy: equilibrium with cancer. J. Leumoc. Biol., 84 (2008), 988-933.
10. Rosenberg SA, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report.NEngl J Med. 1988; 319:1676–1680.
11. Johnson, L. A., Morgan, R. A., Dudley, M. E., Cassard, L., Yang, J. C., Hughes, M. S., Kammula, U. S. et al., Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 2009. 114: 535–546.
12. Tran, E., Turcotte, S., Gros, A., Robbins, P. F., Lu, Y. C., Dudley, M. E., Wunderlich, J. R. et al., Cancer immunotherapy based on mutation- specific CD4+ T cells in a patient with epithelial cancer. Science 2014. 344: 641–645.
13. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor- modified T cells in chronic lymphoid leu- kemia. N Engl J Med 2011;365:725-33.
14. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with che- motherapy-refractory acute lymphoblastic leukemia. Science Transl Med 2013;5: 177ra38.
15. Maude SL, Noelle F, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. NEJM 2014; 371: 1507-17.
16. Bibikova M. Enhancing Gene Targeting with Designed Zinc Finger Nucleases. Science. 2003; 300:764–764.
17. Reyon D, et al. FLASH assembly of TALENs for high-throughput genome editing. Nat Biotechnol. 2012; 30:460–465.
18. Jinek M, et al. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity.Science. 2012; 337:816–821.
19. Vogelstein B, et al. Cancer genome landscapes. Science. 2013; 339:1546–1558.
20. Matano M, et al. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. Nature Medicine. 2015; 21: 256-262.

GENE THERAPY APPLICATIONS IN MODERN GYNECOLOGY AND OBSTETRICS

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Abstract

Gene Therapy is a novel therapeutic approach which allows using the recombinant DNA technology to create a functional gene-expressing unit. This unit can deliver DNA or RNA and allows the replacement of the absent, or integration with diseased DNA of targeted tissues, to change the transcription function responsible for the pathologic expression, and suppress this function or change its function to a non-pathological one.

This chapter is a comprehensive review that focuses on the application of gene therapy in gynecology and obstetrics. However, because numerous challenges remain, including the safety of gene-therapy approaches, it is critical to concisely summarize the current status of the experimental gene-therapy studies conducted in animal models and refer briefly to some of the available clinical trials on gene therapy. In addition, we briefly summarize the recent advances in gene delivery technologies in the context of gene therapy.

Keywords: Gene Therapy, viral vectors, Adeno-associated viruses (AAVs), Plasmid DNA (pDNA), uterine fibroids, endometriosis, premature ovarian failure, ovarian cancer, pelvic adhesions, intrauterine growth restriction (IUGR), Induced pluripotent stem cells (iPSCs).

Introduction

Gene Therapy (GT) allows the use of recombinant DNA technology to create a functional gene-expressing unit that delivers the DNA or RNA nuclear parts and allows its integration with diseased nuclei of targeted tissues to change their transcription function and correct the pathology[1]. The mechanism of gene therapy is its ability to utilize the nucleic acids (DNA or RNA) for the treatment and prevention of human disorders by uploading new genetic codes that will change the current gene function and set up the new gene function to either restore lost function or initiate new targeted function to achieve therapeutic goals[2].

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Gene therapy has several successful applications in clinical medicine reported through successful animal trials, and the concept of gene therapy has become very well established in therapeutic approaches. Examples include treatment of cancer, chronic or progressive diseases such as heart failure, neurodegenerative and metabolic disorders, including Parkinson's disease, and diabetes[3-5].

In addition, there are reasonable gene therapy applications in animal studies in the field of obstetrics and gynecology.

This comprehensive review focuses on the application of gene therapy in gynecology with specific focus on arresting the proliferative pathophysiology of uterine fibroids while briefly highlighting other applications.

Gene delivery methods

Vectors are known as the gene delivery vehicles. There are two classes: DNA (non-viral) vectors and viral vectors (Figure 1). Circular plasmid DNA enters cells in its naked form, or can be treated with chemicals to enhance stability and delivery efficiency. Viral vectors take advantage of the infectious nature and gene-shutting (capable of inactivetion) capability of certain viruses but are deliberately engineered to minimize harm by removing as many viral genes as possible. Both types of vectors can directly deliver genes into the human body. Alternatively, gene transfer may be applied to isolated human cells, which can then be re-infused into patients to function.

DNA (non-viral) vectors

A gene functional unit is typically composed of a promoter that drives gene transcription, the transgene of interest, and a termination signal to end gene transcription(Figure 1). Such an expression unit can be integrated into a plasmid (circularized, double-stranded DNA molecule) as a delivery vehicle. Plasmid DNA (pDNA) can be directly injected in vivo by a variety of injection techniques, among which hydrodynamic injection achieves the highest gene transfer efficiency in major organs by quickly injecting a large volume of pDNA solution and temporarily inducing pores in cell membrane[6].

High delivery can be achieved by using chemicals including cationic lipids and cationic polymers to condense pDNA into lipoplexes and polyplexes, respectively to negatively charge pDNA molecules and facilitate penetration of the hydrophobic cell membranes.

These nanoparticles shield pDNA induce nuclease degradation in extracellular space and facilitate entry into target cells[7].

Following cellular uptake, pDNA travels with cytoplasmic vesicles known as endosomes, where cellular surveillance mechanisms that clear foreign DNA pose significant barriers to achieving efficacious transgene expression and some gene receptors can mount a destructive innate immune response[8].

A major advancement in DNA vector design is mini-circle DNA (mcDNA), which differs from pDNA in the lack of bacteria-derived, CpG-rich backbone sequences[9].

When administered in vivo, mcDNA mediates safer, higher and more sustainable transgene expression than conventional pDNA[10]. Novel methods for large-scale production of mcDNA will boost further evaluation on its therapeutic efficacy[11,12].

Viral Vectors

Viruses are the main gene delivery vehicles for gene therapy. The virus invades the cells by the endocytosis process. In order to start this uptake process, the viral surface proteins have to interact with the correspondent

receptors on target cells. The viruses eventually integrate their DNA into the nucleus for viral gene expression to deliver their genetic information. The most widely used viral vectors in gene therapy studies are the gamma retrovirus, lentivirus, adenovirus (AdV), adeno-associated virus (AAV) and herpes simplex virus (HSV) vectors.

Gamma retrovirus and lentivirus are both retroviruses, with RNA genome. They utilize virus-derived reverse transcriptase and insert their DNA into the host genome (Genome integration).

Lentiviral vector is more favorable in many gene therapy settings because lentivirus can transduce replicating and non-replicating cells, whereas gamma retrovirus can only transduce replicating cells[6].

The genome integration property makes these retroviral vectors more preferred for the stable gene transfer into proliferating cells. The recent advances in GT are adding some modification of these two vectors by engineering their envelope glycoproteins.

These modifications enhance their function; increase vector stability, and expand their receptiveness to wide range of cell types.

To make viral vectors targeted to a specific cell type, it can be either pseudotyped with a viral glycoprotein that recognizes a specific membrane receptor of targeted cell type, or integrated with a ligand protein or antibody to the viral glycoproteins that binds to specific cell type surface molecules[6].

Integration into the host genome, is the distinctive feature of retroviral vectors. Genomic integration ensures the stability of transgene and persistent transgene expression in daughter cells following genome replication and cell division, but its randomness results in the risk of insertional mutagenesis by potentially disrupting tumor suppressor genes or activating oncogenes[6].

One of the biggest challenges that face gene therapy is the massive immune reaction that can develop secondary to the reaction of human immune system against the capsid of the infused adenoviral vector. In recent study one out of the 17 subjects treated died, likely secondary to cytokine storm and anaphylactic - like shock[13].

If adenoviral vector is injected into a tumor mass, AdV vector triggers antitumor immunity inside the tumor, and fight the tumor[13].
Elaborated technologies have been developed to shield the viral capsid proteins from recognition by the host immune system, and to provide clinical trials with non-integrating vectors especially in the area of cancer gene therapy[14].

Ex vivo gene transfer

Ex vivo gene therapy means cell isolation from the patient followed by their genetic modification outside the body and subsequent re-introduction into the patient as an autologous transplant. Ex vivo gene therapy has been used for further improving the safety profile of gene therapy.

It also lowers the risk of unwanted off-target effects, such as toxicity due to ectopic expression of the therapeutic gene in off-target organs and excludes germ-line transmission[15].

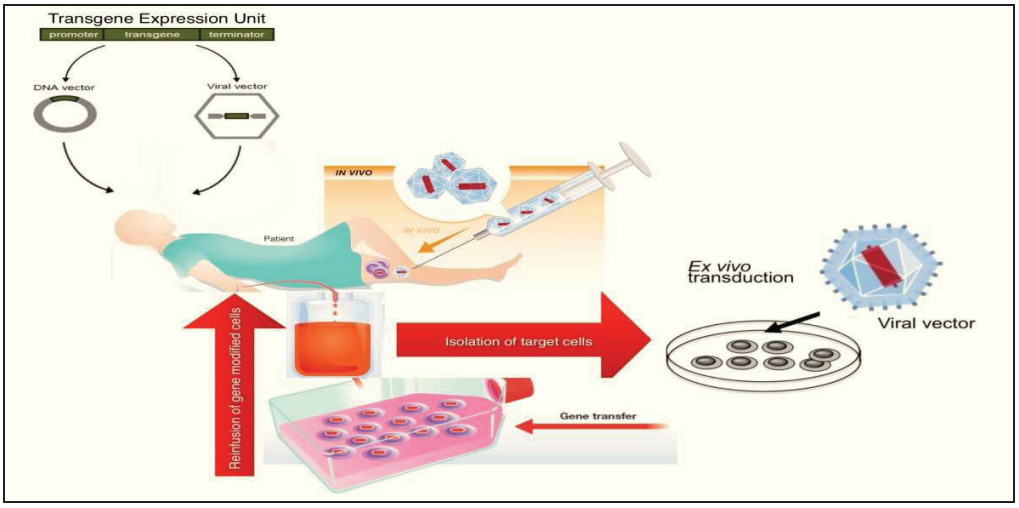

Figure-1:*In vivo and ex vivo gene therapy concepts. Gene therapy vector, the therapeutic transgene expression cassette can be carried by DNA vector or viral vector. In vivo application can be introduced directly into the body (e.g. muscle, liver) of the patient, while for ex vivo applications, patient cells are first isolated from the body, genetically modified outside the body and reintroduced into the patient as an autologous transplant*

Cell-based gene therapy

Cell-based gene therapy often utilizes stem or progenitor cells with an integration of the transgene into a host genome, so that their gene-engineered progeny can induce a long-term therapeutic response. T cell receptors (TCRs) identify specific molecules on the surface of target cells, and mediate target cell-specific killing. This target cell-specific killing capability has been invested in killing tumor cells. This is achieved by armed- T cells with receptors that redirect T cells to target tumor-specific surface molecules[16]. Such receptors include TCRs cloned from natural tumor-targeting T cells and chimeric antigen receptors (CARs) that are engineered by fusing an antibody fragment targeting tumor antigen with intracellular signaling domains triggering T cell activation[17]. After the CAR-armed T cell is re-infused into the human body, the CAR identifies specific molecule on the tumor cell surface. The interaction triggers a cascade of cell signaling event, and eventually allows the T-cell to kill the tumor cell. Several early phase clinical trials have shown the successful use of CARs in recognizing CD19, a B cell surface molecule in B cell lymphomas. The infusion of genetically modified T cells expressing anti-CD19 CAR led to partial remission of advanced-stage lymphoma[18]. Currently, a handful of clinical trials are underway to treat B cell malignancies with anti-CD19 CAR T cells[19]. A recent study has explored the use of ex vivo modified regulatory T cells to dampen immune response and to modulate autoimmune diseases[20].

Induced pluripotent stem cells (iPSCs) are generated by injecting the set of gene transcription factors into ex vivo somatic cells such as skin fibroblasts, which transform somatic cells to pluripotent stem cells that can differentiate into almost all cell types such as muscle, blood, and neuronal cells[22].

Combining the iPSC and ex vivo gene transfer technologies to develop cell-based gene therapy has promoted several studies but none of them are in obstetrics and gynecology. Inserting a functional beta-globin transgene into a “safe harbor” in the genome of a beta-thalassemia patient’s iPSCs, and then differentiating the transduced iPSCs to erythroid lineage yielded therapeutic levels of beta-globin expression[23].

As the targeted genomic editing technology is rapidly evolving, many studies have shown

that iPSCs derived from patients with various diseases are amenable to non-viral or viral vector-mediated genomic editing.
Importantly, patient's iPSCs or their differentiated progeny with a corrected genome demonstrated normal or improved cellular phenotype compared to the uncorrect-ed, diseased counterpart[24].
Adopting the same reprogramming concept, several recent studies have reported the successful development of actively pumping cardiomyocytes by the direct conversion of mouse fibroblasts[25]. In July 2013, Japan has officially approved the world's first clinical trial evaluating the use of iPSCs to treat age-related macular degeneration [Clinical trial ID: JPRN-UMIN000011929][25]. In the future, we would like to see several iPSC applications combined with ex vivo gene transfer in the clinical application of cell-based gene therapy in obstetrics and gynecology.

Integrating vectors, risk of insertional transformation and improved vector design

In molecular biology and genetics, a **promoter** is a regulatory region of DNA that regulates gene transcription. The promoter contains specific DNA sequences that are recognized by proteins known as transcription factors. Promoter activation by transcription factor trigger synthesizes the RNA from the coding region of the gene. The cell regulates the converting DNA to RNA by process called Transcriptional regulation (or transcription).
As mentioned before, genomic integration (virus integration into gene regulatory regions (promoters, enhancers, locus control regions), is the distinctive feature of retroviral vectors. Extensive studies showed that this genomic integration increases the risk of transcriptional dysregulation.
This was attributed to the tendency of gamma retroviral vectors to integrate into gene regulatory regions (promoters, enhancers, locus control regions).
Extensive studies were started to overcome the severe adverse events that were observed in the early gene therapy trials using gamma retroviral vectors. In these trials, the process of retroviral integration in human cell lines and primary human hematopoietic stem cells (CD34.) were tried[26-29].
They found that the probability of dysregulation of gene expression was exacerbated after the discovery of hot spot regions for retroviral integration.
The genomic loci for MDS-EVI1 and LMO2 which are currently known to be the integration hot-spots for gamma retroviral vectors in murine and human HSCs[30-31].
This explained the clonal dominance and the leukemogenesis case that happened in the SCID-X1 and X-CGD trials, in which a strong increase in either LMO2 or EVI1expression was observed due to insertional activation of these genes at their genomic loci[32-33].

These and further observations promoted the creation of transcriptionally inactive LTR by designing the self-inactivating (SIN) retroviral vector with deletions in the U3 region of the 50 LTR. Using an internal heterologous promoter to drive transgene expression has compensated for the lack of promoter activity[34-35]. Although the SIN configuration does not alter the integration profile of gamma retroviral vectors, by measuring transformation in an in vitro immortalization assay, the genotoxicity of vectors was strongly reduced[36].

In contrast to gamma retroviral vectors, the lentiviral vector has lower risk of genotoxicity because their insertion sites are under-represented in regulatory regions with high preference of integration into the body of genes. This was recorded in studies addressing the oncogenic potential of these

vectors either in vitro or in vivo[37-39].

Gene therapy in benign gynecological diseases:

Uterine Fibroids

Uterine fibroids are the most common benign uterine tumors. They occur in nearly 70% of white women and in over 80% of African American women. The majority of fibroids are asymptomatic. Patients with large size fibroids (4 cm or larger)usually present with symptoms such as uterine bleeding, pelvic pain, and infertility. The pathology of uterine fibroid is still unknown. This pathologic condition is benign and the curative treatment can easily be achieved by surgical strategies. Worldwide, fibroids is the leading indication for hysterectomy: 55,000 hysterectomies a year are still performed for fibroids in the United Kingdom and 600,000 in the United States, resulting in a heavy economic burden[40].

Recently, gene therapy as an on-surgical alternative therapeutic approach of the uterine fibroids seems to provide a promising hope in the reduction in the number of hysterectomy-ies, and myomectomies worldwide. Al-Hendy and his group were the first to pursue this goal. Uterine fibroids' growth appears to depend on sex steroids; estradiol plays a vital role in fibroid growth via its receptor ER and progesterone through its receptors PRA and PRB[40,41].This suggested that a therapy based on ER inactivation might be a successful strategy in arresting the growth of fibroids and achieving tumor reduction. Dominant-negative mutants of ER have been used to inactivate ER on estrogen- dependent tumors like pituitary lactotrophes[42]. These mutants bind heterodimers with wild-type ER and change the binding site of estrogen receptor elements (ERE) to make them unable to activate transcription when bound to ERE. Al-Hendy et al 2004, created a model of nude mice by growing rat ELT3 fibroid cells for ex vivo gene therapy application[40]. A nude mouse is a laboratory mouse with inhibited immune system as a result of a genetic mutation that causes absent or degenerated thymus gland with low or absent T cells. To perturb the estrogen-signaling pathway, they transduced nude mice with Dominant-negative estrogen receptor adenovirus (Ad-ER-DN) by direct injection into preexisting fibroid lesions and then injected Adenovirus expressing a marker gene coding for bacterial ß-galactosidase (Ad-LacZ) in a control group. They also assessed the ability of adenovirus to infect fresh 2-3 uterine fibroid tissue disks resected from hysterectomy specimens. The tissues were incubated with an adenovirus expressing a marker gene (Ad-LacZ). In an in vitro system, the Ad-ER-DN treated human and rat fibroids cells demonstrated induced apoptosis. By using the western blot, human fibroids cells and rat fibroids cells infected with Ad-ER-DN demonstrated lower amounts of the anti-apoptotic Bcl-2 protein and a higher amount of the pro-apoptotic Bax protein compared with control with evident apoptosis in the TdT (terminal deoxynucleotidyl transferase)- mediated dUDP nick-end labeling (TUNEL) assay, suggesting marked inhibition of cell proliferation. They also recorded a significant increase in caspase-3 protein levels in Ad-ER-DN treated cells compared with Ad-LacZ treated control groups (P =.001).This denotes that dominant-negative ER works centrally on the nuclear level to induce apoptosis. The caspase-3 is an apoptosis factor that causes degradation of structural and nuclear proteins[43.]

The gene therapy of fibroids works by switching on the apoptosis mechanism through both extrinsic and intrinsic pathways [by delivering apoptosis-inducing ligands, such as the TNF-related apoptosis-inducing ligand (TRAIL), tumor-necrosis factor (TNF), and FasL or by delivering proapoptotic

members of the Bcl-2 family, such as Bax or active caspase molecules]. Angiogenesis, which is essential for fibroids growth is driven by different growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derived endothelial growth factor (PDGF)[44-47]

Al-Hendy group has tried a different method of applying gene therapy called suicide gene therapy described earlier by Vassaux and Martin-Duque [48](Figure-2).Their method was based on transfection with the herpes simplex virus thymidine kinase gene (HSV-TK) followed by ganciclovir (GCV) administration[49].

GCV is phosphorylated to a toxic metabolite "triphosphorylated form (GCVTP) "by HSV1-TK and mammalian cellular kinases[50] which cause cells to die through apoptosis by inhibiting DNA synthesis and blocking the cell cycle[51-52]. The Ad-TK/GCV system has been represented in previous clinical trials as an effective gene therapy strategy in tumor cells eradication in several malignant diseases[49,53-55] and in benign conditions such as vascular smooth muscle proliferative disorder[56]. Using HSV-TK approach has an advantage of "bystander effect" by which the HSV-TK/GCV kills the recipient (HSV-TK$^+$) tumor cells and the surrounding non-recipient (HSV-TK$^-$) tumor cells[57]. Bystander killing effect works mainly on the Gap junctional intercellular communication (GJIC)[58,59] with the special target a protein called "Connexin 43"that forms part of GJIC[57]. This connexin 43is highly expressed in fibroids (leiomyoma) tissues compared with the adjacent normal myometrium[60]. The higher expression of connexin 43 in fibroids provides physiological selectivity for Ad-TK/GCV gene therapy approach. Furthermore, the discrete and well-defined nature of leiomyomas (LM) makes them ideal target for localized adenovirus-mediated herpes simplex virus thymidine kinase (Ad-TK)/GCV gene therapy administration.

The Al-Hendy group's use of the potent bystander killing effect will ensure specific focused delivery of high concentration of Ad-Tk/GCV which compensates for any potential segmental transfection of parts of the fibroid tumor with adenovirus and will lead to significant ablation of most of fibroid lesions[49].

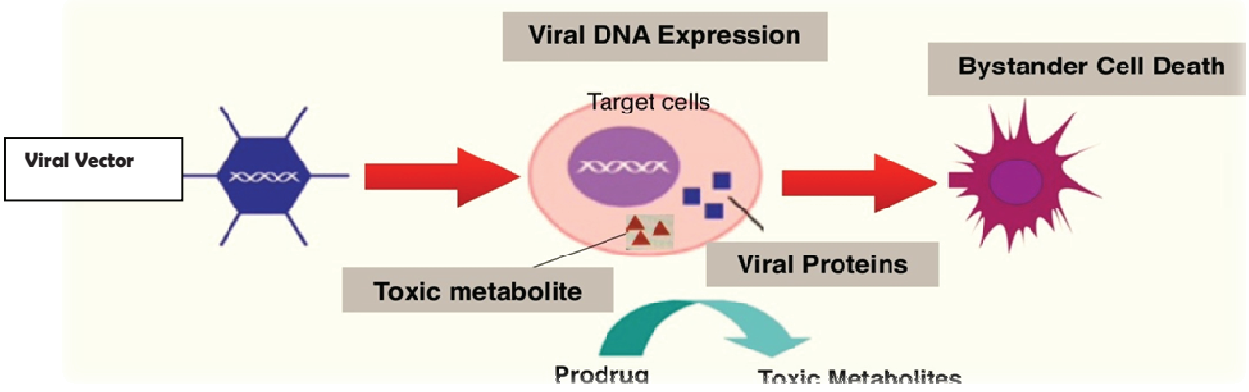

Figure-2: *Transfection with the herpes simplex virus thymidine kinase gene (HSV-TK) is followed by ganciclovir (GCV) administration. GCV is phosphorylated to a toxic metabolite "triphosphorylated form (GCVTP) "by HSV1-TK and mammalian cellular kinases which cause cells to die through the apoptosis by inhibiting DNA synthesis and blocking the cell cycle.*

To test the efficacy of a "bystander killing concept", Salama et al 2007, established a nude mice model with fibroid lesion, by subcutaneous injection of ELT-3 cells[49]. By direct intratumor delivery of the Ad-TK followed by GCV treatment, they found a significant suppression of leiomyoma tumor growth compared with the control groups treated by intratumoral injection of vehicle or Ad-Lac Z vector only, after 1 week of treatment. The sustained effect persisted until the end of a 4-week treatment period. These results demonstrate the efficacy of in vitro application of Ad-TH/GCV as the potential gene therapy approach for the treatment of LM and also demonstrated the significant inhibition of the growth of both ELT-3 cells and the primary human LM cells by using Ad-TK/GCV[49].

The application of gene therapy of human serotype 5 recombinant adenoviruses (Ad5) in clinical trials of tumor gene therapy has some limitations. These include the development of Ad-neutralizing anti-bodies limiting the gene transfer to the target cells[61], the inability of Ad5-based vectors to transduce important therapeutic target cell types[62], short life span of gene expression due to the local immune response against Ad5[63],the paucity of the primary coxsackie/Ad receptor (CAR) in many human tumors, in addition to the virus dissemination to normal tissues that might cause organ toxicity[63-65].So it was necessary to overcome these limitations in order to achieve the highest therapeutic target and maximum volume of focused adenovirus delivery to the pathologic organ with less toxicity. Al-Hendy group, had tested the selectivity of Ad5-luc when injected intratumorally in the Eker rat model of uterine leiomyoma. Forty percent of the Ad5-luc particles were disseminated in the myometrium and 30% in the liver[66].So, to achieve a higher level of tumor cell selectivity, the viruses have been developed using "transcriptional targeted" strategy in which the expression of the gene of interest is placed under the control of a tumor-specific promoter (TSP) to maintain a tumor-on status, a normal tissue-off status and/or liver-off status.

A subgroup C adenovirus (Ad) vectors possess low efficient transduction properties because they express low levels of the high-affinity Coxsackie virus and adenovirus receptor (CAR). Al-Hendy group, in their study, modified the Ad vectors to achieve high therapeutic targeting to human fibroids cells with minimal off target toxicity[67].

They used what is called "CAR-independent pathways", in which they inserted short peptide (21 amino acids) composed of arginine, glycine and aspartate (RGD) to the H1 loop of the wild serotype 5 recombinant adenoviruses (Ad5) fiber knob domain to reroute Ad5 binding to the cellular integrin. The transductionally enhanced Ad5 includes Ad5- Arg-Gly-Asp peptide(Ad5-RGD-luc), Ad5 canine adenovirus serotype 2 (Ad5-CAV2-luc), Ad5-sigma-luc and Ad5/3-luc. The two adenovirus panels used in this study exploit two main strategies: Ad5-transduction targeting and Ad5-transcriptional targeting. Transductional targeting aims at deletion of the broad tropism of Ad5 toward normal epithelial cells and/or enhances virus infectivity of CAR deficient tumor cells.

Efficiency of subsequent gene transfer by standard Ad5-based vectors and Ad5-based vectors was evaluated[67].

Ad5-RGD-luc and Ad5-CAV2-luc showed significantly higher expression levels of luciferase activity in both primary and immortalized human leiomyoma cells when compared with Ad5-Luc. Additionally, these modified viruses demonstrated selectivity toward leiomyoma cells, compared with myometrial cells and exhibited lower liver cell transduction, compared with Ad5-luc, at the same dose levels[67].

Endometriosis

Endometriosis is a devastating benign disease of reproductive-age women that manifests during reproductive years with chronic pelvic pain, dysmenorrhea, deep dyspareunia, dyschasia, and infertility. It is now well recognized that a genetic susceptibility to endometriosis appears probable. The actual pathophysiology has never been clearly established.

A commonly accepted theory is that endometriosis is the result of implantation of retrograded endometrial tissue on the peritoneal surface after menstruation[70].

Several factors are important in the progression of ectopic endometriotic implants such as an impaired immune defense[71], stimulation of endometrial cell proliferation[72], and adherence of the endometrium to the peritoneal surface by the production of adhesive factors and matrix metallo-proteinases[73].

Endometriosis is simply defined as growing endometrial-type mucosa outside the uterine cavity. Since it is endometrial in origin, endometriosis is estrogen-dependent[74], only manifest during the reproductive life and vanishes with menopause[75].

Aromatase enzyme that converts androgens to estrogen[76] and the estrogen receptor isoforms (α and β)[77] are both over expressed locally in the endometriotic peritoneal lesions.

After implantation of endometriotic lesions into the peritoneal cavity, they start to secrete local inflammatory cytokines, especially the vascular epithelial growth factor (VEGF) which is very critical in the angiogenesis process and formation of new vascularization to further the progress of endometriosis. Because angiogenesis plays a critical role in establishing peritoneal implantation and progression of endometriosis, antiangio-genetic therapy would be a potential treat-ment option for this disease. Angiostatin is an endogenous peptide portion of plasminogen and known to have potent anti-angiogenic properties. Angiogenesis is also essential for the normal ovarian physiological function[79]. Administration of Angiostatin in normal cycling mice will cause ovarian dysfunction, defective corpus luteum, and anovulation. It also decreases ovarian, and uterine weight, decreased production of estradiol and progesterone but did not cause complete castration. Dabrosin et al 2002[80], successfully eradicated established endometriosis in estrogen-supplemented ovariectomized mice by transient (10 days) over expression of the angiogenesis inhibitor angiostatin gene. This was accomplished by intra peritoneal delivery of replication-deficient adenovirus vector (Ad-Angiostatin). Ad-Angiostatin is simply an angiogenesis inhibitor which eradicates the endometriosis by inducing apoptosis of the most vascular endothelial cells after treatment, but it has no direct apoptotic effect on the endometriosis cells. The same group have published a recent study, that confirmed the high expression of abiologically active replication-deficient adenovirus vector (Ad-Angiostatin) proteins after delivery by 6 to 10 days in vivo[81]. This is supported by the findings that vascular endothelial growth factor, the most potent and specific angiogenic factor, is found in women with endometriosis at elevated levels in peritoneal fluid and macrophages, where it appears to be regulated by ovarian steroids[82-84].

Angiogenesis plays a vital role for the maintenance of normal physiological function of the female reproductive system[79,85].

These authors further investigated the effect of Ad-Angiostatin on their model mice and compared this effect on normal cycling mice as a control group. They measured the ovarian function, uterine weight, levels of estrogen and progesterone three weeks after administration of Ad-Angiostatin to normal cycling mice. They reported that both the size and weight of the ovaries had decreased up to 50% with overall decrease in secondary

follicles, decreased corpus luteum development, and a loss of stroma on the histological evaluation and quantitative morphometry of ovaries. Serum levels of both estradiol and progesterone were decreased in Ad-Angiostatin-treated normal cycling mice. Control mice showed physiological normal levels of estradiol (46 to 401 pmol/L) versus 60% of normal levels in Ad-Angiostatin-treated mice. Uterine weight is known to be an accurate assessment of systemic estrogen exposure.Uterine weight of normally cycling mice was decreased significantly after Ad-Angiostatin treatment (P 0.0001).[80] Since Ad-Angiostatin treatment lowered the levels of sex steroids, local or targeted delivery of the gene should be considered to avoid prolonged systemic hypo-estrogenic effects and impaired ovarian function.

Othman et al, 2007[86], used the adenovirus vector to deliver an altered estrogen receptor (ER) form called wild-type ER (WT-ER) or dominant negative mutants of estrogen receptors ER1- 536 (DN-ERs) that allow estrogen binding to the transcription factors without activating the estrogen responsive genes. They showed in this study that delivering DN-ER genes to endometriosis cells arrested the cell proliferation, suppressed various cytokine production and apoptosis. In addition to blocking WTER, they found that ad-DN-ER vector mediates its anti-proliferative effect of Ad-DN-ER vector on endometriosis. The Ad-DN-ER transfected endometrial cells secreted less inflammatory cytokines (IL-6, IL-8, IL-10, TNF-α and GM-CSF) in the culture medium in comparison with the untreated endometrial plates. Surprisingly, the treated endometrial plates contained less angiogenic cytokine of vascular endothelial growth factor (VEGF). This means that the Ad-DN-ER possess anti-angiogenic effect through causing a significant suppression of the production of important angiogenic factor (VEGF) that leads to suppression of angiogenesis in endometriotic implants.

The intra peritoneal delivery of replication-deficient adenovirus vectors induces production of angiostatin in vivo within only 10 days. This transient expression was enough to eradicate endometriosis in their mice model[80].

Considering these mechanisms, Adenovirus-mediated expression of mutant Ad-DN-ER vector provides a revolutionary approach for eradication of human endometriosis. It conveys the advantage of blocking estrogenic action in situ at the level of endometriosis cells through functional inactivation of ERs without the unwanted hypoestogenic side effect. The confined nature of endometriosis to the peritoneal cavity allows easy clinical application of gene therapy by injecting the Ad-DN-ER into the peritoneal cavity either as an office procedure through transvaginal ultrasound-guided culdocentesis or as an adjuvant therapy after laparoscopic ablation of visible endometriosis[84].

Adenoviruses can transfer genetic materials effectively in a wide spectrum of dividing and nondividing cells. This is the problem for gene therapy, as this can lead to nonspecific gene transfer and of transgenes into undesired organs or tissues. This, not only can increase toxicity to the organs, but also drains the fraction of the virus that is intended to target the desired origin of the pathology. For a trial to achieve better-targeted desired effects of gene therapy, adenovirus vector should target only the origin of the pathology by targeting strategies called “transduction targeting”[87]. This can simply be achieved by small modifications to the adenovirus capsid proteins (fiber) to reroute through its journey through receptors that are expressed on the pathological target. Transcriptional targeting not only allows for specific targeting to the pathology but also allows delivery of a large number of modified vectors to the targeted organ to achieve better therapeutic results. Trying to apply the transduction-targeting

conception an animal trial. Othman et al, 2008,[87] compared different modifications to the adenovirus to identify which one would sustain a higher gene transfer and expression in human endometriosis cells and would have lower activity in normal organs. They compared the fiber-modified adenoviruses (Ad5-integrin-binding peptide [RGD]-luc, Ad-sigma-luc, and Ad5/3-luc) (as transductional targeted viruses) and replication-defective wild-type adenoviruses with a luciferase reporter gene in the E1 region under the transcriptional control of the corresponding promoters (Ad5-CMV-Luc).

Human endometriotic cells were transfected by targeted adenoviruses expressing luciferase reporter gene. Luciferase activity that was mediated by each virus was expressed as a percentage of adenovirus serotype 5 activity. The transductional activity and efficacy of gene transfer of each virus were reflected by its luciferase transactivation level.

They measured the luciferase activities by the Promega Luciferase Assay System. Ad5-RGD-luc significantly enhanced endometriotic cell transduction with a reporter gene expression 2.5-fold higher, compared with Ad5-CMV-luc ($P < .001$). They also compared the transcriptional activity that was mediated by 3 adenoviruses with tissue-specific promoters (Ad-survivin-luc, Ad-secretory leukocyte protease inhibitor[SLPI]-luc, and Ad-heparanase-luc) to Ad5-CMV-luc (the wild-type adenovirus under the ubiquitously activated cytomegalovirus promoter) in endometriosis cells. The Ad-SLPI-luc– and Ad-heparanase-luc–mediated luciferase activation was 3-fold higher, compared with Ad5-CMV-luc ($P=.008$ and .012, respectively)[87].

The nuclear factor kappaB (NF-kappaB) is a transcription factor playing vital roles in innate immunity and in regulating any other body processes involving cellular proliferation, survival, and differentiation. González-Ramos et al, 2008[88]studywas the first to demonstrate the implication of NF-kappaB pathway in the development of endometriotic lesions in vivo.They proved that NF-kappaB inhibition decreases cell proliferation through reducing ICAM-1 expression, but increases apoptosis of endometriotic lesions, diminishing the initial development of endometriosis in nude animal model. NF-B also mediates its inhibitory effect of proliferation through control of the cell cycle (through reducing cyclin D1 and enhancing G1-to-S-phase progression), which is a vital mechanism in the cellular apoptosis and proliferation. Previous studies also have proven that the Inhibition of NF-B activation decreases cyclin D1 activity, the positive regulator of G1-to-S-phase progression, leading to delayed cell cycle progression which can be used in cancer the treatment[89].

Zhu et al 2015[90], and his group were able to inhibit the development of endometriosis through reducing cell proliferation of ectopic endometrial cells and eliminating angiogenesis through targeting suppressed NF-κB gene expression by NF-κB shRNA. In this study, they injected high specificity adenovirus vector-mediated shRNA targeting NF-κB gene into the endometriosis lesions of the experimental group by laparoscopic surgery while injecting normal saline in the control group. Then endometriotic lesions were taken through laparoscopy after 4 weeks of injection and evaluated for microvessel density in each group using the CD34 immunohistochemistry. Western blot test was used to detect the expression of the NF-κB and proliferating cell nuclear antigen (PCNA)[90].

Premature ovarian failure

Gonadotropins; follicle stimulating hormone (FSH), and luteinizing hormone (LH) are integral parts of the neural (hypothalamus) and endocrine (gonads and pituitary)

regulation that controls steroid hormone synthesis and gamete production[91]. FSH receptor (FSHR) mediated FSH signaling plays a vital role for follicular maturation in females, and for initiation and maintenance of spermatogenesis in males[92]. It is also essential for gamete production in the pre-pubertal stage of male and female life[93].

LH works on its receptors (LHR) on theca cells (females) and Leydig cells (males) to produce testosterone, which in turn is converted to estradiol by the aromatase enzyme in granulosa cells (Two-Cell theory). During ovulation in mammals, LH surge induces a cascade of events that is resumption of the arrested meiotic maturation, cumulus expansion, and follicular rupture [94]. In the LH receptor knock- out mice or LH-subunit-deficient mice, females are infertile. Follicles are able to develop to the antral stage but then fail to continue because they degenerate [95].

The dominant follicle produces the estradiol that triggers the surge of gonadotropins (FSH and LH) which, in turn, stimulates the preovulatory follicle(s) to ovulate and luteinize (form the corpus luteum) which produces progesterone[96]. For gonadotropins (FSH/LH) to exert their hormonal actions on the target cells, they should bind to their G protein-coupled receptors FSHR and LHR[96]. FSH is a glycoprotein hormone, which shares α sub-unit with luteinizing hormone (LH), human chorionic gonadotrophin (hCG) and thyrotrophic hormone. Aittomaki et al, 1995[97],reported the first mutation detected in the FSHR gene. It was an inactivating point or missense mutation (C566T), resulting in an Ala189Val change. This mutation results in a significant decrease in the response to FSH by reduced cyclic adenosine monophosphate production and binding capacity[96]. Males with homozygous mutation of the C566T, show some degree of spermatogenic failure but are not azoospermic[92]. Adult women with the same homozygous mutation are completely infertile due to premature ovarian failure secondary to resistant ovary syndrome (ROS). Their clinical manifestations include hyper-gonadotrophic primary amenorrhea, variable degrees of development of secondary sex characteristics, normal internal and external genitalia and normal karyotype[97].

This heterogeneous disorder is inherited as an autosomal recessive in most cases[98].

Unfortunately, at the present time, there is no effective treatment for these patients, other than symptomatic relief with hormone replacement therapy. Their only hope to get pregnant is with donated oocytes followed by in vitro Fertilization (IVF). However, there is a case report of a successful ovarian stimulation in a patient with ovarian resistant syndrome by using human menopausal gonadotropin[99].

Al-Hendy group[100], had recently created new modification to an adenovirus vector that is capable of expressing full-length normal human FSHR gene (Ad-hFSHR). This new Ad-hFSHR proved to restore FSH responsiveness to cells in various tissues that lack internal FSHR and had shown a successful FSHR responsiveness on inactivating C566T point mutation.

This indicates that Ad-hFSHR responds to FSH and can functionally transcomplement C566T-mutated hFSHR in various cell lines[100].

Adenovirus has been shown to transfect the human granulosa cell line, the target cell for hFSHR expression[101-102]. Al-Hendy et al 2005[102] have shown 100% transfection of murine (KK-15) granulosa cells with Ad-LacZ both in vitro and in vivo. Ghadami's study had shown the ability of adenoviral vectors (Ad-hFSHR) to transfect COS-7 and porcine JC-410 granulosa cell lines and restore the FSHR responsiveness to FSH stimulation and successfully transcomplement the malfunctioning form of human FSHR gene with C566T mutation[100]. As the first step for ovarian failure gene therapy, COS-7, JC-410, JC-410-StAR- scc-luc and JC-410-

P450-scc-luc cell lines were transfected by Ad-hFSHR. This was followed by stimulation with different concentrations of FSH. FSH-dependent cAMP production and luciferase activity measurement indicated that the vector construct used in the study was functionally active to express the hFSHR gene in all transfected cells with 3.7 – 4.6-fold increases in the cAMP generation and 2 – 3-fold increases of luciferase activity in appropriate cells. More importantly, the data from this study demonstrated that the Ad-hFSHR vector is able to correct the Finnish C566T mutation in those cells that express malfunctioning hFSHR with 2.2 – 7.4-fold increases in the cAMP generation. In this study the functional activity of the Ad-hFSHR was tested by measuring cyclic adenosine monophosphate (cAMP) or luciferase activity in response to FSH stimulation. After FSH stimulation, there was a 2–4.6-fold increases in the functional activity of Ad-hFSHR transfected cells compared with Ad-LacZ transfected or untransfected cells (control group), indicating that Ad-hFSHR is functionally active and expressing hFSHR. After FSH stimulation, also there was minimal generation of cAMP in cells expressing mutated hFSHR-T566 only. Co-transfection of Ad-hFSHR in these cells carrying the malfunctioning form of human FSHR caused significant increases of 2.2–7.4-fold in FSH dependent cAMP generation (P= 0.0007). Based on these findings the authors concluded that the adenovirus expressing a normal human FSHR can be used to compensate for the inactivating human FSHR-C566T mutation and can restore FSH responsiveness[100].

Al-Hendy's group had confirmed the positive FSH responsiveness and investigated the effects of bilateral injection of Ad-hFSHR into the ovaries of female mice with deleted FSHR gene called follitropin receptor knockout (FORKO) mouse on the reproductive system and ovarian function[103]. FORKO mouse is an appropriate animal model for studying human hypergonadotropic ovarian dysgenesis and infertility and its FSHR(2/2) phenotype is close to human ROS. Female FORKO mice display high serum level of FSH, low estrogen, thin uteri, and small ovaries and are sterile due to failure in ovarian folliculogenesis at the primary follicle[104]. Intraovarian injection of adenovirus vector was not associated with systemic viral toxicity and has no drawbacks on fecundity or pregnancy outcome[102,105]. One of the major limitations of the vector that was used in this study is that the hFSHR is under the control of the strong constitutive CMV5 promoter which will not allow the expected down-regulation of hFSHR later on in the follicular cycle to support increased expression of LHR, which will induce follicular rupture and successful ovulation. Based on these data, the authors concluded that intra-ovarian injection of Ad-hFSHR vector in FORKO mice was successful in restoring ovarian folliculo-genesis to the antral stage but not to the ultimate ovulation. To overcome such limitation, the autors plan future experiments with an improved Ad-hFSHR vector using the authentic human FSHR promoter[103].

Another method of application of gene therapy in enhancing the steroidogenesis is to try to study the functional action of α-enolase (ENO1) with regard to the processes of goose ovary development and egg laying. Previous studies had shown that the relative expression levels of α-enolase (ENO1) in the ovaries of laying geese increased by 2.34 ± 0.67 folds compared with those of the pre-laying geese[106]. Enolases are glycolytic enzymes that are responsible for ATP-generating conversion of 2-phosphoglycerate to phosphoenolpyruvate.

In vertebrates, there are three different tissue-specific isoenzymes: α-enolase (ENO1), which is expressed in a wide variety of

tissues, whereas β-enolase is expressed in muscle cells and γ-enolase is expressed in neuronal tissues[107].

Post-operative Adhesions

Postoperative adhesions have been a major surgical consequence that causes a lot of morbidity with a recorded high incidence (up to 90%) after open gynecological pelvic surgeries[108].

Common consequences of postoperative adhesions are chronic abdominal and pelvic pain, intestinal obstruction, female infertility, and ectopic gestation[109-111]. There are three possible mechanisms of initiating and maintaining pelvic adhesions induced by local trauma of surgery on the tissue: (1) local tissue trauma causes mesothelium cell injury that results in decreased release of plasminogen activator activity (PAA) which is the key regulator for the fibrinolysis process that is responsible for the rapid healing of the peritoneum without fibrous tissue formation. Decreased plasminogen activator activity leads to inhibition of fibrinolysis, more deposition of extracellular matrix, including collagen and bronectin from the underlying fibroblast,and delay of the intrinsic vascular growth factor[Figure-3]. (2) The trauma, as well as foreign bodies, induce an inflammatory response that causes a surge in the cytokines formation, mainly transforming growth factor-β(TGF-β1), the master factor of tissue fibrosis[113-115]; and other cytokines as interleukin-1 and 6, tumor necrosis factor (TNF), (3) trauma also induces tissue hypoxia as a result of interruption of the blood supply to mesothelial cells and submesothelial fibroblasts, leading to increased expression of hypoxia inducible factor-1 α (HIF-1α)[116,119] and vascular endothelial growth factor (VEGF), which are responsible for collagen formation and angiogenesis[120]. Reduced PAA activity and increased release of plasminogen activator inhibitors PAI-1 and PAI-2 are the main mechanisms for developing a severe adhesion[111-113,116].

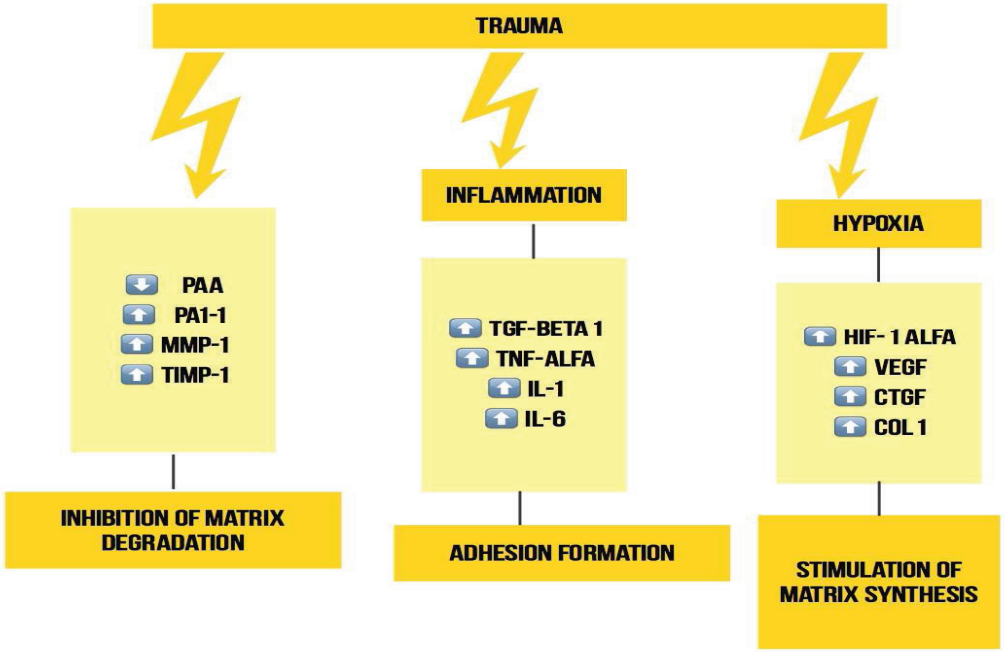

Figure-3: ***The role of trauma, hypoxia, and inflammation in adhesion formation.*** *PAA: Tissue plasminogen activator antigen; PAI-1: Plasminogen activator inhibitors 1; MMP: Matrix metalloproteinase; TIMP: Tissue inhibitors of MMP; TGF-β1: Transforming growth factor-β; TNF-α: Tumor necrosis factor-α; IL: Interleukin; HIF-1α: Hypoxia inducible factor-1α; VEGF: Vascular endothelial growth factor; CTGF: Connective tissue growth factor.*

Adenovirus (Ad) vectors have many characteristics to make them a potential effective solution for postoperative adhesions. Adenovirus is easy to deliver in vivo gene transfer to both dividing and non-dividing cells, has high in vivo stability, and non-integrating nature into the host genome. The infective nature of the virus allows the rapid dissemination to distant organs. The therapy can be applied locally at the end of the surgery before suturing the abdominal wall. This will alter the molecular aberrations (e.g., depressed PAA, elevated PAI-1, TGF-β1, HIF-1α, *etc*.) (Figure-3) through different mechanisms reported by different studies. Two studies attributed the adhesion reduction mechanism to the stimulation of mesothelial cells to proliferate and migrate influenced by the ability of adenovirus to encode the hepatocyte growth factor (HGF) genes itself or its down- stream signaling molecule sphingosine kinase 1 (SK-1)[117,118].
In addition, several studies stated that seven days are enough to achieve gene expression after intra-peritoneal administration in a rat adhesion model[117-119,121].
The latter authors have shown that a first-generation replication-incompetent Ad vector encoding htPA can effectively regulate levels of PAA/PAI without any postoperative complications, and decrease recurrence of peritoneal adhesionsin a rat model[121].

Gene therapy applications in ovarian cancer

Ovarian cancer either originates from the cells on the surface of the ovary (ovarian epithelial carcinomas) or from the egg cells (germ cell tumors). Current therapies used for ovarian carcinoma are ineffective because of delayed diagnosis, resistance to platinum-based chemotherapy, high tumor recurrence, and the side effects of the various chemotherapeutic drugs. Searching for new therapeutic strategies such as gene therapy is necessary, not only to cure cancer, but also to improve the quality of life, reduce recurrence and eliminate the side effect of cytotoxic medications.

Lentiviral vectors are potentially capable of stable transgene expression that is particularly useful for long-term expression and delivery of therapeutic genes in the tumor micro-environment.
In particular, lentiviral vectors could be used to deliver antiangiogenic factors to the tumor microenvironment. Lentiviral vectors are also capable of resting cells transduction in vivo. This feature is theoretically well suited for gene transfers in ovarian cancer cells because all solid tumors contain a certain fraction of cells that are within the G_0 stage of arrest[122].
Lentiviral vectors were used to deliver the enhanced green fluorescent protein (EGFP) gene to ovarian cancer cells to enable its tracking for an evaluation purpose[123]. A study comparing lentiviral and retroviral vectors in gene transfer applications in ovarian cancer cells, showed the therapeutic efficacy of lentiviral vectors. They were efficient in gene transfer both in vitro (both vectors infected ovarian cancer cells under standard culture conditions) and in vivo (intraperitoneal (i.p.) injection of the vector in severe combined immune-deficient (SCID) mice. Using the intraperitoneal route for delivering the gene therapy is well suited in the case of ovarian cancer because the disease remains disseminated in the abdominal cavity[124].
The tissues were evaluated by confocal microscopy analysis of tumor sections, quantitative analysis by flow cytometry and real-time polymerase chain reaction (PCR)[123]. Quantitative analysis by flow cytometry showed 0.05 and 5.6% EGFP(+) tumor cells after administration of the retroviral and lentiviral vector, respectively. In another study, silencing in vivo has been observed after gene transfer by retroviral vectors[125]. To

deal with this, the authors injected tumor cells in SCID mice, which were ex vivo transduced by the two vectors, and expressed EGFP at comparable levels. This indicated that the lentiviral vector was more resistant to in vivo silencing in comparison with the retroviral vector. The therapeutic efficacy of lentiviral vector was assessed by i.p. administration of a murine IFN-α_1-expressing lentiviral vector to deliver this cytokine to ovarian cancer-bearing SCID mice. IFN-α_1 delivered by retroviral vectors was shown to have strong antitumor effects in a breast cancer model in a previous study because of its antiangiogenic activity[123].

Multiple injections of a murine IFN-α(1)-lentiviral vector to ovarian carcinoma-bearing mice significantly prolonged the animals' survival, indicating the therapeutic efficacy of this approach. These promising findings indicated that lentiviral vectors might have a role in future gene therapy of ovarian cancer aimed at achieving long-term expression of therapeutic genes[123].

A randomized Phase II/III trial of p53 gene therapy for use as a first-line treatment of patients with ovarian cancer was conducted by Buller et al[126].

Intra-peritoneal administration of a replication-deficient adenovirus encoding human recombinant wild-type p53(SCH 58500) alone and sequentially in combination with platinum-based chemotherapy substantially reduced serum CA125 levels in patients with recurrent ovarian cancer[126]. SCH 58500 was administered i.p. to three groups of patients with a heavily pretreated recurrent disease. Group 1 (n=17) received a single dose of SCH 58500. Group 2 (n=9) received two or three doses of SCH 58500 given alone for one cycle, and then with chemotherapy for two cycles. A third group (n=15) received a 5-day regimen of a single daily dose of SCH 58500 i.p. alone for cycle 1 and then with intravenous carboplatin/ paclitaxel chemotherapy for cycles 2 and 3. Vector-specific transgene expression in the tumor was documented by RT-PCR in cells from both ascitic fluid and tissue biopsies. Intraperitoneal SCH 58500 was found to be safe and well tolerated, as there was no dose-limiting toxicity after the injections of 236/287 (82.2%) documented doses of SCH 58500.

Few patients reported fever, hypotension abdominal complaints, nausea, and vomiting secondary to adenoviral induced peritoneal inflammation. Despite marked increases in serum adenoviral antibody titers, transgene expression was measurable in 17 of 20 samples obtained after two or three cycles of SCH 58500. Vector was detectable in the peritoneal fluid by 24 hours and persisted for 7 days whereas none was detected in urine or stool. There was a poor correlation between CT scans and CA125 responses indicating that CT scans are not a valid measure of response to i.p. SCH 58500 due to extensive adenoviral-induced inflammatory changes. Serum CA125 decreased greater than 50% from baseline, in 8 of 16 women who completed three cycles of planned chemotherapy regimen. However, the first interim analysis of the study resulted in its closure because it did not show the adequate therapeutic benefits that were expected such as a regression of tumor size on CT scan[126].

The failure of p53 gene therapy might be attributed to several reasons that include the multiple epigenetic dysregulations in cancer that lead to aberrant silencing of genes. It may be that the suitable strategy to treat cancer might be targeting several genes instead of targeting a single gene at a time. One of the strong reasons that could seriously compromise the effectiveness of p53 gene therapy is the dominant negative cross-talk between ectopic wild-type p53 and over expressed dominant p53 mutants, p63 and p73. In addition, the lack of expression of coxsackie-adenovirus receptors and integrin

co-receptors in ovarian tumors also compromised the therapy. The presence of adenovirus-neutralizing antibodies in ovarian cancer-related ascites added a reason for the failure[127].

When the gene for a foreign enzyme (i.e. one from a virus, bacteria, or yeast) is used and delivered to cancer cells, it is called suicide gene therapy (SGT). To apply SGT approach to ovarian cancer therapy a recent study[128] used HPV-16 pseudovirion encoded with HSV-1-TK gene followed by ganciclovir administration into ovarian cancer bearing mice. In this study they used a concept of injecting a vector capable of delivering a specific enzyme-encoding gene that will enable ovarian cancer cells to metabolize a non-toxic prodrug and convert it into a potent cytotoxin, which will be able to kill the infected cancer cells. There were marked therapeutic antitumor benefits in ovarian cancer-bearing mice[128].

The suicide gene/prodrug system works by the use of HSV-thymidine kinase (HSV-tk) combined with ganciclovir (GCV)[129].

HSV-tk catalyzes the first phosphorylation of GCV that can then be tri-phosphorylated by cellular kinases.

Triphosphorylated GCV then can be integrated into replicating DNA, which leads to polymerase inhibition and eventually apoptosis[129-131]. The advantage of using SGT over conventional gene therapies is that it has a bystander effect, which is able to kill the transfected neighboring cells. The active drug can diffuse into neighboring non-infected cells, and also kills them. The dying cells are also able to induce natural killer (NK) cells and T cells to induce a distant bystander effect [128]. In previous studies, DNA plasmids were packaged into the papillomavirus L1 and L2 capsid proteins to generate the 'pseudovirion' that can efficiently deliver the DNA into multiple cell lines[132-134]. In particular, 293TT cells were cotransfected with HPV expression plasmids pShell16 and the plasmids of choice (such as GFP, luciferase or HSVtk) using Lipofectamine 2000 (HPV16-GFP, HPV16-Luc (luciferase), and HPV16-tk (HSVtk Herpes Simplex Virus-Thymidine Kinase) pseudovirions[128]. In this study mice were injected intraperitoneally by luciferaseon day 1 and luciferase activity was examined on day 2 for anumber of the tumors per mouse and for luminescence image acquisition. Mice were injected with HPV16-GFP psV or HPV16-TK psV on day 3. Mice were treated daily with ganciclovir or PBS from day 5 to day 18. Mice were imaged again by luminescence imaging on day 20.

While mice without tumors did not show any luciferase activity, tumor-bearing mice intraperitoneally injected with HPV-16/Luc psV demonstrated significant luciferase activity. The mice without tumors did not show any luciferase activity while the tumor-bearing mice injected i.p with HPV-16/Luc psV demonstrated obvious luciferase activity[128].

These data suggest the HPV pseudovirion preferentially infects the tumor cells in tumor-bearing mice. They further showed that the tumor-bearing mice treated with HPV-16/HSV-tk psV followed by ganciclovir exhibited significantly better therapeutic antitumor effects than mice treated with HPV-16/GFP psV followed by treatment with ganciclovir.

These data indicate HPV-16 pseudovirion can be used to effectively deliver the HSV-tk gene to ovarian tumor cells to render ovarian tumor cells more susceptible to treatment with ganciclovir[128].

The standard systemic chemotherapy of choice for treating primary ovarian cancer with high efficacy is the combination of Docetaxel with a platinum compound (carboplatin) but there are two major issues of this regimen. These are dose-related toxicity and resistance. Studies reported a progressive increase in the number of drug-resistant tumors with cases of recurrent ovarian cancer

with rates around 10–28% [135]. To counteract these challenges in ovarian cancer, in vitro use of combination therapy of docetaxel with a low dose anti-MUC1 monoclonal antibody (MAb C595) on cultured ovarian cancer cells have demonstrated a suppression of ovarian cancer cell proliferationand caused cultured human ovarian cancer cells to undergo apoptosis. This combination may provide a treatment strategy to improve anti-cancer efficacy while reducing the toxicity often seen in patients treated with docetaxel alone. Low-dose MAb C595 combined with docetaxel greatly enhanced killing of MUC1-positive EOC cells[136]. MUC1 is a tumor-associated antigen with high cellular transformation and tumorigenicity. MUC1 is a highly glycosylated type I transmembrane glycoprotein that is over expressed in > 90% of advanced epithelial ovarian cancers. Hence, trials of anti-MUC1 antibody use in combination with docetaxel, theoretically speaking should be able to reduce tumors' growth in a mouse model of ovarian cancer[136]. Tumor-associated MUC1 is an attractive target for gene therapy because it has a reduced glycosylation that is exposing novel regions of the protein core and normally masked epitopeswhich permits the immune system to develop antibodies against the peptide core of the under glycosylated MUC1 antigen (uMUC1) that differentiates normal from adenocarcinoma cells. Enhanced levels of MUC1 expression by cancer cells may cover extra-cellular domains from immune surveillance, conferring a survival advantage on malignant cells and playing an important role in the ability of tumors to invade and metastasize[137]. The immune system produces an IgG_3 called Monoclonal antibody (MAb) C595 against the protein core of human MUC1 (urinary epithelial mucin1)[138]. In a previous study, the ability of C-emitting radioisotope (^{111}In) labeled MAb C595 to localize and identify the tumor in 19 patients with a clinical suspicion of ovarian malignancy was tested. Accuracies of 79% and 64% were achieved compared with conventional MRI and ultrasound [139].Another study demonstrated that MAb C595 is over expressed in over 90% of advanced ovarian cancer sections while no staining was found in normal ovaries[140].

The MAB C595 induced apoptosis of ovarian cancer cultured cells suggests that MAb C595 alone, or combined with docetaxel, is a possible future treatmentstrategy for ovarian cancer. Colony forming assays havefurther confirmed cell death following treatments that was associated with the release of cytochrome c and increased caspase-3 activity[140].

Wang et al 2011[136], have conducted more studies to further evaluate the mechanisms of this combination-mediated apoptosis, they investigated the effectiveness of this combination therapy in vivo by i.p. injection into a mouse model implanted with human ovarian tumor xenografts. Mice were then treated with single MAb C595, docetaxel, combination test (MAb C595 and docetaxel), combination control (negative MAb IgG_3 and docetaxel) or vehicle control i.p for 3 weeks. After sacrificing mice, they assessed ascites fluid volume, tumor weight, CA-125 levels in the ascites fluid and survival rate. Immunohistochemistry was used to evaluate MUC1, CD31, Ki-67, TUNEL and apoptotic proteins in tumor xenografts.

Dose dependent-MAb C595 alone was able to inhibit ovarian tumor growth and decreased ascites production but did not prevent tumor development. However, combination test showed a marked reduction in tumor and ascites volume as well as metastases, reduced CA125 levels in ascites fluid and improved survival of treated mice compared with single agent-treated mice, combination control or vehicle control-treated mice ($P<0.05$)[136].

Another study[141]has delivered bystander effect in vivo through the Intra-peritoneal administration of genetically engineered mesothelial cells expressing herpes simplex

virus thymidine kinase (HSVTK) into in an established mouse model with xenograft ovarian cancer tissue to deliver "bystander" cytotoxic treatment to human primary ovarian cancer cells. Mesothelial cells properties qualify them to be the choice for this technique. They are easily retrievable by a simple needle aspiration, can easily be cultured with previously characterized in vitro growth requirements[142,143], and their re-implantation into the peritoneal cavity is usually successful[134]. This technique has shown that HSVTK-engineered mesothelial cells are sensitive to acyclovir (GCV) killing in vitro leads to substantial suppression of tumor growth and a statistically significant improvement in the survival of animals with ovarian cancer [141].

Virotherapy strategies in treating ovarian cancer utilizing conditionally replicative adenoviruses (CRAd) and advanced generation CRAd agents have been proposed to overcome some of the virotherapy limitations such as imprecise tumor cell replicative specificity and inefficient tumor cell infectivity[144]. These approaches have shown to enhance specificity and infectivity/replication rate in ovarian cancer cells [144].To improve specificity of tumor cell replication, tumor- specific promoters (TSPs) able to drive adenoviral E1 gene expression in tumor cells and thereby accomplish targeted transcription based upon the induced specificity of viral replication[145].The optimal TSP restricts replication to tumor cells alone with the highest activity in tumor cells ("tumor on") and lowest activity in normal cells ("normal off"). Adenovirus vector has a dose-limiting hepatotoxicity due to its predilection to the hepatocytes. The optimum therapeutic index can be achieved when TSP-directed adenovirus recorded the highest "tumor on/liver off" ratio. The CXCR4 promoter is ideal for transcriptional targeting for ovarian cancer gene therapy because, first, it had a "tumor on" and "liver off" phenotype in both in-vitro and in vivo experiments in ovarian cancer[146] and second, CXCR4 gene expression has been undetectable in normal ovarian epithelial cells but markedly expressed in the primary ovarian cancers [147-149]. For enhancing tumor cell infectivity, the adenoviral vector can be used to target tumor cells via the alternative pathway. Tumor is resistant to adenovirus infection due to a relative paucity of the primary receptor CAR on tumor cell surface [150-151]. For that reason, native Ad5 tropism is modified with two capsid proteins (the fiber and the penton base) which bind to the coxsackie–adenovirus receptor (CAR), and to the integrins αvβ3 and αvβ5, respectively to facilitate Ad binding and entry into tumor cells via integrin receptors that are abundantly expressed on tumor cells. They constructed two CRAds (Ad5-CXCR4-RGD and Ad5-CXCR4-F5/3) that control viral replication through the CXCR4 TSP and subjectively enhanced viral infectivity via the capsid modifications (RGD and F5/3)[152,153].

Resistance to chemotherapy is another problem that is observed in numerous cancer types. In this regard, Lui et al[154] used adenovirus-mediated transfer of cytosine deaminase and uracil phosphoribosyl transferase genes that were driven by multidrug resistance gene 1 promoter (MDR1 promoter), They demonstrated a significant targeted killing effect of 5-flurocytosine on taxol-resistant ovarian cancer cells [154].

They concluded that the AdMDR1-CD:UPP in combination with 5-FC is an effective approach to suppress the growth of Taxol-resistant ovarian cancer. In their study, they infected Taxol-resistance (A2780/Taxol, SKOV3/Taxol) ovarian cancer cells and Taxol-sensitive (A2780, SKOV3) ovarian cancer cells with the adenovirus vector carrying the CD: UPP gene that was previously prepared to be driven by the MDR1 promoter. AdMDR1-CD: UPP was delivered through subcutaneous injections

into the xenografts of the nude mice and 5-FC intraperitoneally, and the overall survival and anti-tumor effects were observed. The AdMDR1-CD: UPPcauses a stronger cytotoxicity in A2780/Taxol cells and SKOV3/Taxol cells than in A2780 cells and SKOV3 cells in vitro. The subcutaneous injection of AdMDR1-CD: UPP into the xenografts of mice bearing tumors of A2780/Taxol cells significantly suppressed the tumor growth and prolonged survival as compared with the group of A2780 cells[154].

Gene Therapy and Human Papillomavirus (HPV) Vaccine

Cervical carcinoma remains one of the most common malignancies worldwide. Human papillomavirus type 16 (HPV-16) is the predominant etiologic agent of cervical cancer. HPV possesses three transforming oncogenes, E5, E6, and E7[155-157].T-cell-based immunotherapy of cervical cancer usually targets E6 and E7 oncoproteins because they are consistently expressed on the HPV cell surface. For that reason, E6 and E7 oncogenes products are unique tumor antigens and can be ideally used as tumor vaccines[158]. The HPV antigen-directed immunotherapy against papillomavirus-associated cervical cancer works by different modes of immunization with E7 antigen, such as (i) recombinant vaccinia viruses encode E7 and E6[159-164] (ii) syngeneic cells transfected with E7 oncogene[158,165], (iii) E7 oncoprotein-loaded dendritic cells[166,167] (iv) cytotoxic T lymphocyteepitope-containing peptides[168], (v) E7 vaccine based chimeric papillomavirus virus-like particles [169-171], and (vi) Hepatitis–B virus core loaded by Salmonella enterica serovar typhimurium expressing E7 epitope[172,173].

Adeno-associated virus (AAV), a single-stranded virus, has so many features to make it an attractive virus for gene therapy such as pathogenicity, and targeted integrating capability. In addition, AAV does not express any viral genes; the only gene expressed by AAV vectors is that for the antigen itself. One of the promising studies showed a successful application of gene therapy in the vaccination filed [174].Thetumor vaccine was designed by constructing a chimeric gene containing the HPV-16 E7 cytotoxic T-lymphocyte (CTL) epitope [168,173] and heat shock protein (hsp) DNA. Results showed that intramuscular vaccination could efficiently eliminate tumor cells, indicating that vaccination with this gene could be a therapeutic treatment for cervical cancer containing HPV-16 E7. They used adeno-associated virus (AAV) vectorencoding the viral E7 oncoproteins as the tumor antigens from HPV serotypes 16 (HPV16) and 18 (HPV18).

This study showed the AAV1 vector triggered a HPV-specific cytotoxic response of T lymphocyte (CTL) and secreted an interferon-gamma after a single intramuscular injection when compared with the AAV2 vector.

A study by Zhou et al[174] showed that after prophylactic vaccination with AAV1, 100% of the mice were protected from tumor growth for more than 1 year, in comparison with control mice immunized with either a LacZ vector or saline which continued to grow tumor and died within 6 weeks after inoculation of E7-positivetumor cell line TC-1[174]. In addition, therapeutic immunization with AAV1 inhibited palpable tumor growth and induced tumorsuppression in some mice. Despite lower CTL responses against the E7 antigens, AAV2 vector prophylactic immunization was also sufficient to protect 70-100% of the mice against the tumor[174].

Obstetric applications of Gene Therapy

The main application of gene therapy in obstetrics that is being developed at present is the treatment of intra uterine fetal growth restriction (IUGR) due to placental in sufficiency.

However, with further development of new gene delivery vectors, additional obstetrical disorders such as preterm labor, twin-to-twin transfusion as well as specific fetal anomalies can be novel targets for gene therapy intervention.

Intra-uterine Growth Restriction (IUGR)

One of the most promising recent applications of gene therapy in obstetrics was the intra placental injection of Adenovirus of IGF-1 for the treatment of intra uterine growth restriction (IUGR) caused by placental insufficiency in a rabbit model. The normal weights of fetal liver, and musculoskeletal tissues were restored after intraplacental Ad-IGF-1 gene therapy, with no change in the placental weight[176,177].John et al, 2013, demonstrated that the growth enhancement effect of intra placental Adenovirus-IGF-1 therapy is mediated through the enhancement of GLUT isoform transporter expression and this may be an important mechanism of Ad-hIGF-1 induced correction of placental insufficiency[177].Another opinion interpreted the results shown in these studies as minimal transgene expression that continues to occur in the fetus or mother following placental delivery of gene therapy. Therefore, a recent study[163] was developed the first novel non-viral tissue-specific transgene delivery systems to ensure safe gene therapy application in the developing human placenta.

Despite evidence that nanoparticles and environmental nano-sized materials cross the placental barrier[178], there is no current literature investigating the use of nanoparticles for gene delivery to the placenta. Historically, nanoparticles have been designed for imaging and destruction of unwanted cells such as carcinogenic cells 179. Nanoparticles are unsafe for placental use due to undesired accumulation within the tissue and their toxicity. Controlled release of protein from parenteral formulation can be achieved by a matrix-type delivery system. However, the development of polymer-based biodegradable nanoparticles[180] holds distinct advantages of employing microspheres formulation for delivery of proteins.

The nanoparticles offer stability for rapidly degraded molecules that are otherwise eliminated quickly in vivo.

Encapsulation in microsphere prevents contact of protein (gene) from cells or enzymes such as proteases/ esterases in surrounding tissues, until its release from microsphere. Microsphere formulation can be easily delivered to the target sites via IV, IM or oral route. With recent advances of polymers science, polymers were added to the microspheres that led to the development of controlled release microspheres.

The polymers have no harmful side effects or toxic degradation products and do not alter any pharmacological properties of the active ingredient.

In a recent study[181], the authors employed poly [2-hydroxypropyl) methacrylamide (HPMA), a water soluble polymer that increases delivery of IGF-1 through absorptive endocytosis and is biocompatibile [182] non-immunogenic[183] and specific. Purified plasmid DNA containing a sequence for IGF-1 is complexed by poly2-N,N-dimethy-laminoethyl methacrylate (DMAEMA) complexed to hIGF-1 plasmid DNA under the control of trophoblast-specific promoters (Cyp19a or PLAC1), a tertiary amine that acts as a weak base capable of being protonated at biological pH[184]. Together these co-polymers provide a non-viral nanoparticle alternative for gene transfer into the placenta. The same group had previously characterized IGF-1 complex formation using these co-polymers transfection of pEGFP-C1-containing nanocarriers that was measured via fluorescence microscopy. In vivo transfection was assessed by direct placental-injection into a mouse model of IUGR. Complexes formed

by pHPMA-b-pDMAEMA and CYP19a-923 or PLAC1-modified plasmids, induced trophoblast-selective in vitro transgene expression. Placental injection of PLAC1-hIGF-1 produces measurable RNA expression and enhanced growth of the growth restricted fetusin the mouse model, representing first novel human placental gene therapy[176].

Gene therapy from bench to bedside: Clinical trials

Gene therapy continues to extend from pre-clinical testing in animal models to clinical trials. Application of gene therapy in clinical trials is necessary as not all animal studies will predict the outcomes in clinical applications.
This is attributed to several discrepancies that range from adverse immune response to vector tissue tropism[185,186].
More than 65% of the gene therapy trials have been done in relation to cancer[187]. Various vectors and methods have been employed in gene therapy clinical trials: retroviruses (20%); adenoviruses (18%); adeno-associated viruses (5%); lipofection (6%); and naked/plasmid DNA (18.5%).Out of 1843 cancer gene therapy trials, only 45 have reached Phase III and one is in Phase IV. Based on the outcome, only nine gene therapy clinical trials have been conditionally approved so far. According to the US National Cancer Institute (NCI), a Phase IV r-Ad-p53 gene therapy developed for advanced malignant thyroid tumors is active. The primary objective of this study is to determine the efficacy of intra-tumor injection of rAd-p53 when coupled with radioactive iodine or combined with surgery in advanced thyroid cancers (www.cancer.gov). Preclinical studies employing gene-therapy strategies are encouraging in the case of certain endocrine tumors, and gene therapy is expected to successfully treat these tumors[187].

The challenges ahead

There are a lot of challenges to be addressed in gene therapy before gene-based products enter the routine clinical application to provide safe and affordable therapeutic drugs for otherwise non-treatable chronic diseases.
In particular, nonhuman primate models will continue to play a pivotal role in studying safety, toxicity and biodistribution of gene delivery vectors. Some of the clinical applications require a lot of vectors for efficient genes delivery, such as delivering the efficient viral vector doses in treating muscular dystrophy disorder.
For example how to deliver a high quantity of viral vectors and in the same time ensure their safety still needs a trustworthy study on non-human models. Manufacturing high-quality vectors will significantly expedite clinical translation[188-190].
Some of the challenges are:

1-Safety concerns

Since some patients treated with gamma retroviral vector-mediated gene transfer developed leukemia due to mutagenesis insert[191], the risk of genotoxicity remains a major safety concern particularly for the cell-based gene therapy despite vigorous efforts to improve. This risk is associated with not only integrating gene transfer platforms such as a retroviral vector or transposon/ transposase-based systems but also “non-integrating” gene transfer vectors such as AAV vector that may integrate at a much lower rate[192]. Developing safer gene delivery methods should be coupled with thorough characterization and evaluation of genotoxicity. As ex vivo gene transfer and cell-based gene therapy enters a personalized medicine era, rapidly evaluating genome modification profile by genome-wide sequencing and screening for safe cell clones will be required.
Another safety issue is immunotoxicity that

mainly concerns in vivo gene transfer using viral vectors. The lethal immunotoxicity of AdV vector greatly limits its clinical use[193]. Although the low immunogenicity of AAV vector has not caused acute life-threatening side effects, in several clinical trials it certainly triggered immunotoxicity that could eliminate transduced cells and suppress therapeutic outcome[194-196].
Further understanding of the immune responses against both viral and therapeutic antigens following in vivo gene delivery and deliberately modulating host immune responses will be crucial for developing clinical gene therapy particularly to treat systemic diseases.

2- Transgene expression

To ensure correct application of gene therapy, the more targeted gene delivery with targeted transgene expression and the less off-target delivery is mandatory to guarantee highest therapeutic efficacy, and subsequently, prevent side effects associated with off-target and non-physiological transgene expression.

Currently, most of the gene therapy approaches work by inducing tropism modification through engineering viral vector surface proteins and designing expression cassette of the host cells [197].

3- Nanoparticle Gene therapy

Future studies will be necessary to assess long-term gene expression following nanoparticle delivery.

4- Cell-based Gene Therapy

We encourage the scientists to focus on the application of iPSCs combined with ex vivo gene transfer to broaden the clinical application of cell-based gene therapy.

References

1. Barzon L, Boscaro M, Palu G (2004) Endocrine aspects of cancer gene therapy. Endocr Rev 25: 1-44.
2. *Wirth T1, Parker N, Ylä-Herttuala S. History of the Gene Therapy. Gene. 2013 Aug 10;525(2):162-9. doi: 10.1016/j.gene.2013.03.137.*
3. Elsner M, Terbish T, J ¨orns A, Naujok O, Wedekind D, Hedrich H-J, Lenzen S (2012) Reversal of diabetes through gene therapy of diabetic rats by hepatic insulin expression via lentiviral transduction. Mol Ther 20: 918-926.
4. Jessup M, Greenberg B, Mancini D, Cappola T, Pauly DF, Jaski B, Yaroshinsky A, Zsebo KM, Dittrich H, Hajjar RJ (2011) Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2þ-ATPase in patients with advanced heart failure. Circulation 124: 304-313.
5. LeWitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, Kostyk SK, Thomas K, Sarkar A, Siddiqui MS, et al (2011) AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. Lancet Neurol 10: 309-319.
6. Wang D and Gao G. STATE-OF-THE-ART HUMAN GENE THERAPY: PART I. GENE DELIVERY TECHNOLOGIES.Discov Med. 2014 ; 18(97): 67–77.
7. Jin L, Zeng X, Liu M, Deng Y, He N. Current Progress in Gene Delivery Technology Based on Chemical Methods and Nano-carriers. Theranostics. 2014; 4(3):240–255. [PubMed: 24505233].
8. Barton GM, Kagan JC, Medzhitov R. Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA. Nat Immunol. 2006; 7(1):49–56.
9. Mayrhofer P, Schleef M, Jechlinger W. Use of minicircle plasmids for gene therapy. Methods Mol Biol. 2009; 542:87–104.
10. Gill DR, Pringle IA, Hyde SC. Progress and prospects: the design and production of plasmid vectors. Gene Ther. 2009; 16(2):165–171.
11. Kay MA. State-of-the-art gene-based therapies: the road ahead. Nat Rev Genet. 2011; 12(5): 316–328.
12. Raper SE, Yudkoff M, Chirmule N, Gao G-P, Nunes F, Haskal ZJ, Furth EE, Propert KJ, Robinson MB, Magosin S (2002) A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. Hum Gene Ther 13: 163-175.
13. Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao G-p, Wilson JM, Batshaw ML (2003)

Fatal systemic inflammatory response syndrome in ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol Genet Metab 80: 148-158.
14. Cattaneo R, Miest T, Shashkova EV, Barry MA (2008) Reprogrammed viruses as cancer therapeutics: targeted, armed and shielded. Nat Rev Microbiol 6: 529-540
15. Kaufmann K, Bu¨ning H, Galy A, Schambach A, Grez M. Gene therapy on the move. EMBO Mol Med (2013) 5, 1642–1661.
16. Song XT. Combination of virotherapy and T-cell therapy: arming oncolytic virus with T-cell engagers. Discov Med. 2013; 16(90):261–266. [PubMed: 24333405]
17. Kerkar SP. "Model T" Cells: A Time-Tested Vehicle for Gene Therapy. Front Immunol. 2013; 4:304. [PubMed: 24098300]
18. Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, Maric I, Raffeld M, Nathan DA, Lanier BJ, Morgan RA, Rosenberg SA. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood. 2010; 116(20):4099–4102. [PubMed: 20668228]
19. Kochenderfer JN, Rosenberg SA. Treating B-cell cancer with T cells expressing anti-CD19 chimeric antigen receptors. Nat Rev Clin Oncol. 2013; 10(5):267–276. [PubMed: 23546520]
20. Jethwa H, Adami AA, Maher J. Use of gene-modified regulatory T-cells to control autoimmune and alloimmune pathology: is now the right time? Clin Immunol. 2014; 150(1):51–63. [PubMed: 24333533]
21. Takahashi K, Yamanaka S. Induced pluripotent stem cells in medicine and biology. Development. 2013; 140(12):2457–2461. [PubMed: 23715538]
22. Ellis J, Baum C, Benvenisty N, Mostoslavsky G, Okano H, Stanford WL, Porteus M, Sadelain M. Benefits of utilizing gene-modified iPSCs for clinical applications. Cell Stem Cell. 2010; 7(4):429–430. [PubMed: 20887948]
23. Papapetrou EP, Lee G, Malani N, Setty M, Riviere I, Tirunagari LM, Kadota K, Roth SL, Giardina P, Viale A, Leslie C, Bushman FD, Studer L, Sadelain M. Genomic safe harbors permit high beta-globin transgene expression in thalassemia induced pluripotent stem cells. Nat Biotechnol. 2011; 29(1):73–78. [PubMed: 21151124]
24. Lisa Li H, Nakano T, Hotta A. Genetic correction using engineered nucleases for gene therapy applications. Dev Growth Differ. 2014; 56(1):63–77. [PubMed: 24329887]
25. Efe JA, Hilcove S, Kim J, Zhou H, Ouyang K, Wang G, Chen J, Ding S. Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. Nat Cell Biol. 2011 Mar;13(3):215-22. doi: 10.1038/ncb2164. Epub 2011 Jan 30. PMID: 21278734
26. Cattoglio C, Facchini G, Sartori D, Antonelli A, Miccio A, Cassani B, Schmidt M, von Kalle C, Howe S, Thrasher AJ, et al (2007) Hot spots of retroviral integration in human CD34. hematopoietic cells. Blood 110: 1770-1778
27. Deichmann A, Brugman MH, Bartholomae CC, Schwarzwaelder K, Verstegen MM, Howe SJ, Arens A, Ott MG, Hoelzer D, Seger R (2011) Insertion sites in engrafted cells cluster within a limited repertoire of genomic areas after gammaretroviral vector gene therapy. Mol Ther 19: 2031-2039
28. Derse D, Crise B, Li Y, Princler G, Lum N, Stewart C, McGrath CF, Hughes SH, Munroe DJ, Wu X (2007) Human T-cell leukemia virus type 1 integration target sites in the human genome: comparison with those of other retroviruses. J Virol 81: 6731-6741
29. Mitchell RS, Beitzel BF, Schroder AR, Shinn P, Chen H, Berry CC, Ecker JR, Bushman FD (2004) Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. PLoS Biol 2: E234
30. Cattoglio C, Pellin D, Rizzi E, Maruggi G, Corti G, Miselli F, Sartori D, Guffanti A, Di Serio C, Ambrosi A, et al (2010) High-definition mapping of retroviral integration sites identifies active regulatory elements in human multipotent hematopoietic progenitors. Blood 116: 5507-5517
31. Kustikova O, Fehse B, Modlich U, Yang M, Du¨llmann J, Kamino K, Neuhoff Nv, Schlegelberger B, Li Z, Baum C (2005) Clonal dominance of hematopoietic stem cells triggered by retroviral gene marking. Science (New York, NY) 308: 1171-1174
32. Hacein-Bey-Abina S, von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, et al (2003) LMO2- associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science (New York, NY) 302: 415-419
33. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U, Glimm H, Ku¨hlcke K, Schilz A, Kunkel H, et al (2006) Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 12: 401-409
34. Maetzig T, Galla M, Baum C, Schambach A (2011) Gammaretroviral vectors: biology, technology and application. Viruses 3: 677-713
35. Schambach A, Zychlinski D, Ehrnstroem B, Baum C (2013) Biosafety features of lentiviral vectors. Hum Gene Ther 24: 132-142
36. Modlich U, Bohne J, Schmidt M, von Kalle C, Kno¨ss S, Schambach A, Baum C (2006) Cell-culture

assays reveal the importance of retroviral vector design for insertional genotoxicity. Blood 108: 2545-2553
37. Modlich U, Navarro S, Zychlinski D, Maetzig T, Knoess S, Brugman MH, Schambach A, Charrier S, Galy A, Thrasher AJ, et al (2009) Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors. Mol Ther 17: 1919 -1928
38. Montini E, Cesana D, Schmidt M, Sanvito F, Bartholomae CC, Ranzani M, Benedicenti F, Sergi LS, Ambrosi A, Ponzoni M, et al (2009) The genotoxic potential of retroviral vectors is strongly modulated by vector design and integration site selection in a mouse model of HSC gene therapy. J Clin Invest 119: 964-975
39. Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, Sergi Sergi L, Benedicenti F, Ambrosi A, Di Serio C, et al (2006) Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 24: 687-696
40. Al-Hendy A, Lee EJ, Wang HQ, Copland JA. Gene therapy of uterine leiomyomas: adenovirus-mediated expression of dominant negative estrogen receptor inhibits tumor growth in nude mice. Am J Obstet Gynecol 2004;191:1621–31.
41. Al-Hendy A, Salama S. Gene Therapy and uterine leiomyoma: Review. Human Reproduction Update, 2006.Vol.12, No.4 pp. 385–400.
42. Lee EJ, Jakacka M, Duan WR, Chien PY, Martinson F, Gehm BD, et al. Adenovirus-directed expression of dominant negative estrogen receptor induces apoptosis in breast cancer cells and regression of tumors in nude mice. Mol Med 2001;7:773– 82.
43. Barber GN. Host defense, viruses and apoptosis. Cell Death Differ 2001;8:113-26.
44. Hyder SM, Huang JC, Nawaz Z, Boettger-Tong H, Makela S, Chiappetta C and Stancel GM (2000) Regulation of vascular endothelial growth factor expression by estrogens and progestins. Environ Health Perspect 108,785–790.
45. Gentry CC, Okolo SO, Fong LF, Crow JC, Maclean AB and Perrett CW (2001) Quantification of vascular endothelial growth factor-A in leiomyo- mas and adjacent myometrium. Clin Sci (Lond) 101,691–695.
46. Hong T, Shimada Y, Uchida S, Itami A, Li Z, Ding Y, Kaganoi J, Komoto I, Sakurai T and Imamura M (2001) Expression of angiogenic factors and apoptotic factors in leiomyosarcoma and leiomyoma. Int J Mol Med 8,141–148.
47. DiLieto A, De Falco M, Pollio F, Mansueto G, Salvatore G, Somma P, Ciociola F, De Rosa G and Staibano S (2005) Clinical response, vascular change, and angiogenesis in gonadotropin-releasing hormone analogue-treated women with uterine myomas. J Soc Gynecol Invest 12,123–128.
48. Vassaux G, Martin-Duque P: Use of suicide genes for cancer gene therapy: study of the different approaches. Expert Opin Biol Ther 2004;4:519–530.
49. Salama S.A, Kamela M. Christmand G, Wangb H.Q., Fouad H.M., Al-Hendy A. Gene Therapy of Uterine Leiomyoma: Adenovirus-Mediated Herpes Simplex Virus Thymidine Kinase/Ganciclovir Treatment Inhibits Growth of Human and Rat Leiomyoma Cells in vitro and in a Nude Mouse Model. Gynecol Obstet Invest 2007;63:61–70
50. Tasciotti E, Zoppe M, Giacca M. Transcellular transfer of active HSV-1 thymidine kinase mediated by an 11-amino-acid peptide from HIV-1 TAT. Cancer Gene Ther 2003;10:64–74.
51. Reid R, Mar EC, Huang ES, Topal MD. Insertion and extension of acyclic, dideoxy and Ara nucleotides by herpesviridae, human alpha and human beta polymerase; a unique inhibition mechanism for 9-(1, 3-dihydroxy-2- propoxymethyl) guanine triphosphate. J Biol Chem1988;263:3898–3904.
52. Robe PA, Princen F, Martin D, Malgrange B, Stevenaert A, Moonen G, Gielen J, Merville MP, Bours V. Pharmacological modulation of the bystander effect in the herpes simplex virus thymidine kinase/ganciclovir gene therapy system; effects of dibutyryl adenosine 3′, 5′-cyclic monophosphate, alpha-glycrrhetinic acid, and cystosine arabinoside.
53. Satoh T, Irie A, Egawa S, Baba S: In situ gene therapy for prostate cancer. Curr Gene Ther 2005;5:111–119.
54. Immonen A, Vapalahti M, Tyynela K, Hur-skainen H, Sandmair A, Vanninen R, Lang- ford G, Murray N, Yla-Herttuala S: AdvHSV- tk gene therapy with intravenous ganciclovir improves survival in human malignant glio- ma: a randomised, controlled study. Mol Ther 2004;10:967–972.
55. Ketola A, Maatta AM, Pasanen T, Tulimaki K, Wahlfors J: Osteosarcoma and chondro- sarcoma as targets for virus vectors and her- pes simplex virus thymidine kinase/ganci- clovir gene therapy. Int J Mol Med 2004;13: 705–710.
56. Ohno T, Gordon D, San H, Pompili VJ, Im-periale MJ, Nabel GJ, Nabel EG: Gene thera- py for vascular smooth muscle cell prolifera- tion after arterial injury. Science 1994;265: 781–784.
57. Pope IM, Poston GJ, Kinsella AR: The role of the bystander effect in suicide gene therapy. Eur J Cancer 1997;33:1005–1016.
58. Dilber MS, Abedi MR, Christensson B, Bjorkstrand B, Kidder GM, Naus CC, Gahrton G, Smith CI: Gap junctions pro- mote the bystander effect of herpes simplex virus thymidine kinase in vivo. Cancer Res 1997;57:1523–1528.
59. Matono S, Tanaka T, Sueyoshi S, Yamana H, Fujita H, Shirouzu K: Bystander effect in suicide gene

therapy is directly proportional to the degree of gap junctional intercellular communication in esophageal cancer. Int J Oncol 2003;23:1309–1315.
60. Andersen J, Grine E, Eng CL, Zhao K, Barbieri RL, Chumas JC, Brink PR: Expression of connexin-43 in human myometrium and leiomyoma. Am J Obstet Gynecol 1993;169: 1266–1276.
61. Worgall S, Wolff G, Falck-Pedersen E, Crystal RG. Innate immune mechanisms dominate elimination of adenoviral vectors following in vivo administration. Hum Gene Ther 1997;8:37–44.
62. Stone D, Ni S, Li ZY, Gaggar A, DiPaolo N, Feng Q, Sandig V, Lieber A. Development and assessment of human adenovirus type 11 as a gene transfer vector. J Virol 2005;79:5090–5104.
63. Yang Y, Li Q, Ertl HC, Ertl HC, Wilson JM. Cellular and humoral immune responses to viral antigens create barriers to lung-directed gene therapy with recombinant adenoviruses. J Virol 1995;69:2004–2015.
64. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 1997;275:1320– 1323.
65. Tomko RP, Xu R, Philipson L. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proc Natl Acad Sci USA 1997;94:3352–3356.
66. Hassan MH, Salama S, Zhang D, Al-Hendy A. Towards fibroids gene therapy: adenovirus mediated delivery of dominant negative estrogen receptor shrinks uterine Fibroids tumors in Eker rats. Reprod Sci 2007;14:80A.
67. Hassan M.H, N. Khatoon N, Curiel D.C, Hamada F.H, Arafa H. F and Al-Hendy A. Toward gene therapy of uterine fibroids: targeting modified adenovirus to human Fibroids cells. January 9, 2008. Human Reproduction Vol.23, No.3 pp. 514–524, 2008 doi:10.1093/humrep/dem410.Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissues into the peritoneal cavity. Am J Obstet Gynecol 1927, 14:422– 469
68. Dmitriev I, Krasnykh V, Miller CR, Wang M, Kashentseva E, Mikheeva G, Belousova N, Curiel DT. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. J Virol 1998;72:9706 – 9713.
69. Cripe TP, Dunphy EJ, Holub AD, Saini A, Vasi NH, Mahller YY, Collin MH, Snyde JD, Krasnykh V, Curiel DT et al. Fiber knob modifications overcome low, heterogeneous expression of the coxsackievirus-adenovirus receptor that limits adenovirus gene transfer and oncolysis for human rhabdomyosarcoma cells. Cancer Res 2001;61:2953–2960.
70. Sampson JA: Peritoneal endometriosis due to menstrual dissemina- tion of endometrial tissues into the peritoneal cavity. Am J Obstet Gynecol 1927, 14:422– 469
71. Lebovic DI, Mueller MD, Taylor RN: Immunobiology of endometriosis. Fertil Steril 2001, 75:1–10
72. Surrey ES, Halme J: Effect of peritoneal fluid from endometriosis patients on endometrial stromal cell proliferation in vitro. Obstet Gynecol 1990, 76:792–797
73. Sillem M, Prifti S, Neher M, Runnebaum B: Extracellular matrix remodelling in the endometrium and its possible relevance to the pathogen- esis of endometriosis. Hum Reprod Update 1998, 4:730–735
74. Dizerega GS, Barber DL, Hodgen GD. Endometriosis: role of ovarian steroids in initiation, maintenance, and suppression. Fertil Steril 1980; 33:649 –53.
75. Gurates B, Bulun SE. Endometriosis: the ultimate hormonal disease. Semin Reprod Med 2003;21:125–34.
76. Bulun SE, Imir G, Utsunomiya H, Thung S, Gurates B, Tamura M, et al. Aromatase in endometriosis and uterine leiomyomata. J Steroid Biochem Mol Biol 2005;95:57– 62.
77. Zeitoun KM, Bulun SE. Aromatase: key molecule in the pathophysiology of endometriosis and a therapeutic target. Fertil Steril 1999;72: 961–9.
78. Matsuzaki S, Murakami T, Uehara S, Canis M, Sasano H, Okamura K. Expression of estrogen receptor alpha and beta in peritoneal and ovarian endometriosis. Fertil Steril 2001;75:1198 –205.
79. Hyder SM, Stancel GM: Regulation of angiogenic growth factors in the female reproductive tract by estrogens and progestins. Mol En- docrinol 1999, 13:806–811.
80. Dabrosin C, Gyorffy S, Margetts P, Ross C, Gauldie J. Therapeutic effect of angiostatin gene transfer in a murine model of endometriosis. Am J Pathol 2002;161:909–18.

81. Gyorffy S, Palmer K, Gauldie J: Adenoviral vector expressing murine angiostatin inhibits a model of breast cancer metastatic growth in the lungs of mice. Am J Pathol 2001, 159:1137–1147

82. McLaren J, Prentice A, Charnock-Jones DS, Millican SA, Muller KH, Sharkey AM, Smith SK: Vascular endothelial growth factor is pro- duced by peritoneal fluid macrophages in endometriosis and is reg- ulated by ovarian steroids. J Clin Invest 1996, 98:482–489
83. Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP,

Meng YG, Ferrara N, Jaffe RB, Taylor RN: Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endome- triosis. J Clin Endocrinol Metab 1996, 81:3112–3118

84. McLaren J: Vascular endothelial growth factor and endometriotic angiogenesis. Hum Reprod Update 2000, 6:45–55

85. Ferrara N, Chen H, Davis-Smyth T, Gerber HP, Nguyen TN, Peers D, Chisholm V, Hillan KJ, Schwall RH: Vascular endothelial growth factor is essential for corpus luteum angiogenesis. Nat Med 1998, 4:336– 340

86. Al-Hendy A, Lee EJ, Wang HQ, Copland JA. Gene therapy of uterine leiomyomas: adenovirus-mediated expression of dominant negative estrogen receptor inhibits tumor growth in nude mice. Am J Obstet Gynecol 2004;191:1621–31.

87. Othman E, Salama SA, Ismail N, Al-Hendy A. Gene therapy of endometriosis: adenovirus mediated expression of dominant negative estrogen receptor induces apoptosis in human endometriotic cells. Fertil Steril 2007;88: 462-71.

88. Othman EE, Zhu Z.B, Curiel D, Khatoon N, Salem H, Khalifa E.M., Al-Hendy A. Toward gene therapy of endometriosis:Transductional and transcriptional targeting of adenoviral vectors to endometriosis cells.Am J Obstet Gynecol. 2008 Aug; 199(2): 117.e1–117.e6.

89. González-Ramos R, LangendoncktaA.V, Defrère S, Lousse J C, Mettlen M, Guillet A, Donnez J. Agents Blocking the Nuclear Factor- B Pathway Are Effective Inhibitors of Endometriosis in an in vivo Experimental Model. Gynecol Obstet Invest 2008;65:174–186.

90. Yamamoto Y, Gaynor RB: Therapeutic potential of inhibition of the NF- B pathway in the treatment of inflammation and cancer. J Clin Invest 2001;107:135–142.

91. Zhu F[1], Liu M[2], Pan Y[1], Wang X[1], Chen Y[1]. Zhonghua Fu Chan Ke Za Zhi. Small hairpin RNA targeting inhibition of NF-κB gene in endometriosis therapy of Macaca fascicularis. 2015 Jan;50(1):48-53.

92. Young, E.A., 1995. The role of gonadal steroids in hypothalamic-pituitary adrenal axis regulation. Critical Reviews in Neurobiology 9, 371–381.

93. Tapanainen JS, Aittomaki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat Genet 1997;15:205–206.

94. Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. Endocr Rev 1997;18:739–773.

95. Filicori, M., Cognigni, G.E., Samara1, A., Melappioni, S., Perri, T., Cantelli, B., et al., 2002. The use of LH activity to drive folliculogenesis: exploring uncharted territories in ovulation induction. Human Reproduction Update 8, 543–557.

96. Ma, X.P., Dong, Y.L., Matzuk, M.M., Kumar, T.R., 2004. Targeted disruption of luteinizing hormone beta-subunit leads to hypogonadism, defects in gonadal steroidogenesis, and infertility. Proceedings of the National Academy of Sciences of the United States of America 101, 17294–17299.

97. Richards, J.S., 1994. Hormonal control of gene expression in the ovary. Endocrine Reviews 15, 725–751.

98. Aittomaki K, Lucerna JL, Pakarinen P, Sistonen P, Tapanainen JS, Gromoll J, Kaskikari R, Sankila EM, Lehvaslaiho H, Engel AR. Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell 1995;82:959–968.

99. Simpson JL, Christakos AC, Horwith M, Silverman FS. Gonadal dysgenesis in individuals with apparently normal chromosomal complements: tabulation of cases and compilation of genetic data. Birth Defects Orig Artic Ser 1971;7:215–228.

100. Talbert LM, Raj MH, Hammond MG, Greer T. Endocrine and immunologic studies in a patient with resistant ovary syndrome. Fertil Steril 1984; 42:741 – 744.

101. Ghadami M, Salama SA, Khatoon N, Chilvers R, Nagamani M, Chedrese PJ, Al-Hendy A. Toward gene therapy of primary ovarian failure: adenovirus expressing human FSH receptor corrects the Finnish C566T mutation. Mol Hum Reprod 2008;14:9–15.

102. Somers JP, DeLoia JA, Zeleznik AJ. Adenovirus-directed expression of a nonphosphorylatable mutant of CREB (cAMP response element-binding protein) adversely affects the survival, but not the differentiation, of rat granulosa cells. Mol Endocrinol 1999;13:1364–1372.

103. Al-Hendy A, Wang H, Salama SA. Towards gene therapy of ovarian failure: intraovarian injected adenovirus successfully transduced granulosa and stromal but not germ cells. J Soc Gynecol Investig 2005;12:S-659.

104. Ghadami M, El-Demerdash E, Salama S, Binhazim A, Archibong A, Chen X, Ballard B.R., Sairam M.R., and Al-Hendy A. Toward gene therapy of premature ovarian failure: intraovarian injection of adenovirus expressing human FSH receptor restores folliculogenesis in FSHR(2/2) FORKO mice. Molecular Human Reproduction, 2010, Vol.16, No.4 pp. 241–250.

105. Dierich A, Sairam MR, Monaco L, Fimia GM, Gansmuller A, LeMeur M et al. Impairing follicle-stimulating hormone (FSH) signaling in vivo: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. Proc Natl Acad Sci USA 1998;95:13612–13617.
106. Rhee GS, Lee HJ, Kim SS, Kwack SJ, Lee RD, Seok JH et al. Reproductive safety evaluation and quantification of adenoviral vectors in the mouse. Mol Ther 2004;9:S172.
107. Wang, D. 2010. Research on six genes related to egg-production in the ovaries of Zi Goose. Dissertation, master, Daqing, Heilongjiang, China.
108. Merkulova, T., Dehaupas, M., Nevers, M.-C., Créminon, C., Alameddine, H., Keller, A., 2000. Differential modulation of α, β and γ enolase isoforms in regenerating mouse skeletal muscle. European Journal of Biochemistry 267 (12), 3735–3743.
109. Liakakos T, Homakos N, Fine PM, Dervenis C, Young RL. Peritoneal adhesions: etiology, pathophysiology, and clinical significance. Recent advances in prevention and management. Dig Surg. 2001; 18:260–273. [PubMed: 11528133]
110. Kresch AJ, Seifer DB, Sachs LB, Barrese I. Laparoscopy in 100 women with chronic pelvic pain. Obstet Gynecol 1984; 64: 672-674
111. Sutton C, MacDonald R. Laser laparoscopic adhesiolysis. J Gynecol Surg 1990; 6: 155-159
112. Holmdahl L, Eriksson E, Eriksson BI, Risberg B. Depression of peritoneal fibrinolysis during operation is a local response to trauma. Surgery. 1998; 123:539–544. [PubMed: 9591006]
113. Ivarsson ML, Bergstrom M, Eriksson E, Risberg B, Holmdahl L. Tissue markers as predictors of postoperative adhesions. Br J Surg. 1998; 85:1549–1554. [PubMed: 9823923]
114. Holmdahl L, Falkenberg M, Ivarsson ML, Risberg B. Plasminogen activators and inhibitors in peritoneal tissue. APMIS. 1997; 105:25–30. [PubMed: 9063497]
115. Chegini N, Kotseos K, Zhao Y, Bennett B, McLean FW, Diamond MP, Holmdahl L, Burns J. Differential expression of TGF-beta1 and TGF-beta3 in serosal tissues of human in- traperitoneal organs and peritoneal adhesions. Hum Reprod 2001; 16: 1291-1300
116. Cheong YC, Shelton JB, Laird SM, Li TC, Ledger WL, Cooke ID. Peritoneal uid concentrations of matrix metalloprotein- ase-9, tissue inhibitor of metalloproteinase-1, and transform- ing growth factor-beta in women with pelvic adhesions. Fertil Steril 2003; 79: 1168-1175
117. Molinas CR, Elkelani O, Campo R, Luttun A, Carmeliet P, Koninckx PR. Role of the plasminogen system in basal adhesion formation and carbon dioxide pneumoperitoneum-enhanced adhesion formation after laparoscopic surgery in transgenic mice. Fertil Steril. 2003; 80:184–192. [PubMed: 12849822]
118. Guo Q, Li QF, Liu HJ, Li R, Wu CT, Wang LS. Sphingosine kinase 1 gene transfer reduces postoperative peritoneal ad- hesion in an experimental model. Br J Surg 2008; 95: 252-258
119. Liu HJ, Wu CT, Duan HF, Wu B, Lu ZZ, Wang L. Adenovi- ral-mediated gene expression of hepatocyte growth factor prevents postoperative peritoneal adhesion in a rat model. Surgery 2006; 140: 441-447
120. Segura T, Schmokel H, Hubbell JA. RNA interference tar- geting hypoxia inducible factor 1alpha reduces post-opera- tive adhesions in rats. J Surg Res 2007; 141: 162-170
121. Cahill RA, Wang JH, Soohkai S, Redmond HP. Mast cells facilitate local VEGF release as an early event in the patho- genesis of postoperative peritoneal adhesions. Surgery 2006; 140: 108-112
122. Atta HM, Al-Hendy A, El-Rehany MA, Dewerchin M, Abdel Raheim SR, Abdel Ghany H, Fouad R. Adenovirus- mediated overexpression of human tissue plasminogen ac- tivator prevents peritoneal adhesion formation/reformation in rats. Surgery 2009; 146: 12-17
123. Slingerland, J. M., and Tannock, I. F. Cell proliferation and cell death. In: I. F. Tannock and R. P. Hill (eds), The Basic Science of Oncology, pp. 135–165. New York: McGraw-Hill Health Professions Division, 1998.
124. Indraccolo, S., Gola, E., Rosato, A., Minuzzo, S., Habeler, W., Tisato, V., Roni, V., Esposito, G., Morini, M., Albini, A., Noonan, D. M., Ferrantini, M., Amadori, A., and Chen, S. L., C. P. Han, Y. P. Tsao, J. W. Lee, and C. S. Yin. 1993. Identification and typing of human papillomavirus in cervical cancers in Taiwan. Cancer 72:1939–1945.
125. Matthews KS, Alvarez RD, Curiel DT (2009) Advancements in adenoviral based virotherapy for ovarian cancer. Adv Drug Deliv Rev 61: 836-841.
126. Palmer, T. D., Rosman, G. J., Osborne, W. R., and Miller, A. D. Genetically modified skin fibroblasts persist long after transplantation but gradually inactivate introduced genes. Proc. Natl. Acad. Sci. USA, 88: 1330–1334, 1991.
127. Buller RE, Runnebaum IB, Karlan BY, Horowitz JA, Shahin M, et al. (2002) A phase I/II trial of rAd/p53 (SCH 58500) gene replacement in recurrent ovarian cancer. Cancer Gene Ther 9: 553-566.
128. Zeimet AG and Marth C. Why did p53 gene therapy fail in ovarian cancer? Lancet Oncol 2003 4: 415–22.
129. Hung C-F, Chiang AJ, Tsai H-H, Pomper MG, Kang TH, et al. (2012) Ovarian Cancer Gene Therapy

Using HPV-16 Pseudovirion Carrying the HSV-tk Gene. PLoS ONE 7(7): e40983. doi:10.1371/journal.pone.0040983.
130. Morgan RA (2012) Live and Let Die: A New Suicide Gene Therapy Moves to the Clinic. Molecular Therapy 20: 11–13.
131. Altaner C (2008) Prodrug cancer gene therapy. Cancer letters 270: 191–201.
132. Moolten FL (1986) Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. Cancer research 46: 5276–5281.
133. Peng S, Monie A, Kang TH, Hung CF, Roden R, et al. (2010) Efficient delivery of DNA vaccines using human papillomavirus pseudovirions. Gene therapy 17: 1453–1464.
134. Gordon SN, Kines RC, Kutsyna G, Ma ZM, Hryniewicz A, et al. (2012) Targeting the vaginal mucosa with human papillomavirus pseudovirion vaccines delivering simian immunodeficiency virus DNA. Journal of immunology 188: 714–723.
135. Peng S, Ma B, Chen SH, Hung CF, Wu T (2011) DNA vaccines delivered by human papillomavirus pseudovirions as a promising approach for generating antigen-specific CD8+ T cell immunity. Cell & bioscience 1: 26.
136. M. Harries, S.B. Kaye, Recent advances in the treatment of epithelial ovarian cancer, Exp. Opin. Invest. Drugs 10 (2001) 1715–1724.
137. Wang L, Chen H, Pourgholami MH, Beretov J, Hao J, et al. (2011) Anti-MUC1 monoclonal antibody (C595) and docetaxel markedly reduce tumor burden and asci- tes, and prolong survival in an in vivo ovarian cancer model. PLoS One 6: e24405.
138. O.D.M. Hughes, H. Denley, R.B. Kunkler, G. Denton, M.R. Price, A.C. Perkins, MUC1 mucin expression in transitional cell carcinoma of the bladder, J. Urol. Pathol. 12 (2000) 179–191.
139. M.R. Price, J.A. Pugh, F. Hudecz, W. Griffiths, E. Jacobs, I.M. Symonds, A.J. Clarke, W.C. Chan, R.W. Baldwin, C595 – a monoclonal antibody against the protein core of human urinary epithelial mucin commonly expressed in breast carcinomas, Brit. J. Cancer 61 (1990) 681–686.
140. A.C. Perkins, I.M. Symonds, M.V. Pimm, M.R. Price, M.L. Wastie, E.M. Symonds, Immunoscintigraphy of ovarian carcinoma using a monoclonal antibody (111In-NCRC48) defining a polymorphic epithelial mucin (PEM) epitope, Nucl. Med. Commun. 14 (1993) 578–586.
141. L. Wang, J. Ma, F. Liu, Q. Yu, G. Chu, A.C. Perkins, Y. Li, Expression of MUC1 in primary and metastatic human epithelial ovarian cancer and its therapeutic significance, Gynecol. Oncol. 105 (2007) 695– 702.
142. Rancourt C, Bergeron C, Lane D, Garon G, Piché A (2003) Delivery of herpes simplex thymidine kinase bystander effect by engineered human mesothelial cells for the treatment of ovarian cancer. Cytotherapy 5: 509- 522.
143. Nagy JA, Shockley TR, Masse EM et al. Mesothelial cell- mediated gene therapy: feasibility of an ex vivo strategy. Gene Ther 1995;2:393 •/401.
144. Murphy J-E, Rheinwald JG. Intraperitoneal injection of genetically modified, human mesothelial cells for systemic gene therapy. Hum Gene Ther 1997;8:1867,/79.
145. Rocconi RP, Zhu ZB, Stoff-Khalili M, Rivera AA, LuB, et al. (2007).Treatment of ovarian cancer with a novel dual targeted conditionally replicative adenovirus (CRAd). Gynecol Oncol 105: 113-121.
146. Haviv YS, Curiel DT. Engineering regulatory elements for conditionallyreplicative adeno-viruses. Curr Gene Ther 2003;3:357–85.
147. Zhu ZB, Makhija SK, Lu B, Wang M, Kaliberova L, Liu B, et al. Transcriptional targeting of adenoviral vector through the CXCR4 tumorspecific promoter. Gene Ther 2004;11:645–8.
148. Li F, Zhu HS, Han ZQ, Chen G, Gao QL, Jia P, et al. Effects of chemokine receptor and its ligand on migration of ovarian cancer cells. Ai Zheng 2005;24:23–7.
149. Scotton CJ,Wilson JL, Milliken D, Stamp G, Balkwill FR. Epithelial cancer cell migration: a role for chemokine receptors? Cancer Res 2001;61: 4961–5.
150. Scotton CJ, Wilson JL, Scott K, Stamp G, Wilbanks GD, Fricker S, et al. Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. Cancer Res 2002;62:5930–8.
151. Dmitriev I, Krasnykh V, Miller CR,Wang M, Kashentseva E, Mikheeva G, et al. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. J Virol 1998;72:9706–13.
152. Miller CR, Buchsbaum DJ, Reynolds PN, Douglas JT, Gillespie GY, Mayo MS, et al. Differential susceptibility of primary and established human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer. Cancer Res 1998;58:5738–48.
153. Bauerschmitz GJ, Lam JT, Kanerva A, Suzuki K, Nettelbeck DM, Dmitriev I, et al. Treatment of ovarian cancer with a tropism modified oncolytic adenovirus. Cancer Res 2002;62:1266–70.
154. Suzuki K, Fueyo J, Krasnykh V, Reynolds PN, Curiel DT, Alemany R. A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. Clin Cancer Res 2001;7:120–6.
155. Liu B, Zhang H, Luo X, Xie Y, Hao J, Zhou Q,

Duan X, Wang Y, Zhao W. High-eYciency transfer and expression of AdCMV-p53 in human cervix adenocarcinoma cells induced by subclinical-dose carbon beam radiation. J Cancer Res Clin Oncol (2009) 135:925–932.
156. Chen, S. L., C. P. Han, Y. P. Tsao, J. W. Lee, and C. S. Yin. 1993. Identifi- cation and typing of human papillomavirus in cervical cancers in Taiwan. Cancer 72:1939–1945.
157. Howley, P. M. 1991. Role of the human papillomaviruses in human cancer. Cancer Res. 51:5019s–5022s.
158. Zur Hausen, H., and E. M. de Villiers. 1994. Human papillomaviruses. Annu. Rev. Microbiol. 48:427–447.
159. Chen, L. P., E. K. Thomas, S. L. Hu, I. Hellstrom, and K. E. Hellstrom. 1991. Human papillomavirus type 16 nucleoprotein E7 is a tumor rejection anti- gen. Proc. Natl. Acad. Sci. USA 88:110–114.
160. Borysiewicz, L. K., A. Fiander, M. Nimako, S. Man, G. W. Wilkinson, D. Westmoreland, A. S. Evans, M. Adams, S. N. Stacey, M. E. Boursnell, E. Rutherford, J. K. Hickling, and S. C. Inglis. 1996. A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. Lancet 347:1523–1527.
161. Boursnell, M. E., E. Rutherford, J. K. Hickling, E. A. Rollinson, A. J. Munro, N. Rolley, C. S. McLean, L. K. Borysiewicz, K. Vousden, and S. C. Inglis. 1996. Construction and characterization of a recombinant vaccinia virus expressing human papillomavirus proteins for immunotherapy of cervical cancer. Vaccine 14:1485–1494.
162. Gao, L., B. Chain, C. Sinclair, L. Crawford, J. Zhou, J. Morris, X. Zhu, and H. Stauss. 1994. Immune response to human papillomavirus type 16 E6 gene in a live vaccinia vector. J. Gen. Virol. 75:157–164.
163. Lin, K. Y., F. G. Guarnieri, O. C. K. F. Staveley, H. I. Levitsky, J. T. August, D. M. Pardoll, and T. C. Wu. 1996. Treatment of established tumors with a novel vaccine that enhances major histocompatibility class II presentation of tumor antigen. Cancer Res. 56:21–26.
164. Meneguzzi, G., C. Cerni, M. P. Kieny, and R. Lathe. 1991. Immunization against human papillomavirus type 16 tumor cells with recombinant vaccinia viruses expressing E6 and E7. Virology 181:62–69.
165. Zhu, X., M. Tommasino, K. Vousden, E. Sadovnikava, R. Rappuoli, L. Crawford, M. Kast, C. J. Melief, P. C. Beverley, and H. J. Stauss. 1995. Both immunization with protein and recombinant vaccinia virus can stimulate CTL specific for the E7 protein of human papilloma virus 16 in H-2d mice. Scand. J. Immunol. 42:557–563.
166. Chen, L., M. T. Mizuno, M. C. Singhal, S. L. Hu, D. A. Galloway, I. Hell-strom, and K. E. Hellstrom. 1992. Induction of cytotoxic T lymphocytes specific for a syngeneic tumor expressing the E6 oncoprotein of human papillomavirus type 16. J. Immunol. 148:2617–2621.
167. De Bruijn, M. L., D. H. Schuurhuis, M. P. Vierboom, H. Vermeulen, K. A. de Cock, M. E. Ooms, M. E. Ressing, M. Toebes, K. L. Franken, J. W. Drijfhout, T. H. Ottenhoff, R. Offringa, and C. J. Melief. 1998. Immunization with human papillomavirus type 16 (HPV16) oncoprotein-loaded dendritic cells as well as protein in adjuvant induces MHC class I-restricted protection to HPV16-induced tumor cells. Cancer Res. 58:724–731.
168. Tuting, T., A. B. DeLeo, M. T. Lotze, and W. J. Storkus. 1997. Genetically modified bone marrow-derived dendritic cells expressing tumor-associated viral or “self” antigens induce antitumor immunity in vivo. Eur. J. Immunol. 27:2702–2707.
169. Feltkamp, M. C., H. L. Smits, M. P. Vierboom, R. P. Minnaar, B. M. de Jongh, J. W. Drijfhout, J. ter Schegget, C. J. Melief, and W. M. Kast. 1993. Vaccination with cytotoxic T lymphocyte epitope-containing peptide protects against a tumor induced by human papillomavirus type 16-transformed cells. Eur. J. Immunol. 23:2242–2249.
170. Greenstone, H. L., J. D. Nieland, K. E. de Visser, M. L. De Bruijn, R. Kirnbauer, R. B. Roden, D. R. Lowy, W. M. Kast, and J. T. Schiller. 1998. Chimeric papillomavirus virus-like particles elicit antitumor immunity against the E7 oncoprotein in an HPV16 tumor model. Proc. Natl. Acad. Sci. USA 95:1800–1805.
171. Liu, X. S., I. Abdul-Jabbar, Y. M. Qi, L. H. Frazer, and J. Zhou. 1998. Mucosal immunization with papillomavirus virus-like particles elicits sys- temic and mucosal immunity in mice. Virology 252:39–45.
172. Schafer, K., M. Muller, S. Faath, A. Henn, W. Osen, H. Zentgraf, A. Benner, L. Gissmann, and I. Jochmus. 1999. Immune response to human papillomavirus 16 L1E7 chimeric virus-like particles:induction of cytotoxic T cells and specific tumor protection. Int. J. Cancer 81:881–888.
173. Londono, L. P., S. Chatfield, R. W. Tindle, K. Herd, X. M. Gao, I. Frazer, and G. Dougan, 1996. Immunization of mice using Salmonella typhimurium expressing human papillomavirus type 16 E7 epitopes inserted into hepatitis B virus core antigen. Vaccine 14:545–552.
174. Liu A.W., TSAO Y.P, Kung J.T, Ding Y.A, Sytwu H.K, Xiao X, and Chec S.L. Recombinant Adeno-Associated Virus Expressing Human

Papillomavirus Type 16 E7 Peptide DNA Fused with Heat Shock Protein DNA as a Potential Vaccine for Cervical Cancer, 2000. Journal of virology, Mar.2000, p. 2888–2894.
175. Zhou L, Zhu T, Ye X, Yang L, Wang B, Liang X, Lu L, tSAO YP, Chen SL, Li J, Xiao X. Long-term protection against human papillomavirus e7-positive tumor by a single vaccination of adeno-associated virus vectors encoding a fusion protein of inactivated e7 of human papillomavirus 16/18 and heat shock protein 70. Hum Gene Ther. 2010 Jan;21(1):109-19.
176. Tindle, R. W., G. J. Fernando, J. C. Sterling, and I. H. Frazer. 1991. A "public" T-helper epitope of the E7 transforming protein of human papillomavirus 16 provides cognate help for several E7 B-cell epitopes from cervical cancer-associated human papillomavirus genotypes. Proc. Natl. Acad. Sci. USA 88:5887–5891.
177. Jones HN, Crombleholme T, Habli M. Adenoviral-mediated placental gene transfer of IGF-1 corrects placental insufficiency via enhanced placental glucose transport mechanisms, 2013. PLoS One. 2013 Sep 3;8(9):e74632.
178. Keswani SG[1], Balaji S, Katz AB, King A, Omar K, Habli M, Klanke C, Crombleholme TM. Intraplacental gene therapy with Ad-IGF-1 corrects naturally occurring rabbit model of intrauterine growth restriction. Hum Gene Ther. 2015 Mar;26(3):172-82.
179. Saunders M. Transplacental transport of nanomaterials.Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2009 1(6):671–84. doi: 10.1002/wnan.53 PMID: 20049824
180. Mahmood M, Casciano D, Xu Y, AS Biris Engineered nanostructural materials for application in cancer biology and medicine. J Appl Toxicol. 2012; 32(1):10–19 doi: 10.1002/jat.1718 PMID: 21882206
181. Lu XY, Wu DC, Li ZJ, Chen GQ. Polymer nanoparticles. Prog Mol Biol Transl Sci. 2011; 104:299–323 doi: 10.1016/B978-0-12-416020-0.00007-3 PMID: 22093222
182. Abd Ellah NH, Potter SJ, Taylor L, Ayres N, Elmahdy MM, Fetih GN, et al. Safety and efficacy of aminecontaining methacrylate polymers as nonviral gene delivery vectors. Journal of Pharmaceutical Technology & Drug Research 2014, 3; 2, doi: 10.7243/2050-120X-3-2
183. Liu J. Bauer H. Callahan J. Kopeckova P. Pan H. Kopecek J. Endocytic uptake of a large array of HPMA copolymers: Elucidation into the dependence on the physicochemical characteristics J. Controlled Release 2010, 71–79.
184. Kopecek J. Targetable Polymeric Anticancer Drugs: Temporal Control of Drug Activity Ann. N.Y. Acad. Sci. 1991, 618, 335–344. PMID: 2006794
185. Dubruel P.; Schacht E. Vinyl polymers as non-viral gene delivery carriers: Current status and prospects. Macromol. Biosci. 2006, 6 (10), 789–810. PMID: 17039574
186. Lisowski L, Dane AP, Chu K, Zhang Y, Cunningham SC, Wilson EM, Nygaard S, Grompe M, Alexander IE, Kay MA. Selection and evaluation of clinically relevant AAV variants in a xenograft liver model. Nature. 2014; 506(7488):382–386. [PubMed: 24390344]
187. Pien GC, Basner-Tschakarjan E, Hui DJ, Mentlik AN, Finn JD, Hasbrouck NC, Zhou S, Murphy SL, Maus MV, Mingozzi F, Orange JS, High KA. Capsid antigen presentation flags human hepatocytes for destruction after transduction by adeno-associated viral vectors. J Clin Invest. 2009; 119(6):1688–1695. [PubMed: 19436115]
188. Barar J, Omidi Y (2012) Translational Approaches towards Cancer Gene Therapy: Hurdles and Hopes. Bioimpacts 2: 127-143.
189. Kotin RM. Large-scale recombinant adeno-associated virus production. Hum Mol Genet. 2011; 20(R1):R2–6. [PubMed: 21531790]
190. Segura MM, Kamen AA, Garnier A. Overview of current scalable methods for purification of viral vectors. Methods Mol Biol. 2011; 737:89–116. [PubMed: 21590394]
191. Segura MM, Mangion M, Gaillet B, Garnier A. New developments in lentiviral vector design, production and purification. Expert Opin Biol Ther. 2013; 13(7):987–1011. [PubMed: 23590247]
192. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, Martinache C, Rieux-Laucat F, Latour S, Belohradsky BH, Leiva L, Sorensen R, Debre M, Casanova JL, Blanche S, Durandy A, Bushman FD, Fischer A, Cavazzana-Calvo M. Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med. 2010; 363(4):355–364. [PubMed: 20660403]
193. Kaeppel C, Beattie SG, Fronza R, Van Logtenstein R, Salmon F, Schmidt S, Wolf S, Nowrouzi A, Glimm H, Von Kalle C, Petry H, Gaudet D, Schmidt M. A largely random AAV integration profile after LPLD gene therapy. Nat Med. 2013; 19(7):889–891. [PubMed: 23770691]
194. Lehrman S. Virus treatment questioned after gene therapy death. Nature. 1999; 401(6753):517–518. [PubMed: 10524611]
195. Masat E, Pavani G, Mingozzi F. Humoral immunity to AAV vectors in gene therapy: challenges and potential solutions. Discov Med. 2013; 15(85):379–389. [PubMed: 23819952]
196. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood. 2013
197. Wang D, Zhong L, Nahid MA, Gao G. The potential of adeno-associated viral vectors for gene delivery to muscle tissue. Expert Opin Drug Deliv. 2014; 11(3):345–364. [PubMed: 24386892].

ONCOGENESIS OF THYROID CANCER

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Abstract

Thyroid neoplasms encompass a variety of lesions that range from benign adenoma to malignant tumors. Malignant thyroid tumors can be well-differentiated, poorly differentiated or undifferentiated (anaplastic) carcinomas. More than 95% of thyroid cancers are derived from thyroid follicular cells, while 2-3% of thyroid tumors (medullary thyroid cancers) are derived from the calcitonin producing C-cells. Over the last decade, investigators have developed a clearer understanding of the genetic alterations underlying thyroid carcinogenesis. A number of point mutations and translocations are involved in thyroid cancer, not only in its tumorigenesis, but also as being potentially useful as diagnostic and prognostic indicators and therapeutic targets. These point mutations and translocations occur in genes for several important signaling pathways, in particular the mitogen-activated protein kinase (MAPK) pathway.

Medullary thyroid carcinoma (MTC) is a distinct thyroid carcinoma that originates in the parafollicular C cells of the thyroid gland. These C cells produce calcitonin. Sporadic (isolated) MTC accounts for 75% of cases, and inherited MTC constitutes the rest. Inherited MTC occurs in association with multiple endocrine neoplasia (MEN) type 2A and 2B syndromes, but non-MEN familial MTC also occur. Advances in genetic testing have revolutionized the management of MTC, with prospects of genetic screening, testing and early prophylactic thyroidectomy. Ethical concerns of these advances will be addressed.

Keywords: thyroid cancer, mutations, undifferentiated thyroid cancer, differentiated thyroid cancer, medullary thyroid cancer, genetics

Introduction

Thyroid cancer is the most common type of endocrine malignancy with an incidence that has steadily increased for the past three decades. Deaths from thyroid cancers alone account for more deaths than all of the other endocrine malignancies combined[1]. In the U.S., 62,450 new cases and 1,950 deaths are estimated for 2014 [2].

The increased incidence of thyroid cancer diagnoses has been attributed, in part, to improved detection of small or subclinical thyroid nodules by thyroid ultrasonography and by other imaging techniques; however, increased incidence of thyroid tumors of all sizes has also been reported[3].

Thyroid cancer typically presents as a thyroid nodule. However, thyroid nodules are commonly found incidentally and may be seen in up to 50% of patients older than 60 years of age. Only 5% of thyroid nodules are malignant[4].

Epithelial malignant cancers of the thyroid

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arise from two different types of parenchymal cells, follicular and parafollicular. Follicular cells line the colloid follicules, concentrate iodine and are predominantly involved in production of thyroid hormones. From these cells arise well differentiated and anaplastic thyroid cancers. The parafollicular or C cells, which are spread among the thyroid follicules, are responsible for the production of calcitonin and from these cells arise medullary thyroid cancers[5].

Well differentiated thyroid carcinomas (DTC) account for 90% of all thyroid cancers, while medullary thyroid carcinomas (MTC) account for 2-3%, anaplastic carcinomas and poorly differentiated carcinomas for the remaining 7 to 8%. Well differentiated carcinomas are further subdivided histologically as papillary thyroid cancer (80–85%), follicular thyroid cancer (10–15%) and Hurtle cell carcinoma (3–5%). Overall, DTCs have a very good prognosis with long term disease free survival close to 95% for papillary thyroid cancers (PTC) and 80% for follicular thyroid cancers (FTC).

Medullary thyroid cancers are clinically classified as sporadic or familial cancers. Sporadic MTCs occur as localized cancers with infrequent lymph node involvement (unifocal) and correspond to 70% of all cases, while familial cancers are typically diagnosed as advanced disease (multifocal) in the remaining 30% of the cases[6]. Familial MTCs have been described as part of the multiple endocrine neoplasia (MEN 2a) syndrome which includes the presence of pheochromocytoma and parathyroid hyperplasia, and of the MEN 2b syndromes that also include pheochromocytoma and mucosal neuromas and/or gastrointestinal ganglioneuromas.

Most thyroid cancers are well-differentiated papillary carcinomas or follicular carcinomas and are associated with a low mortality rate, particularly in patients with stage I or II disease (survival rate more than 98%). However, a subset of these patients will have recurrent disease. In addition, patients who present with higher-stage disease or distant metastases and patients with poorly differentiated or anaplastic thyroid cancer have higher mortality rates[7]. Accurate identification of subsets of patients with risk factors for aggressive disease and higher mortality rates can help to guide management and prevent overtreatment of patients with low-risk disease.

In addition to environmental factors, genetic factors are involved in thyroid cancer predisposition. Aside from the well-characterized familial forms of medullary thyroid cancer, an individual whose first degree relative is diagnosed with non-medullary thyroid cancer has a fourfold to tenfold higher risk than those in the general population[8,9].

The last several years have seen dramatic advances in our understanding of the genomics of thyroid cancer. Major technological advances have allowed scientists to interrogate DNA and RNA changes at a depth and speed that were previously impossible. Several genetic abnormalities have been involved in the pathogenesis of thyroid cancers, that induce dysregulation of the MAPK and phosphatidylinositol-3 Kinase (P13K)/AKT signaling pathways[10]. (fig.1)

Mutations

1. RET/PTC rearrangements

The RET proto-oncogene is a 21-exon gene located on chromosome 10q11-2 that encodes a membrane tyrosine kinase receptor. It is expressed in thyroid C cells, but not in normal follicular cells[11].

The RET gene can be activated by fusion with various partners, in which the 3' or tyrosine kinase domain of the RET gene is fused with the 5' domain of a foreign gene. The foreign gene is constitutively expressed, resulting in a permanent expression of the rearranged RET gene.

These rearranged genes have coiled-coil domains that activate ret kinase through permanent dimerization. Because these rearrangements were originally found only in papillary thyroid cancers they were called RET/PTC[12,13].

Point mutation of the RET proto-oncogene results in an unregulated dominant activation of receptor.

RET mutation and medullary thyroid carcinoma

Somatic and germ-line mutations of the RET gene play an important role in the development of sporadic and familial forms of medullary thyroid carcinoma, respectively. Genetic diagnosis has an important role in differentiating sporadic from familial MTC. Furthermore, depending on the location of the mutation, patients can be classified into risk classes. Therefore, genetic screening of the RET gene plays a critical role not only in diagnosis but also in assessing the prognosis and course of MTC[14].

MTCs are characterized by activating mutations of RET proto-oncogene which occur prevalently as point alteration of codons. In 98% of MEN 2a families, a germ-line activating RET mutation can be detected. Germ-line RET mutations indicate hereditary MTC and determine the lifetime risk for developing MTC, which is nearly 100% for RET mutation carriers. A high prevalence of "*de novo"* RET mutations (over 50%) has been identified in MEN 2b patients, and to a lesser extent in MEN 2a/FMTC patients[15].

Also, germ-line RET mutations are frequently detected in apparently sporadic MTC patients, indicating the importance of genetic testing in all MTC patients, even without a clear indication for hereditary disease. Somatic RET mutations can be detected in tumor tissue of 40-60% of sporadic MTC patients. The contribution to tumor development of somatic RET mutations in MTC pathogenesis is unclear. Somatic RET mutations are not consistently distributed within primary tumors and metastases, indicating that the mutation can occur during progression of the tumor or that MTC is a disease of polyclonal origin [(15)] . Probably in these cases, somatic RET mutations merely contribute to the disease phenotype instead of causing it. (fig.2)

2. RAS mutations

The RAS genes (H-RAS, K- RAS and N- RAS) encode a 21 kD protein (p21) involved in signal transmission from cell membrane receptors to growth factors to the nucleus to both the MAPK and PI3K/AKT pathways. (fig.3)

The RAS gene is activated by point mutations mostly in codon 12 or 61 and sometimes in codon 13 or 59. In thyroid cancers, point mutations are found in the three RAS genes, with N-RAS being the most frequently mutated. RAS mutations are found in 10-20% of papillary thyroid carcinoma and in 25% of poorly differentiated thyroid cancers. RAS-mutated papillary thyroid carcinomas typically are of the follicular variant. RAS mutations are also seen in 20-40% of follicular adenomas[16].

RAS mutation mostly HRAS, KRAS and NRAS have been found in 68%of sporadic MTC without RET mutation[17].

3. Mutations in the P13K/AKT pathway

P13K/AKT pathway may be driven by activating RAS point mutation. Inactivating mutations or deletions of the tumor suppressor gene PTEN activate the P13K/AKT pathway and are the genetic basis for follicular thyroid cell tumorigenesis in Cowden's syndrome[18]. Activating mutations of PIK3CA are common in follicullar thyroid carcinoma, poorly differentiated thyroid carcinoma and anaplastic thyroid carcinoma. AKT1 mutations were only found in metastatic thyroid cancers. Promoter methylation of PTEN, which is consistent with the loss of its expression is associated with genetic alterations of P13K-AKT pathway in follicular thyroid carcinoma and anaplastic thyroid carcinoma, including mutations of RAS, PIK3CA and PTEN[19,20]. Over expression of proteins involved in this pathway is frequently found in aggressive thyroid cancers.

4. BRAF

The *BRAF* gene provides instructions for making a protein that helps transmit chemical signals from outside the cell to the cell's nucleus. This protein is part of a signaling pathway known as the RAS/MAPK pathway, which controls several important cell functions. Specifically, the RAS/MAPK pathway regulates the growth and division (proliferation) of cells, the process by which cells mature to carry out spe-

cific functions (differentiation), cell movement (migration), and the self-destruction of cells (apoptosis). Chemical signaling through this pathway is essential for normal development before birth[21,22].

Point mutation in BRAF are found in approximately 45% of papillary thyroid cancers(PTC) and less frequently in poorly differentiated and in anaplastic thyroid cancers .In most cases, the V600E mutation causes constitutive activation of this serine/threonine kinase. In few cases other BRAF mutations such as the K601E mutation, small in frame insertions or deletions, or BRAF rearrangement can occur[23,24].
BRAF mutations are identified in 60% of classic PTC , 80% of tall cell varient PTC, and only 10% of follicular variant PTC.

Many studies demonstrated a significant association of BRAFV600E with poor clinicopathological outcomes of PTC, including aggressive pathological features, increased risk of recurrence, loss of radioiodine avidity, treatment failures and mortality. However, other small number of studies have not confirmed the prognostic impact of BRAF mutation[25,26].

In addition to serving as a driver mutation in papillary thyroid cancer, activation of the MAPK pathway via BRAF mutation results in decreased expression of sodium iondine symporter, TSH receptor, and thyroglobulin resulting in relatively iodine refractory state[27].

5. Other genetic abnormalities

Mutations in hTERT have been found in follicular cell derived thyroid cancers, but were absent in benign lesions and in medullary thyroid cancers[28]. These hTERT mutations have a significantly higher prevalence in aggressive thyroid tumors including widely invasive oncocytic carcinoma and anaplastic thyroid carcinoma.

In papillary thyroid cancer, a hTERT mutation has been more frequently found in advanced tumors and in tumors with a BRAFV600E mutation, and the rate of recurrence is 8 times greater in tumors with both mutations, as compared to patients who lack both mutations[29].
Other important genes that are mutated in thyroid tumorigenesis include β-catenin (CTNNB1) that is involved in Wnt signaling, TP53 a tumor suppressor.These mutations have been mostly found in poorly differentiated and anaplastic thyroid cancer, in particular the TP53 mutations that may participate to dedifferentiation of these tumors[30,31].
Mutations in thyroid stimulating hormone receptor (TSH-R) are found in 40% to 60% of benign hyperfunctioning adenomas. Activating mutations of the TSH-R have also been found in the rare hyperfunctioning follicular carcinomas with high radioiodine uptake and thyrotoxicosis. The role of TSH-R and gsp mutations in the tumorigenesis of some hypofunctioning thyroid tumors is unclear[32].
In Hűrthle-cell thyroid carcinoma, mutations of NADH dehydrogenase (ubiquinone) 1α subcomplex 13 (NDUFA13 or GRIM19) are fairly common but classical genetic alterations frequently found in other thyroid cancer subtypes, such as RET/PTC, RAS or BRAF mutations are not found[33].
Oncogenic gene amplification or copy-number gains are more prevalent in poorly differentiated and anaplastic than in differentiated thyroid carcinomas, suggesting that these genetic alterations are important for the progression and aggressiveness of thyroid cancer. This is particularly the case for genes encoding receptor tyrosine kinases (RTKs) and for the genes encoding P13K-AKT pathway members. Many of the genes with copy-number gains are proto-oncogenes, and an increased protein expression will induce activation of the signaling pathways in which they are involved[34].
TRK rearrangements are found in 1-5% of papillary thyroid carcinomas and at higher frequencies in patients with a history of radiation exposure[35].

Anaplastic lymphoma kinase (ALK) gene was found in 9% of poorly differentiated thyroid cancers, 4% of anaplastic thyroid cancers, and 1% of papillary thyroid cancers[36]. The

PAX8/PPARγ rearrangement is found in 30-40% of follicular carcinomas. It is also found, at lower prevalence, in the follicular variant of papillary thyroid carcinoma and in follicular adenomas[37,38].

Altered Signaling Pathways in Thyroid Cancer

The MAPK and P13-1AKT signaling pathways

The importance of the MAPK pathway has been well established in the tumorigenesis of papillary thyroid cancer. The MAPK pathway is driven by activating mutations, including BRAF and RAS mutations, by RET/PTC, TRK or ALK rearrangements. One of these mutations was found in 70-80% of papillary thyroid cancers and genetic abnormalities are found in over 95% of cases by using next generation sequencing (NGS). These driver mutations are mutually exclusive, indicating that this mutation may be responsible for the occurrence of the papillary thyroid cancer. Activation of this pathway induces the activation of phosphatases, resulting in feedback mechanisms that will limit the activation of the pathway[39].

MAPK-mediated thyroid tumorigenesis involves a wide range of secondary molecular alterations that synergize and amplify the oncogenic activity of this pathway, such as genome-wide hypermethylation and hypomethylation and altered expression of miRNAs. Upregulation of various oncogenic proteins can occur that drive cancer cell proliferation, growth, migration and survival, as well as tumor angiogenesis, invasion and metastasis. These include chemokines, vascular endothelial growth factor A (VEGFA), C-MET, nuclear factor κB (NF κB), matrix metalloproteinases (MMPs), hypoxia-inducible factor 1α (HIF1α), transforming growth factor β1 (TGFβ1). Many of these proteins are key constituents of the extracellular matrix microenvironment[40,41].

All these alterations permit the distinction of two main groups of papillary thyroid cancers: one group includes tumors that harbor a RAS mutation and another group includes tumors that harbor a BRAF mutation. This second group is heterogeneous but in general tumors have a more aggressive and less differentiated phenotype.

The MAPK and P13K-AKT pathways are primarily involved in differentiated papillary and follicular thyroid carcinoma, respectively. As genetic alterations accumulate and both pathways become activated, the tumor progresses into poorly differentiated and anaplastic thyroid carcinoma[42]. The coexistence of multiple genetic alterations to members of the MAPK pathway also occurs, as exemplified by the simultaneous presence of BRAFV600E mutation, RAS mutations and RET-PTC in some aggressive recurrent papillary and anaplastic thyroid cancers. In metastatic tissues, the dominant genetic abnormalities present in the primary tumor are usually found with additional abnormalities in some.

Impairment of the Iodide-Handling Machinery

Aberrant activation of the MAPK pathway has a crucial role in the impairment of the iodide uptake and metabolism. In addition to serving as a driver mutation in papillary thyroid cancer, activation of the MAPK pathway via BRAF mutation results in decreased expression of NIS, TPO, TSH-R, and Tg resulting in a relatively iodine refractory state. Inhibition of the activated MAPK pathway with a BRAF inhibitor or a MEK inhibitor was able to restore the effectiveness of RAI therapy in mouse thyroid cancers with BRAF activation, and in small cohorts of RAI refractory differentiated thyroid cancer patients. Activation of the PI3K-AKT pathway was also shown to down regulate the iodide uptake and metabolism in thyroid cells both in vitro and in vivo[43].

Clinical implications:

Remarkable advances in the translation of molecular findings in thyroid cancer to the clinic have occurred recently.

Diagnosis of thyroid cancer

The diagnostic accuracy for thyroid nodules that are otherwise diagnostically indeterminate by

conventional cytology assessment is improved by the search for genetic markers in fine needle aspiration biopsy (FNAB) samples, including BRAF mutation, RAS mutations, RET-PTC and PAX8-PPAR rearrangements. More recently the diagnostic performance of this strategy was further improved by the use of NGS.
Also, the use of a gene-expression classifier permits to rule out malignancy in half of the nodules with indeterminate cytology results[44,45].

The differentiation of radiation induced thyroid tumors from thyroid tumors occurring in the absence of radiation exposure may benefit from mutation analysis. Rearrangements are more frequently and point mutations less frequently found after radiation exposure. Also the study of the expression of a panel of genes helps in this differentiation.

Prognosis

The prognostic application of BRAFV600E mutation has also been the subject of many clinical studies. It appeared that BRAFV600E is associated with poorer clinicopathological outcomes even in conventionally low-risk patients. However, the use of mutation status in clinical practice is unclear and most patients are treated according to their risk of death and of recurrence; furthermore in patients with refractory advanced disease, the RAS or BRAF mutation status did not appear to be an independent factor of survival or a predictive factor of response to anti-angiogenic therapy[42,46].

Medullary thyroid carcinoma:

MTC accounts for approximately 5-8% of all thyroid cancer. Clinically, MTC is mainly sporadic (70-80%), but hereditary pattern is present in 20-30% of cases, transmitted as an autosomal dominant trait[12]. The sporadic form of MTC is observed in 60-70 years old patients with a palpable thyroid nodule indistinguishable from any other thyroid nodule. Neck lymph node metastases are detected in at least 50% of patients and may reveal the disease. Metastases outside the neck, in liver, lungs or bones, are initially present in 10 - 20% of the cases. Hereditary MTC may be part of "multiple endocrine neoplasia type 2" (MEN2) and is divided into three clinical forms:
A) MEN2a is characterized by the presence of MTC in combination with pheochromocytoma and/or hyperparathyroidism. Cutaneous lichen amyloidosis has been observed in some families[13] and Hirschsprung's disease has been observed in a few families with MEN2a[47] .The typical onset of this condition is in the third or fourth decade of life. Nearly the 100% of gene carriers will develop MTC, but this depends on the mutation[14].
The risk of developing unilateral or bilateral pheochromocytoma is as high as 57% (both for germ-line mutations of codon 918 and 634), and 15– 30% of codon 634 mutations carriers will develop hyperparathyroidism.
B) MEN2b in which MTC is accompanied by pheochromocytoma, multiple mucosal neuromas and marfanoid habitus. MEN2b is the rarest and most aggressive form of MEN 2 based on its development of MTC earlier in life, usually before the age of 5-10 years. It is frequently associated with extension beyond the thyroid capsule, with lymph node and distant metastasis at the time of diagnosis. Patients also have chronic constipation and colonic cramping due to the presence of megacolon disorder. More than 50% of cases are "*de novo"* germline mutations. The higher mortality rate of MEN 2B reflects its more advanced stage at presentation, rather than the tumour behavior once established[46,47].
C) FMTC (Familiar Medullary Thyroid Carcinoma) occurs when MTC is the only clinical feature. Clinical presentation of cancer is at a later age and a relatively more favorable prognosis. The most rigid definition is multigenerational transmission of MTC in which no family member has pheochromocytoma or hyperparathyroidism; a less rigid definition is the presence of MTC in four affected family members without other manifestations of MEN 2A. Histology is peculiar in hereditary MTC; C-cell hyperplasia is always associated with hereditary MTC with bilaterality and multicentricity as a consequence when the patients over 5-years old, carry the codon 918 or codon 634 mutations.

Whereas, sporadic MTC generally presents as a single tumor confined to one thyroid lobe, except for a 5- 9% of patients. Tumor metastasizes early to paratracheal and lateral cervical lymph nodes; lymph nodes metastases are found in 20-30% of patients with MTC < 1 cm in diameter, in 50% with tumor > 2 < 4 cm and in up to 90% of the patients with tumors that are more than 4 cm in diameter or infiltrating surrounding tissues. The prognosis of MTC is intermediate between well differentiated and anaplastic thyroid cancer. Its prognosis is worse compared to papillary and follicular thyroid cancer and better than the prognosis in anaplastic thyroid cancer. Therefore, an early diagnosis is fundamental for a good prognosis in these patients[15]. Genetic abnormalities are present in MTC. Hereditary forms are characterized by germline mutations while sporadic MTC showed somatic alterations in 40-60% of patients[48,49].

Recently a French study has demonstrated that the prognostic factor of disease free survival after surgery in young patients with RET germline mutation is best predicted

by TNM staging and preoperative basal CT level below 30 pg/ml. Basal CT, class D genotype, and age constitute key determinants to decide preoperatively timely surgery[50]. Moreover, genetic screening of germline RET mutations permits the identification of unsuspected FMTC in apparently sporadic MTC patients. Therefore, RET genetic screening of patients with apparently sporadic MTC represents a major tool for the preclinical diagnosis and early treatment of unsuspected affected family members and allows the identification of a relevant percentage of hidden FMTC [(56)]. Given the high chance of a RET gene carrier of developing MTC at some point during their life, these patients should be offered prophylactic thyroidectomy. In cases of MEN 2a/FMTC mutations of ATA level C risk, prophylactic total thyroidectomy should be carried out before 5 years of age. In patients with RET mutations of ATA level A and B risk, prophylactic thyroidectomy may be delayed beyond the first 5 years in the setting of a normal basal and/or stimulated serum CT and normal annual cervical ultrasound starting at 5 years of age. Prophylactic level VI central compartment neck dissection may not be necessary in patients with MEN 2a/FMTC who undergo prophylactic thyroidectomy within their first 3-5 years of life unless there is clinical or radiological evidence of lymph node metastases, thyroid nodules >5 mm in size or a serum basal CT >40 pg/ml in a child >6 months old . Children with MEN 2b (ATA level D risk) should have thyroidectomy as soon as possible, preferably within the first year of life. Prophylactic level VI neck dissection may not be necessary in patients with MEN 2b, unless there is clinical or radiological evidence of lymph node metastases. In RET-mutation-positive patients, screening for pheochromocytoma, including annual plasma metanephrines and normetanephrines, or 24-h urine collection for metanephrines and normetanephrines, begins by 8 years of age in carriers of the RET mutation associated with MEN 2b and codons 630 and 634, and by 20 years of age in carriers of other MEN 2a RET mutations. Patients with RET mutation associated with FMTC alone should be screened periodically from 20 years of age. Abdominal imaging is not indicated in the absence of symptoms or biochemical data. Screening for hyperparathyroidism should be carried out with the same interval by measuring serum calcium and parathyroid hormone[51].

Molecular targeted therapy

The MAPK and P13-AKT pathways, from tyrosine kinase receptors in the cell membrane to the various downstream signaling relay molecules, such as BRAF, MEK, AKT and mTOR, are therapeutic targets that are being actively tested for treatment of RAI refractory thyroid cancer with novel small-molecule protein-kinase inhibitors. A promising therapeutic strategy is genetic-based targeting of thyroid cancer, as supported by many preclinical studies demonstrating the selective inhibition of BRAFB600E-mutant thyroid cancer cells targeting of the P13K-AKT pathway may also be genetically guided, as genetic alterations that activate this pathway confer thyroid cancer cells with remarkable increased sensitivities to AKT and mTOR inhibitors[52].

The involvement of multiple signaling pathways in aggressive thyroid cancer suggests that it may be necessary to target them simultaneously for effective treatment. Synergistic effects of simultaneously targeting the MAPK and P13K-AKT pathways were even more pronounced in cells that harbored genetic alterations in both pathways[53].

Summary and Conclusions:

From the 1990s, when pathogenesis of only approximately 25% of thyroid cancers was understood, to the present, when genes involved in the pathogenesis of over 90% of thyroid cancers have been described, much progress has been made in elucidating the molecular mechanisms underlying thyroid cancer. This progress provides the basis upon which new diagnostic and prognostic markers, as well as new targeted therapies, have been developed.

The molecular pathogenesis of differentiated thyroid cancer, with specific signaling pathways and activating point mutations, has been successfully elucidated. Some of the specific genes tend to be mutated in more aggressive and advanced thyroid cancer. Other discoveries include mutations in thyroid stimulating hormone receptor (TSHR) that were shown to play a role in thyroid tumorigenesis.

Medullary thyroid carcinoma (MTC), whether sporadic or hereditary, with or without other combinations, have specific genetic abnormalities in the RET proto-oncogene system. The issue of prophylactic thyroidectomy, and its timing, have been outlined and debated.

Ethical and psychosocial considerations of predictive testing for mutations have been discussed and debated.

Hereditary MTC, as part of MEN2, is one of the first hereditary syndromes to be described. The syndrome has been utilized as an example for understanding and analyzing the prediction of testing for gene carriers and timing of prophylactic thyroidectomy at the proper age in childhood.

Related ethical issues, including, the ethics and legality of genetic testing prior to employment and insurance, and involving other family members in hereditary cancer syndrome screening and management have been debated. Ethicists advocate safeguarding individuals and family members by strict measures of privacy and confidentiality.

The possibility of performing reimplementation genetic diagnosis (PGD) to identify genetic mutations in fertilized ova has not been discussed, so far, in medical-jurisprudence or *fatwa* forums, and should properly addressed.

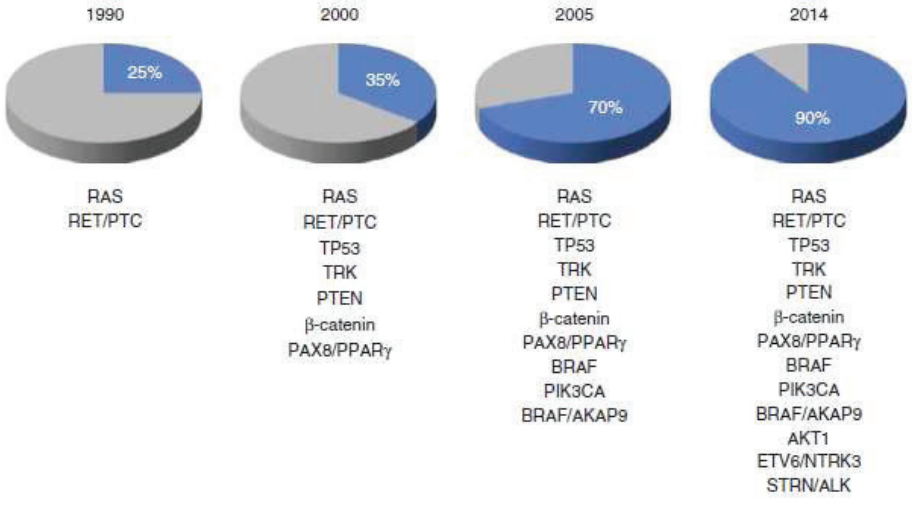

Figure 1
Progress in identifying mutational markers in thyroid cancer.

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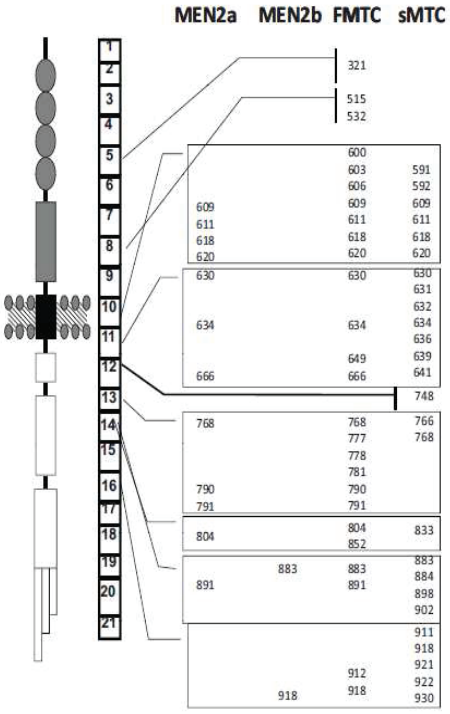

Fig. (2). Shows the RET receptor, the gene with its exons and the most common mutations in hereditary and sporadic MTC.

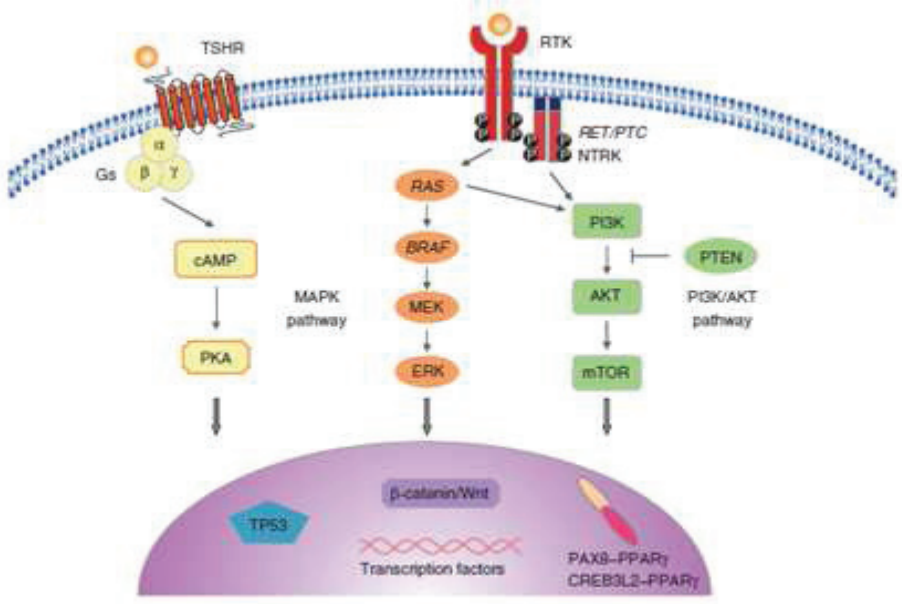

Fig.3 Molecular pathway in thyroid cancer. The molecular pathogenesis of thyroid cancer includes dysregulation of the MAPK, phosphatidylinistol-3kinase (PI3KYAKT, and the TSHR cAMP signaling pathway. Thae MAPK pathway is frequently activated in thyroid cancer through point mutation of the BRAF and RAS and RET/PTC.
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References:

1. Davies L & Welch HG 2006 -Increasing incidence of thyroid cancer in theUnited States, 1973–2002. Journal of the American Medical Association 295 2164–2167
2. Gabriella Pellegriti, Francesco Frasca, Concetto Regalbuto Worldwide Increasing Incidence of Thyroid Cancer: Update on Epidemiology and Risk Factors -Journal of Cancer Epidemiology Volume 2013 (2013), Article ID 965212 (American Cancer Society (ACA)2014)
3. Albores-Saavedra J, Henson DE, Glazer E & Schwartz AM 2007- Changing patterns in the incidence and survival of thyroid cancer with follicular phenotype – papillary, follicular, and anaplastic: a morphological and epidemiological study. Endocrine Pathology 18 1–7.
4. Brito JP, Yarur AJ, Prokop LJ 2013- Prevalence of thyroid cancer in multinodular goiter vs. single nodule: a systematic review and meta-analysis. Thyroid 23 449–455.
5. Burgess JR & Tucker P 2006- Incidence trends for papillary thyroid carcinoma and their correlation with thyroid surgery and thyroid fine-needle aspirate cytology. Thyroid 16 47–53.
6. Kloos RT, Eng C, Evans DB, Francis 2009 - Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid 19 565–612.
7. Hansford JR & Mulligan LM 2000 -Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis. Journal of Medical Genetics 37 817–827.
8. Moses W, Weng J& Kebebew E 2011 - Prevalence, clinicopathologic features, and somatic genetic mutation profile in familial versus sporadic nonmedullary thyroid cancer. Thyroid 21 367–371.
9. Jung CK, Little MP, Lubin JH 2014- The increase in thyroid cancer incidence during the last four decades is accompanied by a high frequency of BRAF mutations and a sharp increase in RAS mutations. Journal of Clinical Endocrinology and Metabolism 99 E276–E285.
10. Frich L, Glattre E & Akslen LA 2001- Familial occurrence of nonmedullary thyroid cancer: a population-based study of 5673 first-degree relatives of thyroid cancer patients from Norway. Cancer Epidemiology, Biomarkers & Prevention 10 113–117.
11. Fenton CL, Lukes Y, Nicholson D 2000 - The ret/PTC mutations are common in sporadic papillary thyroid carcinoma of children and young adults. Journal of Clinical Endocrinology and Metabolism 85 1170–1175.
12. de Groot JW, Links TP, Plukker JT 2006 -RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. Endocrine Reviews 27 535–560.
13. Santoro M, Dathan NA, Berlingieri MT 1994 - Molecular characterization of RET/PTC3; a novel rearranged version of the RETproto-oncogene in a human thyroid papillary carcinoma. Oncogene 9 509–516.
14. Agrawal N, Jiao Y, Sausen M, Leary R, Bettegowda C. 2013 Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in RET and RAS. Journal of Clinical Endocrinology and Metabolism 98 E364–E369.
15. A.Taccaliti*, F. Silvetti, G. Palmonella and M. Boscaro Genetic Alterations in Medullary Thyroid Cancer: Diagnostic and Prognostic Markers Current Genomics, 2011, 12, 618-625.
16. Wells SA, Robinson BG, Pacini F, et al. Hereditary medullary thyroid carcinoma. J Clin Endocrinol Metab. 2013; 98: 3148-3164
17. Marques AR, Espadinha C, Catarino AL.2002- Expression of PAX8–PPARg1 rearrangements in both follicular thyroid carcinomas and adenomas. Journal of Clinical Endocrinology and Metabolism 87 3947–3952.
18. Dahia PL, Marsh DJ, Zheng Z, Zedenius J. 1997- Somatic deletions and mutations in the Cowden disease gene, PTEN, in sporadic thyroid tumors. Cancer Research 57 4710–4713.
19. Garcia-Rostan G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R 2005 -Mutation of the PIK3CA gene in anaplastic thyroid cancer. Cancer Research 65 10199–10207.
20. Hou P, Liu D, Shan Y, Hu S, Studeman K. 2007- Genetic alterations and their relationship in the phosphatidylinositol 3-kinase/Akt pathway in thyroid cancer. Clinical Cancer Research 13 1161–1170
21. Chiosea S, Nikiforova M, Zuo H, Ogilvie J. 2009 A novel complex BRAF mutation detected in a solid variant of papillary thyroid carcinoma. Endocrine Pathology 20 122–126.
22. Ciampi R & Nikiforov YE .2005 Alterations of the BRAF gene in thyroid tumors. Endocrine Pathology 16 163–172.
23. Kim TY, Kim WB, Song JY. 2005 -The BRAFV600E mutation is not associated with poor prognostic factors in Korean patients with conventional papillary thyroid microcarcinoma. Clinical Endocrinology 63 588–593.
24. Adeniran AJ, Zhu Z, Gandhi M. 2006- Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. American Journal of Surgical Pathology 30 216–222.

25. Ito Y, Yoshida H, Maruo R, Morita S, Takano T. 2009- BRAF mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. Endocrine Journal 56 89–97.
26. Liu RT, Chen YJ, Chou FF. 2005 -No correlation between BRAFV600E mutation and clinicopathological features of papillary thyroid carcinomas in Taiwan. Clinical Endocrinology 63 461–466.
27. Ricarte-Filho JC, Ryder M, Chitale DA. 2009- Mutational profile of advanced primary and metastatic radioactive iodinerefractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Research 69 4885–489
28. Landa I, Ganly I, Chan TA, Mitsutake N. 2013 - Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. Journal of Clinical Endocrinology and Metabolism 98 E1562–E1566
29. Liu T, Wang N, Cao J, Sofiadis A, Dinets A. 2013- The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. Oncogene.
30. Garcia-Rostan G, Camp RL, Herrero A.2001 b-Catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis.American Journal of Pathology 158 987–996.
31. Dobashi Y, Sugimura H, Sakamoto A.1994 Stepwise participation of p53 gene mutation during dedifferentiation of human thyroid carcinomas. Diagnostic Molecular Pathology 9–14.
32. Donghi R, Longoni A, Pilotti S. 1993- Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. Journal of Clinical Investigation 91 1753–1760.
33. Nishihara E, Amino N, Maekawa K. 2009- Prevalence of TSH receptor and Gsa mutations in 45 autonomously functioning thyroid nodules in Japan. Endocrine Journal 56 791–798.
34. Liu Z, Hou P, Ji M. 2008- Highly prevalent genetic alterations in receptor tyrosine kinases and phosphatidylinositol 3-kinase/akt and mitogen-activated protein kinase pathways in anaplastic and follicular thyroid cancers. Journal of Clinical Endocrinology and Metabolism 93 3106–3116.
35. Leeman-Neill RJ, Kelly LM, Liu P. 2014- ETV6–NTRK3 is a common chromosomal rearrangement in radiationassociated thyroid cancer. Cancer 120 799–807.
36. Kelly LM, Barila G, Liu P, Evdokimova VN. 2014 -Identification of the transforming STRN–ALK fusion as a potential therapeutic target in the aggressive forms of thyroid cancer. PNAS 111 4233–4238.
37. Leeman-Neill RJ, Brenner AV, Little MP 2013- RET/PTC and PAX8/PPARg chromosomal rearrangements in post-Chernobyl thyroid cancer and their association with iodine-131 radiation dose and other characteristics. Cancer 119 1792–1799.
38. Xing M, Westra WH, Tufano RP. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *J Clin Endocrinol Metab*. 2005;90:6373–6379
39. Bongarzone I, Vigneri P, Mariani L, Collini P, Pilotti S & Pierotti MA 1998 RET/NTRK1 rearrangements in thyroid gland tumors of the papillary carcinoma family: correlation with clinicopathological features. Clinical Cancer Research 4 223–228.
40. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA. 2003- High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC–RAS–BRAF signaling pathway in papillary thyroid carcinoma. Cancer Research 63 1454–1457.
41. Liu X, Bishop J, Shan Y. 2010- Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocrine-Related Cancer 20 603–610.
42. Musholt TJ, Musholt PB, Khaladj N. 2000- Prognostic significance of RET and NTRK1 rearrangements in sporadic papillary thyroid carcinoma. Surgery 128 984–993.
43. Xing, M., BRAF mutation in papillary thyroid microcarcinoma: the promise of better risk management. Ann Surg Oncol, 2009. **16**(4): p. 801-3.
44. Baloch ZW, LiVolsi VA, Asa SL. 2008- Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagnostic Cytopathology 36 425–437.
45. Bartolazzi A, Orlandi F, Saggiorato E. 2008- Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: a prospective multicentre study. Lancet Oncology 9 543–549.
46. Verrienti A, Carbone A, Bellitti P, Fabiano MC, De Rose RF, Maranghi M, et al. Anovelduoble mutation VAL648ILE and VAL 804 LEU of RET protooncogene in multiple endocrine neoplasia type 2. *Endocr Pract*. 2015 Aug 6
47. Kluijfhout WP, van Beek DJ, Verrijn Stuart AA, Lodewijk L, Valk GD, van der Zee DC, et al. Postoperative Complications After Prophylactic Thyroidectomy for Very Young Patients With Multiple Endocrine Neoplasia Type 2: Retrospective Cohort Analysis. *Medicine (Baltimore)*. 2015 Jul. 94 (29):e1108

48. Lallier M, St-Vil D, Giroux M, et al. Prophylactic thyroidectomy for medullary thyroid carcinoma in gene carriers of MEN2 syndrome. *J Pediatr Surg*. 1998 Jun. 33(6):846-8
49. Machens A, Lorenz K, Dralle H. Progression of Medullary Thyroid Cancer in RET Carriers of ATA class A and C Mutations. *J Clin Endocrinol Metab*. 2013 Dec 2.
50. Ainahi Abdelhakim, Barlier Anne, Roche Catherine[2]Journal of Cancer Research and Therapeutics, Vol. 5, No. 3, July-September, 2009, pp. 198-202
51. Cote GJ. RAS mutations in medullary thyroid cancer. ICE/ENDO 2014, Newly-Identified Gene Rearrangements & Mutations in Thyroid Cancer Symposium, , June 23, 2014
52. Solomon B, Rischin D. Progress in Molecular Targeted Therapy for Thyroid Cancer: Vandetanib in Medullary Thyroid Cancer. *J Clin Oncol*. 2011 Oct 24
53. Tufano RP, Teixeira GV, Bishop J. 2012- BRAF mutation in papillary thyroid cancer and its value in tailoring initial treatment: a systematic review and meta-analysis. Medicine 91 274–286

STEM CELLS AND HUMAN CLONING

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Abstract

Recent advances in stem cell and cloning research have made steady progress and created hopes to cure disease entities deemed incurable in the past.
In view of ethical concerns, stem cell-based research and possible clinical applications have witnessed new hopes with new reprogramming technologies that produced induced pluripotent (iPSCs) stem cells from various somatic tissues. These cell lines could be further programmed to form the desired cell types, with promise in regenerative medicine and pharmacogenetics.
Other significant hopeful stem cell types are human adult (non-embryonic) and animal stem cells, which have already been used clinically.
Human cloning is currently more based on the technologies of somatic cell nuclear transfer (SCNT). Reproductive human cloning has reached a closed end, in view of both ethical considerations and technological failures.
Therapeutic cloning, on the other hand, has witnessed steady progress with promise in regenerative medicine, disease prevention and organ transplantation.

This presentation will highlight updates in these two inter-related issues from the aspects of technology, research, clinical and ethical dimensions.

Keywords: Stem cells, reproductive cloning, therapeutic cloning, regenerative medicine, ethics.

Stem cells

Stem cells (SCs) are primitive, pluripotent or multipotent cells that have the capability of differentiating into specialized, committed cells of various body tissues and organs.
Advances in stem cell research have progressed steadily over the past three decades, with tens of thousands of publications across the world[1].
More than 700 companies are working worldwide in areas of SCs utilization in regenerative medicine, which have witnessed significant recent advances[2].
Impressive therapeutic products have been marketed to cure diseases considered incurable in the past.
Other hopes include gene therapy, functional genomics and pharmacogenomics.
Reviewing recent literature, the following types of stem cells, related to their origin, have been described:

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Human embryonic stem cells (hESC)

They are obtained from the earliest stages of embryos: the morula and the inner cell mass of the blastula, within the first 5-7 days after fertilization. Special media and feeder cells were designed to grow and maintain hESCs, to acquire the capacity of self-renewal, and to maintain their characteristic of being flexible pluripotent cell lines[3-5].
Compared to other types of stem cells, hESCs are the most stable (immortal), with distinguished pluripotency and capability of indefinite, unlimited expansion in vitro, as undifferentiated colonics.
It is also possible to produce disease-specific[5] stem cells by the technology of nuclear transfer from patients with cancer and other diseases.

HESCs could be genetically manipulated by transfecting them with DNA constructs[6] for specific desired properties.
HESCs have been produced from aborted fetuses but primarily from excess human embryos in in-vitro fertilization (IVF) centers.
Optimization and sophistication of culture conditions of hESCs is important for safety, and proper differentiation into the desired specific cell lines. Prevention of contamination or infection, with minimal human handling, is an important part of the current culture techniques.
Moreover, for research purposes, disease-specific hESCs were derived from embryos with diagnosed mutations by pre-implantation genetic diagnosis (PGD), such as Fanconi disease, cystic fibrosis[7].....etc.
HESCs are considered one of the most clinically promising sources of SCs, but their use may result in tumor formation. HESCs have been tested in research centers[8] as a cure for retinal diseases which so far, are incurable, but were reported to pose risk of teratoma formation. While hESCs have great potential of curing diseases and advancing regenerative therapy in humans, their use raises major ethical concerns because they cannot be obtained without causing destruction of a human embryo[1].
HESCs are considered by some as morally equivalent to a human being, especially by the Roman Catholic Church[9]. Followers of many other faiths and ethical denominations do not share this view, and accept using them for research and in regenerative medicine[10,11].

Induced pluripotent stem cells (iPSCs)

Human-induced pluripotent stem cells (iPSCs) could be derived from various somatic tissue sources, by reprogramming. Most past work was on fibroblasts, whereby somatic cells could be "instructed" to become pluripotent. Their use circumvents ethical and immunological concerns related to hESCs.
They have the capacity of unlimited self-renewal and are pluripotent[12,13].
IPSCs could be programmed to form the desired cell types in vitro, and represent promise in cell therapy for many diseases[14].

Human (adult) non-embryonic stem cells

To avoid ethical concerns, somatic or adult human stem cells have been resorted to.

Adult stem cells normally exist in all body tissues for continuous repair and regeneration albeit in very small numbers. Sources of adult stem cells used in therapy include bone marrow[15], peripheral blood, cord blood[16], mesenchymal cells from adipose tissue[17], exfoliated deciduous teeth[18], and other tissue types.

These stem cell types are easily accessible, and pose no ethical concerns.

Genetic engineering of stem cells

All types of stem cells could be differentiated into various cell types in the body. Special bioreactors have been designed to provide for the desired tissue-specific functions[19].
In molecular cloning, a DNA sequence of interest is isolated, and multiple copies are obtained in vitro, with increasing uses in microbiology in the context of recombinant vaccines, antigens, cytokines…etc. These advances are currently utilized in screening for HIV,HCV, HBV and CMV[20].

Stem cells derived from somatic cell nuclear transfer (SCNT)

This has been recently developed and holds promise in regenerative medicine[21].
SCNT-derived stem cells share the same totipotent capabilities of embryonic stem cells. Their use, however, raises ethical concerns in view of their source from cloned embryos which, in view of some ethicists, share a common moral status as natural embryos. If applied with interspecies, (iSCNT) could be instrumental in production of transgenic animals to cure human disease and produce therapeutics. Moreover, it could be used for conservation of endangered animal species[22].

Animal stem cells

Genetically modified pig mesenchymal stromal stem cells (PMSCs) could potentially be utilized in xenotransplantation[23]. They could also be used in regenerative medicine because of their anti-inflammatory and immunomodulatory effects. They are easy to obtain in large quantities, and their genetic modification can be related to the therapeutic goal of MSCs.

Hematopoietic stem cell transplantation

This type of transplantation was one of the main successes of stem cell-based therapy. Significant achievements were acquired in many hematological malignancies[24]. One of the challenges is the behavior of the immune system in the transplant recipients, particularly the development of graft versus host reaction that could be serious. Strategies to deal with this possibility and to improve graft function are being further developed.
Other non-malignant disorders, including hemoglobinopathies, inborn errors of metabolism, some autoimmune conditions and others, have been treated with stem cell transplantation. Stem cells for these trials can be derived from bone marrow, peripheral blood or umbilical vein cord blood[25].

Ethical, Moral and Religious Concerns

The Roman Catholic Church, and many ethicists in the West, have strong objections on stem cell research and its clinical applications, considering it as aggression on early human life[9].
Islamic ethical perspectives of stem cells in research and medical applications were addressed by several forums and individual scholars[26-28].
The Council of Islamic*Fiqh* Academy- Muslim World League, in its 17th session held in Makkah 2003 (1424H) issued the following decree[29]:
After due consideration of scientific research and opinion of members and experts on this subject matter, the following decision was adopted:

First:

It is permissible to obtain stem cells, to culture and use them in research, with the intention of curing human disease, or to perform permissible scientific research, if their sources are duly permitted. The following are examples of permitted sources of stem cells:
1.Adults, if they consented, provided that it will not cause harm to them.

2.Children, with consent of their parents or guardians, provided that it will not cause harm to them.
3.Placenta and umbilical cord, with parents' consent.
4.Spontaneously aborted fetuses, or those fetuses which were aborted for an approved therapeutic reason, with parents' consent.
5.Surplus fertilized ova, if they existed in centers of assisted conception, with parents' consent, with strict assurance that such ova will never be used to induce illegal pregnancies.

Second:

It is not permissible to obtain stem cells and utilize them if their source is impermissible.

Examples:
1.Inducing unapproved abortion with the aim of obtaining stem cells.
2.Intentionally fertilizing human donated ova-sperm with the aim of producing stem cells.
3.Therapeutic human cloning.

This jurisprudence decision was a significant landmark in the area of stem cell research. It opens the doors in Islamic countries for ethical stem cells research.

It is pertrinent to state that recent advances and technologies in areas of adult (non-embryonic), induced pluripotent (iPSCs) and animal stem cells, that have been used, or have potential uses to prevent or cure diseases, make it incumbent on scientists and Muslim Jurists to undertake further discussions to arrive to new *Shari'ah*-approved stand points.

Human Cloning

Cloning of plants, animals and other organisms has been going on for many years, aiming at production of food materials and medical products in desired quantities and qualities.

The announcement of the successful cloning of the sheep "Dolly" in 1997 heralded a worldwide attention from amongst the scientific, ethical, legal communities and the public at large.

In summary, mammalian cloning is based on the technology of somatic cell nuclear transfer (SCNT). The nucleus of a somatic cell is transferred into an enucleated ovum, under specific conditions. This ovum becomes "fertilized" by the somatic nucleus, acting similarly to an ovum fertilized by a sperm, enters into the usual cascade of cell division ending up as an embryo[30-31].

This scientific and technological breakthrough has stirred worldwide debate in view of its possible application to human cloning.

Cloning of embryos has been used in the breeding of cattle and sheep since the late 1980s. The technique started by producing monozygotic (identical) twins, triplets or quadruplets[32]. Subsequently, ambitious researchers became interested in the area of human cloning using the technology of SCNT.

Despite governmental and ethical groups' limitations and objections, some researchers did not conceal their plans to enter the arena of human cloning to produce adult individuals.

Richard Seed, of Illinois- USA, announced that his human cloning clinic was close to attempting the cloning of humans[33].

The South Korean veterinarian Woo Suk Hwang, published his first paper in February 2004[34], and a second one in May 2005[35], in which he reported the first astounding success in creating human stem lines from cloned human embryos[36].

In November 2005, amid increasing evidence of falsification of data, Hwang admitted that he had lied[37], and in January 2006, Hwang's human cloning research was deemed fraudulent by an investigation committee from his university (SNO).

Reproductive cloning

This technique uses SCNT technology to fertilize eggs with somatic cells which could later develop into a human being. Philosophically, it is not possible to clone a human individual, with identical genetic and phenotypic qualities to the person from whom the somatic cells were derived[37].
Cloning of the genome of an individual is possible, despite significant technical obstacles, but the individual as a whole cannot be cloned. This is in view of the many crucial influences of environment, lifestyle, education, and society as well as the individual's own intellectual abilities, moral and religious values, and other characteristics acquired by his or her experience, learning, and imitation through life from conception to death[37].
Another hurdle is the immense technological obstacles. The group of Ian Wilmut of the Roslin Institute in Scotland, succeeded with "Dolly" only after more than 270 attempts, that induced only 13 pregnancies, and only one culminated into a live birth, Dolly.
The success rate for cloning animals has notably improved over the years, but without ever reaching 100%. Presently, cloned animals include mice, rats, goats, dogs, sheep, cows, pigs, horses and others.
The great majority of pregnancies end in spontaneous abortion[38]. Moreover, fetal death occurs close to term.
If animals survive after birth, serious health problems are encountered, such as gross obesity, disfigured limbs, dysfunctional immune systems, with maladies of liver, kidney and other systems[37].
It is significant to note that "Dolly" had to be euthanized in 2013 at 6 years of age in view of severe health deterioration with progressive lung disease and arthritis[39,40].
The cloning technologies have not yet been well developed to an extent that would produce a healthy human individual[37].However, the low success rate of cloning may improve in the future through technical advances.

Therapeutic cloning

This technique is also termed nuclear transplantation therapy. Contrary to reproductive cloning, which is inefficient and error-prone, therapeutic cloning does not suffer these major handicaps, because the process depends on the selection of functional pluripotent stem cells with the potential to differentiate into any of the three germ layers characteristic of humans and other animals: endoderm, ectoderm and mesoderm[41].
Using the technology of somatic cell nuclear transfer (SCNT), it generates autologous embryonic stem cell (ESC) lines derived from cloned embryos for the purpose of tissue replacement, thus eliminating immune rejection as the transplanted tissue or organ is derived from the same individual and has the same DNA.
The process, moreover, could be repeatedly conducted in view of its renewable source.
This technology is very promising in:
-Regenerative medicine.
-Therapy or prevention of diseases.
-Organ and tissue transplantation.

Subsequently, researchers started to combine nuclear cloning with gene and cell therapy.
Nuclear-transfer ESCs have been derived from mouse cumulus or fibroblast cells which could be programmed, or induced (iPSCs) into becoming somatic cells of the desired tissues to be treated or replaced.
It has to be stated that SCNT is more effective and less costly than the iPSCs.

At the present time, therapeutic cloning is widely used in bone marrow transplantation. Blood stem cells are used in the therapy of sickle cell anemia.
Promising utilizations of therapeutic cloning include[42]:

- Organs grown for transplantation by using stem cells that have the genome of the recipient.
  Therapeutic cloning using stem cells that have the genome of the recipient of organ/tissue transplantation needs further progress to overcome two hurdles: the possibility of immune rejection, and the availability of organs from suitable donors[37].
- Another anticipated regenerative application is therapeutic growth of nerve cells.

Currently, the one gene therapy that can be practiced is mitochondrial replacement therapy (MRT)[43], which has been recently legalized in the UK. This issue has been addressed in a separate chapter in this volume of FIMA yearbook.
MRT, however, has limited success rate in view of heteroplasmy effects, and also if utilized for genetic conditions that appear in older age.
Very recently, the technology of mesenchymal stem cells has shown possible uses in regenerative medicine. These cells could be obtained, with relative ease, through noninvasive methods from adult tissues. They have promising uses for autologous cell transplantation, and regenerative medicine, without the risk of immune rejection.
These SC have the important features of high yield, plasticity, immunomodulatory and anti-inflammatory effects, and lack of tumorigenicity.
Another recent development is the technology of induced pluripotent stem cell (iPSC) production. These cells can be derived from somatic cells through gene programming, and could be used in regenerative medicine and other utilizations of therapeutic cloning. This technology avoids embryo destruction to generate the needed pluripotent cells, an undertaking that avoids ethical implications related to embryonic stem cells therapy.
Like mesenchymal stem cells, iPSC scan be used for the same medical purposes[44].

Ethical, social and religious perspectives of cloning

Apart from the limitations and shortcomings of cloning technology, it has provoked worldwide controversies and opposition from the scientific, legal, ethical, religious communities and the public at large[45,46].
Opposition to human cloning research began soon after the birth of "Dolly" in 1997[41, 42].
In June 1997 and 1999 the Southern Baptists Annual Conventions passed resolutions supporting a US governmental ban on funding human cloning and asked that such cloning be prohibited.
In August 2000 the Roman Catholic Church issued a ban on human cloning, considering any form of experimenting on human embryos, ova or sperms as unapproved aggression on early human life[9].
Many countries have prohibited human cloning[47-48].
On March 12, the Canadian Parliament passed legislation banning human cloning, the sale of sperm, and payment to egg donors and surrogate mothers.

On July 9, 2004 the French Parliament adopted a new bioethics law considering human cloning a "crime against the human species" with prison punishment of up to 20 years.

In UK, the Human Fertilization and Embryology Act of 2008 explicitly prohibited reproductive cloning.

In the US, 13 states banned reproductive cloning and 3 states prohibited use of public funds for research on reproductive cloning[49]. Surprisingly, some researchers seemed not to be deterred by these measures, or by the reported high mortality, deformities and other genetic disorders in animal cloning, and human cloning research is going on overtly and covertly.

In the Islamic World, every Islamic seminar, *Fatwa* council or individual scholar had considered cloning procedures aiming at producing human clones as not permissible. The majority of scholars considered it *Haram* (forbidden) in all its details (on its own features). Others considered it *Haram* as a way to prevent a cause of harm, an opinion keeping possible exceptions to be entertained in the future, in case new information about benefits becomes more clear and approved by *Shari`ah*. Such exceptions, when and if they arise, should be discussed, as per case basis, in the light of the *Shari`ah*.

There was discussion of the case of using nuclear transfer technology between the husband and wife. Even this issue was not approved as per the following reasons mentioned below,for the purpose of reproductive cloning.

All scholars unanimously approved the prohibition of introducing any third party element upon the marriage relationship, whether this party is a womb, ovum, sperm, or somatic cell for cloning[50-53].

The Basis for Prohibition (*Tahrim*)

1. The basic concept in reproduction is to abide with the Shari`ah -approved system of legally binding marriage, through the union of sperm and ovum.
2. Human cloning is against the natural process (*Fitrah*) of human relationships of marriage and reproduction.
3. The major harms far exceed any benefits. Such harms include disturbance and impurity of lineage, family relations, social structure, and disruption of many Shari`ah's principles dependent on lineage. There is major difficulty in classifying the "cloned" person, as a son (or daughter), or identical twin of the person from whom the somatic nucleus was derived.
4. The issues of identical features of the clones, and their social, moral, psychological and legal implications.
5. The problem of safety: There is no guarantee that cloned humans will be normal, either shortly after birth, or later in life. In cloned animals, mortality, deformities and genetic disorders and premature ageing were reported. There is no reason to assume that such problems will not take place in human clones. Another safety concern is the likelihood of concentration and proliferation of certain genetic illnesses, mutations…etc, which are expected from the lack of neutralization of lethal genes as a result of human cloning. It is known that natural union of ovum-sperm neutralizes or abolishes many lethal genes.
6. The likelihood of disturbance of the time-honored balance between males and females, when left to the wishes of people.

Concluding remarks

Stem cell research and medical applications have progressed significantly over the past few years.

In some medical disorders, suchas hematopoietic malignancies, other malignant and non-malignant diseases, stem cell transplantations have been very successful.

In most other hoped-for clinical applications, they are still at investigational or pre-clinical stages.
Human ESCs are more flexible, stable, durable and pluripotent than other types of SCs. Ethical concerns of hESCs research have been largely minimized by careful derivation form excess fertilized ova, or from pre-implantation human embryos in IVF centers. This allows for more research towards clinical applications.
Technologies of other types of SCs, including human non-embryonic and animal stem cells have progressed with promise in several research and medical applications, with significantly limited ethical obstacles.
There are various ethical/religious opinions related to SC research and its medical applications, which range from complete rejection to liberal (unquestioned) approval. Islamic bioethics in general, and specifically in regards to stem cell research and cloning,is based on flexible interpretation of *Shari`ah* (Divine Law). Jurisprudence (*fiqh*) is characterized by careful intellectual balancing (*Ijtihad*) with broad understanding of benefits of relieving human suffering, quest for new knowledge, versus the need for proper regulation/governance. That is exposing and barring of irresponsible aggressive undertakings to change human genetic blueprint and tampering with human non-pathologic qualities.
Moreover, Islamic guidance prohibits any form of monopolization of technologies and therapies that will create and widen gaps among individuals, social classes and nations.

Human reproductive cloning is out of the picture, technologically, ethically and legally.
As for therapeutic cloning, ethical and religious debates are still intense.
In the Islamic World, this issue was discussed in scientific-jurisprudence forums, and clear rulings were issued and published, as outlined earlier.

It should be stated, however, that all Islamic *Shari`ah* opinion was formulated prior to the successes of newer technologies that pose no aggression on early human embryos, namely the technologies of induced pluripotent stem cells (iPSCs) and their uses in therapeutic cloning. This issue needs further discussions between scientific and jurisprudence scholars.

References

1. De Miguel- Beriain B, The ethics of stem cell revisited. Advanced Drug Delivery Reviews 82-83 (2015)" 176-180.
2. Alliance for Regenerative Medicine Annual IndustryReport, http://alliancerm.org/news/download-must-have-industry-report2014 (last accessed: 28 August 2014).
3. Thomsom J, Itskovitz-Eldor J, Shapiro S et al: Embryonic stem cell lines derived from human blastocytes. Science 1998, 282(5391): 1145-7.
4. Susan Okie: Stem cells research- Signposts and Roadblocks. N Engl J Med 2005; 353" 2728-b. www.NEJM.org
5. Alan Trounson. The production and directed differentiation of human embryonic stem cells. Endocrine Review 2006; 27(2): 208-219.
6. Eiges R, Rchuldiner M, Drukker M, et al. (2001) Establishment of human embryonic stem cell transfected clones carrying a marker for undifferentiated cells. CurrBiol 11:514-518.
7. Pickering SJ, Minger SL, Patel M, Taylor et al. (2005) Generation of a human embryonic stem cell line encoding the cystic fibrosis mutation delta F508, using preimplantation genetic diagnosis. Reprod Biomed Online 10:390-397.
8. Alonso-Alonso MI, Srivastava GK, Current focus of stem cell application in retinal repair.

World J Stem Cells. 2015, April 26; 7(3): 641-8. doi:10.4252/wjsc.v7.i3.641
9. Sacks J: Ethical Issues at the start of life. Clinical Medicine (JRCP), London, 2001, 1,(5): 401-406.
10. Suarez A, is this cell entity a human being? Neural activity, spiritual soul, and the status of the inner cell mass and pluripotent stem cells, in: A. Surarez, J. Huarte (Eds). Is this cell a human being? Springer, 2011, pp 171-192.
11. Devolder K, Savulescu J. The moral imperative to conduct embryonic stem cell and cloning research, Camb. Q. Health. Ethics 15(1) (2006) 7-21.
12. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131:861-72.
13. Sun N, Panetta NJ, Gupta DM, et al. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. ProcNatlAcadSci USA 2009; 106:15720-5.
14. Palmo AB, Lucas m, Dilley RJ, et al: The power and the promis of cell reprogramming: personalized autologous body organ and cell transplantation. J Clin Med. 2014 Apr4;3(2):373-87.doi:10.3390/jcm3020373
15. Liao HT, Chen CT. Osteogenic potential: comparison between bone marrow and adipose-derived mesenchymal stem cells. World J Stem Cell. 2014, Jul 26; 6(3): 288-95. Doi:10.4252/wjsc.v6.i3.288
16. Solh M. Haploidenticalvs cord blood transplantation for adults with acute myelogenous leukemia. World J Stem Cells. 2014Sept26;6(4):371-9. doi:10.4252/wjsc.v6.14.371.
17. Tsuji w, Rubin JP, Marra KG. Adipose-derived stem cells: Impliations in tissue regeneration. World J Stem Cells. 2014 July 26,6(3):312-21.Doi: 10:10.4252/wjsc.v6.i3.312
18. Annibali S, Cristali MP, Tonoli F et al. Stem Cells derived from human exfoliated deciduous teeth: A narrative synthesis of literature. Euro Rev Med Pharmacol Sci. 2014, Oct; 18(19): 2863-81.
19. Liu M, Liu N, Zang R. et al. Engineering stem cells niches in bioreactors. World J Stem Cells. 2013 Oct 26; 5(4): 124-35. Doi:10.4252/wjsc.v5.i4.124
20. Sharma K, Mishra AK, Mehraj V, et al. Advances and applications of molecular cloning in clinical microbiology. Biotechnol Genet Eng Rev. 2014 Oct 30(1-2): 65-78. Doi:10.1080/02648725.2014.921501
21. Baker M., Stem Cells made by cloning adult humans. Cell lines made by two separate teams could boost the prospects of patient- specific therapies, Nature (April 28 2014). http://dx.doi.org/10.1038/nature.2014.15107
22. Verma G, Anora JS, Seth: RS. et al. Handmade cloning: Recent advances, potential and pitfalls, J AnimBiotechnol. 2015 Oct. 15; 6:43. doi; 10.1186/s40104-015-0043-Y. ecollection 2015.
23. Li J, Ezzelarab MB, Ayares D, et al. The potential role of genetically-modified pig mesenchymal stromal cells in xenotransplantaiton. Stem Cell Rev. 2014 Feb; 10(1): 79-85. doi:10.1007/s12015-013-9478-8
24. Corey Cutler and Joseph H Antin.An overview of Hematopoietic Stem Cell Transplantation. Clinics in Chest Medicine, Vol 26, Issue 4, December 2005.
25. Negrin R. Sources of hematopoietic stem cells, UpToDate 2007. http://www.uptodate.com/retrievedApril 2007
26. Islamic Organization For Medical Sciences (IOMS)-Kuwait. The International Seminar on: Dilemma of Stem Cells: Research, Future and Ethical Challenges. November 3-5, 2007. http://www.islamset.com
27. Albar MA. Stem cells, Ethical and Jurisprudence Issues. Publisher: Al-Dar al-Saudiyyah, Jeddah, Saudi Arabia, 1423H/2002.
28. Fadel HE: Developments in stem cell research and therapeutic cloning: Islamic ethical positions, A Review. Bioethics.2012, Mar;26(3):128-35. http://dx.doi.org/10.111/j.1467_8519.2010.01840
29. The Council of Islamic Fiqh Academy-Muslim World League, 17th Session held in Makkah, 13-17 December, 2003.
30. Campbell Kh, McWhir J, Ritchic WA, Wilmut I: Sheep cloned by nuclear transfer from a cultured cell line Nature 1996; 380:64-6
31. Wilmut I, Schnickc AE, McWhir J, Kind A, and Campbell K, Viable offspring derived from fetal and adult mammalians cells Nature 1997; 385:810-3.
32. Ethical aspects of human cloning: What is embryo cloning? http://ww.religioustolerance.org/clo_intr.htm
33. Ayala FJ (1982) Population and Evolutionary Genetics. A Primer (Benjamin/Cummings, Menlo Park, CAa).
34. Hwang, W.S. et al. Science 303, 1669-1674 (2004).
35. Hwang, W.S. et al. Science 308, 1777-1783 (2005).

36. 36.Lee, B.C. et al. Nature 438, 536-537 (2005).
37. 37.Ayala FJ. Cloning humans? Biological, ethical and social considerations. Proc-NatlAcadSci-USA (PNAS), July 21, 2015. V.112 (29)8879-86. www.pnas.org/cgi/doi/10.1073/pnas.1501798112
38. Jabr F (2013 Will cloning ever save endangered animals? Sci Am. Available at www.scientificamerican.com/article/cloning-endangered-animals/. Accessed March 1,2015.
39. Highfield R (2007). Dolly creator Prof. Ian Wilmut shuns cloning. Daily Telegraph. Prof-Ian-Wilmut-huns-cloning.html. accessed March 1,2015.
40. Shiels PG, et al. (1999) Analysis of telomere lengths in cloned sheep. Nature 399(6734):316-317.
41. Carpenter MK, Inokuma MS, Denham J, et al. Enrichment of neurons and neural precursors from human embryonic stem cells. ExpNeurol 2001; 172:383-97.
42. Hochedlinger K, Jaenisch R. nuclear Transplantion, Ebryonic Stem cells, and the Ptoential for cell N Engl J Med 2003; 349: 275-86.
43. Schuldiner M, Yanuka O, Itskovitz-Eldor J, et al. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. ProcNatlAcad USA 2000; 97:11307-12.
44. Zomer HD, Vidane AS, Goncalves NN, et al. Mesenchymal and induced pluripotent stem cells: General insights and clinical perspectives. Stem cell cloning. 2015 Sep. 28'8:125-34. Doi:10.2147/SCCAA.S88036.
45. Human Cloning: Recent developments, 1997 to the present time. http://www.religioustolerance.org/clo_rece.htm
46. Adult DNA Cloning topics. http://www.religioustolerance.org/clo_rece.htm
47. Writer S (2008) Mps support embryology proposais. BBc News Online.Available at news.bbc.co.uk/2/hi/uk_news/politics/7682722.stm.accessed March 1,2015.
48. UK Statute Law Database (2001) Text of the human fertilization and embryology (research purposes) regulations 2001 (no.188). Available at www.legislation.gov.uk/uksi/2001/188/contents/made. Accessed March 1,2015.
49. NCSL (2008) Human cloning laws. National conference of state legislatures (NCSL).Available at www.ncsl.org/research/health/human-cloning-laws.aspx.Accessed March 1,2015.
50. An Islamic perspective on some contemporary medical Issues: cloning. Published in 1999: The Islamic Organization For Medical Sciences (Kuwait), p 503-515.
51. Contemporary Biomedical Issues in Light of Islamic *Shari`ah*, Volume II, September 2000. Published by the Society of Islamic Medical Sciences in Jordan (Arabic), p 156-158.
52. Council of International Islamic *Fiqh* Academy-OIC, 10th session held in Jeddah, June 28-July 3, 1997, Decision #100/2/D10.
53. Council of Islamic *Fiqh* Academy-Muslim World League, 15th session held in Makkah, October, 1998.

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Example: The Glorious Qur'an: Mariam: 19: 54.

n. *Hadith* from printed volume:
Example: Ibn Hajar al-'Asqalani. Fath al-Bari bi-Sharh Sahih al-Bukhari (The Creator's Inspiration in Interpreting the Verified Collection of al-Bukhari).Cairo, Egypt: Al-Bahiyyah Egyptian Press; 1930. Vol 11, p. 405.

o. *Hadith* from database:
Example: Sahih Al-Bukhari, Book 79, Kitaab al-Tibb, Chapter 1, Hadith 5354. [on-line] Available from: http://www.muhaddith.org.

11. Number the tables consecutively, and use Arabic numerals. Each table must be cited in sequence at an appropriate point in the text. Each table has to have a caption. These should be brief yet indicate closely the purpose or content of the table. Each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table.

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