Introduction

James Watson was quoted as saying “we used to think that our fate was in our stars, but now we know, in large measures, our fate is in our genes”. Genes, the functional unit of heredity, are specific sequences of bases that encode instructions to make proteins. Although genes get a lot of attention, it is the proteins that perform most life functions. When genes are altered, encoded proteins are unable to carry out their normal functions, resulting in genetic disorders. Gene therapy (use of genes as medicines) is basically to correct defective genes responsible for genetic disorder by one of the following approaches:

- A normal gene could be inserted into a nonspecific location within the genome to replace the Nonfunctional gene (most common)
- An abnormal gene could be swapped for a normal gene homologous recombination
- An abnormal gene could be repaired through selective reverse mutation
- Regulation (degree to which a gene is turned on or off) of a particular gene could be altered

Gene therapy states and remains an experimental discipline and many researches remain to be performed before the treatment will realize its potential. Majority of the gene therapy trials are being conducted in the United States and Europe, with only a modest number in other countries including Australia. Scope of this approach is broad with potential in treatment of diseases caused by single gene recessive disorders (like cystic fibrosis, hemophilia, muscular dystrophy, sickle cell anemia etc), acquired genetic diseases such as cancer and certain viral infections like AIDS, as shown in Figure 1.

Fig. 1: Proportion of protocol for human gene therapy trials relating to various types of diseases

- Infectious diseases: 10%
- Monogenic diseases: 14%
- Other diseases: 6%
- Cancer: 70%

Other gene therapy projects are targeted at conditions such as heart disease, diabetes mellitus, arthritis and Alzheimer’s disease, all of which involve genetic susceptibility to illness. Table 1 shows a summary of approved current clinical gene therapy protocols.

Historical Perspectives

Since the earliest days of plant and animal domestication, about 10,000 years ago, humans have understood that characteristics traits of parents could be transmitted to their offspring. The first to speculate about how this process worked were ancient Greek scholars, and some of their theories remained in favor for several centuries. The scientific study of genetics began in 1850s, when Austrian monk Gregor Mendel, in a series of experiments with green peas, described the pattern of inheritance, observing that traits were inherited as separate units we know as genes. Mendel’s work formed the foundation...
for later scientific achievements that heralded the era of modern genetics. But little was known about the physical nature of genes until 1950s, when American biochemist James Watson and British biophysicist Francis Crick developed their revolutionary model of double stranded DNA helix. Another key breakthrough came in the early 1970s, when researchers discovered a series of enzymes that made it possible to snip apart genes at predetermined site along a molecule of DNA and glue them back together in a reproducible manner. Those genetic advances set the stage for the emergence of genetic engineering, which has come in the early 1970s, when researchers discovered a series of enzymes that made it possible to snip apart genes at predetermined site along a molecule of DNA and glue them back together in a reproducible manner. Those genetic advances set the stage for the emergence of genetic engineering, which has produced new drugs and antibodies and enabled scientists to contemplate gene therapy. A few years after the isolation of genes from DNA, gene therapy was discovered in 1980s.6

**Process of Gene Therapy**

**Approach**

The process of gene therapy remains complex and many techniques need further developments. The challenge of developing successful gene therapy for any specific condition is considerable. The condition in question must be well understood, the undying faulty gene must be identified and a working copy of the gene involved must be available. Specific cells in the body requiring treatment must be identified and are accessible. A means of efficiently delivering working copies of the gene to the cells must be available. Moreover diseases and their strict genetic link need to be understood thoroughly.

**Types of gene therapy**

There are 2 types of gene therapy.

1. **Germ line gene therapy:** Where germ cells (sperm or egg) are modified by the introduction of functional genes, which are integrated into their genome. Therefore changes due to therapy would be heritable and would be passed on to later generation. Theoretically, this approach should be highly effective in counteracting genetic disease and hereditary disorders. But at present many jurisdictions, a variety of technical difficulties and ethical reasons make it unlikely that germ line therapy would be tried in human beings in near future.

2. **Somatic gene therapy** where therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only and will not be inherited by the patients offspring or any later generation.

**Gene delivery**

In most gene therapy studies, a normal gene is inserted into the genome to replace an abnormal, disease causing gene. Of all challenges, the one that is most difficult is the problem of gene delivery i.e. how to get the new or replacement gene into the patient’s target cells. So a carrier molecule called vector must be used for the above purpose.9 The ideal gene delivery vector should be very specific, capable of efficiently delivering one or more genes of the size needed for clinical application, unrecognized by the immune system and be purified in large quantities at high concentration. Once the vector is inserted into the patient, it should not induce an allergic reaction or inflammation. It should be safe not only for the patient but also for the environment. Finally a vector should be able to express the gene for as long as is required, generally the life of the patient .

Two techniques have been used to deliver vectors i.e. ex-vivo and in-vivo.10 The former is the commonest method, which uses extracted cells from the patient. First, the normal genes are cloned into the vector. Next, the cells with defective genes are removed from the patient and are mixed with genetically engineered vector. Finally the transfected cells are reinfused in the patient to produce protein needed to fight the disease. On the contrary, the latter technique does not use cells from the patient’s body. Vectors with the normal gene are injected into patient’s blood stream to seek out and bind with target cell (Figure 2).

Some of the vectors used in gene therapy include:
A. Viral Vector

One of the most promising vectors currently being used is harmless viruses. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the viral genome and replace them with working human gene. This altered virus can then be used to smuggle genes into cells with great efficiency. Some of the viruses insert their genes into the host genome, but do not actually enter the cell. Others penetrate the cell membrane disguised as protein molecule and enter the cell. Once the transplanted gene ‘is switched on’ in the right location within the cell of an infected person, it can then issue instructions necessary for the cell to make the protein, that was previously missed or altered.

Some of the different types of viruses used as gene therapy vectors:

- **Retrovirus**

  First viruses to be used as vectors in gene therapy experiments were retroviruses. They belong to a class of viruses (RNA as genetic material) which can create double stranded DNA copies with the enzyme reverse transcriptase. These copies of its genome can be integrated into the chromosome of host cell by another enzyme carried the virus called integrase. Now the host cell has been modified to contain a new gene. If such modified host cells divide later, their descendants will contain the new genes. Although retroviruses have been used in most gene therapy experiments so far, they present problems. One such problem is that integrase enzyme can insert genetic material of the virus into any arbitrary position in the genome of the host, which can lead to insertional mutagenesis (if insertion is in the middle of the gene) or uncontrolled cell division (if gene happens to be one regulating cell division) leading to cancer. This problem has recently begun to be addressed by utilizing zinc finger nuclease or by including certain sequences such as beta globin locus control region to direct the site of integration to specific chromosome.

  Gene therapy trial using retroviral vector to treat X-linked severe combined immune deficiency represent the most successful application till date.

- **Adenovirus**

  To avoid problem of inserting genes at wrong sites, some researchers have turned to other types of viruses. A class of virus with double stranded DNA genome that can cause respiratory, intestinal and eye infection (especially the common cold). When these viruses infect a host cell, they introduce their DNA molecule into the host. The genetic material of the adenovirus is not incorporated into the host cell’s genetic material. The DNA molecule is left free in the nucleus of the host cell, and the instructions in this extra DNA molecule are transcribed just like any other gene (Figure 1). Adenovirus also can infect a broader variety of cells than retrovirus, including cells that divide more slowly, such as lungs cells. However, adenovirus also are more likely to be attacked by the patient’s immune system, and the high levels of virus required for treatment often provoke an undesirable inflammatory response. Despite these drawbacks, this vector system has been promoted for treating cancer of liver and ovaries and indeed the first gene therapy product to be licensed to treat head and neck cancer is Gendicine, p53 based adenoviral product. Concern about the safety of the above vectors was raised after the 1999 death of Jesse Gelsinger while participating in a gene therapy trial. Since then, work using adenovirus vector has focused on genetically crippled version of the virus.

- **Adeno-associated viruses (AAVs)**

  One of the most promising potential vectors is a recently discovered virus called the AAV, which infects a broad range of cells including both dividing and non dividing cells. AAVs are small viruses from the Parvovirus family with a genome of single stranded DNA. It can insert genetic material at a specific site on chromosome 19 with near 100% certainty. Researchers believe that most people carry AAV which do not cause disease and do not provoke an immune response. Scientists have demonstrated the animal experiments using AAV to correct genetic defects. It is now being used in preliminary studies to treat hereditary blood disease hemophilia, muscle and eye disease. Also clinical trials have been initiated to use AAV vectors to deliver genes to brain as the virus can infect nondividing cells like neurons in which their genome are expressed for a long time.

  The chief drawback of AAV is that it is small, carrying only 2 genes in its natural state. Its payload therefore is relatively limited. It can produce unintended genetic damage because the virus inserts its genes directly into host cell’s DNA. Researchers have also had difficulties in manufacturing large quantities of the altered virus. The production problem has recently being solved by Amsterdam Molecular Therapeutics. The recombinant virus...
DNA, which does not contain any viral genome and only the therapeutic gene, does not integrate into the genome, instead fuses at its end to form circular, episomal forms which are predicted to be the primary cause of long term gene expression.

- **Herpes simplex virus (HSV)**
  
  It is a human neurotropic virus, which is mostly used for gene transfer in nervous system. It has a large genome compared to other viruses, which enable scientist to insert more than one therapeutic gene into a single virus, paving the way for treatment of disorders caused by more than one gene defect. HSV makes an ideal vector as it can infect a wide range of tissues including muscle, liver, pancreas, and nerve and lung cells. The wild type of HSV-1 virus is able to infect neurons which are not rejected by immune system. Antibodies to HSV-1 are common in humans, however complications due to herpes infections are somewhat rare.

## B. Non-Viral Methods

Simplest method of non-viral transfection is direct DNA transfer. Clinical trials to inject naked DNA plasmids have been performed successfully. There have been trials with naked PCR products, which have had greater success. Research efforts have yielded several non-viral methods gene transfer such as electroporation (creation of electric field induced pores in plasma membrane), sonoporation (ultrasonic frequencies to disrupt cell membrane), magnetofection (use of magnetic particle complexed with DNA), gene guns (shoots DNA coated gold particles into cells by using high pressure) and receptor mediated gene transfer are being explored. Each method has its own advantages and disadvantages.

Among the several nonviral approaches, receptor mediated gene transfer currently holds the most promise. This application involves the use of DNA conjugated with specific proteins (viral structural protein), or with liposome, or both. Under experimental ex-vivo conditions, liposomes containing DNA have been shown to undergo cellular uptake through endocytosis, with subsequent transient exogenous gene expression. Nevertheless, the application of this approach will likely be limited until methods for the stable integration of the endocytosed DNA are devised and improvement in target ability, transfection efficiency and DNA carrying capacity are developed.

Recently several chemical methods like use of synthetic oligonucleotides (to inactive defective genes by using antisense specific to target gene), lipoplexes (made up of anionic and neutral lipids) and polypelexes (complex of polymers with DNA) have been used to facilitate delivery of the DNA into cell. Recently there have been some hybrid methods developed that combine two or more techniques. For example- vibrosomes that combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cell than either viral or liposomal method alone. Other hybrid methods involve mixing viral vectors with cationic lipids. Researchers are also experimenting with introducing a 47th (artificial human) chromosome into target cells. This chromosome would exist autonomously alongside the standard 46, not affecting their workings or causing any mutation. It would be a large vector capable of carrying substantial amount of genetic code, and scientists anticipate that, because of its construction and autonomy, the body’s immune system would not attack it.

The advantage of direct transfer of non-complexes or protein- complexed DNA by chemical, mechanical, electrical, particle bombardment method or artificial chromosome method include the possibility of transferring relatively large DNA fragments. However, these processes are still inefficient, are limited to ex-vivo gene transfer and have undefined cytotoxic effects.

### Target tissues for gene therapy

Hematopoietic cells derived from bone marrow (BM) may be readily obtained and manipulated ex-vivo in a variety of tissue culture system. Furthermore, therapies based on transfer of genetically modified hematopoietic cells are potentially applicable to a wide range disorders, including the hemoglobinopathies, AIDS and cancer. For these reasons, BM cells are attractive targets in gene therapy research.

Gene transfer into human hematopoietic stem cells capable of proliferating in-vivo for long period of time, giving rise to large number of progeny expressing the desired product, remains an elusive goal. The main limitation is related to the fact that hematopoietic cells greatest potential for proliferation are generally quiescent and resistant to retrovirus mediated gene transfer. Meanwhile, the utility of genetically modified hematopoietic cells, with limited but predictable proliferative potential continues to be elevated.

Research into the usefulness of other cell types as gene transfer vehicle remains very active. The known regenerative properties of the liver make it an attractive gene transfer target. Disorders theoretically amenable to this form of therapy include familial hypercholesterolemia, hemophilia, urea cycle defect, -1-antitrypsin deficiency and phenylketonuria. Other potential targets for therapy include skeletal muscle cells for amelioration of muscular dystrophies, respiratory epithelial cells for the treatment of respiratory insufficiency in cystic fibrosis, and central nervous system tissue for degenerative neurologic disorders. The utility of carrier cells expressing exogenous genes in nonantigenic porous microcapsules has also been demonstrated, and this method promises long term gene delivery using “universal donor” cell lines. In a more radical experimental approach, implantable “neo-organs” made up of genetically modified carrier cells bound to collagen coated synthetic fibres have been shown to have the capacity to become vascularized intraperitoneally and to secrete proteins.

### Journey of Clinical Trials in Gene Therapy [1980 – 2010]

There are several early speculations on the method of gene therapy. In 1966, Tatum predicted that viruses could be used to convert genes in theoretical studies in somatic-cell genetics. The first attempt in genetics was done in Pecking ducks which were injected with DNA extracts from Khaki Campbell ducks and expressed some of the characteristics of the said duck but another trial on albino rat by DNA extracts of pigmented rat did not produce any significant result. In 1970, American doctor Stanfield Rogers tried to treat two sisters, suffering from arginemia (that lacks enzyme arginase, a type of protein) by injecting shope papilloma virus containing an arginase gene but this gene therapy was unsuccessful to raise the above protein levels higher. In 1977, scientists were able to use gene therapy technique to deliver a gene into cells of mammals.
In 1980, Mercola and Cline undertook the first human gene therapy trial to treat β thalassemia patients by transfecting β globin gene into human bone marrow cells. This protocol lacked appropriate ethical review, was widely reviewed as premature on scientific grounds and was eventually stopped. Two important points emerged from this study. One was the fact that highly regulated and coordinated expression of both α-like and β-like globin genes would likely be required for successful gene therapy of the hemoglobinopathies. Other was the need to address the safety and ethical concern adequately in clinical trials of gene therapy.

In 1990, American doctor Anderson performed one of the first successful gene therapy study on a 4 year old girl named Ashanti DeSilva, with a rare genetic immune system disorder called severe combined immuno-deficiency (SCID). The lack of production of adenosine deaminase (ADA), had made her immune system weak, so she had become susceptible to many severe diseases. Anderson and his colleagues extracted her WBCs, implanted genes producing ADA into WBCs and then transferred the cells back to her body. The WBCs strengthened the girl's immune system made it possible for her to survive. The effects were only temporary, but successful.

In 1992, Claudio Bordignon of Italy performed the first procedure of gene therapy using hematopoietic stem cells as vectors to deliver genes intended to correct hereditary disease.

In 1993, a new born baby Andrew Gobea, with SCID, was treated by gene therapy technique using retrovirus vector carrying ADA gene. Blood was removed from his placenta and umbilical cord immediately after birth, containing stem cells. Retrovirus (carrying ADA gene) and stem cells were mixed, after which they entered and inserted the gene into stem cells' chromosome. Stem cells containing the working ADA enzyme were also given weekly. For next few years, WBCs (produced by stem cells), made ADA enzyme using ADA gene. After then further treatment was needed.

In 1999, gene therapy suffered a major setback with the death of 18 year old Jesse Gelsinger who participated in a gene therapy trial for ornithine transcarboxylase deficiency. He died from multiple organ failure 4 days after starting the treatment. His death was believed to have been triggered by a severe immune response to the adenovirus carrier.

In 2002, French researcher Alain Fischer tried to cure children suffering from X-linked SCID (also known as bubble boy) by inserting retrovirus carrying normal gene into children's blood stem cell. This clinical trial was questioned when 2 of them developed a leukemia-like condition. However another major blow came in Jan 2003, when the “FOOD and DRUG ADMINISTRATION” (FDA) placed a temporary halt on all gene therapy trials using retrovirus vector in blood stem cells. Then in April 2003, FDA eased the ban after regulatory review of the protocol in USA, UK, France, Italy and Germany since the treatment had benefitted a large number of children.

Researchers at Case Western Reserve University and Copernicus Therapeutics have been able to create DNA nanoballs (tiny liposomes 25nm) that can carry therapeutic DNA through pores in nuclear membrane. Moreover gene therapy approach repairs errors in messengerRNA derived from defective genes. This technique has the potential to treat thalassemia, cystic fibrosis, and some forms of cancers.

In 2003, Los Angeles research team inserted genes into brain using liposome coated in a polymer called polyethylene glycol. The transfer of gene into brain is a significant achievement because viral vectors are too big to get across the blood brain barrier. This method has potential for treatment for Parkinson’s disease.

Scientists at the National Institute of Health (Bethesda, Maryland) have successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells. This study constitutes one of the first demonstrations that gene therapy can be effective in treating cancer. In another development, gene therapy may be used to Huntington’s disease. Short interfering RNAs (siRNAs) are designed to match the RNA, copied from a faulty gene and to produce abnormal product of that gene. This RNA interference or gene silencing may be used in gene therapy to switch off Huntington’s disease.

In 2005, scientists were able to repair deafness in guinea pig by using adenovirus vector. Atoh1 gene (which stimulates hair cell’s growth) was delivered to cochlea resulting in regrowth of hair cells and so regaining 80% of original hearing threshold. This study may pave the way to human trials of gene therapy in such cases.

In 2006 (March), an international group of scientists announced the successful use of gene therapy to treat two adult patients for a disease affecting myeloid cells. Study published in Nature Medicine, is believed to be the first to show that gene therapy can cure disease of myeloid system. In May 2006, a team of scientists from Italy, reported a breakthrough for gene therapy in which they developed a way to prevent immune system from rejecting a newly delivered gene with the use of micro RNAs, whose natural function could be used to selectively turn off the identity of the therapeutic gene. The researchers were successful in mice experimentation. This work will have important implication for the treatment of hemophilia and other genetic disease by gene therapy. In August 2006, researchers successfully reengineered immune cells, called lymphocyte, to target and attack cancer cells in patients with advanced metastatic melanoma. This is the first time that gene therapy is used to successfully treat cancer in humans. In November 2006, Preston Nix from the University of Pennsylvania School of Medicine reported on VRX496, a gene-based immunotherapy for the treatment of human immunodeficiency virus (HIV) that used a lentiviral vector for delivery of an antisense gene against the HIV envelope. Patients responded to the above therapy showing stable and increased immune response (CD4 T cell count). This was the first evaluation of a lentiviral vector administered in U.S. Food and Drug Administration-approved human clinical trials for any disease. Data from an ongoing clinical trial were presented at CROI (conference on retrovirus and opportunistic infection).

In 2007, a team of British doctors from Moorfield’s Eye Hospital and University college of London, announced the world’s first gene therapy trial to test a revolutionary gene therapy treatment for a type of inherited retinal disease i.e. Leber's congenital amaurosis, which is caused by mutation in the RPE65 gene. Sub-retinal delivery of recombinant AAV carrying RPE65 yielded positive result, with patient having modest increase in vision, and more importantly, no apparent side-effect.

In 2009 (March), the School of Pharmacy in London tried nanotechnology based gene therapy (which delivers genes wrapped in nanoparticles) to target and destroy hard-to-reach cancer cells. In September 2009, journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichromatic vision to squirrel monkeys.
using gene therapy. This could have a significance on future treatment for colour blindness in humans. In November 2009, the journal Science reported that researchers succeeded at halting a fatal brain disease, adrenoleukodystrophy, using a vector derived from HIV to deliver the gene for the missing enzyme.

In 2010, a paper by Komaromy et al. published in April 2010, deals with gene therapy for a form of achromatopsia (complete colour blindness) in dogs. It is presented as idle model to develop gene therapy directed to cone photoreceptor. Cone function and day vision have been restored for at least 33 months in two young dogs with achromatopsia. However, the therapy was less efficient for older dogs.

**Disadvantages of Gene Therapy**

No therapy, established or experimental, is without some associated risks. As human gene therapy is still a relatively new procedure, there are still many risks associated with it. Scientists do not yet understand all of the risks and there has not been enough time to complete detailed studies on how gene therapy works and the problems that it poses. Safety will appropriately remain an important consideration as the field of gene therapy evolves. Some of the problems of gene therapy include:

- **Short-lived nature of gene therapy**: Before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and cells containing the therapeutic DNA must be long-lived and stable. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy. Moreover, the new gene fails to express itself or the virus does not produce the desired response.

- **Immune response**: Anytime a foreign object is introduced into human tissues, the immune system has evolved to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a possibility. Furthermore, the immune system’s enhanced response to invaders makes it difficult for gene therapy to be repeated in patient.

- **Problem with viral vectors**: Viruses, while the carrier of choice in most gene therapy studies, present a variety of potential problems to the patients- toxicity, immune and inflammatory response and gene control and targeting issues. In addition, there is always the fear that viral vector, once inside the patient, may recover its ability to cause disease.

- **Multigenic disorders**: Conditions or disorders that arise from mutation in a single gene are best candidates for gene therapy. Unfortunately, some of the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer’s disease, arthritis and diabetes, are caused by the combined effects of variations in many genes. Multigenic or multifactorial disorders would be especially difficult to treat effectively using gene therapy.

- **Insertional mutagenesis**: The main problem that geneticists are encountering is the virus may target the wrong cells. If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. This has occurred in clinical trials for X-linked SCID patients in which hematopoietic stem cells were transduced with a corrective transgene using a retrovirus, and this led to the development of T cell leukemia in 3 of 20 patients.

**Ethical and Social Consideration**

Gene therapy is a powerful new technology that might have unforeseen risks, scientists first develop a proposed experiments i.e. protocol, that incorporates strict guidelines. After the approval from FDA, the organization continues to monitor the experiment. In the course of a clinical trial, researchers are required to report any harmful side effects. Critics and proponents all agree that risks of gene therapy must not be substantially larger than the potential benefit. Gene therapy poses ethical considerations for people to consider. Some people are concerned about whether gene therapy is right and it may be used ethically.

Some of the ethical considerations for gene therapy include:

- Deciding what is normal and what is a disability;
- Deciding whether disabilities are diseases and whether they should be cured;
- Deciding whether searching for a cure demeans the live of people who have disabilities;
- Deciding whether somatic gene therapy is more or less ethical than germ line gene therapy

Initial experiments using gene therapy have been conducted primarily in patients for whom all other treatments have failed, so that the risks are small. Many people feel that because gene therapies use altered genes and potentially dangerous viruses, those treatments should be tested more extensively.

**Conclusion**

Most scientists believe the potential for gene therapy is the most exciting application of DNA science, yet undertaken. How widely this therapy will be applied, depends on the simplification of procedure. As gene therapy is uprising in the field of medicine, scientists believe that after 20 years, this will be the last cure of every genetic disease. Genes may ultimately be used as medicine and given as simple intravenous injection of gene transfer vehicle that will seek our target cells for stable, site-specific chromosomal integration and subsequent gene expression. And now that a draft of the human genome map is complete, research is focusing on the function of each gene and the role of the faulty gene play in disease. Gene therapy will ultimately play Copernican part and will change our lives forever.

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