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Cystic Fibrosis: Biological and Ethical Considerations

Cystic fibrosis (CF) is a progressive, multisystem disease whose etiology is a genetic

mutation in the CF gene product, cystic fibrosis transmembrane conductance regulator

(CFTR). The disorder affects all exocrine glands, with common symptoms involving the

lungs and pancreas. Although the CF gene and its protein product have been identified,

two aspects of the disease make CF particularly difficult to diagnose and manage: (a)

variability in both degree and pattern of the mutation in different individuals and (b) lack

of information regarding the precise molecular and cellular mechanisms responsible.[1] Let

us begin by examining the pathogenesis and pathophysiology of the disease and current

diagnosis, treatment, and research in gene therapy.

EPIDEMIOLOGY

Cystic fibrosis, the most common fatal genetic disorder among Caucasians, affects some

30,000 people in the United States.[2] The disease occurs equally in both genders and,

although most common among Caucasians, appears in virtually every race (Table 1).

One Caucasian in 28 carries the CF gene. In the United States the median survival has

increased from approximately 20 years in 1970 to 31.3 years in 1996.[3] Adults with the

disease increased from 4,418 in 1986 to 7,436 in 1996.[3] At the end of 1996, there were

2,274 CF patients in the United States 30-years-of-age or older and 74 patients 50-years-

of-age or older.[3] This success is likely the result of advances in diagnosis, access to

medical treatment, and respiratory therapy.[2]

PATHOPHYSIOLOGY

Cystic fibrosis is characterized by disrupted function of the exocrine glands, which release their secretions through ducts. Exocrine glands perform highly specialized functions in a variety of organs (i.e., in the skin, respiratory tract, gastrointestinal tract, and reproductive system); therefore, the number of possible symptoms and complications in cystic fibrosis is large (Figure 1).

The CFTR protein acts as a chloride ion (Cl⁻) channel in the apical membrane of exocrine glands and is involved in the regulation of both sodium and chloride flux. The movement of water by osmosis across the cellular membrane is linked to ion transport. Chloride secretion is one means of hydrating the mucosal surface of exocrine glands.[4] CF patients possess a malformed CFTR protein which causes abnormal electrolyte transport—primarily reduced ability of the membrane to excrete chloride—leading to impaired water secretion and abnormally thick, viscous exocrine secretions.[5,6] Luminal obstruction by highly viscous mucus and other secretions in CF is a major factor in progression of the disease, particularly at the pulmonary and pancreatic levels.[7]

The highest concentrations of CFTR are in the submucosal glands of the airways, pancreas, salivary glands, sweat glands, intestines, and the reproductive tract. The primary clinical manifestations of CF are concentrated in these areas and are summarized in Figure 1. Table 2 provides a more inclusive list of the effects and complications in each organ system, illustrating that cystic fibrosis is a multisystem disease.

Respiratory System

The respiratory pathology of CF is in part traceable to defective clearance of thick, highly viscous mucus from the bronchial passages, leaving the lungs susceptible to infection and consequent inflammation.[7,8] The lower airways of patients with CF are most commonly colonized with *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[9] Repeated bacterial, viral, and fungal infections trigger an exaggerated immune response that brings inflammatory cells to the airways. These cells, neutrophils and macrophages, release proteases that contribute to the destruction of lung tissue by increasing mucus secretion, slowing the mucus-clearing movement of cilia, and directly damaging lung tissue.[7] Respiratory disease in CF is characterized by a reduced rate of mucus clearance, consequent accumulation of purulent sputum, recurrent respiratory infections, and progressive loss of pulmonary function, which can ultimately result in respiratory failure.[10] Problems in the lower respiratory tract cause more than 90 percent of the morbidity and mortality associated with CF.[11]

Digestive System

Pancreatic function becomes increasingly abnormal in most CF patients as the pancreatic ducts become progressively obstructed by thick, viscous secretions from the exocrine portion of the organ; pancreatic enzymes that are trapped within the ducts lead to the autodestruction of the pancreas.[9] In 85 percent of patients with CF, partial or complete blockage of the ducts leading from the pancreas to the small bowel results in the impairment of fat and protein digestion and the subsequent malabsorption of nutrients.[7]

Patients are prone to failure to thrive and undernutrition, which can lead to a number of gastrointestinal conditions (see Table 2). In advanced stages of the disease, pancreatic fibrosis, sometimes causes obliteration of the islets of Langerhans and, consequently, diabetes mellitus.[9] CF also affects the liver and bilary tract. Here, too, the primary mechanism appears to be obstruction of ducts by abnormally viscid secretions.[9]

Reproductive System

Except for an increase in viscosity in cervical mucus, no consistent pathologic changes occur in the female reproductive tract in patients with CF.[9] In the male reproductive tract, however, the vas deferens, seminal vesicles, or epididymides are either atretic or absent at birth.[7] Approximately 98 percent of males with CF are infertile.[9]

Sweat Glands

The sweat glands of CF patients manifest no distinctive histological changes.[9] Nonetheless, their function is abnormal. These abnormalities include reduced reabsorption of sodium and chloride ions, leading to high concentration of these electrolytes in sweat, causing salty sweat and excessive loss of fluid.[12] Salt depletion and heat stroke are consequent dangers.

DIAGNOSIS

The Cystic Fibrosis Foundation National CF Patient Registry data from 1996 indicate that the diagnosis of CF is usually made in the first year of life (71% of cases).[13] However, in 8 percent of patients the diagnosis is not established until after 10 years of age, and the

diagnosis is now being made in an increasing number of adults.[13] It is essential to confirm or exclude the diagnosis of CF in a timely fashion and with a high degree of accuracy to avoid unnecessary testing, to provide appropriate therapeutic interventions and prognostic and genetic counseling, and to ensure access to specialized medical services.[14] A recent consensus document defined the specific criteria for CF diagnosis as 1) the presence of one or more typical clinical features (Table 3), 2) a history of CF in a sibling, or 3) a positive newborn screening test result plus laboratory evidence of a CFTR abnormality (i.e. the demonstration of an elevated [>60 mmol/L] sweat chloride concentration).[14]

Sweat Testing

Of the several methods for sweat testing, the quantitative Gibson-Cooke test is currently the only uniformly accepted method.[15] A 30-minute sweat sample ($\geq$75 mg) is obtained by applying pilocarpine and mild electrical stimulation to a small area of the skin of the forearm or back. The test measures the chloride and sodium levels. A sweat-chloride concentration greater than 60 mmol/L is consistent with CF.[15]

Genetic Screening

Genetic testing has made it possible for prospective parents with family histories of CF to find out whether they are likely to be carriers of CF and to learn whether a fetus has two mutated copies of the gene, and thus will have CF. The current general-screening test can detect several major mutations, including ΔF508, but because there are over 500 known mutations of the CFTR gene, it is impossible at this point for laboratories to test for every

mutation.[16]   The idea of screening the general population for the CF gene is controversial.   While carrier screening has improved to 85-90 percent, most experts do not consider this high enough to justify screening the general population.[17]   There are many questions about the usefulness and cost of screening, ethical and social implications, insurance coverage, and how to educate and counsel people about CF. While screening the general population is not now recommended, screening in families with a history of CF is appropriate, in part because it is more accurate.[18]

GENETICS

In 1936 cystic fibrosis was described for the first time by the Swiss pediatrician Guido

Fanconi. But the official history of CF began in 1938 when Dorothy Anderson, a

pediatric pathologist at the Columbia University Babies Hospital and Institute for

Pathology in New York, published the paper which provided the first comprehensive

description of the symptoms of cystic fibrosis and its effects in various organs. Anderson

also gave the disease a name, calling it "cystic fibrosis of the pancreas," on the

microscopic features she observed in pancreatic tissue.[29] By 1946 researchers deduced

that cystic fibrosis was a recessive condition after examining the pattern of disease

inheritance in families. Over the next few decades extensive clinical work resulted in the

development of more accurate diagnostic methods and treatments that control the

symptoms of the disease.

The Cystic Fibrosis Gene and Its Distribution

In 1989, the gene responsible for the disease was isolated by a large group of

collaborators, led by Lap-Chee Tsui and John R. Riordan of the Hospital for Sick

Children in Toronto and by Francis S. Collins, now the Director of the Human Genome

Project at NIH.[30] The CF gene codes for the cystic fibrosis transmembrane regulator

(CFTR) protein. This gene was identified with an approach known as positional cloning,

which permitted mapping of the gene without prior knowledge of the biochemical defect

through the use of polymorphic DNA markers. A single locus was found on the long arm

of chromosome 7.[20] Following a series of molecular cloning experiments, which included

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"chromosome walking" and "jumping," a candidate gene was identified. Formal proof that this was the CF gene came in 1990 when a normal CFTR gene was inserted in CF cells *in vitro*, resulting in the correction of the Cl⁻ secretory defect.[31]

The most common CF mutation and the first to be described is a three-base pair deletion in exon 10 that causes a deletion of the amino acid phenylalanine from position 508 (ΔF508) of the CFTR glycoprotein (Figure 2). This mutation accounts for 66 percent of CF mutations. More than 600 mutations have now been reported, however, and the list continues to grow. In addition, numbers of benign sequence variations have been described. A listing of the most common mutations and their relative frequency is included in Table 4.

Inheritance of Cystic Fibrosis

Every person has a pair of genes, one contributed by each parent, that define each hereditary trait. Cystic fibrosis is an autosomal recessive genetic disorder. A heterozygous individual, one with one normal and one defective copy of the gene, will not have the disorder. These individuals are carriers of the defective gene and can pass it to their children.. However, a homozygous person, who has received two copies of the mutated gene (one from each parent), will have CF. So, if both parents are carriers, the risk of having a child with CF is 1 in 4, regardless of the outcome of previous pregnancies. The odds of a single pregnancy resulting in CF, based on the genetic status of the parents, are shown in Figure 3.

THE MOLECULAR BASIS OF CF

CF is attributed to abnormalities in salt metabolism due to the absence or malfunction of the Cystic Fibrosis Transmembrane Regulator (CFTR) protein, which is essential in the passage of the chloride ion in and out of the plasma membrane. Research and characterization of the CFTR protein as it relates to channel regulation has given us greater insight into the manifestations of the disease and subsequent treatment options.

Molecular Findings in CF Research

The movement of chloride across epithelial surfaces plays an important physiological role in salt and water balance. In the infancy of CF research, it was first discovered that individuals with CF have an elevated sweat chloride concentration.[15] Electrophysiologists determined that some gene defect resulted in defective function of a cAMP-activated chloride channel in epithelial cells of sweat glands and bronchial mucosa.[19] Figure 4 shows a model of this chloride channel in a cellular context. To further characterize the chloride abnormality, several investigators studied individual chloride channel proteins present in the plasma membranes of CF cells. An effort toward understanding the molecular physiology of cystic fibrosis evolved from clinical observations of abnormal glandular function to the identification of a single chloride channel abnormality in the disease. Riordan, Tsui, and Collins employed direct molecular techniques in identifying the gene responsible for cystic fibrosis,[20] discovering that individuals with cystic fibrosis possessed a mutation resulting in the deletion of a phenylalanine residue at position 508 (ΔF508).

The CFTR Protein

The gene responsible for CF spans 250 kilobases and contains 27 exons that, once spliced, form a messenger RNA of approximately 6500 nucleotides encoding a 1480 amino acid polypeptide.[19] Anticipating its role, this protein was named CFTR for cystic fibrosis transmembrane conductance regulator. The structure of the protein, shown in Figure 5, is predicted to be a membrane glycoprotein consisting of two repeated elements, each containing a domain capable of spanning a membrane six times (transmembrane domain or TMD) and a region containing protein sequences known to bind nucleotides (nucleotide-binding domain or NBD). The two halves of the protein are separated by a large highly charged domain referred to as the regulatory or R-domain.[21] The sequence and structure of the TMDs and NBDs are analogous to a known group of proteins called ABCs, or ATP-binding cassette proteins. Characteristics of this family include the ability to bind ATP and transport substances across membranes.[21]

In deducting the function of the CFTR protein, scientists had to answer one important question: Was the CFTR a chloride channel or a regulator of chloride channels? Subsequent investigations provided evidence that CFTR is a chloride channel.[22, 23, 24] As shown in Figure 6, CFTR is phosphorylated *in vivo* by the cAMP-dependent protein kinase A (PKA). PKA-directed phosphorylation is a necessary priming event for channel opening. Once phosphorylated, CFTR requires ATP binding and hydrolysis for activation,[19] which is probably bound by NBD1. When CFTR is activated and in the open state, chloride movement is controlled by its own electrochemical gradient.[21] That is, no more ATP is then required because passive diffusion takes place.

CFTR Dysfunction

The phenotype of CF arises due to a wide array of mutations, which are scattered through the CFTR channel. Out of the approximately 500 identified CF mutations, about 67 percent of all instances of cystic fibrosis are caused by the ΔF508 mutation. Four distinct classes of mutations have been identified and are represented in Figure 7.[21]

The Class I mutations are scattered throughout the CFTR gene and produce premature termination signals because of splice site abnormalities, frameshift mutations, or nonsense mutations. In some cases, such mutations result in an unstable mRNA and no detectable protein. In other cases, a truncated protein may be synthesized and quickly degraded. All mutants in Class I are expected to produce little or no full-length CFTR protein, causing a loss in Cl⁻ channel function in affected epithelia.[25]

The most common mutant, ΔF508, falls in the Class II mutations, which involve defective protein processing. In recombinant cells, CFTR ΔF508 fails to mature into the fully glycosylated form.[25] These proteins fail to be transported to the apical membrane. Until recently, the reason for failure had not been established. New research indicates that the F508 deletion causes the inability of the mutant protein to fold correctly and transit to the apical membrane.[26] Therefore, phenylalanine in position 508 makes crucial contacts during the folding process.

Class III mutations include a defect in the nucleotide-binding domains and are seen rarely.[25] Because the opening of the CFTR Cl⁻ channel is an energy dependent process, the proper binding of ATP is critical. Normal regulation but reduced current flow

characterize Class IV mutations. These mutations affect the membrane-spanning domain of CFTR and result in reduced ion flow.[25]

Genotype and Phenotype Correlations

The categorization of these mutations are occasionally helpful in forming a relationship between an individual's genotype and phenotype. However, there are many unexplained exceptions. For example, all Class I and II mutations result in a severe pancreatic-insufficient phenotype.[25] These patients have pancreatic failure, requiring pancreatic enzyme supplementations. In contrast, Class III and IV mutations are associated with less-severe phenotypes.[25] These patients retain significant pancreatic function and require little or no pancreatic enzymes. Most dramatically, some individuals with a Class III mutation do not exhibit classic CF symptoms at all but simply have isolated male infertility due to the absence of the vas deferens.[27] Presumably, the differences in phenotype relate to the degree of abnormal regulation, but its molecular basis is not yet understood.

Physiology of Secretory Pathways Involved in Cystic Fibrosis

Transport physiology in CF has been extensively studied, especially prior to the discovery of the CF gene. Historically, one of the first symptoms of CF is a salty taste discovered on an infant's skin due to elevated sweat chloride concentrations. Essentially, the secretory segment of the human sweat gland secretes isotonic NaCl, and, as this solution transverses the distal two-thirds of the gland, NaCl is reabsorbed in excess of H_2O, yielding a hypotonic sodium chloride solution, which is sweat. In CF, the defect

lies in the distal part of the gland, and secreted NaCl is not reabsorbed, resulting in an increased concentration of salt in the sweat.[21]

More significant is the defective chloride transporter in the pancreas. Shown in Figure 8 is a model of solute transport in the pancreatic duct. Chloride is secreted by the ductal cells through CFTR and is reabsorbed by a chloride/bicarbonate exchanger with the net effect of bicarbonate secretion. The bicarbonate (HCO_3^-) secretion obligates sodium movement, and thus $NaHCO_3$ appears in the ductal lumen. More importantly, the net secretion of $NaHCO_3$ causes H_2O movement across the epithelium of the duct, resulting in isotonic pancreatic secretions. In CF, the chloride secretion through CFTR is inhibited and results in decreased $NaHCO_3$ and water secretion. The latter is of particular consequence, and ductal dehydration leads to concentration of pancreatic secretions which accounts for the pancreatic insufficiency in CF individuals.[21] The same effect can be seen in the epithelia of the lungs as well. An absent or malfunctioning CFTR channel allows the chloride concentration to build up in the cell, causing water to be drawn in from the mucus outside, which leaves the mucus thick, sticky, and prone to bacterial infections.[28]

TREATMENT

The identification of the CF gene led to considerable progress in the understanding of the

molecular basis of cystic fibrosis and the CFTR protein. This improved understanding of

the structure and function of the protein continues to advance novel approaches toward

treatment. There is no cure for cystic fibrosis. Thus, management goals revolve around

the amelioration of the respiratory and gastrointestinal manifestations of the disease.

Although management in general consists of many components applied in combination,

some of the individual components of therapy are discussed separately below.

Respiratory Management

Chronic progressive pulmonary disease accounts for most of the morbidity associated

with cystic fibrosis. The goals of respiratory management are to reduce airway

obstruction by increasing the clearance of secretions and to reduce pulmonary infection.

One of the primary methods to increase mucociliary clearance in CF patients is chest

physiotherapy (CPT), which requires vigorous percussion (by using cupped hands) on the

back and chest to dislodge thick mucus from the lungs. Patients with few pulmonary

symptoms may require only a single daily treatment, but others may require three

sessions a day.[32] Since the conventional form of CPT requires someone to help with

percussion, many adolescents and adults have switched to an inflatable vest that vibrates

the chest rapidly and increases mucus clearance.[33] Patients also incorporate the use of

several types of breathing exercises to increase mucociliary clearance. Some physicians

believe that aerobic exercise can substitute for CPT.[7] However, a regular exercise

program has not been shown to improve pulmonary function or reduce morbidity and mortality, although it does increase maximal oxygen consumption, exercise tolerance, respiratory muscle endurance, and psychological well-being.[34]

Lung transplantation has become an accepted therapeutic option for patients with CF who have end-stage lung disease. These patients are good candidates for transplantation if (1) the life-threatening problems associated with CF are confined to the lungs, (2) they are relatively young when they are considered for transplantation, and (3) they have shown that they can comply with complex treatment regimens. Bilateral lung transplantation is the rule, because unilateral transplants would rapidly become infected by the remaining lung. Over 700 people with cystic fibrosis have received some form of lung transplant, and researchers are beginning to report long-term outcomes of these procedures. According to transplant specialists at the 11th Annual North American Cystic Fibrosis Conference, survival rates are rising, infection rates are falling, post-operative exercise performance are improving, and problems of transplant rejection are decreasing.[35] Most patients survive the first year after surgery, and about half survive five years or longer. Over one-third of patients are alive and doing well eight years after transplantation, and this proportion is increasing because of improvements in techniques and treatments. The two biggest problems facing lung transplantation for CF patients are a shortage of donor organs and obliterative bronchiolitis (OB). About 50 percent of patients on the transplant waiting list die before donor lungs become available. OB, which is a form of chronic rejection, is the main cause of transplant-related disability or death that occurs years after transplantation.[35]

Nutritional Support

Pancreatic insufficiency affects an estimated 85 percent of patients with CF and, if untreated, results in individuals who are undernourished and, therefore, do not grow at normal rates.[2] The mainstay in managing pancreatic insufficiency is use of pancreatic enzymes which are ingested along with any food that contains protein, fat, or complex carbohydrates. Other nutritional interventions include education and dietary counseling and vitamin supplementation. Patients are encouraged to eat a normal diet to promote adequate caloric intake with an emphasis on high fat content. They may require 120 percent to 150 percent of the Recommended Daily Allowance (RDA) for calories. Salt supplementation is necessary for infants, and may be used for patients who engage in strenuous physical activity, especially in the summer.[36]

Although it is true that pulmonary function is the predominant factor in determining morbidity and mortality in CF, it is becoming increasingly clear that overall patient status is closely tied to nutritional status. Data from the national CF Registry indicate that 40 percent of patients with CF are below the fifth percentile of weight for age and that mortality is increased in this group.[9]

Pharmacologic Therapy

Pharmacologic therapy primarily involves medications to manage the GI (Table 5) and pulmonary (Table 6) manifestations of CF. During the past few decades of CF treatment, antibiotics have proved to be the key element responsible for increased survival. Antibiotics fight common pathogens such as *Pseudomonas aeruginosa* and

Staphylococcus aureus that cause respiratory tract infections. Why are CF patients more prone to bacterial infections? First, the CFTR gene defect raises salt concentrations in the fluid lining the airways of the lung. This high salt level inactivates factors that would normally help kill invading bacteria. The lung infections that cause so much damage in cystic fibrosis have long been blamed on sticky mucus, which creates a comfortable place for bacteria to nest. However, surprising new work shows that the bacteria are not just passive guests. These bacterial agents work in three ways: (1) they interfere with the body's normal ways of clearing out mucus, (2) they increase production of mucus in airways, and (3) they cause inflammation, which also increases mucus secretion.[37] Despite aggressive physiotherapy and the use of antibiotics, the pulmonary status in a patient with CF often remains impaired because of thick, viscous sputum and bronchiectasis.

Corticosteroids and nonsteroidal anti-inflammatory drugs play a role in palliative therapy for CF lung disease, presumably because they suppress the inflammatory response to chronic infection that contributes to lung destruction. Bronchodilators aid in the reversal of bronchospasms.[9] Another therapeutic approach highlighted in Table 5 involves DNA. It was found that DNA is present in large amount in bronchiopulmonary secretions and greatly contributes to its increased viscosity. Recombinant human deoxyribonuclease is an enzyme that is currently being studied for its ability to digest extracellular DNA and work to decrease mucus viscosity.[19] Basically, any therapy that improves pulmonary status will also improve the overall health of a patient with CF.

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HUMAN GENE THERAPY

Gene therapy is the introduction of genetic material into cells for therapeutic purposes. Gene therapy's goal is to correct the genetic source of the disease, whereas therapies mentioned above only alleviate the symptoms. Gene therapy is a novel form of drug delivery that enlists the patient's own cells to produce a therapeutic agent. Using the body to treat its own disease eliminates the need for repeated administration of proteins (as in hemophilia) or drugs (as in hereditary hypercholesterolemia) and reduces the difficulties in complying with exogenous-drug regimens.[38] Applications of gene therapy are not limited to rare hereditary disorders, but potentially extend to common acquired disorders, such as cancer, heart disease, and acquired immunodeficiency syndrome.

The first approved clinical protocol for somatic gene therapy started trials in September 1990.[39] Since then, more than 300 clinical protocols have been approved worldwide and over 3000 patients have carried genetically engineered cells in their body.[40] The conclusions from these trials are that gene therapy has the potential for treating a broad range of human diseases and that the procedures for gene delivery appear to have relatively low adverse effects. However, the efficiency for gene transfer and expression still remain low. Except for anecdotal reports of individual patients being helped, there is still not conclusive evidence that a gene-therapy protocol has been successful in the treatment of human disease.[40]

In this section, I hope to introduce the most common gene delivery methods and address some of the problems faced by gene therapy. There are various categories of gene

therapy, distinguished by the mode of delivery of the gene to the affected tissue (see Box 1). At present, gene therapy is being contemplated only on somatic (non-reproductive) cells. Although many somatic tissues can receive therapeutic DNA, the choice of cell usually depends on the nature of the disease. Sometimes a clear definition of the target cell is needed. For example, the gene that is defective in CF has been identified, and clinical trials to deliver DNA as an aerosol into the lung have already begun. Although cystic fibrosis is manifest in this organ, it is still not clear that delivery of a correcting gene by this method will reach the right type of cell.[41] It is also unclear how much of the therapeutic protein or gene should be delivered.

The biggest challenge to the success of gene therapy is the development of a safe and efficient gene delivery system. This goal is more difficult to achieve than many investigators had predicted years ago because our immune system protects us from an onslaught of environmental hazards, including the incorporation of foreign DNA into its genome.

There are two categories of delivery vehicles, or vectors. The first is comprised of non-viral vectors, such as DNA mixed with polylysine or cationic lipids that allow the gene to cross the cell membrane. Most of the current gene-therapy approaches make use of the second category - viral vectors. Importantly, the viruses used have been disabled of any pathogenic effects. The use of viruses is a powerful technique, because many of them have evolved a specific machinery to deliver DNA to cells.[42] Although the viruses are not intact, the body still recognizes them as foreign and reacts with a typical immune response. This host response serves as a major impairment to efficient gene delivery.

Retroviral Vectors

Retroviruses are a group of viruses whose RNA genome is converted to DNA in the infected cell. The genome comprises three genes termed *gag, pol,* and *env,* which are flanked by elements called long terminal repeats (LTRs). LTRs are required for integration into the host genome and define the beginning and end of the viral genome. They also serve as enhancer-promoter sequences - that is, they control viral gene expression. Recombinant human CFTR cDNA is the genetic material used for CF gene therapy. The transgene, in this case, the gene responsible for CFTR production, is inserted into the vector. Transcription of the transgene may be under the control of viral LTRs, or enhancer-promoter elements can be engineered in with the transgene. Retrovirus vector design, production, and gene transfer is outlined in Figure 9.

Retroviruses were initially chosen as the most promising gene-transfer vehicles.[43] Currently, about 60 percent of all approved clinical protocols utilize retroviral vectors.[40] Murine leukaemia virus (MuLV) has traditionally been used as the retroviral vector of choice for clinical gene-therapy protocols. This vector can accept up to about 8 kilobases (kb) of exogenous DNA. The use of retroviruses is advantageous because they are easily generated, the infected viruses can be extensively characterized in tissue culture before being injected into patients, and theoretically, the stable integration of the virus into the host chromosome ensures its retention by the cell.

However, there are some critical limitations of retroviral vectors. One limitation is their inability to infect non-dividing cells. This limitation can be useful in some situations, for

example, when a toxin gene is being inserted into dividing cancer cells and not into the normal non-dividing cells. But, most tissues in adults, such as those that make up muscle, brain, lung, and liver tissue, consist primarily of non-dividing cells. For this reason, retroviruses are primarily used in *ex vivo* gene delivery. Another formidable challenge is the efficiency of transplantation of the infected cells. As of now, it is not possible to get a large number of vector particles to the desired cell type *in vivo*. The viral particles would bind to as many cells they encounter and, therefore, would be diluted out before reaching their target. What is needed is a retroviral particle that will preferentially bind to its target cells and can be manufactured at a high titre.[40]

Another problem is the possibility of the random integration of vector DNA into the host chromosome. This could lead to the activation of oncogenes or inactivation or tumor-suppressor genes. Although the theoretical probability of such an event is quite low, it is of some concern.[42]

DNA Virus Vectors

The DNA virus used most widely for *in situ* gene transfer vectors is the adenovirus (specifically serotypes 2 and 5). Adenoviral vectors have several positive attributes. They are large and can therefore potentially hold large DNA inserts (up to 35 kb); they are also human viruses and are able to transfect a large number of different human cell types at a very high efficiency (often reaching nearly 100% *in vitro*). Adenoviruses can insert genetic material into non-dividing cells and they can be produced at very high titres in culture.[40] This DNA virus has been the vector of choice for several laboratories trying

to treat the pulmonary complications of cystic fibrosis, as well as for a variety of protocols attempting to treat cancer.

Figure 10 outlines adenovirus vector design, production, and gene transfer. These vectors do not usually integrate into the host DNA. Instead, they are replicated as episomal (extrachromosomal) elements in the nucleus of the host cell, thus avoiding the risks of permanently altering the cellular genotype or of insertional mutagenosis.[44] As Figure 11 explains, the E1 region is deleted in the adenovirus preventing viral replication. Expression is short-lived, usually lasting only 5-10 days.[40]

One of the main problems faced with adenoviruses is that they evoke nonspecific inflammation and antivector cellular immunity. The immune reaction is potent, eliciting both the cell-killing 'cellular' response and the antibody-producing 'humoral' response. In the cellular response, virally infected cells are killed by cytotoxic T lymphocytes. [45, 46] The humoral response results in the generation of antibodies to adenoviral proteins, and it will prevent any subsequent infection by an adenovirus.[40] Unfortunately for gene therapy, most of the human population will probably have antibodies to adenovirus from previous infection with the naturally occurring virus. There are still considerable immunological problems to be overcome before adenoviral vectors can be used to deliver genes and produce sustained expression.

Another DNA virus used in clinical trials is the adeno-associated virus (AAV). It is a non-pathogenic virus that is wide-spread in the human population (about 80% of humans have antibodies directed against AAV).[40] Initial interest in AAV arose because it is the

only known mammalian virus that shows preferential integration into a specific region in the genome (into the short arm of human chromosome 19). Unfortunately, the present AAV vectors appear to integrate in a nonspecific manner[47], although it has been suggested that vectors could be designed that retain some specificity.[48]

The adeno-associated virus contains two genes (*cap* and *rep*) that are sandwiched between inverted terminal repeats that define the beginning and end of the virus. These genes contain the packaging sequence.[49] The *cap* gene encodes viral capsid (coat) proteins, and the *rep* gene encodes four proteins responsible for viral replication and integration. To produce an AAV vector, the *rep* and *cap* genes are replaced with a transgene. AAV is often called a defective or dependent virus because it requires a helper virus (usually adenovirus or herpes simplex virus) to generate a productive infection.[50]

The features that make AAV an attractive potential gene therapy vector include nonpathogenicity, the ability to remove all of the viral genes without loss of infectivity, and more sustained expression in non-dividing cells.[50] Indicators from preclinical studies suggest that a primary difference between AAV and adenoviral vectors appears to be the lack of immune response to AAV-transduced cells *in vivo*. This is due to the fact that AAV vectors carry no viral genes, whereas current adenoviral vectors still retain about 30 kb of viral coding sequences.[51] However, latent AAV vector in the genome could theoretically be mobilized or rescued by coincident infection by wild-type AAV and adenovirus outbreaks in the community.[52]

A main disadvantage of the AAV vector is the fact that its genome is small, only allowing

room for about 4.8 kb of DNA. The wild-type human CFTR cDNA sequence is roughly

4.5 kb, leaving little room for promoter sequence in the AAV genome.[50] Other

limitations include vector production and purification, which are still very labor intensive.

Non-Viral Vectors

Although viral systems are potentially very efficient, two factors suggest that non-viral

gene delivery will be the preferred choice of the future: safety, and ease of

manufacturing. The most thoroughly tested nonviral vectors are based on cationic lipids.

For CF, the lipid formulations vary from study to study but have included the cationic

lipids DOTMA (N-[1-2,3-dioleyloxypropyl]-N,N,N-trimethylammonium chloride),

DMRIE (1,2-dimyristyloxypropyl-3-dimethylhydroxyethylammonium bromide) or DC-

CHOL (D(3βN[N'N'-dimethylammonoethane)carbomyl] cholesterol), complexed with

DOPE (dioleoylphosphatidylethanolamine).[41] The DNA component is a plasmid

containing the CFTR cDNA with a strong heterologous promoter. These complexes

mediate gene transfer by a largely unknown mechanism. Once inside the nucleus, the

plasmid DNA most likely remains episomal.

The design and gene transfer of a typical lipid:DNA complex is shown in Figure 11.

These complexes transfect a variety of cell types, are easy to prepare, and can

accommodate and deliver genes of unlimited size.[41] Relative to viral vectors, they are

minimally toxic, but the efficiency is much lower and the complexes are generally not

stable. Current efforts to improve these limitations include optimization of the lipid

formulation and enhancement of the formation and stability of the lipid:DNA complex.[53]

Conclusions

Almost ten years have elapsed since clinical trials for gene therapy began. The promises

are still great, but several major deficiencies still exist, including poor delivery systems,

immunological responses, and poor gene expression after genes have been delivered. All

of these problems are surmountable, but each will take time to solve (see Box 2 for

description of ideal vector). However, the logic underlying the potential usefulness of

human gene transfer is compelling. The Human Genome Project will provide 80,000 to

100,000 human genes that could be used for human gene transfer. Despite our present

lack of knowledge, gene therapy will almost certainly revolutionize the future practice of

medicine. The ability to give the patient therapeutic genes offers extraordinary

opportunities to treat, cure, and ultimately prevent a wide range of inherited and acquired

diseases.

Box 1 The three categories of somatic cell gene therapy

- The first is *ex vivo* where cells are removed from the body, incubated with a vector and the gene-engineered cells are returned tot he body. This procedure is usually done with blood cells because they are the easiest to remove and return.

- The second is *in situ*, where the vector is placed directly into the affected tissues. Examples are the infusion of adenoviral vectors into the trachea and bronchi of patients with CF, the injection of a tumor mass with a vector carrying the gene for a cytokine or a toxin, or the injection of a vector carrying a dystrophin gene directly into the muscle of a patient with muscular dystrophy

- The third is *in vivo*, where a vector could be injected directly into the bloodstream. There are no clinical examples of this third category as yet, but if gene therapy is to fulfill its promise as a therapeutic option, *in vivo* injectable vectors must be developed.

Box 2 The Ideal Vector

The ideal vector should have:
- High concentration ($>10^8$ viral particles per ml) allowing many cells to be infected;
- Convenience and reproducibility of production;
- Ability to integrate in a site specific location in the host chromosome, or to be successfully maintained as a stable episome;
- A transcriptional unit that can respond to manipulation of its regulatory elements;
- Ability to target the desired type of cell;
- No components that elicit an immune response.

ETHICAL CONSIDERATIONS

This paper has explored the use of somatic cell gene therapy (SCGT) in correcting the

CFTR defect in patients with cystic fibrosis. Somatic cell gene therapy for the treatment

of serious disease is now accepted as ethically appropriate.[40] In fact, many equate SCGT

to common replacement therapy in medicine. For example, there is no ethical difference

between injecting a diabetic with a gene for producing insulin and injecting him with

insulin itself (the gene product). The side effects from gene therapy protocols have been

so minimal that the danger now exists that genetic engineering may be used for non-

disease conditions, that is for functional enhancement or cosmetic purposes.

September 1997 marked the first Gene Therapy Policy Conference organized by the

NIH Recombinant DNA Advisory Committee (RAC) which focused exclusively on the

enhancement issue. The conclusion was that enhancement engineering is inevitable and

could slip through the regulatory process if RAC and the FDA are not watchful. For

example, a US biotechnology company has developed the technology for transferring

tyronase genes into hair follicle cells.[54] Presently, they are looking for genes to promote

hair growth, specifically to reverse hair loss in cancer patients undergoing chemotherapy.

When licensing the product, the FDA would list chemotherapy-induced alopecia as the

product indication. The risk-benefit analysis would be favorable in this case. However,

once a product is licensed for any indication, it can be prescribed by physicians for any

use that is 'clinically justified.' The result could be millions of balding men receiving

gene therapy to treat their hair loss. The conference concluded that the DNA should use a

risk-benefit analysis that takes into account the extensive 'off-label' usage for cosmetic reasons that could take place.[40]

Using gene therapy to treat baldness is not an issue in itself, but is an example of how some fear that society may be heading towards a 'slippery-slope' where genetic engineering could be used for a broad range of enhancement purposes. In SCGT, the added gene is not inherited by offspring of the patient being treated. Although I believe this area should be approached with caution, using a risk-benefit analysis as its basis, no argument has been provided to show why SCGT differs in any morally significant way from cosmetic plastic surgery.[55] The main concerns here should be safety issues and feasibility in a time of limited medical resources. Although I may prefer that plastic surgery be used to repair serious problems, I do not claim that plastic surgery is unethical when used to further enhance the appearance of those without serious problems.

A far more important issue is raised by the development and use of germ-line gene therapy (GLGT) in which eggs, sperm, or very young embryos are genetically engineered. If preimplantation screening were to show a zygote to be positive for CF, the parents could choose gene therapy to insert a functional allele in the stem cells. Not only is this type of gene therapy permanent, but the transgene also becomes heritably transmitted to future generations of the affected individual. The main concerns surrounding GLGT fit into three categories. First is the "slippery slope" argument which says that we should not proceed with any GLGT protocol because of the ambiguous distinction between positive and negative gene therapy. The second concern is an evolutionary one, arguing that elimination of certain deleterious genes, which could have

some survival value, would eventually be detrimental to the human species. Third, there is an iatrogenic concern, meaning that scientists who carry out GLGT to cure serious genetic maladies may cause even greater maladies for the offspring of the treated individual.

Positive and Negative Gene Therapy

Gene therapy used to cure or treat serious genetic maladies, such as cystic fibrosis, can be referred to as negative gene therapy. The goal of positive gene therapy is to improve or enhance certain characteristics, such as intelligence, in an otherwise normal individual.

When discussing positive and negative gene therapy, it is most helpful to begin with a definition of genetic malady. A generic definition by Clouser, Culver, and Gert, is as follows:

> A person has a malady if and only if he has a condition, other than his rational beliefs and desires, such that he is suffering, or is at increased risk of suffering, a harm or an evil (death, pain, disability, loss of freedom or opportunity, loss of pleasure) in the absence of a distinct sustaining cause.[56]

Genetic conditions, such as CF, hemophilia, and muscular dystrophy, fit the definitional criteria of malady. A genetic condition that does not meet the definitional criteria of a malady should not be considered a malady, and gene therapy for such conditions constitutes positive gene therapy. Examples of nonmaladies might include eye color, blood type, or freckles. It is inevitable that the above definition will be vague to some extent. Some conditions are considered "borderline" because their malady status is questionable. Regarding a borderline condition, such as obesity, it is unclear whether the harm suffered is universal or is primarily due to cultural conditions. Also, there is so

little we know about the extent of environmental influence on some genetic diseases. Even now, it is difficult to distinguish between serious disease, minor disease, and genetic variation. Most can agree that there are extreme cases that clearly represent serious disease. As one moves into the gray area beyond serious disease, those conditions will not be realistic candidates for gene therapy, at least initially.[55]

The fundamental concern comes down to whether positive germ line gene therapy is in some way an unethical practice in itself. If it is not, then our society may decide that GLGT used to enhance a child's intelligence, performance, and appearance is acceptable. One proponent of this view is constitutional scholar John Robertson, who wrote in his 1994 work *Children of Choice*:

> Although it may not count as part of a core procreative liberty, non-therapeutic enhancement may nevertheless be protected. A case could be made for prenatal enhancement as part of parental discretion in rearing offspring. If special tutors and camps, training programs, even the administration of growth hormone to add a few inches to height are within parental rearing discretion, why should genetic interventions to enhance normal offspring traits be any less legitimate? As long as they are safe, effective, and likely to benefit offspring, they would no more impermissibly objectify or commodify offspring than postnatal enhancement efforts do.[57]

W. French Anderson is considered to be one of the preeminent gene therapy researchers in this country, maintaining a lab at both the NIH and UNC. He represents the other side of the argument, strongly and repeatedly cautioning against germ-line enhancements. He says that they do not belong in the same class as "postnatal enhancement efforts," as ballet classes and after-school tutors. In his article published in the *Hastings Center Report,* he wrote:

> First, it could be medically hazardous, i.e., the risk could exceed the potential benefits and could therefore cause harm, and second, it would be morally precarious, i.e., it would

require moral decisions that our society is not now prepared to make and which could lead to an increase in inequality and an increase in discriminatory practices.[58]

The Slippery-Slope Argument

The argument put forth by some is that, if we use negative gene therapy to cure serious disease, we will be unable to prevent positive gene therapy from occurring. Because we will not be able to draw a nonarbitrary line between negative and positive gene therapy, we should protect ourselves from the latter by prohibiting the former. Those that believe GLGT is not an acceptable method for making societal decisions see it as a "slippery slope" to modern eugenics, which cannot be justified. The abuse of power that societies have historically demonstrated in the pursuit of eugenic goals is well-documented. By starting with small "improvements," some say we will slide into a new age of eugenic thinking.

However, others including myself, hold a less extreme view. With proper education, careful foresight, and open moratoriums, society can learn from its past and will be able to apply genetic information and technology responsibly. I believe that gene therapy research has, thusfar, proceeded with caution. As a society, we should be working to prevent discrimination against individuals who do or do not elect to participate in gene therapy.

Evolutionary Concerns

A major concern voiced by critics is evolutionary in nature. Some deleterious alleles that would be systematically eliminated by gene therapy may have some unknown survival

benefit to future offspring. Genetic variation affords an evolutionary advantage in adapting to new and perhaps unforeseen conditions. A good example is found in populations where sickle cell anemia is prevalent. An individual who is homozygous for the recessive gene develops sickle-cell anemia. However, heterozygotes (those who carry the sickle-cell allele and a normal allele) have a high-resistance to malaria. The relative immunity of sickle-cell carriers to malaria is thought to occur because some of the red blood cells of the heterozygotes are sickle-shaped, a state that inhibits infection by the malarial parasite.

The evolutionary concern is misleading for two reasons. First, when discussing maladies based on the inheritance of recessive alleles, it is important to remember that it is not the presence of two mutant alleles that causes the malady, it is rather the absence of a normal allele. In other words, as long as the normal allele is present, the mutant allele will have no effect. So, as long as a normal allele is introduced into the genome, gene therapy will work for recessive conditions. The mutant and nonfunctional genes may still remain. Therefore, in theory, gene therapy will not lead to a loss of allelic variation.

Secondly, it seems very unlikely that there will be any serious attempt to totally eradicate an allele from the human gene pool. The technology required will be expensive and will probably be applied on an individual basis, with rather limited accessibility. Although many couples might qualify for gene therapy, only a small number would elect to participate. It is also well known that the majority of mutant alleles are kept in the gene pool through a heterozygous carrier. There would be no reason to perform gene therapy on heterozygotes, so the frequency of alleles would still remain at a high level. For

example, if germ-line gene therapy could be developed for Tay-Sachs disease and was

used to treat all homozygous Tay-Sachs embryos (which occur at a frequency of 1/2000),

the frequency of the Tay-Sachs allele in the entire population would decrease only from

0.01000 to 0.0099 in one generation.

Iatrogenic Concerns

Germ line gene therapy would not only affect the treated individual, but would also affect

the individual's offspring. Since embryos cannot give informed consent, the couple

involved would be responsible. If GLGT had no associated risks, there would be no

problem with the parents giving consent. However, GLGT does carry risk that may affect

generations to come. There is so little we know about how genes act - a single gene can

have multiple effects on the body. Moreover, genes do not act alone. Their effects can

be amplified, diminished, or counterbalanced in ways we do not fully understand. W.

French Anderson in his article said:

> Medicine is a very inexact science. We understand roughly how a simple gene works that
> there are many thousands of house-keeping genes, that is, genes that do the job of running
> the cell. We can predict that there are genes which make regulatory messages that are
> involved in the overall control and regulation of the many housekeeping genes. Yet we
> have only limited understanding of how a body organ develops into the size and shape it
> does. We know many things about how the central nervous system works --- for we are
> beginning to comprehend how molecules are involved in electric circuits, in memory
> storage, in transmission of signals. But we are a long way from understanding thought
> and consciousness. And we are even further from understanding the spiritual side of our
> existence.
> Even though we do not understand how a thinking, loving, interacting organism can be
> derived from its molecules, we are approaching the time when we can change some of
> those molecules. Might there be genes that influence the brain's organization or structure
> or metabolism or circuitry in some way so as to allow abstract thinking, contemplation of
> good and evil, fear of death, awe of a "God"? What if in our innocent attempts to improve
> our genetic makeup we alter one or more of those genes? Could we test for the alteration?
> Certainly not at present. If we caused a problem that would affect the individual or his or
> her offspring, could we repair the damage? Certainly not at the present. Every parent who

has several children knows that some babies accept and give more affection than others, in the same environment. Do genes control this? What if these genes were accidentally altered? How would we know if such a gene were altered?[58]

The list of unknown risks could not be longer. A major known risk emerges from the fact that, with our present techniques, a transgene cannot target a specific region of chromosome. The danger in random insertion lies in the capability of insertional mutagenesis, which has been documented in a number of cases. In theory, the insertion of foreign DNA may disrupt some important basic gene function if the site of insertion were to be near or within the affected gene. If a transgene were to be inserted near or within a protooncogene or tumor suppressor gene, cancer could result. Even if GLGT were to become an acceptable goal, there would have to be precise guidelines that reflect the limitations of technology. These limitations could restrict the use of GLGT to treating only very serious life-threatening genetic conditions.

CONCLUSIONS

I believe that there are many convincing reasons why germ-line gene therapy should not

be pursued. First and foremost is the safety issue. We have such a lack of knowledge

about how genes work and the long-term results of gene insertion, especially regarding

the effects of protooncogenes and tumor suppressor genes. In fact there was a concern a

few years back when lymphomas developed in monkeys exposed to a retrovirus in a gene

therapy experiment. In the May, 1997, issue of *Proc Natl Acad Sci USA* (1997;94:5837),

Carsten Munk and colleagues from Germany, a country where gene therapy controls are

especially tight, recorded spongiform encephalomyelopathy in mice inoculated with

amphotrophic murine leukaemia virus. The insertional risk involved in SCGT is certainly

present, and is even greater for GLGT.

The other major concern is the possibility of using GLGT for enhancement purposes. In

a simple risk-benefit analysis, I can see no justification for using GLGT for enhancement

purposes. We might be willing to expose a patient to great risk to treat a grave disease,

but subjecting someone to the same risk is ethically unacceptable if the person seeking

treatment is healthy. The use of gene therapy for enhancement is only speculative and

unlikely at this time. Because genetic enhancement might reinforce irrational social

prejudices, we must look ahead and consider the social harm that might result.

I am not suggesting that germ line gene therapy should not be developed at all, just not at

this time. Now, research should be directed toward discovering the short- and long-term

effects of the gene insertion in general, and should be working toward eliminating

iotrogenic risk. In time, like any other medical procedure, germ-line gene therapy must be proven safe within an animal model before human trials are even considered. In the case of grave disease, where extreme suffering and loss of life are inevitable, human trials (to ensure the safety of the procedure) and risk associated with the procedure itself, are both justifiable.

When considering somatic cell gene therapy, I believe that direct efforts in improving its problematic aspects should continue. Improving vector delivery, efficacy, and safety should remain at top priority. I see no ethical reason why SCGT should not be embraced. However, I believe that a moratorium needs to be held to discuss issues of SCGT enhancement. We need to decide what enhancements we consider unacceptable and prevent their use. A helpful model is the moratorium that scientists imposed on themselves in the early 1970s, when they had just discovered how to manipulate genetic material through recombinant DNA techniques.

Genetic medicine should continue to be an empowering, not an exclusionary science. It needs to be viewed as a clinical tool employed to help people increase their length and quality of life, not as a public-health device designed to benefit the population. Gene therapy has the potential to treat, cure, and ultimately prevent diseases like cystic fibrosis. Both somatic cell and germ line gene therapy should be approached cautiously, guidelines should be established regarding enhancement, and our long-term judgment must not be blurred by short-term benefits.

GLOSSARY OF ABBREVIATIONS

ATP	adenosine triphosphate ; a high energy molecule
AAV	adeno-associated virus
AV	adenovirus
cDNA	complementary DNA; used in DNA cloning, usually made by reverse transcriptase; complementary to a given mRNA
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CPT	chest physiotherapy
DC-CHOL	D(3βN[N'N'-dimethylammonoethane)-carbomyl] cholesterol
DMRIE	1,2-dimyristyloxypropyl-3-dimethylhydroxyethylammonium bromide
DNA	deoxyribonucleic acid
DOPE	dioleoylphosphatidylethanolamine
DOTMA	N-[1-2,3-dioleyloxypropyl]-N,N,N-trimethylammonium chloride
ΔF508	most common CF gene mutation; absence of three bases (one cytosine and two thymines), resulting in the deletion of phenyalanine (single letter abbreviation F) at position 508 of the CFTR protein
GI	gastrointestinal
kb	kilobase; unit of measurement for genetic material
LTRs	long terminal repeats; flank each end of retroviral genome
MuLV	murine leukaemia virus; most common retroviral vector used in current gene-therapy protocols
NBD	nucleotide-binding domain
NIH	national institutes of health
OB	obliterative bronchiolitis
PKA	enzyme protein kinase A
RAC	recombinant DNA advisory committee
RNA	ribonucleic acid; genetic material from which proteins are synthesized
RT	reverse transcriptase; used to convert retroviral RNA to proviral DNA
TMD	transmembrane domain

Table 1

incidence of cystic fibrosis (per live births)	
Whites	1 in 3,200
Hispanics	1 in 9,500
Native Americans	1 in 11,200
Blacks	1 in 15,000
Asians	1 in 31,000

Table 2

clinical conditions associated with cystic fibrosis

Respiratory
Airway reactivity
Allergic bronchopulmonary
 aspergillosis (ABPA)
Atelectasis
Bronchiectasis
Bronchitis
Chronic cough
Cor pulmonale
Fibrosis
Dyspnea
Hemoptysis
Hyperinflation of the lungs
Nasal polyps
Pneumonia
Pneumothorax
Progressive loss of pulmonary
 function
Pulmonary hypertension
Recurrent exacerbations of
 pulmonary infection
Sinusitis
Sputum production
Tachypnea
Wheezing

Pancreatic
Abnormal glucose tolerance
CF-related diabetes mellitus
 (CFRDM)
Pancreatitis
Pancreatic enzyme insuffi-
 ciency/malabsorption
Pancreatic obstruction

Gastrointestinal
Abdominal pain/distension
Distal intestinal obstruction
 syndrome (meconium ileus
 equivalent)
Excessive flatus
Fibrosing colonopathy
Gastroesophageal reflux
Intussusception
Meconium ileus
Meconium plug syndrome

Peptic ulcers
Rectal prolapse
Steatorrhea
Volvulus
Weight loss

Hepatobiliary
Ascites
Cholangitis
Cholecystitis
Cholelithiasis
Cirrhosis of the liver
Edema
Fatty liver
Focal biliary fibrosis
Jaundice
Portal hypertension
Prolonged neonatal jaundice

Reproductive
Azoospermia
Delayed puberty
Hydrocele
Infertility
Inguinal hernia
Thickened vaginal secretions

Sweat abnormalities
Dehydration
Heat stroke
Hyponatremia
Salty sweat

Other
Arthritis (episodic)
Bruising
Digital clubbing
Failure to thrive
Hypoprothrombinemia
Impaired growth
Metabolic alkalosis
Otitis media (chronic)

Table 3   Phenotypic features consistent with a diagnosis of CF

1.   Chronic sinopulmonary disease manifested by
  a. Persistent conlonization/ infection with typical CF pathogens including
Staphylococcus aureus, nontypeable *Haemophilus influenzae*, mucoid and nonmucoid
Pseudomonas aeruginosa, and *Burkholderia cepacia.*
  b. Chronic cough and sputum production
  c. Persisten chest radiograph abnomalities (e.g. bronchiectasis, atelectasis, infiltrates,
hyperinflation)
  d. Airway obstruction manifested by wheezing and air trapping
  e. Nasal polyps; radiographic or computed tomographic abnormalities of the paranasal
sinuses
  f. Digital clubbing

2.   Gastrointestinal and nutritional abnormalities including:
  a. Intestinal: meconium ileus, distal intestinal obstruction syndrome, rectal prolapse
  b. Pancreatic: pancreatic insufficiency, recurrent pancreatitis
  c. Hepatic: chronic hepatic disease manifested by clinical or histologic evidence of
focal biliary cirrhosis or multilobular cirrhosis
  d. Nutritional: failure to thrive (protein-calorie malnutrition), hypoproteinemia and
edema, complications secondary to fat-soluble vitamin deficiency

3.   Salt loss syndromes: acute salt depletion, chronic metabolic alkalosis

4. Male urogenital abnormalities resulting in obstructive azoospermia (CBAVD)

Table 4

Most Common CFTR Mutations in the World		
Name of Mutation	Frequency (%)	Population with High Prevalence
ΔF508	28,948 (66)	
G542X	1062 (2.4)	Spanish
G551D	717 (1.6)	English
N1303K	589 (1.3)	Italian
W1282X	536 (1.2)	Jewish-Ashkenazi
R553X	322 (0.7)	German
621+1G→T	315 (0.7)	French-Canadian
1717-1G→A	254 (0.6)	Italian
R117H	133 (0.3)	
R1162X	125 (0.3)	Italian
R347P	106 (0.2)	
3849+10kbC→T	104 (0.2)	Jewish-Ashkenazi, Hispanic
Δ1507	93 (0.2)	
394delTT	74 (10–30)*	Nordic, Finnish
G85E	67	
R560T	67	
A455E	62	Dutch
1078delT	57	Celtic
2789+5G→A	54	Spanish
3659delC	54	
R334W	53	
1898+1G→T	53	
711+1G→T	49	French-Canadian
2183AA→G	49	Italian
3905insT	38 (6–17)*	Swiss, Amish, Acadian
S549N	30	
2184delA	29	
Q359K/T360K	(87.5)*	Jewish-Georgian
M1101K	(69)*	Hutterite
Y122X	(48)*	French, Reunion Island
1898+5G→T	(30)*	Chinese, Taiwan
3120+1G→A	(11)*	African-American
I148T	(9.1)	French-Canadian

Table 5

drugs used for gastrointestinal manifestations

Pancreatic enzyme supplements

Examples: pancreatin, pancrelipase

- Supplements the pancreatic enzymes amylase, lipase, protease
- Enables patients to digest food
- Needed in about 85% of patients
- Goal of enzyme replacement is to maintain near-normal, well-formed stools (avoiding steatorrhea) and adequate weight gain (goal is 1–2 stools/day)
- Amount of enzyme needed varies with patient age and size and the fat content of meals
- The usual dose is 500–2500 lipase units/kg/meal. If patients require more than 2000–2500 lipase units/kg/meal, they should be evaluated to determine if causes other than malabsorption are producing symptoms
- Enzyme supplements may be given mixed in prepared foods
- In infants and young children, ensure that no enzyme beads remain in contact with the mucous membranes of the mouth, to prevent skin breakdown
- Frequent diaper changes, good perianal hygiene, and application of petroleum jelly will help prevent skin breakdown
- Try not to overdose or underdose enzyme; maintain a careful balance between diet and supplement

Antacids

Examples: aluminum or magnesium hydroxide

- May be useful in patients with ulcers or gastro-esophageal reflux
- H_2 blockers are preferable to the long-term use of antacids, which may interfere with the absorption of some antibiotics

H₂ blockers

Examples: cimetidine, ranitidine

- Used for patients with gastroesophageal reflux, ulcers
- May increase the effectiveness of pancreatic enzyme supplements by decreasing stomach acidity

Antihyperglycemic agents

Examples: insulin, oral agents (eg, glipizide)

- Indicated for patients with CF-related diabetes
- Most patients with CF do not have Type I diabetes although they may be treated with low doses of insulin

Table 6

drugs used for managing pulmonary symptoms

Antibiotics	aminoglycosides	erythromycin
	(eg, tobramycin)	clindamycin
	penicillins	quinolones
	cephalosporins	(usually not used in young children)

- Antibiotics are the cornerstone for the treatment of respiratory tract infections
- Common pathogens are *Pseudomonas aeruginosa* and *Staphylococcus aureus*; less common are *Haemophilus influenzae*, *Burkholderia cepacia*, *Candida,* and *Aspergillus*
- Once established, infection usually cannot be eradicated, only controlled
- No consensus exists on the use of prophylactic antibiotics
- Oral antibiotics may be used in patients with mild exacerbations, but IV therapy is usually required
- Aerosolized antibiotic therapy has recently become available

Corticosteroids	hydrocortisone	triamcinolone
	prednisone	flunisolide
	beclomethasone	dexamethasone

- Used to treat airway obstruction secondary to bronchospasm and mucosal inflammation
- Studies to date indicate that the incidence of serious side effects with systemic corticosteroids outweighs their benefits in routine use in patients with CF
- Administration of corticosteroids by inhalation could mitigate their adverse effects; efficacy studies of inhaled corticosteroids are under way
- Alternate-day use for 1–2 years may be considered in selected patients

Bronchodilators

- Routes of administration: oral, subcutaneous, inhaled
- Used to treat airway hyperactivity and to reverse bronchospasm
- While common, use of bronchodilators by patients with CF remains controversial
- Long-term benefit in CF has not been established

Cough suppressants

- Not generally used in CF because coughing is desirable to clear mucus

Nonsteroidal anti-inflammatory drugs (eg, ibuprofen)

- In patients with CF and mild lung involvement, long-term high-dose ibuprofen may slow the progression of lung disease
- Lab monitoring of BUN and creatinine levels is required

Mucolytic

acetylcysteine

- Inhalation preferred over oral route of administration, but inhaled acetylcysteine can trigger bronchospasm
- Use in CF controversial; controlled trials indicate little benefit

Enzyme

recombinant human deoxyribonuclease (rhDNase)
In vitro, this enzyme decreases mucus viscosity by digesting extracellular DNA in the mucus In vivo, dornase alfa

- Improves pulmonary function
- Reduces incidence of exacerbations of infection that require parenteral antibiotics in patients ≥ 5 years of age whose FVC is ≥ 40% of predicted
- Improves quality of life

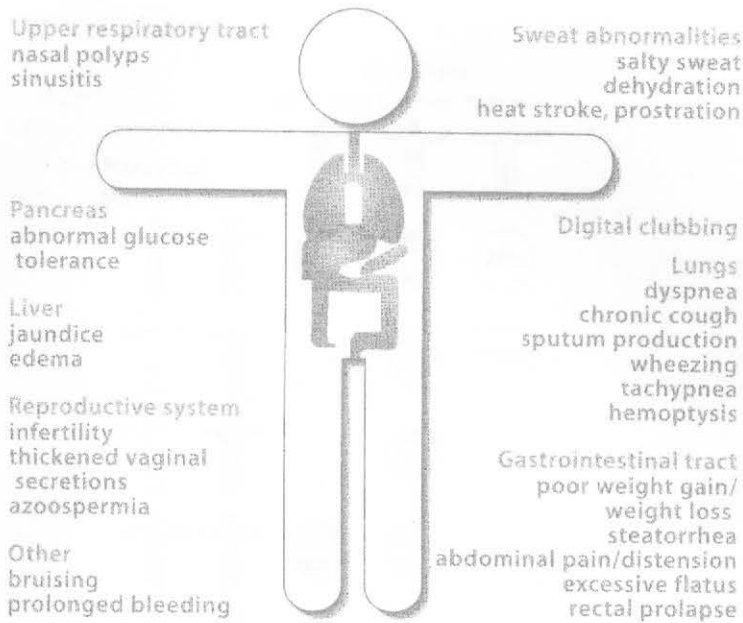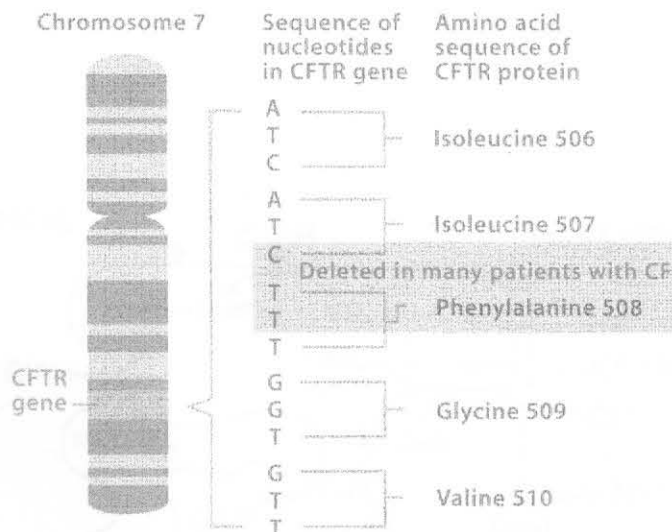Figure 1. Clinical manifestations of CF

Figure 2. The ΔF508 mutation in chromosome 7 that is most commonly associated with Cystic Fibrosis
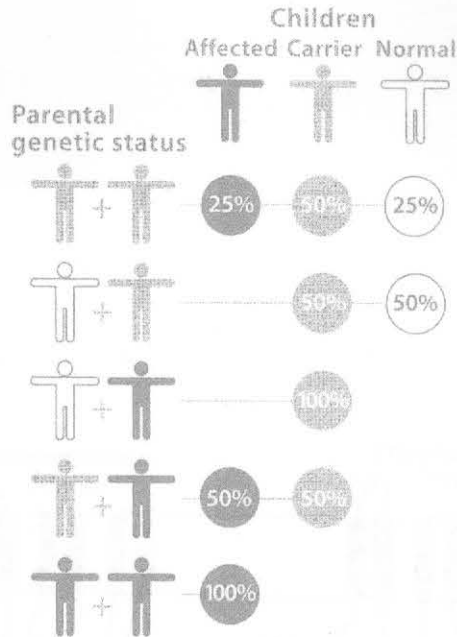
Figure 3. Possible outcome of a pregnancy according to the genetic status of the parents
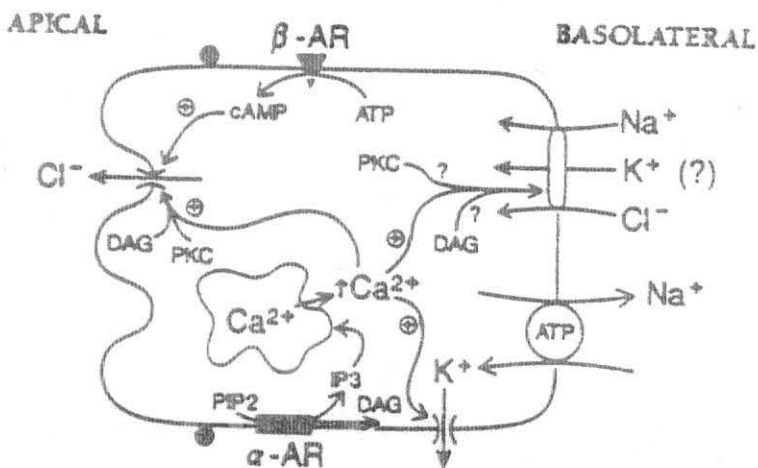

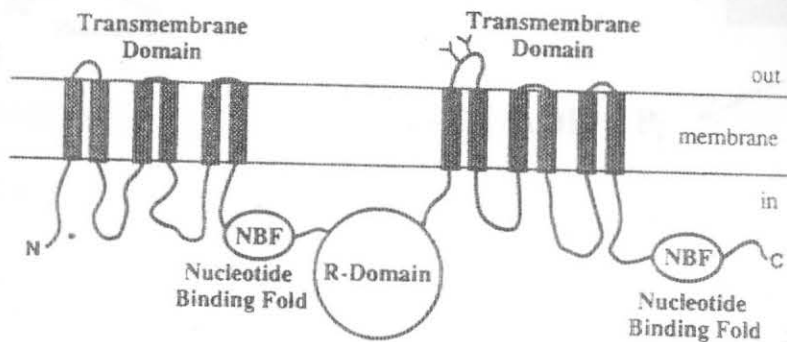Figure 4. Model of cAMP-regulated chloride secretion

Figure 5 Model of CFTR. The protein is shown inserted into a lipid bilayer. Potential glycosylation sites are represented as branched structures on the fourth extracellular loop. Nuclear Binding Domain (NBD) also called Nuclear Binding Fold (NBF)
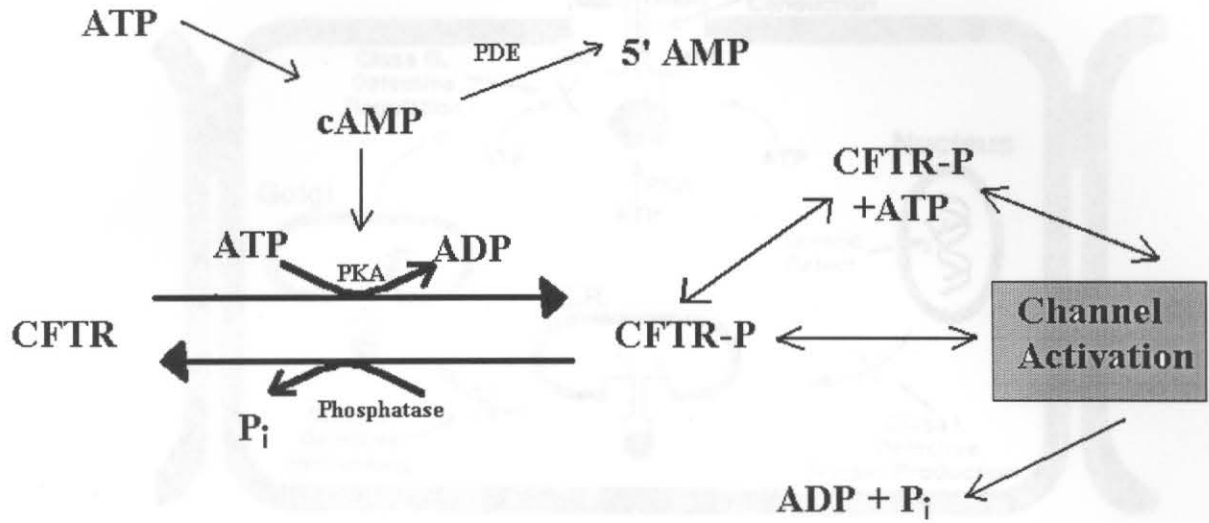
Figure 6. Multistep kinetic model of CFTR activation. Chloride channel opening requires two step: (a) CFTR phosphorylation by cAMP-dependent PKA and (b) ATP binding and hydrolysis. PKA = protein kinase A; PDE = phosphodiesterase; cAMP = cyclyc AMP; P_I = inorganic phosphate
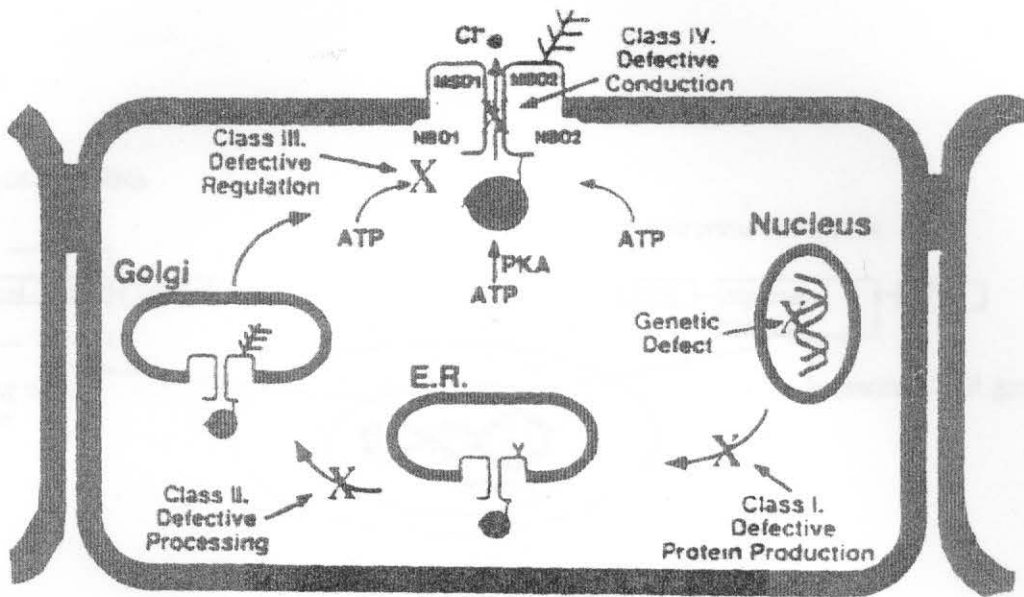
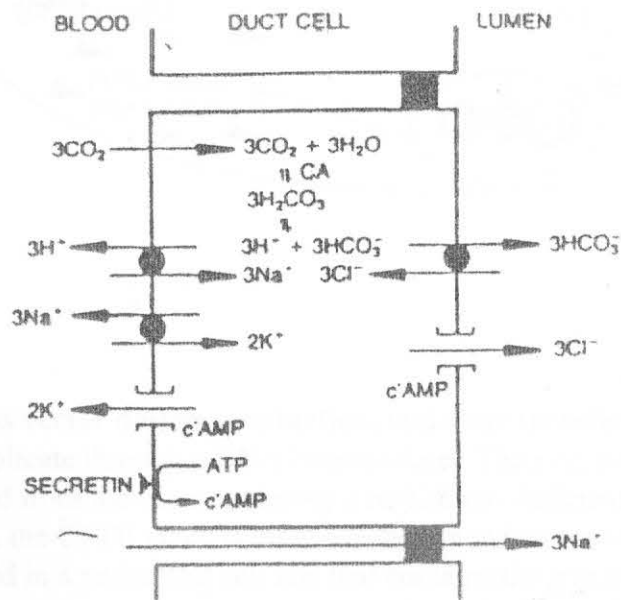Figure 7. Biosynthesis and function of CFTR in an epithelial cell

Figure 8. Cellular model of bicarbonate secretion by pancreatic duct cell.
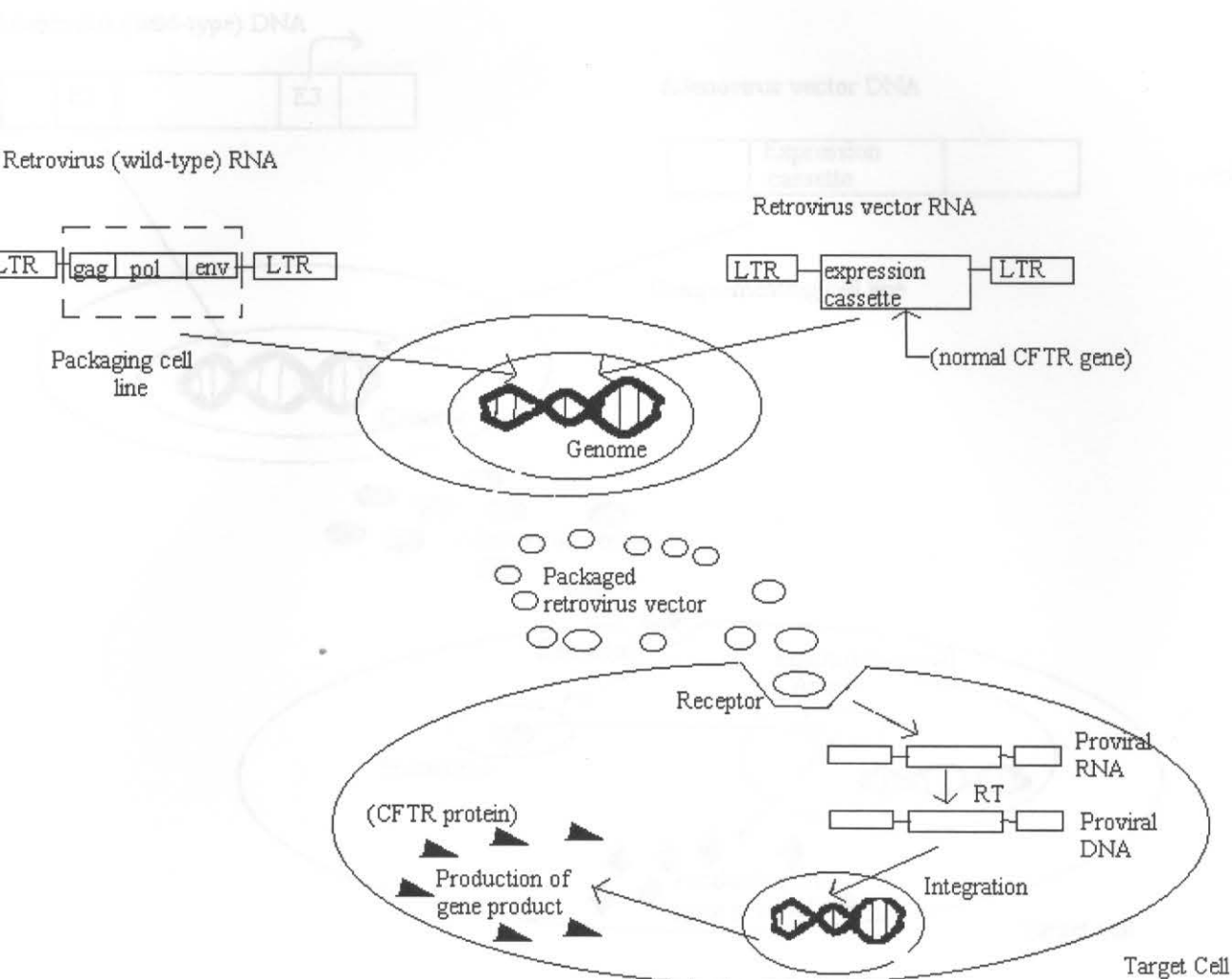CA = carbonic anhydrase

Figure 9. **Retrovirus vector design, production, and gene transfer.** Retroviruses are RNA viruses that replicate through a DNA intermediate. The *gag, pol,* and *env* sequences are deleted from the virus rendering it replication-deficient. The expression cassette, in this case, the CFTR gene, is inserted, and the infectious replication-deficient retrovirus is produced in a packaging cell line that contains the *gag,pol,* and *env* sequences that provide the proteins necessary to package the virus. The vector with its expression cassette enters the target cell via a specific receptor. In the cytoplasm, reverse transcriptase (RT) carried by the vector converts the vector RNA into the proviral DNA that is randomly integrated into the target cell genome, where the expression cassette makes its product, in the case of cystic fibrosis - the functional CFTR protein.
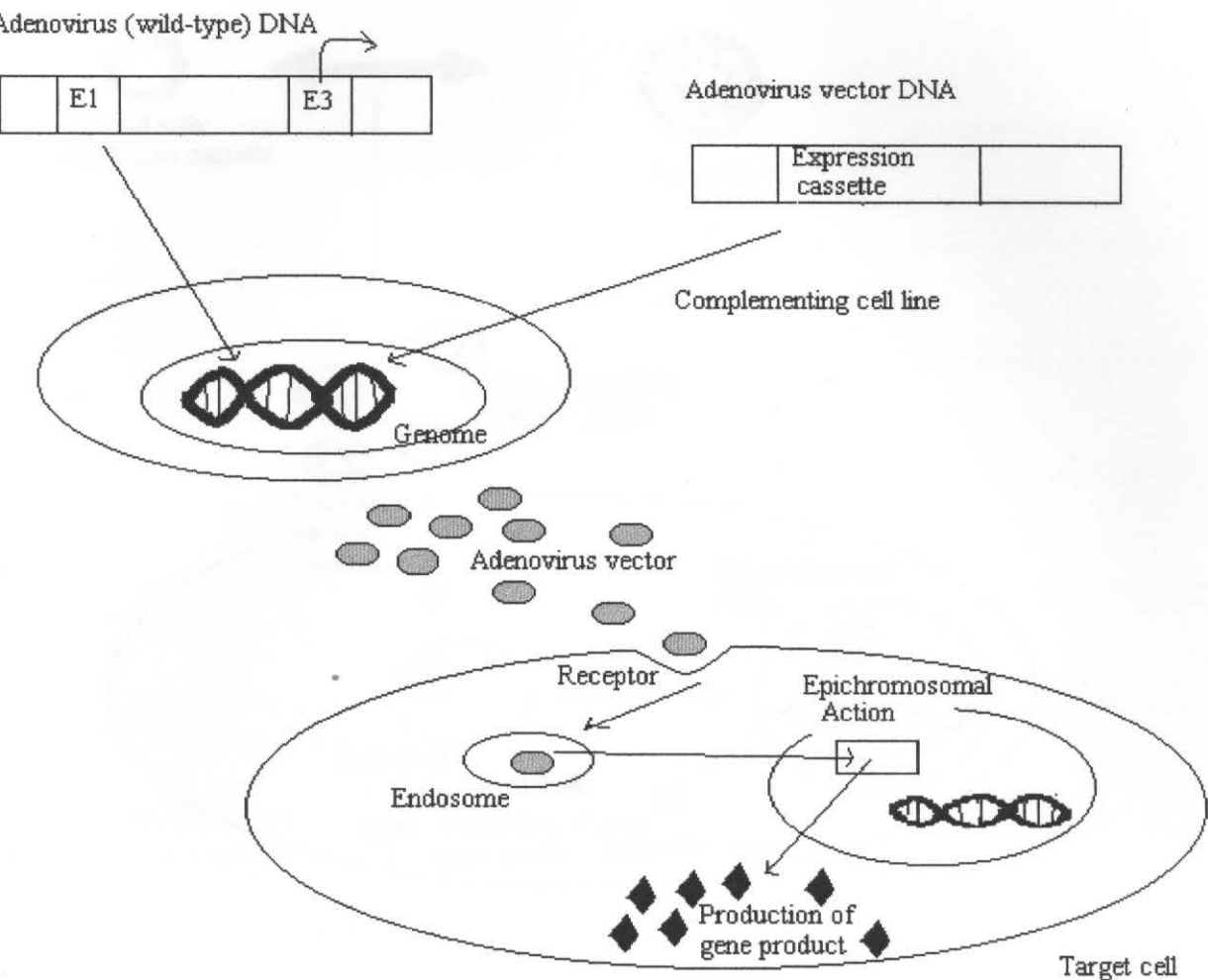
Figure 10. **Adenovirus vector design, production, and gene transfer**. Adenoviruses are DNA viruses with a 36-kb genome. The wild-type adenovirus genome is divided into early (E1 to E4) and late (L1 to L5) genes. All adenovirus vectors administered to humans use adenovirus serotypes 2 or 5 as the base. The ability of the adenovirus genome to direct production of adenoviruses is dependent on sequences in E1. To produce an adenovirus vector, the E1 sequences (and E3 sequences if the space is needed) are deleted. The expression cassette is inserted (in our case, the CFTR gene), and the vector DNA is transfected into a complementing cell line with E1 sequences in its genome. The adenovirus vector with its expression cassette is E1‾ and thus incapable of replicating. The vector binds to the target cell through an interaction of the adenovirus fiber and penton, each to a specific receptor, moves into a cytoplasmic endosome, and breaks out and delivers its linear, double-stranded DNA genome with the expression cassette into the nucleus where it functions in an epichromosomal fashion to direct the expression of its product.
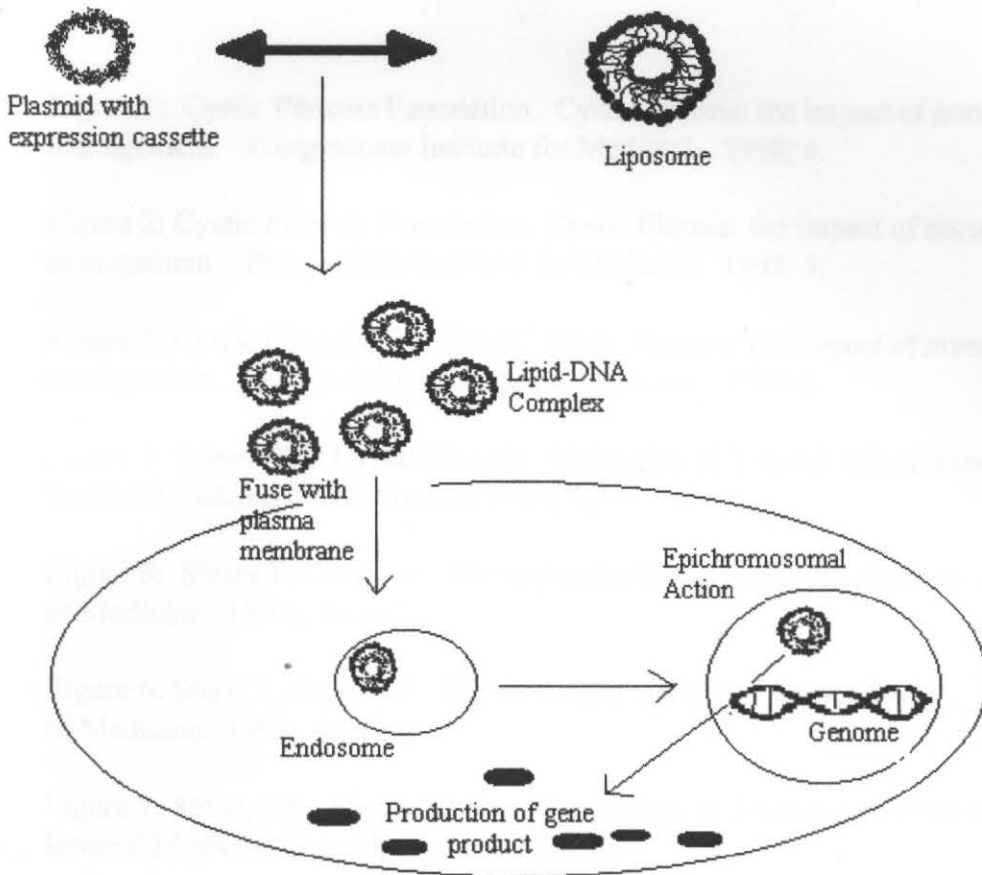
Figure 11. **Lipid:DNA complex design and gene transfer**. The liposomes used in human gene transfer trials have various compositions, but typically include synthetic cationic lipids. The positively charged liposome is complexed to the negatively charged plasmid with its expression cassette, in cystic fibrosis gene therapy – the CFTR gene. The complexes enter the target cell by fusing with the plasma membrane. The vector does not have an inherent macromolecular structure that conveys information to enable efficient translocation of the plasmid to the nucleus. Consequently, most of the newly introduced genetic material is wasted as it is shunted to the cytoplasmic organelles. When used *in vivo*, it is likely that most, if not all, of the plasmids that reach the nucleus will function in an epichromosomal fashion.

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