

Prospects for gene therapy in cystic fibrosis

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Since the discovery of the gene encoding cystic fibrosis (CF), there has been much excitement about the possibility of gene therapy, which has now reached the stage of phase I clinical trials in adults. The baby born with CF has normal lungs at birth, but evidence of inflammation and lung changes are present as early as 4 weeks of age. Ideally, future novel treatments such as gene therapy should begin before the onset of airway damage. We review the pathophysiology of CF, including current molecular understanding, and set out the issues involved in gene therapy for children with CF.

Pathophysiology

CF is the commonest lethal inherited disease in white people in the UK, with an incidence of ~ 1/2000 live births. The median estimated life expectancy of children born in the 1990s is 40 years, which represents a doubling in the past 20 years. It is estimated that in the next millennium half of all patients will be adults.

In 1989, the gene responsible for CF was localised to the long arm of chromosome 7. This gene encodes a protein called the CF transmembrane conductance regulator (CFTR), which is situated in the apical membrane of epithelial cells and functions as a chloride channel regulated by a cAMP dependent protein kinase. It also modulates the activity of other ion channels including the downregulation of the amiloride sensitive sodium channel.

More than 800 mutations of the gene have been identified and they are categorised into five classes on the basis of CFTR protein alterations. Class I mutations result in either unstable mRNA or an abnormal protein that is rapidly degraded. Class II mutations result in faulty processing of the protein and failure of the protein to traffic to the apical membrane. This class includes the $\Delta F508$ mutation, which is a codon deletion resulting in the loss of a phenylalanine residue at position 508 in the first nucleotide binding fold. Approximately 70% of all patients with CF carry this deletion but there is great geographical variation and $\Delta F508$ is less common in non-white races.¹ Class III mutations result in a protein that is correctly localised but has altered regulation of its chloride channel activity. Class IV mutant proteins are correctly localised and regulated but have altered rates at which they allow chlo-

ride ions to flow. Class V mutations result in reduced synthesis of CFTR and these patients tend to have mild disease, borderline sweat tests, and residual intestinal chloride secretion.

Role of CFTR in the developing and growing lung

The role of CFTR in the developing human lung is unclear. Animal work, predominantly in lambs, indicates that there is active chloride secretion in the lung early in gestation leading to the production of fetal lung liquid. In the days immediately before birth, fluid secretion falls and after birth the lung absorbs rather than secretes fluid, primarily through sodium absorption.² In humans, the gene encoding CFTR is expressed throughout gestation, becoming more localised to the proximal airway in late pregnancy, a distribution resembling that seen in the adult.³ Postnatally, most CFTR expression occurs in the serous cells of the submucosal gland. In the first 2 years of life, the relative percentage of serous cells increases and few new glands are formed, the increase in gland area being the result of an increase in gland complexity. The gland does not approach the form characteristic of the adult until 13 years of age.⁴ This is of relevance if the submucosal glands are to be targeted by gene therapy in children. At present, however, it is the epithelial cells of the airway that are targeted, and it is estimated that as little as 6% of these cells need to be transfected to correct the chloride defect.⁵

Role of CFTR in lung pathology

Defects in the CFTR protein lead to abnormal host defence and lung damage by a variety of proposed mechanisms. The thick sputum typically seen in CF patients may be a result of poor hydration of the airway surface fluid and defective ciliary clearance. The resultant airway plugging and bacterial colonisation induces inflammatory responses that destroy surrounding lung tissue. Secondly, it has been shown that high sodium and chloride concentrations inactivate naturally occurring antimicrobial peptides present in the airway surface liquid.⁶ The composition of the airway fluid is controversial, but if such changes in salt concentrations are present in vivo, this may link the ion channel defects to the increased bacterial colonisation. Furthermore, it has been suggested that CFTR can itself act as a receptor for

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binding, endocytosing, and clearing *Pseudomonas aeruginosa*,⁷ a function that may be missing in CF. Finally, defective CFTR leads to an increase in binding sites for bacteria, commonly isolated from CF subjects, with a consequent increase in bacterial adherence.⁸ The relative importance of these, or other yet unknown mechanisms, is at present uncertain.

CF gene therapy trials to date

The current principle behind gene therapy in CF is the insertion of DNA encoding normal CFTR into respiratory cells, which should overcome the abnormalities seen in CF. The use of a vector as a delivery vehicle increases gene transfer. So far, there have been five phase I clinical trials using adenovirus as a vector, one using adeno associated virus (AAV), and four with liposomes.⁹ There are at least seven other phase I trials currently under way or recently completed. Recombinant adenoviruses have the advantage of high transduction efficiency and are potentially excellent vectors. However, clinical trials have shown that they elicit an immune response, as demonstrated by changes on computed tomography and the production of antibodies; this limits repeated application. AAV has the potential for site specific integration into the host genome, which could lead to prolonged expression, although this has not been shown clearly in the airways in vivo. Although less efficient than viruses, synthetic vectors such as cationic liposomes are less toxic, thus allowing repeated administration and delivery of large quantities of DNA. Modifications in the way the liposome is structured should increase efficiency in the future.

Because the trials to date have been primarily for proof of principle, there has been no expectation of clinical benefit, such as improvement in lung function or quality of life. Most of the studies have concentrated on the nose as a model for the lower airway because it is easier to access for measurements and is likely to be safer. A novel approach is the use of the maxillary sinus in those patients with an antrotomy.¹⁰ The importance of studying the lower airway is becoming more evident and such studies have been undertaken both with adenovirus¹¹ and more recently with liposomes.¹²

Which end points?

During the phase of mild to moderate inflammation, when gene therapy would be expected to have an effect, survival curves are generally unhelpful as an outcome measure, and surrogate end points need to be used. These might be molecular, such as the demonstration of mRNA or CFTR protein. Another approach is to measure functional changes, such as the potential difference response across the airway epithelium. This can be used both as a diagnostic tool¹³ and experimentally.¹⁴

Using a fluorescence marker that quantifies chloride secretion, CFTR function can be further assessed in vitro in cells brushed from the airway.¹⁵ A recent development in assessing CFTR function is an assay that measures the adherence of *P aeruginosa* to CF airway epithel-

lia. This is reduced following CFTR gene transfer.⁸ In the nose trials, approximately half of the patients show molecular evidence of gene transfer and about one third demonstrate a degree of correction of the chloride defect. Recently, using bronchial potential difference measurements, a significant correction of the chloride defect was shown in the lungs after nebulisation with a liposome-CFTR cDNA complex.¹² No trial has demonstrated correction of the sodium hyperabsorption.

Potential for gene therapy in children with CF: the ethical dilemma

To date, the only gene therapy trials to involve children have been in adenosine deaminase (ADA) deficiency.¹⁶ One of the important aspects in the development of all potential treatments is that of safety. This is particularly so in gene therapy, where there are controversial ethical implications, and trials have had to adhere to stricter ethical guidelines than most other clinical trials. In our lung trial, only male patients with proven sterility could be recruited, because of the hypothetical risk of incorporation of the delivered gene into the somatic cell line. Different ethical rules apply to children, and before gene therapy is used on children society has to deem it acceptable. This is made even harder when one considers that the predicted median survival is 40 years and children are no longer expected to die early in life such as is the case in ADA deficiency. Future gene therapy trials in children are likely to require invasive procedures, such as repeat bronchoscopies. In the beginning, gene therapy trials in children are likely to be carried out to demonstrate safety and proof of principle, and it is unlikely that they will derive clinical benefit. There is also the potential that this relatively well group of patients may be made less well, because it is unknown what effect gene delivery will have on the growing lung. In addition, there are the general ethical implications about conducting non-therapeutic trials in children who are unable to consent. The Royal College of Paediatrics and Child Health (formerly the British Paediatric Association) has published ethical guidelines on research in children.¹⁷ They are centred around six basic principles:

- research involving children is important for the benefit of all children and should be encouraged
- children are not small adults and have unique additional needs
- research involving children should be carried out if comparable research on adults could not answer the same question
- a research procedure that is not intended to benefit the child directly is not necessarily unethical
- proposals involving children should be submitted to local research ethics committees
- valid consent should be obtained from the child, parent, or guardian as appropriate.

When is the right time to start in children?

As with the development of any drug treatment, it is usual to start in adults and progress to children. So when is the appropriate time to commence trials in children? Proof of principle in both the nose and lung has been achieved in adults; however, trials have yet to provide evidence of clinical benefit, although they have proved to be relatively safe. As with all treatments, the potential risks must be weighed against potential benefits. Ideally, trials in children would commence after clinical benefit has been shown in adults. However, this approach, which protects children from the potential harms of research, might deny them potential benefits.

In addition, it might be difficult to show clinical benefit in adults with already established disease. Furthermore, adult and paediatric lungs might behave differently. Work in the growing lungs of animals with adenoviral and AAV vectors has shown that expression of the CFTR gene after gene therapy is comparatively better than in adult lungs.¹⁸⁻²¹ Conversely, using a liposomal vector, preliminary work in mice suggests that the reverse is true.²²

It is likely that the medical profession and society will be more comfortable with gene therapy trials in children if complete and repeatable correction of the ion defects is demonstrated. However, at present, it is unknown whether the correction of the chloride ion defect alone is sufficient for clinical improvement.

A more contentious issue is that of fetal gene therapy. This has the potential advantage of avoiding immune sensitisation, targeting stem cells that are not accessible after birth, and providing an alternative to termination after antenatal diagnosis. Animal work with viral vectors has shown that in utero gene transfer to the fetal lung is possible,^{23 24} and a controversial study by Larson *et al* suggested that in utero gene transfer to a CF knockout mouse resulted in a permanent reversal of the abnormal phenotype.²⁵ However, at present, none of the vectors meets the requirement of a single safe application to the relevant stem cells and the potential risk to the fetus is too great.²⁶

Cost

Ultimately, there will be financial issues, as with all new treatments. Developing gene therapy is expensive and millions of pounds are being invested in this area so that it is unlikely that the final product will be cheap. However, we can hope that gene therapy may ultimately lead to savings in hospital admissions, drugs, and medical time. There will undoubtedly be arguments over who is responsible for payment of the treatment, such as we are now seeing with recombinant human DNase. This underscores the need for agreed central funding for expensive treatments, so that they can be distributed on the basis of need not region.

Conclusions

Since the discovery of the gene responsible for CF there has been a huge increase in our knowledge about the molecular pathophysiology of CF. The aim must be that future innovative treatments should begin before the onset of airway damage—this means that babies with CF should receive gene therapy either in utero or soon after birth. Now is the time to consider trials in children and, for the public to accept this, there has to be rational dialogue between scientists, doctors, and laymen. In the meantime, we must ensure that patients are diagnosed early and get the best possible treatment available by referral to specialist units.²⁷ The new developments in CF treatment are very exciting. Since the discovery of the gene encoding CF, over 100 patients have received the gene in gene therapy trials, but it must be remembered that the increase in median survival has resulted from good nutrition, aggressive use of antibiotics, meticulous physiotherapy, and a better understanding of the disease. This must be borne in mind when families ask: “how long until gene therapy is available doctor?”

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