LETTER TO THE EDITOR

Two CF patients, one homozygous for the 621 + 1G>T splice mutation, the other homozygous for the 1898 + 1G>A splice mutation

Cystic fibrosis (CF) is regarded as the most common severe autosomal recessive disorder in the white population, with an estimated incidence of 1/2500. Since the characterisation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and the most common mutation ($\Delta F508$) in 1989, over 400 additional mutations have been identified. Many groups, including our own,¹ have tried to associate disease phenotype with the genotype. For the rarer mutations this is greatly hindered by the small numbers of cases, and the often unknown influence of the CF mutation on the other chromosome. The occurrence of rare homozygotes for uncommon mutations is therefore of great help in assigning a phenotype to a particular allele. To date, investigators have described homozygotes for G542X,² R553X,³ G85E,⁴ S549N,⁵ R117H,⁶ 2184delA,⁷ R1162X,⁸ and W128X.⁹ We report here two patients, one homozygous for 621 + 1G>T, the other homozygous for 1898 + 1G>A.

621 + 1G>T homozygote. This 24 year old man of Welsh/English ancestry was diagnosed to have CF on clinical grounds at 7 years of age, sweat test results having been equivocal before then. Initial problems were malabsorption and failure to thrive. Later he developed moderate lung disease and *Pseudomonas aeruginosa* colonisation. He has no evidence of liver disease or diabetes. At 24 years of age he is on the 75th centile for height and the 3rd centile for weight; his lung function tests are FEV1 24%, FVC 41%, and PEFR 41%, of the predicted values. Oxygen saturation is 90% in air. He is clubbed with signs of chronic lung disease. Abdominal examination showed faecal loading but no hepatosplenomegaly. Radiological examination of his chest (Chrispin Norman score 18/38) showed widespread bronchial wall thickening, atelectasis, and some ring shadows.

1898 + 1G>A homozygote. This 18 year old man of Welsh/English ancestry, with a family history of CF, presented with meconium ileus. A positive sweat test confirmed the diagnosis. Pancreatic insufficiency from birth, he developed overt liver disease by 5 years of age; liver transplantation was carried out at 14 years. Respiratory disease has been mild despite colonisation with *Pseudomonas aeruginosa*. At 16 years, height and weight were below the 3rd centile; lung function was FEV1 80%, FVC 95%, and PEFR 80% of the predicted values. By 18 years his lung function had improved, with normal FEV1 and FVC.

His height and weight are now on the 25th centile. The chest x ray (Chrispin Norman score 7/38) shows mild bronchial wall thickening and suggests hyperinflation.

The effects of these mutations at the RNA level have been well characterised. Zieliński *et al*¹⁰ and Hull *et al*¹¹ showed that the 621 + 1G>T mutation can cause both alternative splicing within exon 4 and skipping of the whole exon, and Strong *et al*¹² and Hull *et al*¹¹ have shown that 1898 + 1G>A causes skipping of exon 12. Since all three of the aberrant transcripts maintain an open reading frame, it is possible that mutant CFTR proteins are produced. However it has been proposed that none of these would be functional.^{10,12} Expression analysis studies on these CFTR variants will help resolve this issue; however it is interesting to note that expression of CFTR variants lacking either exon 5 or exon 9 in HeLa cells does not result in cAMP mediated chloride transport.¹³

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BOOK REVIEWS

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Gene Therapeutics. Methods and Applications of Direct Gene Transfer. Ed Jon A Wolff (Pp 417; £75.00.) Boston: Birkhauser Publishers. 1994.

The first successful clinical trials of gene therapy followed more than 30 years of theoretical and practical developments. Clinical gene therapy trials are now targeting inherited disorders, cancer, and infectious disease. Seventy-three clinical gene therapy protocols are now under consideration, and likely to be in full swing before the second half of 1994. Jon Wolff's book is a very timely contribution, composed of 22 chapters and divided into three sections (Background, Methods and Mechanisms, and Applications).

A fascinating historical introduction traces the roots of the current explosion in mammalian gene transfer experiments, and the technology leap towards gene therapy of human disease. This is followed by a chapter on genetic animal models of human diseases, and two chapters which discuss endogenous cis acting long range regulatory sequences, as well as post-transcriptional regulation of gene expression. These two chapters provide important insights into areas where much development will occur within the next few decades as many gene expression systems are currently limited by the lack of long term, high level, and cell type specific expression.

The Methods and Mechanisms section covers a wide variety of gene transfer strategies, that is, direct "naked" DNA and oligonucleotides, adenovirus-polylysine-DNA complexes, liposomes, receptor targeted complexes, calcium-phosphate, particle bombardment, and electroporation. The chapters on calcium-phosphate and electroporation mediated gene transfer present a particularly well reviewed and discussed account of these techniques. It is clear from these chapters that today's technology includes at least 10 to 20 different techniques to transfer genes into target cells. However, a general conclusion stemming from most chapters in this section is that most transferred DNA is lost between its entry into the cell and its delivery to the nucleus. In spite of the existence of several methods to deliver DNA to cells both in vivo and in vitro, a low efficiency transit of DNA from the cytoplasm or endosomes to the nucleus is thus still the major limiting step to achieving overall efficient gene transfer and expression. This will undoubtedly become an important area for future research.

Viral vectors (retroviruses, herpes simplex