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. Zielenski *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

virus 1, adenovirus), as well as methods to achieve localised delivery by physical means, are presented in section III. Only one chapter describes actual clinical trials, namely the treatment of severe combined immunodeficiency and cerebral tumours using different types of retroviral vectors. While this certainly reflects the state of clinical gene therapy trials when the book went to press, advances are proceeding at a very fast pace. Very recently (*Nature Genetics* 1994;6:335-41) Jim Wilson's group has published data on treatment of familial hypercholesterolaemia, and further publications of clinical trials using adenovirus and liposomes for the treatment of cystic fibrosis should become available very soon. This section also describes various pre-clinical applications of HSV-1 vectors in the treatment of brain tumours, the potential use of adenovirus for the treatment of cystic fibrosis and inherited metabolic disorders. The potential of adeno- and retroviruses for the treatment of Duchenne muscular dystrophy are also elegantly reviewed. Two further very original contributions present the current status and future perspectives of gene transfer into the cardiovascular system, and the treatment of arthritis. A detailed chapter on herpes simplex virus 1 outlines the future development of truly non-toxic long term expression vectors. Such vectors will have an enormous impact on future developments of neurological gene therapy.

This book will be of much interest to researchers interested in gene transfer and gene therapy independently of their actual fields of interest, molecular geneticists interested in methods and applications of gene transfer, molecular biologists, and clinicians interested in basic aspects of gene therapy.

A series of valuable chapters are by industrial contributors (pharmacokinetics of DNA based drugs, receptor mediated gene transfer, among others). This is of vital importance since industry is playing a major role in developing these new technologies. Another important aspect of this book is the concentration on techniques that can be eventually applied to in vivo models of disease and thus possible clinical applications. Most of the chapters are short, filled with information, and easy to read. References available for most chapters are also very up to date; most reviews contain 1993 references.

Although all possible methods to achieve gene transfer into cells are not included (for example, adeno-associated virus and Semliki forest virus vectors, yeast (human?) artificial chromosomes) Professor Wolff has done an excellent job in concentrating on those methods which today show the greatest promise for their application to human gene therapy. Nevertheless, many methods that so far have not been used in human gene therapy clinical protocols, will undoubtedly be used in the next decade.

With respect to the organisation of the book, I felt that a detailed overview explaining and discussing the state of the field and the techniques presented would have been very useful. Also, chapters covering similar topics were placed in different sections of the book, leading to some repetition in the introductory statements and many of the discussions. Much of this repetition could have been avoided to enhance the flow of the contents. Also, only one chapter provides experimental methods. A discussion on the future of gene therapy at the end of the book would also have been important in pointing the way forwards to the readers and non-experts.

In summary, this is a timely and outstanding contribution to a very rapidly advancing field of research and clinical application. It succeeds in identifying and describing 95% of gene transfer vectors and applications in current use, as well as the problems that future generations of gene therapists will have to overcome to make this technique applicable to more diseases. Also, it provides chapters by industrial contributors, which will have a very important role to play in the future of gene therapy. In short, a highly readable and dynamic book, full of useful information and tips on the current status of, and where to look for, the important future developments in one of the most rapidly advancing fields within clinical and experimental medicine.

P R LOWENSTEIN

The Inherited Metabolic Diseases. 2nd edition. Editor J B Holton. (Pp 596; £70.00.) Edinburgh: Churchill Livingstone. 1994.

Those familiar with *Metabolic basis of inherited disease* of Scriver, Stanbury, and their colleagues will be curious about the niche filled by a much smaller text, particularly as there are other short texts already available on the inherited metabolic diseases.

This multiauthored book, written by clinicians, biochemists, and molecular biologists aims to be a concise source of the background information needed to understand the clinical presentation, diagnosis, and management of this diverse group of disorders. Eighteen European authors have written on subjects in which they are known to have a major interest. The topics cover the major accepted subdivisions within the subject and the editor has focused on ensuring the book has an appeal for students, clinicians, and scientists seeking a quick and reasonably comprehensive review of inherited metabolic diseases. The book, now in its second edition, has been extensively rewritten to include recent molecular genetic advances, especially those which allow further insights into disease pathogenesis as well as genotype-phenotype correlates.

One practical problem for the reader is that a diversity of approach is evident within many of the sections. This is likely to be reflecting the different backgrounds of the authors; however, it creates difficulties when considering the book's likely audience. Some sections, for example those covering the disorders of carbohydrate metabolism, the amino acid disorders as well as the porphyrias, comprehensively cover the clinically important aspects which will appeal to many practising clinicians. Other sections are approached more through the biochemical classification, with a greater stress placed on defining the diseases within the framework of the chosen classification. Sometimes, this emphasis lessens the clinical utility of the topic. Another difficulty arises when considering some of the topics that are included in and excluded from the book. For example, while the inherited disorders of steroid biosynthesis have been included, those involving thyroid hormone biosynthesis and a number of other hormone biosynthetic defects are absent.

Despite these aspects, the book is a comprehensive, yet concise account of the vast

majority of inherited metabolic diseases. It has been written with the student in mind and each section has an extensive bibliography directing the reader to more comprehensive references. Overall, the compact nature of the book, and the broad multidisciplinary coverage of topics makes this book useful for those studying biochemistry, chemical pathology, and molecular genetics as well as for medical postgraduates seeking a concise overview of a large topic.

D RAVINE

Medical Genetics: Principles and Practice. 4th edition. Editors J J Nora, F C Fraser, J Bear, C R Greenberg, D Patterson, D Warburton. (Pp 467; £33.50.) UK: Waverly Europe Ltd. 1994.

Nora and Fraser come close to achieving their stated objective of producing a book covering what a medical student wants and needs to know in an introductory course. Twenty-eight chapters cover the scientific background to human genetics, introduce the reader to a variety of monogenic and multifactorial disorders, and discuss the various "special topics" in differing levels of detail.

On the whole the chapters are individually well structured and easy to read, with helpful diagrams and clear, appropriate photographs. I was particularly pleased to see several useful, although short, discussions of the ethical issues associated with both genetic screening and mapping of genetic loci. I am not sure that dermatoglyphics deserved a separate chapter, and as a topic it certainly does not merit a chapter of equal length to the "genetics and cancer" section. The minimalistic referencing is reflected in some of the details being rather out of date; Marfan syndrome is most certainly more common than the 1 in 60 000 frequency quoted, for example.

This book undoubtedly has value as a teaching aid when supplementary information is most likely to be provided. As long as it is recognised that referencing is minimal, then other associated health workers should find the book offers helpful summaries of many of the important areas in medical genetics.

J GRAY

Genome Rearrangement and Stability. Genome Analysis Volume 7. Editors K E Davies, S T Warren. (Pp 165; \$49.00.) New York: Academic Press. 1994.

Over the last 10 years, our comforting picture of the human genome as a stable repository of pristine information has been replaced by an image of an unsettlingly dynamic entity, the information hostage to its chemical composition and the imperfections of the enzymes which act upon it. The recent revelations of the unprecedented fluidity which underlies the trinucleotide repeat expansion mutations have spawned a series of books and review articles which strive to describe and explain these phenomena.

Although this book carries the rather general title of "Genome Rearrangement and Stability", the tenor of its contents page and