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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 (ΔF508) 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 insufficient 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 electrotransfection. 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

the provenance of its editors will ensure that most prospective readers of the volume expect a discussion of matters specifically pertinent to the role of genomic instability in human genetic disease.

Five of the eight chapters indeed fit together to form an eclectic but coherent core to the book. These comprise a fairly up to date review of human disorders associated with triplet repeat expansions (Nelson), a rigorous examination of potential mechanisms involved in minisatellite polymorphism (Armour *et al*), a description of factors regulating simple sequence stability in yeast (Lustig and Petes), and interesting discussions of the possible roles of the physical properties of DNA (Wells and Sinden, although their figure 2 appears to depict some unusual 3′→5′ polymerase activity) and homology driven interactions (Radman *et al*) in genomic rearrangement. Although the editors do not attempt a synthesis (the preface differs from the contents page only in its word count), this collection of disparate pieces of work into a single volume is thought provoking.

The remaining three chapters struck me as outliers to this central core. One, a retelling of the discovery of the Huntington's disease gene as a detective story (MacDonald *et al*), while interesting and useful in its own right, covers many aspects of the disorder which are rather tangential to the mechanisms of mutagenesis, the latter being adequately reviewed in Nelson's chapter. A chapter on recombination mediated generation of variability in trypanosome surface glycoprotein genes (Eisen and Strand) is too specialised to have a significant bearing on other matters raised in the book, and the subject is burdened by an arcane nomenclature. A chapter concerning the role of repetitive sequences in leukaemia translocations (Stallings *et al*) starts as a useful review but then descends into an obsessively detailed account of a family of chromosome 16 specific low abundance repeats whose role in translocations is unclear. This chapter also seems to merit the only colour plate in the book (FISH analysis of the aforementioned repeats).

The selection of authors for this volume betrays its origins as "The Book of the Meeting" (a Banbury Conference at Cold Spring Harbor), and the editors would probably have assembled an entirely different cohort given the title alone. Nevertheless, the circumstances are probably to some extent responsible for the profitable constellation of the five "core" chapters. This book will serve as a useful and readable briefing in mechanisms of genomic instability for those casting about for possible explanations of unusual mutational phenomena.

ROLAND G ROBERTS

Molecular Medicine. An Introductory Text for Students. R J Trent. (Pp 239.) Edinburgh: Churchill Livingstone. 1993.

Most clinicians are aware of the changes occurring in clinical medicine because of the ever quickening advances in molecular biology. For those keeping abreast of the progress within their own specialities, it is fast becoming clear there is a need to keep up or catch up with what DNA technology has on offer. The problem for many, in a time when information overload looms in from every side, is how to get the necessary information without being too diverted by a field that has seemingly limitless boundaries.

This reassuringly brief but informative book has a useful position in a market where there is a need for concise introductory information about the practical application of current DNA technology in clinical practice. The scope of the text is wide ranging and covers the major developments that have influenced the fields of medical genetics, fetal medicine, medical microbiology, medical oncology, therapeutics, and forensic medicine. Each topic is introduced assuming little previous knowledge, and considerable effort has been directed towards ensuring the reader is left with a clear understanding of important principles and techniques. Molecular biology comes with its own lexicon that is unfamiliar to many undergraduate students of medicine and foreign to a sizeable proportion of postgraduates. Important terms are explained as they arise and there is also a glossary at the back of the book covering the terms that are likely to be unfamiliar to many. Many useful cartoons have been included to complement the text.

The author points out that a major difficulty with any rapidly advancing field is the problem of a book becoming outdated even before publication. With this in mind, there has been an emphasis placed on the various applications of recombinant DNA technology in medicine. Descriptions of diseases have been used as examples to highlight the principles involved, knowing there will almost certainly be a fast game of musical chairs played out by many diseases. This approach is successful and the easily readable style and layout will help ensure a longer shelf life before another edition becomes necessary.

This book, written by a clinician with a broad understanding of molecular biology, is successful in its aim of providing a succinct survey of the current and likely future impact of recombinant DNA technology on the practice of medicine. While written for medical students, it will also appeal to many clinicians who wish to update their knowledge of the principles of molecular biology.

D RAVINE

NOTICES

International Genetic Workshop on Crouzon and Other Craniofacial Disorders

This workshop will be held in Pittsburgh, Pennsylvania, USA on 10 and 11 March, 1995. The purpose is to synthesise the rapid interdisciplinary research progress that has been made on the genetic mapping and the molecular cloning and characterisation of genes and proteins that cause selected craniofacial-synostosis syndromes. Further research collaborations will be discussed. Proffered abstracts about current results will be considered for poster or platform presentations. Workshop Organisers are John J Mulvihill, J Christopher Post, and Garth D Ehrlich. Contact: University of Pittsburgh Medical Center, Center for Continuing Medical Education, Attn: Trish Smith, Nese-Barkan Building, Fifth Floor, 3811 O'Hara Street, Pittsburgh, PA 15213–2593, USA. Tel 412–647–8126; fax 412–647–8222; email cepsmith @dvs.nbupmc.edu.

IV International Fetal Genetic Pathology Workshop

This workshop will be held at Malelane Lodge, Kruger National Park, South Africa on 31 March to 2 April 1995. Main focus: "Craniofacial Development and Malformation." Other topics will be presented. Enquiries and further information from Lesley Stephenson, Conference Office, PO Box 327, WITS 2050, South Africa. Tel: 27 11 716 5091. Fax: 27 11 339 7835.

Please note: The South African Society of Human Genetics, 6th Congress, 27 to 29 March 1995, Cape Town, South Africa.

36th Annual Short Course in Medical and Experimental Mammalian Genetics, Bar Harbor, Maine, USA, 17–28 July 1995

A joint undertaking of The Jackson Laboratory and Johns Hopkins University, this course consists of 52 hours of lectures on chromosome structure and function, molecular genetics, biochemical genetics, immunogenetics, population genetics, developmental genetics, clinical genetics, etc, and 22 hours of workshops on molecular genetics, cytogenetics, biochemical screening and patient evaluation, computers in the management of genetic data, linkage analysis, transgenic methods, and mouse models, as well as a medical genetics clinic with patient presentations. Supported by: The March of Dimes Birth Defects Foundation, National Institute of Child Health and Human Development, NIH. The Course is limited to 120 participants and the registration fee is $475.00. Application can be made to either of the co-directors of the course: Edward H Birkenmeier, MD, The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609–0800, USA, or Victor A McKusick, MD, Center for Medical Genetics, Johns Hopkins Hospital, Baltimore, MD 21287–4922, USA.

The Fragile X Syndrome and Inherited Mental Handicaps: to understand in order to help

This conference will be held in the Palais des Congrès, Caen (Calvados), France on 19, 20, and 21 October 1995. Organised by the French Fragile X Syndrome Foundation 'Le Goëland'. Further details from Coordination Congrès, Association 'Le Goëland', Capucines No 2, Les Fleurs, 61100 Flers, France. Tel: (33) 33 64 95 17.