LETTERS TO THE EDITOR

FMRI fully expanded mutation with minimal methylation in a high functioning fragile X male

I refer to the paper by Wang et al entitled “FMRI fully expanded mutation with minimal methylation in a high functioning fragile X male” published in J Med Genet 1996;33:376-8, in which the authors state that the case described “represents one of fewer than 10 documented which show this mutation pattern.” I would like to draw your and the authors’ attention to the fact that in 1993 (Am J Hum Genet 1993;53:1064-73) we presented a similar case which was not referred to in the above paper. If these had been added to the list, and the possibility of general under-representation of such males in the total sample of fragile X males ascertained through affected probands were considered, this might have somewhat changed the view expressed in the paper that the discrepancy between the size of CGG repeat and methylation in fragile X is “an extremely rare molecular genetic pattern”.

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This letter was shown to Dr Taylor and colleagues, who reply as follows.

We thank Dr Loesch for her comments on our paper by Wang et al and apologise for our oversight in omitting reference to her article. The incidence of unmethylated full mutations among fragile X males is not known, but this molecular finding may be somewhat less rare than initially supposed. At the time of submitting the article by Wang et al, there were few cases published. Since then there have been several additional reports (for example, by Kambouris et al, Am J Hum Genet 1996;64:404-7 and by Lachiewicz et al, Ann Hum Genet 61:263-7) presented at the 1996 Fragile X Conference in Portland Oregon in August). It is of particular interest that all cases have been males with mild manifestations of fragile X syndrome and IQs in the normal range. It is likely that with increasing awareness of the existence of high functioning, non-retarded fragile X males, and of the importance of considering fragile X testing in borderline retarded or learning disabled males, more cases will be identified with minimally methylated or unmethylated fully expanded mutations.

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Raising the sensitivity of fetal RhD typing and sex determination from maternal blood

The fact that all currently available methods for tissue sampling carry procedure related risks has been a motivation for many years to develop less invasive techniques for prenatal diagnosis, ideally by studying fetal cells in the maternal circulation. Methods directly to PCR amplifying sequence specific for the fetal Y chromosome (important in the case of X chromosomal recessive diseases) or for fetal RhD status (to manage rhesus sensitisation) without previous prior enrichment of fetal DNA from maternal blood have been successfully established.1 However, these methods have so far not reached the precision required for routine application, since rates of false results, about 2%, are prior observed.1 On test PCR based sex determination and RhD typing in over 100 samples of amniotic cells, chorionic villi, cord blood, or peripheral blood, we obtained no false results. Y chromosome specific PCR was performed by a dual amplification procedure using the primers Y1.5 and Y1.6 in a first round PCR. One micro litre of the first round product was reamplified by nested PCR with negative rate by increasing the sensitivity of the PCR isolation. We separated the PCR products on an agarose gel and transferred them on nylon membranes by Southern blotting.1 After UV fixation the blots were hybridized with a biotinylated radioactively labelled sense primers, washed, and exposed to X-ray films at -70°C using intensifying screens.1 Artificial dilution of fet al DNA in adult DNA by this method we were able to increase the sensitivity of detection from one fetal cell in a negative background of 100 000 cells to 1 in 10 000 000 cells. Accordingly, using this approach to analyse maternal blood samples the rate of false negative results decreased from about 20% to less than 3% for RhD as well as for Y chromosome sequences. Our data indicate that false negative PCR results from maternal blood are mostly likely based on detection limits rather than on more physiological reasons and our methods increase the power of PCR to prevent less invasive prenatal diagnosis.

However, a major drawback for PCR based prenatal mal formation from whole maternal blood is the fact that a PCR setting which is highly sensitive can be used here, is also very likely to give false positive results. Such false positive results on fetal sex and RhD status determination from fetal cells have also been observed by other groups.1,2,4 In our hands, the RhD status determination, general concerns about discrepancies between serological typing and PCR derived RhD type have been raised.1 Recently, a large scale evaluation of the accuracy of PCR in the diagnosis of RhD status using DNA derived from adult blood samples was performed. Two of 632 RhD positive subjects were characterised as RhD negative and seven of the 133 RhD negative subjects were characterised as RhD positive.5,6 The interpretation of these results could also lead to the interpretation that not all RhD negative subjects exhibit total loss of the RhD gene. Our data do not support this interpretation. Taking precautionary steps to avoid sample contamination and by including positive and negative controls, we did not obtain any false results doing PCR analysis of over 100 samples of amniotic cells, chorionic villi, cord blood, or peripheral blood. However, when we analysed fetal sex and RhD status by PCR from maternal blood samples, we obtained a relatively high rate of false positive results. The assumption that these false positive results are not the result of inaccuracy in our PCR technique is further supported by the observation that analysing artificial DNA dilutions from a male donor with chromosome DNA or the RhD gene with excess of negative DNA did not lead to these rates of false positives. An alternative interpretation might be that these results represent a fundamental biological phenomenon because of fetal cells in the maternal circulation from previous pregnancies or a previous maternal blood transfusion.7 In the cases presented here we could not explain the observed high rate of false positive results this way.

In summary, the data presented here describe a successful approach to increasing the sensitivity of fetal PCR diagnosis from maternal blood by reducing the rate of false negative results. However, as long as the PCR setting is not optimised to diminish the observed positive results, one might wonder whether this diagnostic approach can routinely be used without previous enrichment of fetal cells.

Work in MH’s laboratory is supported by grant number PO983-MED from the "Förderung der wissenschaftlichen Forschung" and by the "Kommission Onkologie" of the Medical Faculty of the University of Vienna.}" and our work on the isolation by genetic screening of chromosome DNA or the RhD gene with excess of negative DNA did not lead to these rates of false positives. An alternative interpretation might be that these results represent a fundamental biological phenomenon because of fetal cells in the maternal circulation from previous pregnancies or a previous maternal blood transfusion. In the cases presented here we could not explain the observed high rate of false positive results this way.

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BOOK REVIEWS

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This relatively expensive book is an interesting addition to the expanding body of publications dealing with familial predisposition to cancer. The subject is broad and covered fairly comprehensively. However, the book left me with the impression of a collection of papers written in isolation by experts in the same broad field. This leads to some repetition of basic themes from chapter to chapter. I was unsure at whom the book is aimed. Read as a book from cover to cover the contents would give the reader a good idea of the controversies and variation in clinical practice which is the inevitable result of the still limited clinical research data available in much of this field. The subject matter, of course, leaves ample room for individual opinion as it is an evolving field. Although perhaps not the primary aim of the book, the differences in opinions and the differences in clinical practice in different health care systems came across well by reading the entire book. However, I felt using this as a reference text for clinical practice would be difficult. The book led me to reflect once more on the differences between health care in different countries.

Certainly there is a wealth of information here with some excellent chapters and some perhaps less accessible chapters. Many of the contributors balance appropriately their own protocols for best clinical practice in their setting with contrary views, such as the unknown benefit and harm of the advocated screening activities provoked by identification of a cancer predisposition in a person. In some situations where the natural history is understood and the screening strategy is proven to reduce mortality (such as for familial adenomatous polyposis), screening strategies and prophylactic surgery are generally accepted and agreed. In, for example, genetic predisposition to breast cancer the case remains to be proven for either screening or prophylactic surgery. More emphasis on the need for collaborative studies to address some of these issues would redress the balance.

There are chapters covering some basic laboratory techniques in which the genetic terminology would present difficulties for a reader unfamiliar with basic molecular biology. There are some good balanced overview chapters. However, since there is no apparant specific target audience and the arrangement of the information does not lend itself to use as a reference text, as the field moves on and expands even further, a subsequent edition may need a slightly different approach.

There are some interesting and wide ranging chapters, for example, on the legal and ethical issues raised by the discovery of cancer predisposition genes, which add interest to a wide range of potential readers. Although this book may be a useful addition to the departmental library, the high cost and the layout of the book would make me hesitate to recommend it for personal use.

DIANA M ECCLES


This book is a typical product of the proceedings of a meeting that was held in Charleston, South Carolina, in April 1994 and sponsored by the National Down Syndrome Society. The theme of the meeting was to discuss the latest developments on the aetiology and pathogenesis of Down syndrome, which is a compilation of phenotypes resulting from three copies (instead of two) of genes that map to human chromosome 21. The field of aneuploidy and gene dosage is fascinating, challenging, and ready for discoveries. The exploration of the genome of chromosome 21 will undoubtedly provide some answers to the aetiology and pathophysiology of certain phenotypes. However, the full understanding of the gene to phenotype process will probably come a long time after the cloning and characterisation of all chromosome 21 genes.

The book contains chapters from numerous authors or groups of authors that vary widely in topic and quality. Some of the chapters describe the results of many years of investigations and the data could well be part of textbook discussions. Other chapters, in particular those that describe mapping infor-

mation, are outdated because of the extensive amount of new information that accumulates rapidly. The chapter on the cognitive abilities in Down syndrome is an idiosyncratic contribution to this book and deals with a topic that the methodological biologist does not usually find in his/her publications. The methodological chapters provide a nice account of approaches to familial trisomy in model organisms or mammalian cells.

The book is interesting to those who are students of aneuploidy and gene imbalance. It is also of interest to neurologists, geneticists, biologists, and behavioural paediatricians. I particularly liked the closing chapter (epilogue) that contained a personal view (that of Dr C Epstein) of how far we have come and how far we can reasonably expect to go. I fully agree with his predictive remarks. It is my impression that after the understanding of the majority of monogenic disorders, trisomy 21 will again come to a prominent position in the stage of problems that the human biologist would tackle with the tools of whole genome analysis.

STYLIANOS E ANTONARAKIS


This book aims to provide practical guidance to lay people or genetics professionals on starting a genetic support group or working with an existing one. A key theme is the importance of partnership between professionals and families based on mutual respect and the effective use of their different contributions. Weiss is the director of the US Alliance of Genetic Support Groups, and the authors take a realistic and practical approach to the subject, with an understanding of both the potential and the pitfalls.

An overview is given of the benefits and limitations of genetic support groups. The authors stress their value in breaking down isolation, particularly after diagnosis, and in enabling communication with people who have gone through similar experiences. They also emphasise that groups cannot solve all problems, and the importance of referral to professionals when needed. They examine common difficulties and conflicts, which can limit a group’s ability to give support, and how to deal with these.

There is practical advice on how to organise a genetic support group. This covers getting started, setting goals, finding people, assessing people’s need (with a useful checklist), developing group leadership, and avoiding burnout and other problems. The book gives guidance on developing a group charter; the role of professionals in working with the group; communication and publications; organising meetings and support sessions; fundraising; and taking part in research.

While written from a US perspective, this clearly written basic guide is a useful resource for anyone involved in setting up or working with a genetic support group. It includes a comprehensive listing of US genetic support groups, generic services, and other resources.

JANE BELMAN