Methionine synthase and neural tube defects

Accumulated evidence implicates an abnormality of folate metabolism in the genetic aetiology of neural tube defects (NTD). Periconceptional folic acid can prevent NTD and many studies of fetuses with NTD display high levels of blood homocysteine; a low maternal vitamin B<sub>12</sub> is another independent risk factor for NTD. These observations suggest the enzyme methionine synthase (MS), which is central to folate metabolism and which catalyses the conversion of homocysteine to methionine in a vitamin B<sub>12</sub>-dependent reaction, as a target for study. As yet there has been no investigation of the idea that mutations in the MS gene might contribute to NTD susceptibility, but the recent cloning of this gene<sup>1</sup> now makes allele association studies possible. We have investigated DNA samples from British NTD families which include 36 affected subjects, 31 with spina bifida (SB) and five with SB and aneuploidy, and Dutch NTD families which include 32 with SB, two with SB and aneuploidy, and one with encephalocele.

The MS gene has been mapped to chromosome 1q43 and shown to encode a protein of 140 kDa comprising 1265 amino acids.<sup>1</sup> Two MS polymorphisms have been reported: an Arg<sup>111</sup>Leu polymorphism found in North American samples<sup>2</sup> and an Asp<sup>919</sup>Gly polymorphism which occurs with a frequency of 0.15 for the less common allele in a French/Canadian population.<sup>3</sup> The Arg<sup>111</sup>Leu variation was not detected in our British and Dutch control groups or among 25 affected subjects and their families. However, the Asp<sup>919</sup>Gly variation occurred in both control groups with a very similar frequency: Dutch Gly<sup>919</sup>0.19 and British Gly<sup>919</sup>0.17.

We have compared the frequencies of Asp<sup>919</sup>Gly and Gly<sup>919</sup> homozygotes in normal controls of British origin (n=72) and unrelated subjects attached to marriage by Dutch NTD families (n=47) with those for the NTD cases, their mothers and fathers; no evidence for an association between either of the MS<sup>919</sup> alleles and the occurrence of NTD was found (table 1). The risk of NTD owing to abnormalities in folate metabolism may be influenced by maternal genotype or by a combination of the maternal and fetal genotype. The Gly<sup>919</sup> homozygote frequency for mothers is 5.9% and for NTD offspring is 4.4% compared with 3.4% for controls (table 1); while slightly increased, these differences were not significant.

The transmission test for linkage disequilibrium (TDT)<sup>4</sup> was used to look for allele association. We used data from 45 heterozygous parents who transmitted 49 alleles to their NTD offspring. The Asp<sup>919</sup>Gly allele was transmitted on 22 occasions and the Gly<sup>919</sup> on 27 occasions (table 2). The calculated \( \chi^2 = 0.51 \), \( p = 0.25 \), showed that this difference was not significant. The transmission of alleles was relatively more asymmetrical in the Dutch NTD group when they were considered separately (23 transmissions, eight

<table>
<thead>
<tr>
<th>Allele frequency % (No)</th>
<th>Asp/Asp</th>
<th>Asp/Gly</th>
<th>Gly/Gly</th>
</tr>
</thead>
<tbody>
<tr>
<td>British (72)</td>
<td>66.7 (48)</td>
<td>31.9 (23)</td>
<td>1.4 (1)</td>
</tr>
<tr>
<td>Dutch MS (47)</td>
<td>68.1 (32)</td>
<td>25.5 (12)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Combined (119)</td>
<td>67.2 (80)</td>
<td>29.4 (35)</td>
<td>3.4 (4)</td>
</tr>
</tbody>
</table>

The TDT for the MS<sup>919</sup>Gly allele is not significant; but it might not be excluded that the MS<sup>919</sup>Gly locus is in linkage disequilibrium with another more significant marker (table 2).

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Asp</th>
<th>Gly</th>
</tr>
</thead>
<tbody>
<tr>
<td>British</td>
<td>0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>Dutch MS</td>
<td>0.81</td>
<td>0.19</td>
</tr>
<tr>
<td>Combined</td>
<td>0.82</td>
<td>0.18</td>
</tr>
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Unrelated subjects married in to NTD families (MI) were used as the Dutch control population.

Limbgirdle muscular dystrophy or spinal muscular atrophy: a source of diagnostic confusion?

We examined 95 patients with a clinical diagnosis of limb-girdle muscular dystrophy to determine whether diagnostic confusion with spinal muscular atrophy was common. Analysis for deletions in the SMN and NAP1 genes showed only one family in which a misdiagnosis had been made. Our results suggest that


there is unlikely to be major confusion between the two groups.

The limb-girdle muscular dystrophies (LGMD) are a group of autosomal inherited progressive myopathic diseases. Eight different genetic types of LGMD have so far been identified, of which two are dominantly inherited and the other six recessively inherited. Of the genes so far identified, three encode structural proteins of the dystrophin associated glycoprotein complex and one encodes the muscle specific calpain. Currently, there are insufficient data so far to distinguish between the different forms of LGMD.

In the past, LGMD has been confused with other forms of muscle disease. Patients previously diagnosed as suffering from LGMD have in some cases been shown by molecular analysis to have a dystrophinopathy (X linked) or mitochondrial or metabolic disease.

Given the extreme heterogeneity of LGMD, it is important to ensure that resources are applied to track down the primary genetic defect in any particular family or case and that appropriately designed, carefully evaluated investigation of all possible alternative diagnoses is performed. It has been postulated that the milder forms of spinal muscular atrophy (type III SMA) may be a source of diagnostic confusion in recessive dystrophy. Phenotypically, both diseases show proximal muscle weakness and wasting, creatine kinase levels may be raised in SMA, and EMG and muscle biopsy analyses may show conflicting or confusing results.

It is now possible to perform molecular tests for the genetic faults which are associated with chromosome 5 linked SMA. We examined a panel of 95 patients with a diagnosis of limb-girdle muscular dystrophy. These patients were from a variety of different sources both in the UK and abroad. Some referred themselves to our department because of our research interest in LGMD, others were referred from recognized neuromuscular units. All, according to the information available, had clinical characteristics and investigations which were consistent with a diagnosis of LGMD according to the latest diagnostic criteria.

We analysed DNA samples for deletions of exons 7 and 8 of the survival motor neurone (SMN) gene (deleted in approximately 94% of milder SMA cases) and also for deletions of exons 5 and 6 of the neuronal apoptosis inhibitory protein (NAIP) gene (deleted in about 67% of SMA type 1 cases and 42% of type 2 and 3 cases). We found deletions in SMN and NAIP in only one family. Haplotype analysis confirmed that the affected sibs in this family did share the chromosome 5 region containing the SMN and NAIP genes. The three children from this family had childhood onset of a predominantly proximal muscle weakness and wasting diagnosed clinically and on investigation as a limb-girdle muscular dystrophy. Recent re-evaluation of serum creatine kinase showed that the level remained high (576 IU/l normal up to 180 IU/l) even many years after the onset. All had relatively slow progression of disease and normal intelligence.

We conclude that spinal muscular atrophy associated with deletions of SMN and NAIP is not a common source of confusion in the diagnosis of LGMD. The investigation may, however, be of some use in families in which there is genuine diagnostic confusion on the basis either of equivocal creatine kinase levels or conflicting results from EMG or muscle biopsy.

We are very grateful to the patients and clinicians who have helped us collect the samples for this and other studies. Financial support has been provided by the Medical Research Council of Great Britain, the Muscular Dystrophy Group of Great Britain and Northern Ireland, and the British Medical Association.

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It has been predicted that in the coming decade cell based therapies will begin to rival traditional pharmaceuticals in many areas of medicine but especially oncology. This timely book largely succeeds in its goal of providing a snapshot of this rapidly expanding field. The more than 70 contributors are authorities in the field and this compilation is a comprehensive compendium for the inevitable variation in the up date material is.

The book is organised into sections covering overlapping scientific, technical, and clinical aspects of haematopoietic cell therapy and immunotherapy introduced by succinct reviews of relevant aspects of the haematopoietic and immune systems by Moore and Slavin. The main body of the book is devoted to laboratory and clinical aspects of haematopoietic cell therapy starting with excellent reviews of progenitor assays, flow cytometry, and genetic transformations. Mobilisation of peripheral blood progenitor cells, perhaps the major advance in haematology in the last decade, is well covered in a series of chapters by Shippal, To, and Sheridan and this is followed by reviews of cell selection, purging, and expansion by some of the innovators and authorities in the field. The development of these technologies is, of course, that they facilitate dose intensification strategies in the treatment of malignant disease, and approaches to this involving growth factor and cellular support in a variety of diseases are covered next. Discussion of future applications of haematopoietic cell therapy is restricted to gene therapy and cellular therapy of HIV disease in which success to date has been modest to say the least. The techniques for the generation of dendritic cells, perhaps one of the most promising new developments, are scarcely mentioned and this must count as a major omission. The final section of the book concentrates on experimental and clinical aspects of immunotherapy with coverage of neuronal transfer, tumour vaccines, and adoptive immunotherapy with autologous T and natural killer cells and allogeneic cells in the context of bone marrow transplantation.

Irrelevantly some relevant topics are not covered, notably neural progenitor cells which are only mentioned in passing and a chapter on T cell depletion, perhaps the commonest type of "graft engineering" currently performed, would have been helpful. Regulatory issues do vary from country to country but there are common safety concerns and discussion of these would also have been useful. Overall, however, these omissions do not detract from the value of the book which will be of interest to scientists, clinicians, and those in the biotechnology and pharmaceutical industries.

STEPHENV. DEVEREUX


This book is intended for biologists wishing to understand more about molecular biology databases and methods of sequence analysis. As the editors state in their preface, it is 10 years since the publication of the seminal work entitled "Nucleic Acid and Protein Sequence Analysis" in this series and a new volume is certainly welcome. There are very few books covering this and as a result the information is scattered in original papers and software manuals. On the technical side, one of the most noticeable changes in working methods has been the use of the World Wide Web to access databases and software. I felt that while the preface emphasised this, some of the earlier chapters that concentrated heavily on software were rather dated, partly as a result of the time taken to publish such a book. It would have been helpful to have a longer introduction to the book to tie together the material. For example, there was no explanation of why one chapter was devoted to software for the Macintosh (10 years ago a similar chapter referred to the PC). There is a wide variation in style between authors and in the prior mathematical and computing knowledge assumed. Some, for example, undoubtedly feel that much of this book is about theory and not practice, though this is very much a feature of the subject.

Many of the later chapters give a better balance between the theoretical background and the software to perform the analyses. This was especially true of the chapter on Phylogenetic Estimation by Dr N Goldman, J Med Genet. First published as 10.1136/jmg.34.11.958-a on November 1997. Downloaded from http://jmg.bmj.com/ on September 14, 2023 by guest. Protected by copyright.