Heterotaxia syndromes and 22q11 deletion

In a recent issue of your journal we read with interest the very accurate review by Penman Split et al on defects of left-right asymmetry. The authors correctly reported that in patients with heterotaxia (asplenia and polysplenia syndromes), conotruncal defects are one of the more frequent heart malformations. It is well known that 22q11 deletion has been described in a subgroup of patients with conotruncal anomalies in the setting of Di-George4,5 and velocardiofacial syndromes. In the paper of Penman Split et al5 is was reported (personal communication to the authors) that the same microdeletion has been found in two patients, one with dextrocardia and one with left isomerism (polysplenia syndrome).

Since 1993 we have performed clinical and molecular evaluation of all patients with conotruncal anomalies observed at our hospital,6 including 20 cases with heterotaxia. Fifteen had asplenia syndrome and five polysplenia. All patients underwent phenotypic and cardiac examinations. Fluorescent in situ hybridisation was used for detecting 22q11 deletion.

No patients had phenotypic features of Di-George or velocardiofacial syndromes, and the genetic study did not show 22q11 deletion in any case. Our experience suggests that the conotruncal anomalies in the setting of heterotaxy syndromes are not related to 22q11 deletion, and are probably secondary to distortion of cardiac looping or to the anomaly of the situs itself. Different gene(s) and different developmental mechanisms may be involved in the pathogenesis of conotruncal anomalies in patients with situs solitius and in those with laterality defects.

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First report of three cystic fibrosis patients homozygous for the 1717-1G→A mutation

We report the identification for the first time of three cystic fibrosis (CF) patients homozygous for the 1717-1G→A mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

The clinical presentation of CF varies widely, the most common characteristics being chronic obstructive lung disease, raised electrolyte and pancreatic insufficiency (PI).2 About 15% of patients display pancreatic sufficiency (PS).3

The isolation of the CFTR gene4,5 has made it possible to identify the main disease causing mutation, AF508, accounting for about 70% of molecular defects in the world population,4 and over 600 rare presumptive mutations (CF Genetic Analysis Consortium). Among these, the 1717-1G→A mutation is implicated in a G→A base change at the 3' end of the consensus sequence of intron 10. It was first reported in a patient of Celtic origin1 and since then it has been detected in other populations, having an overall frequency of 1.1%.7

To date, a clinical correlation for this mutation has been defined only in patients who are compound heterozygotes for AF508, who display a similar pancreatic and pulmonary phenotype to that of homozygotes for AF508.8

In this report we describe the first three patients found to be homozygous for the 1717-1G→A mutation. They showed early pancreatic insufficiency (osteoporosis and ileus) and two of them had early onset of respiratory symptoms, but with subsequent minimal lung involvement progression. These clinical findings suggest that this mutation might pre-dispose to a milder respiratory course.

Two patients (cases A and B) regularly attended the Milan CF Centre at the Department of Pediatrics, University of Milan; the third patient (case C) is followed at the Naples CF Centre, Pediatrics Department, II University of Naples.

The three 1717-1G→A homozygous patients included: case A, female born on April 20, 1988 (birth weight 3550 g) to healthy, non-consanguineous parents. At birth, she presented with meconium ileus, which was surgically treated with a 10 cm ileal resection. She had a high immune reactive trypsinogen (IRT) value at 5 days of life (173 ng/ml, normal value <40 ng/ml) and CF was confirmed by a positive polyclonal immunoperoxidase sweat test (112 mmol/l chloride).9 Regular treatment for CF was started at 2 months with pancreatic enzyme supplementation (Pancreas® and chest physiotherapy (positive expiratory pressure technique). At the latest clinical visit, she was normal on examination, weight was 14 kg (75th centile), height 94 cm (97th centile). Good nutritional status was obtained with a low dose of cystic fibrosis diet (1785 U/kg/day of pancrelipase) associated with a high fat content diet. A chest x ray showed only minimal thickening of the bronchial walls in the lower lobes. Staphylococcus 38 kg and height 139 cm. Steatorrhea was absent and fat absorption coefficient was 93%, with 1061 U/kg/day of pancrelipase and a high fat content diet. A chest x ray showed only basal bronchial wall thickening. Staphylococcus aureus was consistently detected from sputum samples. He needed only one therapeutic antibiotic course per year for upper respiratory tract infection and the clinical course was mild, with no further hospital admission after starting treatment at our CF Centre following diagnosis. Lung function tests were always in the normal range. As compliance with chest physiotherapy was poor, daily sporting activities were encouraged, and nutritional indices were ever noted. Liver function tests were normal until July 1995, when serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), and glutamyltransferase (GGT) were slightly elevated (227 ALT, 147 U/l, and 53 U/l, with normal values less than 37 U/l, 41 U/l, and 49 U/l). Ultrasonic liver examination showed early signs of liver disease, so ursodeoxycholic acid therapy was prescribed.

The third patient, case C, a female, was born to healthy, non-consanguineous parents. Both paternal and maternal ancestors came from the same small city near Naples. Cystic fibrosis presented early with failure to thrive, malabsorption, and bronchiolitis, and she had afebrile upper respiratory tract infection admissions at 1 and 3 months of age. CF was confirmed by a positive polyclonal immunoperoxidase sweat test (93 mmol/l chloride). Regular follow up at the CF Center and treatment was started with pancreatic enzyme...
supplementation, mucolytic and bronchodi-
larloger aerosols, antibiotics, oral almo-
philline, and intensive chest physiotherapy.

The clinical courses of the two CF patients
(cases A and B) homozygous for the 1717-
1G→A mutation might also have developed
only a mild lung phenotype.

The data reported here for three 1717-
1G→A homozygous patients show an
association of early onset of pulmonary symp-
toms (two patients) and pancreatic involvement
(three patients) with a surprisingly slow pro-
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be associated with mild to minimal lung disease,
even if prediction of slow, mild, progressive
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patients needs further confirmation both in a
larger survey and over time when longitudinal
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Table 1: Anamnestic, clinical, and laboratory data for the three 1717-1G→A homozygous patients

<table>
<thead>
<tr>
<th>Case A</th>
<th>Case B</th>
<th>Case C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Onset of symptoms</td>
<td>Birth</td>
<td>1 mth</td>
</tr>
<tr>
<td>Meconium ileus</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Data at age of diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>Birth</td>
<td>6 mth</td>
</tr>
<tr>
<td>Sweat chloride (mmol/l)</td>
<td>112</td>
<td>105</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pulmonary symptoms</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Data at last visit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2 yr 4 mth</td>
<td>9 y</td>
</tr>
<tr>
<td>Weight/weight centiles</td>
<td>75/97</td>
<td>97/90</td>
</tr>
<tr>
<td>Cole's index*</td>
<td>95%</td>
<td>128%</td>
</tr>
<tr>
<td>Chest x ray score†</td>
<td>5.38</td>
<td>9.38</td>
</tr>
<tr>
<td>Pseudomonas colonisation</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>FEV1 (% of predicted)§</td>
<td>ND§</td>
<td>ND§</td>
</tr>
<tr>
<td>FVC (% of predicted)§</td>
<td>93</td>
<td>96</td>
</tr>
</tbody>
</table>

* Weight expressed as a percentage of the ideal weight for height.
† With enzyme supplementation.
§ Not determined (patients were not able to perform reproducible tests).

Table 2: Clinical data of patients according to genotype

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Genotype</th>
<th>ΔF508/AF508</th>
<th>ΔF508/1717-1G→A</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients (male)</td>
<td>23 (62%)</td>
<td>21 (62%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>15±7.9</td>
<td>14±7.3</td>
<td>11±7.3</td>
<td>0.478 NS</td>
</tr>
<tr>
<td>Age at diagnosis (y)</td>
<td>1.3±2.6</td>
<td>2.4±3.2</td>
<td>0.271 NS</td>
<td></td>
</tr>
<tr>
<td>Sweat chloride (mmol/l)</td>
<td>106±12.4</td>
<td>115±36.1</td>
<td>1.3±2.4</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>39±15.7</td>
<td>39.5±13.2</td>
<td>0.904 NS</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>145±20.3</td>
<td>146±3.1</td>
<td>19.7</td>
<td>0.88 NS</td>
</tr>
<tr>
<td>Chest x ray score</td>
<td>7.0±3.6</td>
<td>8.8</td>
<td>0.926 NS</td>
<td></td>
</tr>
<tr>
<td>FEV1, % of predicted (Range)</td>
<td>67±29.2</td>
<td>80.7±26.7</td>
<td>0.158 NS</td>
<td></td>
</tr>
<tr>
<td>FEV1, % of predicted (Range)</td>
<td>79±29.3</td>
<td>90±2.6</td>
<td>0.158 NS</td>
<td></td>
</tr>
<tr>
<td>No of patients (male)</td>
<td>21 (100%)</td>
<td>19 (90.5)</td>
<td>0.468 NS</td>
<td></td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>27 (100%)</td>
<td>19 (90.5)</td>
<td>0.65 NS</td>
<td></td>
</tr>
<tr>
<td>Meconium ileus</td>
<td>2 (0.5)</td>
<td>4 (15.4)</td>
<td>0.5 NS</td>
<td></td>
</tr>
<tr>
<td>Nasal polyps</td>
<td>1 (0.5)</td>
<td>2 (9.2)</td>
<td>1 NS</td>
<td></td>
</tr>
<tr>
<td>DIOS</td>
<td>2 (0.5)</td>
<td>0</td>
<td>0.46 NS</td>
<td></td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver disease</td>
<td>5 (23.8)</td>
<td>8 (38.1)</td>
<td>0.5 NS</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (14.3)</td>
<td>3 (14.3)</td>
<td>1 NS</td>
<td></td>
</tr>
<tr>
<td>ARPA</td>
<td>4 (19.0)</td>
<td>5 (23.8)</td>
<td>1 NS</td>
<td></td>
</tr>
</tbody>
</table>

FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; DIOS = distal intestinal obstruction syndrome; ARPA = allergic bronchopulmonary aspergillosis. NS = not significant.

In our group, the severity of pulmonary disease associated with the 1717-1G→A mutation is highly variable in compound heterozygous patients, but although their mean FVC and FEV1 values are higher than those in ΔF508 homozygotes, the differences are not statistically significant (table 2).

The clinical courses of the two CF patients (cases A and B) homozygous for the 1717-
1G→A mutation showed that both had early pancreatic involvement and the second an
early onset of respiratory symptoms. Con-
versely, the progression of lung involvement
was minimal in both patients, who only re-
quired a small amount of therapy. The clinical

course is different from that observed in 1717-
1G→A/ΔF508 compound heterozygotes and
ΔF508 homozygotes, even if ΔF508 homozy-
gotes may exist who display clinical features
similar to those of our patients, taking into
account their young age.

The general clinical pattern for patient C
was slightly more severe than for the other two
patients, but the association between GER and
acute and chronic respiratory disease in infants
and children is well known. In addition, GER
has been reported increasingly often in CF and
it is known that pulmonary function may be
worse in CF patients presenting with GER.16
Thus, we cannot exclude that in the absence
of GER this patient homozygous for the 1717-
1G→A mutation might have developed
only a mild lung phenotype.

The data reported here for three 1717-
1G→A homozygous patients show an
association of early onset of pulmonary symp-
toms (two patients) and pancreatic involvement
(three patients) with a surprisingly slow pro-
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BOOK REVIEWS

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As the Editor will attest, it has taken me far too long to find the time to read this book and write a review. In large part, this is because life is far too fraught and hectic for scientists these days to sit back and reflect on the origins and evolution of their subject and the science they practice. Yet, ultimately, it was a most rewarding experience to be guided by the deliberately erudite Henry Harris through this fertile terrain which con-stitutes the still evolving history of somatic cell genetics. After all, I myself had been a participant in some of the crowded scenes, and had met or heard lectures delivered by many of the eminent figures whose photographs grace the second half of the book, if not their predecessors in the first half.

Before somatic cells could be used to explore biology, their credentials had to be painstakingly established. The kind of cell differentiation and transformation that are currently a familiar part of cancer research has been described. Among the principal reasons for the success of cancer research, this book clearly shows, is that somatic cell genetics has gone beyond the simple demonstration of species specificity to the nurture of cell differentiation. The discovery of intraspecies somatic cell hybrids in which species-specific chromosome loss (still unexplained) occurred led to the development of the more narrowly defined concept of some cell genetics that, with paraxial chromosome segregation can be harnessed for the beginning of gene assignment and mapping. This proved to be a particular bonus for human genetics which had lagged so far behind other organisms where breeding experiments were permissible. Many refinements were introduced, for example, the fragment technique of characterization species can be used to allow gene order determination (by Steven Goss in Henry Harris’s laboratory in the mid-1970s). This approach has recently been revisited on a grand scale when development of a combination of automated molecular techniques and statistical analysis have made possible the mapping to a very high density of DNA markers, as a prelude to the final human genome sequencing effort which is now in progress.

Gene mapping is only one area revolutionized through the use of somatic cell genetics. Initially, before DNA level analysis by “molecular biology” was made possible through the encirclement of restriction enzymes, Southern blotting, and eventually the polymerase chain reaction (PCR)—each step carefully traced by Harris—all gene mapping was through analysis of expression functions. Of course, many functions expressed in vivo are not expressed in cell culture. Even when obvious differentiated functions, such as melanin production or serum albumin synthesis, are seen in culture, early fusions between cells from different stages of development show that differentiated functions were frequently extinguished after fusion but subsequently re-expressed as more chromosome loss took place, removing genes which presumably mediate extinction. Cell culture methods have been improving gradually as new growth factors are defined; cells from different tissues can now be grown under conditions where the control of expression of the spectrum of differentiated functions can be studied. The development of monoclonal antibody technology in Cambridge in the late 1970s heralded a major step in harnessing the ability of appropriately chosen somatic cell partners to express specific products of differentiation. Another area in which Harris and co-workers make some seminal contributions is in our understanding of the mechanisms of tumorigenesis. Many aspects of current ideas in this field were developed using somatic cells: from the examination of specific chromosomal rearrangements in cells derived directly from patients. Harris, in particular, has shown that, in a first glance, that malignant and normal cells fused together initially produce normal hybrids, although malignancy may be re-expressed as certain specific chromosomes are lost. This work, together with some other explorations, led to the concept of tumour suppressor genes. DNA transfection studies to define oncogenes were also done in somatic cells.

Many years ago it was estimated that it would take a mouse chimaeras to another area that has been elaborated into the great industry of making transgenic mice and specific gene knockouts which are providing so much insight into developmental control and gene interaction. Somatic cells continue to provide new approaches to unravelling biological function. Reading this scholarly account of their history is well worth the effort, not only to provide insights, but quite likely foresight and new ideas.

VERONICA VAN HEYNINGEN


Fundamental to cell differentiation and development is the regulation of gene expression in a spatial and temporal specific manner. This is brought about by unique combinations of specific binding sites and trans-acting factors in conjunction with RNA polymerase II. This little book is, in essence, a catalogue of the eukaryotic transcription machinery components. It is one of a series called "Essential Data", of which most are concerned with laboratory equipment and methods.

Chapter 1 consists of a brief account of the various polypeptide subunits of the RNA polymerase II enzyme complex. It then lists the subunits and defined interacting transcription factors in yeast and other organisms, along with molecular weight, brief comments, and references.

Chapter 2 consists of a very brief introduction to transcription factors and a 40 page long comprehensive list of DNA binding sites, factors which bind to them, the family or families to which the factor belongs, and a very brief comment on each, including references.

Chapter 3 provides a detailed and informative account of RNA polymerase I and II transcription factors. The tables in this chapter include GenBank accession numbers, which are absent in the previous two chapters.

Chapter 4 consists of charts of the major transcription factor families, indicating the relative frequency and importance of specific members at each position. The authors would do well to avoid looking at these charts. The book is full of jargon, much of which is not explained. For example, despite a section of text on UBFI, I failed to find out what UBFI was an abbreviation of.

It is clear then that this book is intended for transcription factor aficionados with a desire to have a little information on all transcription factors and the references finding the details they require. For those wishing to know more about transcription factor molecular biology, this book is not for you. The Royal use of philosophical discussions on transcription factors would be a better bet.

ROSS HAWKINS