A new deletion of 18q23 with few typical features of the 18q− syndrome

Maija Kohonen-Corish, Gordon Strathdee, Joan Overhauser, Timothy McDonald, Vapinder Jammu

Abstract
We report on a patient with a deletion of 18q23. At both 2 and 4 years of age, she displayed few of the facial features or other clinical features associated with the 18q− syndrome. Fluorescent in situ hybridisation and microsatellite marker and RFLP analysis were performed to characterise the extent of the deletion and a terminal deletion of 18q23 was confirmed. The deleted region includes the gene for myelin basic protein, suggesting that hemizygosity of this gene does not invariably lead to mental and developmental delay. The clinical presentation of this patient suggests that either she is not deleted for the genes involved in the 18q− clinical phenotype or this patient represents one end of the spectrum of the clinical variability seen with 18q terminal deletions.

Key words: 18q− syndrome; FISH; RFLP/microsatellite markers.

The 18q− syndrome is a well described disorder resulting from a partial deletion of the long arm of chromosome 18. Cytogenetically the breakpoint varies greatly, but no terminal deletions extending proximal to 18q21.2 have been detected. The clinical features associated with 18q− syndrome include growth deficiency, specific facial features, deformed limbs and skeletal system, genitourinary malformations, and various central nervous system problems, such as hypotonia, seizures, deafness, and mental and physical retardation. The incidence of these clinical symptoms in patients with 18q deletions is also variable.12 Over 100 cases of the 18q− syndrome have been published but only recently are the deletions being characterised at the molecular level.12

Improvements in cytogenetic methods enable the detection of smaller deletions than was previously possible, resulting in the identification of patients who may be helpful in further localisation of the chromosomal regions that are involved in the aetiology of the syndrome. With respect to the 18q− syndrome, the region 18q22-q23 has been implicated as the critical chromosomal region.23

Despite recent concerted efforts to construct a high resolution genetic map for chromosome 18,15 few genes causing diseases have been mapped to the distal region of 18q. Genes mapping to this region include the myelin basic protein (MBP) that is associated with brain myelin disorders in the mouse1 and the cytochrome b5 gene that is associated with methaemoglobinemia.7

In an effort to identify chromosomal regions that may contain genes implicated in human disease, we describe the molecular genetic characterisation of a new deletion in the 18q23 terminal band in a 4 year old child. Both fluorescent in situ hybridisation (FISH) and microsatellite marker and restriction fragment length polymorphism (RFLP) analysis were used to characterise the extent of the deletion. The implications with respect to the critical chromosomal region associated with the 18q− syndrome are discussed.

Case report
The patient is a female who was born after an uneventful pregnancy and normal delivery by caesarian section. At birth the baby was diagnosed as having dysplastic pulmonary and tricuspid valves and severe persistent pulmonary hypertension which required prolonged ventilation and paralyse for three weeks and hospitalisation for three months. She was hypotonic for several months and remained on cardiac medication until 12 months. Cytogenetic analysis was carried out from peripheral blood lymphocytes and part of the 18q23 band was found to be deleted (fig 1). The deletion was ascertained at the 550 band level. The parents' chromosomes were normal, indicating that this deletion is de novo origin.

The child's progress has been closely monitored. Her congenital heart defect was initially severe but at 4 years of age she has a persistent pulmonary murmur of no haemodynamic significance. The child's height and weight centiles remained below the 3rd centile until 3 years of age, but since then they have followed the 10th to 25th centile. She showed some gross motor

Figure 1: Partial karyotype of the patient showing del (18)(q23).
delay and expressive language delay, but has caught up to normal at 4 years of age. Griffith’s test at the age of 2 was within normal limits for age. Preschool assessment at age 4 years 9 months showed some minor problems with attention span, but otherwise she is functioning in the normal range. Table 1 summarises which of the typical features of the 18q– syndrome were found in the child at 2 and 4 years of age. Parental consent for pictures could not be obtained.

**Methods and results**

**FLUORESCENT IN SITU HYBRIDISATION**

FISH was performed as previously described from an EBV transformed cell line established from peripheral blood lymphocytes. Lambda phage clones previously mapped to the distal end of chromosome 18 were labelled with biotin and used as probes on metaphase spreads to determine the extent of the deletion. Fig 2A shows the DNA probe D18S231 that hybridises to both the normal 18 homologue and the deleted homologue, indicating that it maps outside the deletion. In contrast, fig 2B shows the DNA probe D18S259 that hybridises only to the normal 18 homologue, indicating that it maps within the region that is deleted. The deletion was further characterised by determining whether chromosome 18 specific subtelomeric fragments could be detected on the deleted 18 homologue. This was analysed using a yeast artificial chromosome that contains an insert derived from the end of 18q. This DNA clone (obtained by labelling the entire yeast artificial chromosome RM2050) did not hybridise to the deleted 18 as shown in fig 2C, indicating that the deletion was not an interstitial deletion.

**GENETIC MARKER ANALYSIS**

To obtain further information about the extent and parental origin of the deletion, lack of heterozygosity of genetic markers mapping to 18q was investigated. Genomic DNA was pre-
pared from buffy coat cells. Approximately 10 μg of DNA was digested with RsaI, run in a 0.8% agarose gel for 19 hours, alkaline blotted onto nylon membrane, and hybridised with 32P labelled probes. The cDNA clones pERT25 (D18S11, ATCC 59472) and CRI-pLI159-2 (D18S17, ATCC 61800) were obtained from the American Type Culture Collection. EcoG cDNA, a clone containing part of the MBP gene, was received from K Boylan (University of California School of Medicine, San Francisco). D18S27 oligohybridisation was carried out by P McNicholas (Genetic Technologies, Sydney).

By analysing both the child’s and the parents’ DNA, it could be shown that RFLP markers D18S11, D18S17, and D18S27 were present in only one copy, as the child did not have an allele inherited from the father (fig 3). The EcoG probe marker for MBP also detected only one allele in the child, but as both parents shared the same allele, this analysis was uninformative. The RFLP data of fig 3 have also been incorporated into table 2.

Microsatellite marker analysis for D18S43, D18S50, MBP, D18S380, D18S58, D18S70, D18S554, D18S461, and D18S462 was carried out as previously described. For the Genethon markers, PCR was performed at 94°C for 40 seconds and 59°C for 25 seconds (35 cycles), after an initial denaturation step of five minutes and final elongation step of two minutes at 72°C. D18S380 was amplified at 94°C for 15 seconds, 58°C for 15 seconds, 72°C for 25 seconds (five cycles) and then at 94°C for 15 seconds, 54°C for 15 seconds, 72°C for 25 seconds (30 cycles). MBP, D18S43, and D18S50 were amplified at 94°C for one minute, 55°C for two minutes, 72°C for one minute (35 cycles).

With microsatellite markers D18S43 and D18S58, two alleles were seen in the child, but for D18S70, D18S461 and D18S462 only one allele inherited from the mother was present. D18S50, MBP, D18S554, and D18S380 were uninformative. Table 2 summarises the composite results obtained with the RFLP and microsatellite marker analysis which show that the deletion is of paternal origin.

Discussion

We have described a patient with a small deletion of 18q who has few of the clinical features of the 18q—syndrome. This deletion appears to include the chromosomal region that is thought to be involved in the appearance of the clinical features associated with the 18q—syndrome. The smallest deletion that was reported by Silverman et al2 was a 9 Mbp deletion in patient BP18. This determination was made using bivariate flow karyotyping. Unfortunately, the extent of the deletion using molecular markers was not reported, making comparisons with our patient difficult. However, a cell line derived from patient BP18 was obtained and the deletion breakpoint in this patient was found to map between DNA probes D18S239 and D18S309 (J Overhauser, personal communication), a region that is located about 4 cM proximal to D18S58, a DNA probe that is present in two copies in our patient. Thus we can infer that the size of the deletion in our patient is about 5 Mbp.

No apparent reason for the clinical variation of the 18q—syndrome phenotype has yet been determined. Kline et al3 concluded that the size of the deletion correlated with the severity of the phenotype, on the basis of a detailed molecular analysis of seven patients. Later studies with a larger number of patients, however, failed to confirm this correlation, as two patients with a slightly larger deletion than described here, including the deletion of D18S231, have many of the typical features of the 18q—syndrome, such as mental retardation and developmental delay.

All previously described patients with a clinical phenotype of the 18q—syndrome have the deletion of a least the whole chromosomal band 18q23 in common. Only two genes, cytochrome b510 and MBP,11 have been physically mapped to this chromosomal region. Because of the central nervous system problems often associated with this syndrome, it has been suggested that the deletion of one copy of MBP is crucial. Although we could not unequivocally show this in our patient, it is likely that one allele of MBP is deleted. The markers shown in table 2 are ordered based on their physical location (J Overhauser, personal communication), and the MBP gene maps distal to D18S462, a marker that was present in only one copy. Furthermore, FISH with YAC clones that contain the MBP gene failed to hybridise with the deleted 18 homologue (data not shown).

Table 2 Microsatellite marker and RFLP marker alleles

<table>
<thead>
<tr>
<th>Marker</th>
<th>Father</th>
<th>Mother</th>
<th>Child</th>
<th>No. of alleles*</th>
</tr>
</thead>
<tbody>
<tr>
<td>D18S43</td>
<td>80,72</td>
<td>84,72</td>
<td>84,72</td>
<td>2</td>
</tr>
<tr>
<td>D18S55</td>
<td>148, (148)</td>
<td>162,148</td>
<td>152</td>
<td>U</td>
</tr>
<tr>
<td>D18S380</td>
<td>152,148</td>
<td>152,148</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D18S554</td>
<td>226,216</td>
<td>216,216</td>
<td>216</td>
<td>U</td>
</tr>
<tr>
<td>D18S50</td>
<td>184,180</td>
<td>180,180</td>
<td>180</td>
<td>U</td>
</tr>
<tr>
<td>D18S462</td>
<td>191,187</td>
<td>183,183</td>
<td>183</td>
<td>1</td>
</tr>
<tr>
<td>MBP</td>
<td>228,216</td>
<td>216,216</td>
<td>216</td>
<td>U</td>
</tr>
<tr>
<td>EcoG</td>
<td>A,B</td>
<td>A(A)</td>
<td>A</td>
<td>U</td>
</tr>
<tr>
<td>D18S461</td>
<td>166,164</td>
<td>164,162</td>
<td>162</td>
<td>1</td>
</tr>
<tr>
<td>D18S27</td>
<td>C,D</td>
<td>A,B</td>
<td>B</td>
<td>1</td>
</tr>
<tr>
<td>D18S11</td>
<td>A,C</td>
<td>B,D</td>
<td>B</td>
<td>1</td>
</tr>
<tr>
<td>D18S57</td>
<td>A,B</td>
<td>C(C)</td>
<td>C</td>
<td>1</td>
</tr>
<tr>
<td>D18S70</td>
<td>124, (124)</td>
<td>120,112</td>
<td>112</td>
<td></td>
</tr>
</tbody>
</table>

*Number of marker alleles in the child.
1: one allele deleted.
2: both alleles present.
U: marker uninformative for deletion.
The results obtained here also further help in localising several genetic markers to 18q23. The location is confirmed for D18S11 and D18S70 and further refined for D18S17 and D18S27. The two Genethon markers, D18S461 and D18S462, can also be localised to 18q23 with our analysis, providing a new link between the linkage map and the physical map. These markers can now be used in the identification of further genes in this chromosomal area that may be important for the development of the clinical phenotype of the 18q—syndrome.

We thank Michelle Simpson-Radoslovich for technical assistance, the Lifecodes Corporation and Dr Paul McNicholas for the D18S27 hybridisation, and Drs Richard Straub, Steve Gerken, and Kevin Boylan for supplying PCR primers and DNA probes.

14 Giordano SJ, You M, Ward DC, Bhatt M, Overhauser J, Stiegler AW. The human cytochrome b5 gene and two of its pseudogenes are located on chromosome 18q23, 1q43-32.1 and 20q11.2 respectively. Hum Genet 1993;92:615-18.
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