Carrier detection of Hunter syndrome (MPS II) by biochemical and DNA techniques in families at risk

Winnie Schröder, Lothar Petruschk, Manfred Wehnert, Marlies Zschiesche, Günther Seidlitz, John H Hopwood, Falko H Herrmann

Abstract
DNA based and biochemical diagnosis of MPS II was performed on 13 unrelated families using Southern blotting. The 35S-sulphate accumulation in cultured fibroblasts was investigated and the iduronate-2-sulphatase (IDS) activity in the serum determined. Sixteen patients and 36 females at risk were screened for structural aberrations and by RFLP analysis using the intragenic probe pc2S15 and probes VK23B, VK21A, and II-10 for the flanking loci DXS297, DXS296, and DXS466. Structural alterations were found in the DNA of two patients. One of them showed a major deletion including the whole coding sequence of the IDS gene. An aberrant Southern fragment occurred in the HindIII/pc2S15 blot of the other patient suggesting a new HindIII restriction site by point mutation in an IDS gene intron. Twenty-nine females were confirmed as carriers, and for five women the heterozygous state could be excluded. Prenatal diagnosis can be offered to 27 women if requested.

Results
The diagnosis of MPS II was confirmed by enzyme analysis in 18 families of German origin. The families were investigated by biochemical methods and counselled by offering prenatal diagnosis for the women at risk. The figure shows examples of the pedigrees of

Methods
The IDS activity in serum and fibroblasts was determined with the natural substrate (L-O-α-iduronic acid 2-sulphate)-(1→4)-D-O-2,5-anhydro[1-3H]mannitol 6-sulphate) by a modification of the method of Archer et al. The 35S-sulphate accumulation in cultured cells in the presence of F-1-p was measured as described by Petruschk et al.12 By this method the presence of cells expressing the deficient phenotype was indicated by the increased ratio (F+/F−) of sulphate incorporation by cells cultured with or without F-1-p and an increased difference (Δ) in the accumulation factors between these cultures (table 1).

Genomic DNA for the Southern analyses was prepared by standard methods from peripheral white blood cells or fibroblast cultures of patients and women at risk13; 10 μg of genomic DNA was completely digested by the appropriate restriction enzymes (AGS, Boehringer Mannheim). After agarose gel electrophoresis, DNA was transferred onto nitrocellulose filters (Amersham). DNA probes were radiolabelled with deoxyctydine 32P-phosphate using an oligolabelling kit (Amershaw/Buchler), and hybridisation was performed at 42°C in the formamide system as previously described.

For the genomic diagnosis of MPS II families we used the intragenic probe pc2S15 (StuI/pc2S15) and the flanking probes VK23B, VK21A, and II-10 (XmnI/VK23B, TaqI/VK21A, TaqI/II-10). For deletion screening the additional probe VK18 (DXS295) was used.

References
14,18
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Table 1 Results of carrier detection by biochemical tests and genomic analysis in obligate carriers for MPS II.

<table>
<thead>
<tr>
<th>Pedigree No</th>
<th>F-1 p test</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1</td>
<td>3.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Family 2</td>
<td>1.2</td>
<td>0.043</td>
</tr>
<tr>
<td>Family 3</td>
<td>1.0</td>
<td>0.094</td>
</tr>
<tr>
<td>Family 4</td>
<td>1.7</td>
<td>0.091</td>
</tr>
<tr>
<td>Family 5</td>
<td>1.6</td>
<td>0.057</td>
</tr>
<tr>
<td>Family 6</td>
<td>2.4</td>
<td>0.057</td>
</tr>
<tr>
<td>Family 7</td>
<td>3.0</td>
<td>0.057</td>
</tr>
<tr>
<td>Family 8</td>
<td>5.0</td>
<td>0.057</td>
</tr>
<tr>
<td>Normal controls</td>
<td>0.03</td>
<td>0.216</td>
</tr>
</tbody>
</table>

NI = not informative. ND = not determined. HZ = heterozygote for one or more RFLPs.

MPS II families with the results of pedigree, biochemical, and molecular analysis. In 13 families which we investigated extensively, 18 females were obligate carriers for the MPS II gene. The results of the different carrier detection tests, namely IDS activity in serum, sulphate incorporation in cultured skin fibroblasts, and RFLP analysis, are summarised in table 1.

Although the enzyme activity in the serum of MPS carriers is about 50% or less compared with the average IDS activity in the serum of healthy persons, there is a broad overlap in the range of specific activities. A cutoff limit of 0.5 for \( \Delta \) and of 1.5 for the ratio \( F/\Delta \) was fixed for the sulphate accumulation test. Only one (family 3, I-1) of the 16 obligate carriers investigated by this method gave an ambiguous result.

Thirty-six females, including the 18 obligate carriers, were investigated by DNA based analysis. Of the 18 potential carriers, 11 were proven to be heterozygous for the MPS II gene and in five women the heterozygous state could be excluded. In two cases RFLP analysis was not informative. These results agree well with those of the biochemical carrier detection tests. As a result of these investigations DNA based prenatal diagnosis could be offered to 27 women (14 of them with the intragenic Stu1/pc2S15 RFLP) (table 2).

We found structural aberrations in only two of the 16 patients. One patient was found to have a major deletion including the entire coding region and the extragenic locus DXS466 (table 3). In another patient a normal 9-4 kb fragment disappeared while an aberrant fragment of 3-5 kb occurred in the HindIII/pc2S15 blot. Normal Southern patterns occurred in the PsI and TaqI/pc2S15 blots suggesting a new HindIII restriction site by point mutation in an IDS gene intron. This marker fragment was also detected in the Southern blot of the mother.

Discussion

The detection of female carriers by enzymatic methods is sometimes equivocal owing to the wide range of enzyme activity in the serum of normal or heterozygous females. According to our experience and to results published by others, about 10% of false positive or negative results can be assumed for this test. The sulphate accumulation test produces a more accurate diagnosis. So far no false positive results have been reported (L. Petruschka, unpublished data), but even with this method about 10% of potential carriers cannot be detected.

The molecular characterisation of a specific mutation in a patient would allow direct segregation analysis of this defect in the family. Using Southern blotting for deletion screening we found only one deletion in 13 families. The suggested new HindIII restriction site in patient G-117 is localised in the 9-4 kb fragment of the IDS structural gene and should result in two smaller fragments. A 3-5 kb fragment containing exon sequences can be detected by the IDS cDNA. The second expected fragment of 5-9 kb contains only intron sequences and was therefore not detectable by the cDNA probe used. The aberrant fragment can be detected in the Southern blot of the patient’s mother as well, so it can be used as a direct intragenic segregation marker for MPS II in this family.

In most cases the molecular defect cannot be detected by Southern blotting. The method generally followed for DNA based diagnosis in such families is gene tracking using intragenic and intergenic RFLPs closely linked to the mutation site. The reliability of this indirect genotype analysis is dependent on the cross-over rate between the mutation and RFLP sites.

Using the intragenic marker Stu1/pc2S15 the error rate caused by recombination should be less than 1%. Recombination fractions between the IDS locus and flanking loci were determined by multipoint analysis. No recombination was observed between the IDS locus and the flanking loci DXS296 or DXS466.

The results of the DNA based diagnosis in MPS II families show good agreement with the results of the biochemical diagnosis. About 90% of females are informative for one or more RFLPs and 50% of them are informative for the intragenic RFLP and another 10% for the closely linked intergenic TaqI/VK21A or TaqI/II-10 RFLPs or both.

At present, the use of the intragenic IDS cDNA probes and flanking probes available so far allows carrier detection in MPS II families.
Segregation analysis in two families at risk for MPS II for carrier detection using one intragenic and three extragenic RFLPs. The carrier status was confirmed by biochemical and genomic diagnosis.

Table 2 Heterozygosity of intragenic and intergenic RFLP of the IDS gene in female heterozygotes for MPS II.

<table>
<thead>
<tr>
<th>RFLP</th>
<th>Informative genotypes</th>
<th>Total No of probands</th>
<th>Heterozygosity found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>StuI/pC2S15</td>
<td>14</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>XmnI/VK23B</td>
<td>14</td>
<td>22</td>
<td>64</td>
</tr>
<tr>
<td>TaqI/VK21A</td>
<td>4</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>TaqI/II-10</td>
<td>6</td>
<td>27</td>
<td>22</td>
</tr>
<tr>
<td>StuI/pC2S15</td>
<td>18</td>
<td>29</td>
<td>62</td>
</tr>
<tr>
<td>+ XmnI/VK23B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ TaqI/VK21A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ TaqI/II-10</td>
<td>27</td>
<td>29</td>
<td>90</td>
</tr>
</tbody>
</table>

with the same accuracy as, or a little more than, the investigation of IDS activity in serum or of sulphate accumulation in cultured cells. Although genomic analysis will be the method of choice in the future, the application of biochemical methods to carrier detection may be helpful in selected cases.

We are grateful to Dr G R Sutherland, Adel- laide, for providing the probes VK21A, VK23B, and VK18 and to Dr T Hulseboes, Amsterdam, for providing the probe II-10. This work was supported by a grant from Deutsche Forschungsgemeinschaft No He 1885/1-1.

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doi: 10.1136/jmg.30.3.210

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