MPS II in females: molecular basis of two different cases

EDITOR—Hunter disease or mucopolysaccharidosis type II (MPS II, MIM 309900) is an X linked recessive disease resulting from deficiency of the lysosomal enzyme iduronate-2-sulphatase (IDS, E.C.3.1.6.13). The IDS cDNA has been isolated and the genomic region containing the IDS gene and pseudogene has been completely sequenced. Phenotypic expression of X linked disorders in females may be the result of an X chromosome anomaly or homozygosity for the mutated gene, but is most frequently the result of skewed X chromosome inactivation. We describe two affected girls, case 1 and case 2, with a mild and a severe form of MPS II, respectively. Both have a normal karyotype but increased dermal sulphate and heparan sulphate excretion in urine, a marked deficiency of IDS activity, and normal α-L-iduronidase, β-D-gluconuridase, and arylsulphatase A activities in leucocytes and cultured skin fibroblasts ruling out MPS I, MPS VII, and multiple sulphatase deficiency. Molecular studies showed that case 1 is the first case of female MPS II with two mutated IDS genes and that case 2 has a de novo gene rearrangement on the paternal allele (a 3254 bp deletion from intron 7 to intron 8 with an insertion of 20 bp) asso-

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<tr>
<th>Primers or oligoprobes (IDS) used for PCR, sequencing, mutagenesis, or ASO hybridisation</th>
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<tr>
<td><strong>Primer</strong></td>
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<td>PCR/sequencing</td>
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ASO: the mutated base is in bold.
her mother by analysing the methylation status at three
polymorphic loci, MAOA, HUMARA, and DXS255. At the
DXS255 locus (fig 3A), case 1’s allele 1 is resistant
completely digested by Hha I, while the paternal allele is
uncut; in her mother and sister, 50% of both alleles are cut.

The three other tested loci (MAOA, HUMARA, and DXS255).

No IDS activity was observed in L41P transfected Lβ del. An increase in IDS activity was observed in COS cells
and normal fibroblasts transfected by L41P, 3.8% and
28.8% of the IDS activity produced by the wild type
cDNA, respectively (table 2). This residual activity of the
L41P mutation expressed in cells with a basal IDS activity
(COS and normal fibroblasts) may be caused by a delayed
proteolysis of the mutant precursor in the presence of the
normal protein, as previously described for a
proteolysis of the mutant precursor in the presence of the
wild type cDNA or a delayed degradation of the
precursor. (C) Normal skin fibroblasts transfected by L41P cDNA
produced only the unstable 76 kDa precursor. (D) Normal skin fibroblasts transfected by L41P cDNA
produced only the unstable 76 kDa precursor.

The presence or absence of the IDS polypeptide is indicated by + or −.

Table 2 IDS activities in μmol.h−1.mg−1 in the different cell types transfected by the wild type cDNA (L41L), the mutant cDNA (L41P) or not transfected (NT). The basal level of IDS activity in COS cells and normal fibroblasts was subtracted.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Activity in μmol.h−1.mg−1</th>
<th>76 kDa</th>
<th>90 kDa</th>
<th>55 kDa/45 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fib</td>
<td>L41L</td>
<td>4.00 ± 1.21 (n=3)</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>L41P</td>
<td>1.15 ± 0.38 (n=3)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>0.23 ± 0.03 (n=3)</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Lβ del</td>
<td>L41L</td>
<td>4.83 ± 0.12 (n=2)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>L41P</td>
<td>0.00 (n=2)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>0.00 (n=2)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>COS</td>
<td>L41L</td>
<td>48.93 ± 21.4 (n=3)</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>L41P</td>
<td>1.85 ± 0.07 (n=3)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>1.53 ± 0.03 (n=3)</td>
<td>−</td>
<td>−</td>
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</table>

The age of 3 years 6 months, she had a coarse face, hearing loss,
hepatosplenomegaly, severe thoracolumbar kyphosis, limitations of
joint mobility, mental retardation, and mitroaorti-
valvular dysplasia, but no corneal clouding. She had a
severe form of the disease and died at the age of 10 years.

A unique IDS transcript with a complete deletion of
exon 8, resulting in the omission of 58 of the 550 amino
acids of the IDS protein, was identified in fibroblasts.
The exon 8 skipping was caused by a 3254 bp deletion in
genomic DNA, extending from intron 7 (nt 17419,
L35485) to intron 8 (nt 20692, L35485) with an insertion of
20 nucleotides (5′-CCACACAGGCATGAGCCATG)
at position 17418: 17419del3254/17418ins20 in her
family, in agreement with the results of the enzymatic
assays.

At the DXS255 locus (fig 3B), case 2’s maternal allele is
defined by Hha I, while the paternal allele is
uncut; in her mother and sister, 50% of both alleles are cut.
X inactivation is skewed in case 2 and only the paternal X
cromosome is active. X inactivation is random for her
mother and her sister. The three other tested loci (MAOA,
HUMARA, and FMR-1(14) confirmed these results except for the MAOA locus, which was not informative for her mother. As the father does not carry the mutation in available somatic cells (leucocytes, skin fibroblasts), somatic cell hybrids with patient fibroblasts were established to check if the patient’s active X chromosome carries the deletion.

Cell fusion of a 6-thioguanine ouabain resistant variant of Chinese hamster ovary (CHO) cells (RJK88 from Coriell Cell Repositories) with patient fibroblasts was performed with polyethylene glycol.(15) Selection for hybrids was carried out by maintaining the cultures in Dubelcco’s medium. The HAT-ouabain resistant clones were harvested six weeks later by scraping and expanded for DNA analysis. The RJK88-case 2 somatic cell hybrids were tested for the deletion by genomic PCR primed with DB5 (exon 8, table 1) and DB6 (exon 8, table 1) resulting in a 173 bp fragment. These primers are included in the deletion, so no PCR product would be obtained if only the mutated allele is present. The parental origin of the X chromosome in hamster/human somatic hybrid clones was established by testing 15 microsatellites surrounding the IDS gene (DXS8028, DXS8045, DXS1200, DXS998, DXS8091, DXS1215, DXS1193, DXS8084, DXS8043, DXS8086, DXS8011, DXS8103, DXS8069, and DXS1113)(16) and one intragenic StuI polymorphism (nt 20274 in intron 8, primers del S and del AS, table 1).

Nine of the polymorphic markers were informative for case 2 and two were selected (DXS1113 and DXS8086) for the analysis of CHO patient somatic cell hybrids (X number, parental origin, and mutation). Four hamster-patient (RJK88-case 2) somatic cell hybrid clones were obtained. Two clones retained only one X chromosome of paternal origin as shown by DXS1113 and DXS8086. These two clones were carrying the complex mutation 17419del3254/17418ins20 (no DB5–DB6 PCR product).

Case 2 had a skewed X inactivation and her active X was carrying the severe complex mutation. This deletion appeared de novo on the paternal X chromosome (unaffected father, study of somatic cell hybrids) and case 2 was the only carrier in the family. She was also the only female in this family who had skewed X inactivation. The unbalanced X inactivation was probably identical in almost all her cells as she presented with a severe phenotype.

Our two female patients presented with skewed X inactivation. Plenge et al.(17) reported a heterozygous C to G mutation at position 6433 in the XIST (X inactivation specific transcript) minimal promoter (U50908) on the inactive X chromosome in two unrelated females with familial skewed X chromosome inactivation. They suggested that a substitution in the XIST promoter could play a role in the X inactivation process. The XIST’ promoter region from our patients was amplified and sequenced. We identified four differences from the published sequences on both chromosomes of our two patients: a G to T substitution at 6028 (U50908) and three insertions (311insT, 652insT, and 700insG: M97168). These four sequence differences were also present in all the control X chromosomes tested (30 normal females and 19 normal males). Thus, they were not involved in the process of X inactivation and probably represent sequencing errors in U50908 and M97168. Our patients were both negative for the mutation C6433G described by Plenge et al(17) and no specific nucleotide substitution could be identified but there was no familial context of skewed X inactivation in these two cases.

These two MPS II affected females illustrate two different mechanisms by which females can be affected by recessive X linked disorders. The skewed X inactivation of the normal X chromosome in a carrier female has been documented in four other cases of MPS II,(18) as in many other X linked diseases. Our patient I is the first case of an affected MPS II female with two mutated IDS genes.

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MPS II in females: molecular basis of two different cases

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