A 38.8 kb Deletion Mutation of the Iduronate-2-Sulfatase Gene in a Patient with Hunter Syndrome

Yen-Yin Chou,1 Sheau-Chiou Chao,2 Pao-Lin Kuo,3 and Shio-Jean Lin1

Abstract: Mucopolysaccharidosis type II (Hunter syndrome) is an X-linked lysosomal storage disorder. A novel gross deletion in the iduronate-2-sulfatase (IDS) gene was found in a 6-year-old boy with Hunter syndrome. The phenotype of the patient was severe, including joint stiffness, kyphosis, hepatomegaly, hypertrophic cardiomyopathy, moderate mental retardation, and bilateral hearing loss. The 38.8 kb gross deletion involves exons 1-7, the proximal breakpoints lying in intron 7, at position 1307880 (GenBank NT:019686), and the distal deletion breakpoint was located at position 1346697. The large deletion correlated with the severe phenotype of this Hunter syndrome patient.

Key words: DNA mutational analysis; Iduronate sulfatase; Mucopolysaccharidosis II; Sequence deletion

In this study, we describe a novel 38.8 kb deletion extending from upstream region to exon 7 of the IDS gene in a boy with severe Hunter syndrome.

Case Report

The proband was a 6-year-old boy with developmental delay: head lifting at 5 months, rolling over at 12 months, walking at 24 months, and speaking only a few words at 35 months. His mother was Vietnamese and his father was Taiwanese. On examination at 35 months, coarse face with macrognathia, short neck, low hair line, short thumbs, joint stiffness, kyphosis, hypertensive, moderate mental retardation, and bilateral hearing loss were noted. Quantitative analysis of urinary glycosaminoglycans (GAG) done at the Department of Medical Genetics in National Taiwan University Hospital showed elevated level (702.23 mg GAGs/g creatinine) [range, 10.74-112.02]. Diagnosis of Hunter syndrome was established by the finding of low leukocytes IDS activity (0.03 mmol/h/mL) (control, 3.37 mmol/h/mL), based on tests done by the Willink Biochemical Genetics Unit of the Royal Manchester Children’s Hospital. From age 3 to 6 years,
he was admitted 6 times due to pneumonia and chronic paranasal sinusitis. He also had mild hypertrophic cardiomyopathy. At age 6 years, he lost the ability to speak, was hyperactive and occasionally aggressive, and suffered from obstructive sleep apnea. Peripheral blood was collected from the patient and his parents after informed consent was obtained. Polymerase chain reaction (PCR) amplification of the IDS gene was performed on genomic DNA using 9 sets of primers spanning all 9 exons and the upstream and downstream flanking sequences. The primers used were designed for use in our laboratory and their sequences are shown in the Table. For PCR amplification, approximately 200 ng of genomic DNA, 15 pmol of each primer, 10 µmol deoxyribonucleoside triphosphate, 0.25 U of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, NJ, USA) and 1 µL dimethyl-sulphoxide were used in a total volume of 50 µL. The amplification conditions were 94°C for 5 min, followed by 40 cycles of 94°C for 45 s, annealing temperature of 72°C for 45 s, and extension at 72°C for 10 min. The PCR products were examined on 2% agarose gels and subjected to direct automated sequencing using an ABI 377 automated sequencer (ABI Advanced Biotechnologies, Columbia, MD, USA).

Amplified products could only be detected for exon 8 and 9, whereas all other exons failed to be amplified. This result suggests the deletion of exons 1-7. In order to determine the distal deletion breakpoint, several sets of primers were designed to amplify

**Table.** Oligonucleotide primers used for polymerase chain reaction amplification of the iduronate-2-sulfatase (IDS) gene.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence Location</th>
<th>Product size (bp)</th>
<th>AT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDS-1R IDS-1L</td>
<td>5'-AGCACCGGAGGATAGGAGAT-3' 5'-TGCAGTCATTCCATGGGTTC-3'</td>
<td>Exon 1 of the IDS gene</td>
<td>460 55</td>
</tr>
<tr>
<td>IDS-2R IDS-2L</td>
<td>5'-CACAGCACGACCACTCACATGTAAG-3' 5'-TGTCCTCCCTCGAGCCCACCCT-3'</td>
<td>Exon 2 of the IDS gene</td>
<td>438 57</td>
</tr>
<tr>
<td>IDS-3R IDS-3L</td>
<td>5'-GAGCTGAAAGACCAACACTCACATGTAAG-3' 5'-CATGACTCAGCTACAAACTGTAAG-3'</td>
<td>Exon 3 of the IDS gene</td>
<td>547 50</td>
</tr>
<tr>
<td>IDS-4R IDS-4L</td>
<td>5'-TGAGTGTCAGGTTGTGCTCCTCTATGTAAG-3' 5'-GTACTGCGACCTCCATGTAAG-3'</td>
<td>Exon 4 of the IDS gene</td>
<td>490 53</td>
</tr>
<tr>
<td>IDS-5R IDS-5L</td>
<td>5'-CTGACACTGAGGAGCAGGACTACGTAG-3' 5'-CATGACTCAGCTACAAACTGTAAG-3'</td>
<td>Exon 5 of the IDS gene</td>
<td>464 60</td>
</tr>
<tr>
<td>IDS-6R IDS-6L</td>
<td>5'-CAACACTGCTGTGCTCCTCATAA-3' 5'-CTGACTCAGCTACAAACTGTAAG-3'</td>
<td>Exon 6 of the IDS gene</td>
<td>480 53</td>
</tr>
<tr>
<td>IDS-7R IDS-7L</td>
<td>5'-GTGTCAGGACTACCACTGTAAG-3' 5'-GAGGAGATGCAAGCAGGACTACCACTGTAAG-3'</td>
<td>Exon 7 of the IDS gene</td>
<td>438 53</td>
</tr>
<tr>
<td>IDS-8R IDS-8L</td>
<td>5'-TCTGACTCAGCTACAAACTGTAAG-3' 5'-CTGACTCAGCTACAAACTGTAAG-3'</td>
<td>Exon 8 of the IDS gene</td>
<td>506 50</td>
</tr>
<tr>
<td>IDS-9R IDS-9L</td>
<td>5'-ACTCCTCTGACTACCACTGTAAG-3' 5'-GTGTCAGGACTACCACTGTAAG-3'</td>
<td>Exon 9 of the IDS gene</td>
<td>611 58</td>
</tr>
<tr>
<td>IDS-1348R IDS-1348L</td>
<td>5'-TCTGACTCAGCTACAAACTGTAAG-3' 5'-GACACCAAAAAACTGCCACACG-3'</td>
<td>Proximal to IDS-2</td>
<td>654 50</td>
</tr>
<tr>
<td>IDS-1346R IDS-1346L</td>
<td>5'-CGAGGAGATTGAGAAGGAGGAGGAGG-3' 5'-CAGGAGGAGGAGGAGGAGGAGGAGG-3'</td>
<td>Proximal to IDS-2</td>
<td>514 53</td>
</tr>
</tbody>
</table>

**Fig.** Structure and organization of the iduronate-2-sulfatase (IDS) gene, the IDS-2 locus and gene W in Xq27.3-28 (upper panel). The exons are indicated as vertical boxes, while horizontal boxes illustrate homologous sequences presented in the IDS gene and the IDS-2 locus. The middle panel shows a 38.8 kb deletion from intron 7 to the upstream. Primers used for polymerase chain reaction amplification of the deletion breakpoint are indicated by arrows. The lower panel shows the sequence of the deletion junction.
the upstream sequences of the *IDS* gene. The results showed that products for primers distal to IDS-1348 (see Table and Fig. for the location) could be amplified. The deletion junction was then amplified using a combination of primers IDS-8R and IDS-1348L (Fig.), giving rise to a product of 2.8 kb. The PCR product was sequenced and the proximal deletion breakpoint was located at position 1307880 (GenBank NT:019686) and the distal deletion breakpoint was located at position 1346697, giving rise to a 38.8 kb deletion (Fig.). A 2.8 kb product was also found in his mother, which confirmed that the deletion was indeed inherited from his mother.

**Discussion**

Major structural alterations of the *IDS* gene account for approximately 10% of mutations in Hunter syndrome patients and result in more severe phenotypes.4,7-10 Point mutations and other small structural changes may result in either mild or severe phenotypes, which depend on the 3-dimensional model of *IDS* protein.5,11,12 Recently, an *IDS*-related locus, termed the *IDS*-2 locus, was found to be 20 kb telomeric to the *IDS* gene.13,14 This locus harbors sequences homologous to exons 2 and 3, and introns 2, 3, and 7 of the *IDS* gene. *IDS*-2 locus is frequently involved in recombination with the *IDS* gene, resulting in an inversion of the intervening DNA.14,15 One of the most common rearrangements in the *IDS* region is a 40 kb inversion which is caused by homologous recombination between closely related sequences which are located in intron 7 of the *IDS* gene and in the *IDS*-2 locus.15 The homologous sequences might also generate intragenic deletions in the *IDS* gene.

In our patient there was no evidence of recombination and inversion. There were also no homologous short repeated sequences flanking the break points. There is no previous report of gross deletion in Taiwanese Hunter patients.16 The mother is a carrier of the deletion and was from Vietnam. There may be differences in mutation spectrums between ethnic groups.

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**References**


