Assessment of iduronate-2-sulfatase mRNA expression in Hunter syndrome (mucopolysaccharidosis type II)

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Abstract. Eight unrelated patients with Hunter syndrome were investigated for expression of iduronate-2-sulfatase (IDS) mRNA by reverse transcription (RT) linked to polymerase chain reaction (PCR), or RT-PCR. The entire coding region was studied by amplification of two overlapping segments of 0.7 and 1.1 kb. Seven children with Hunter syndrome had PCR products indistinguishable in size from normal. One patient, with clinically severe disease, did not produce either IDS product although mRNA for a control gene was readily amplified. This method rapidly identifies patients having absent or qualitatively abnormal IDS mRNA and may be useful in investigating genotype-phenotype relationships.

Introduction

Mucopolysaccharidosis type II (MPS II), or Hunter syndrome, is an X-linked disorder of glycosaminoglycan (GAG) metabolism resulting from systemic deficiency of lysosomal iduronate-2-sulfatase (IDS) enzymatic activity (Hopwood and Morris 1990; Neufeld and Muenzer 1989). In normal individuals IDS cleaves sulfate from iduronic acid residues of dermatan sulfate and heparan sulfate.

While all patients are thought to have a defect at the same locus, there is considerable phenotypic variation ranging from mild to severe (Young et al. 1982a,b). Children with severe Hunter syndrome exhibit gross skeletal deformity, airway obstruction, cardiac disease, progressive mental retardation, and die before age 15. In contrast, those with milder forms may have normal intellectual function and survive into adulthood with relatively few physical limitations (Young and Harper 1982). To provide prognostic information, and especially in considering the possibility of early high-risk treatment by

bone marrow transplantation (Warkentin et al. 1986), an early indicator of severity would be extremely helpful. However, no clinical criteria or biochemical tests that predict severity are available.

Characterization of the specific molecular defects may be useful in predicting severity in newly diagnosed patients. As a means of evaluating this hypothesis, we developed a rapid method to identify major defects based on examining the integrity of IDS transcripts.

Materials and methods

Patients were diagnosed on the basis of clinical features of MPS disease, excessive excretion of urinary GAG, and deficiency of plasma IDS enzyme activity (Wasteson and Neufeld 1982).

Total cellular RNA was isolated (Chomczynski and Sacchi 1987) from peripheral leucocytes or from Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines.
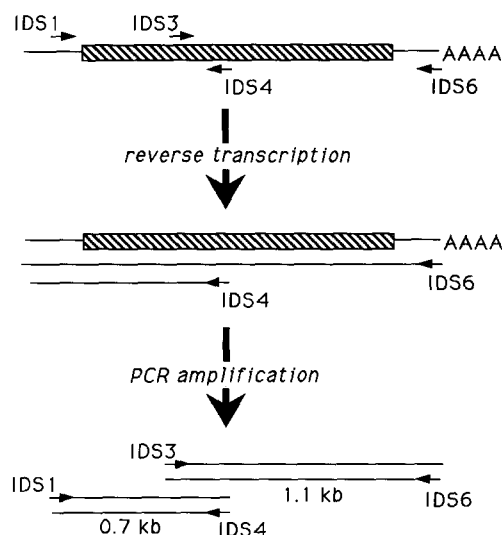
Fig. 1. Strategy for RT-PCR amplification of iduronate-2-sulfatase (IDS) mRNA with location of oligonucleotide primers

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The entire coding region of IDS was reverse transcribed and amplified in two separate but overlapping segments (Fig. 1). For the 3' end, designated 3'-IDS, the RT reaction was primed by IDS6 (5'-TCTAGATCCTCCTCTCACCA-3', nucleotide position +1700 to +1719 numbered from the first base of the initiation codon) with subsequent amplification after the addition of IDS3 (5'-ATGAAAACGTCAGCCAGTCC-3', nucleotide position +635 to +654). For the 5' end, designated 5'-IDS, the RT reaction was primed by IDS4 (5'-TTGGGGTATCTGAAGGGGAT-3', nucleotide position +689 to +708) with subsequent amplification after the addition of IDS1 (5'-CGCGTCGAAGCCGAAATGCC-3', nucleotide position −14 to +5).

As a positive control, the β-glucuronidase (GUS) mRNA was reverse transcribed and amplified in parallel. For GUS we used an antisense primer (5'-CAGTAGCGACTTTCATGCCAACTC-3') designed in this laboratory and a sense primer (5'-TGCATCAC-CACATGCAGGTG-3') described by Tomatsu et al. (1991).

Amplification products were separated by electrophoresis on a 1% agarose gel and visualized after staining with ethidium bromide. Partial direct sequencing of polymerase chain reaction (PCR) products by the dideoxy technique (Sanger et al. 1977) was performed in some cases to verify the identity of the amplified products. To confirm that products were derived from RNA and to rule out contamination, controls were performed in which all steps were identical except that the reverse transcriptase enzyme was omitted. In all cases, this latter control was appropriately negative.

Results and discussion

Eight patients with Hunter syndrome and ten normal individuals were studied (Table 1). Both ends of the gene, the 5'-IDS and 3'-IDS segments, were amplified from total cellular RNA from all ten normal subjects and from seven of the eight patients with Hunter syndrome. The PCR products in these seven patients were indistinguishable in size from those obtained from normal individuals indicating that most of these mutations are likely to be single-base substitutions or small deletions or insertions. In contrast, no amplification of either fragment of IDS cDNA was seen for patient 8 despite successful amplification of GUS cDNA (Fig. 2). Thus, this child was considered to have a mutation that did not allow production of a normal, stable IDS mRNA.

The possibility that patient 8 might have a gene deletion was further investigated. Amplification of genomic DNA from normal individuals using primers IDS3 and IDS4 consistently resulted in a product of approximately 73 base pairs (bp), the size anticipated from the published cDNA sequence (Wilson et al. 1990) if there were no intervening introns; thus, this region is contained within a single exon. However, no product could be amplified using genomic DNA from this patient as template, indicating at least a partial gene deletion. Although he is relatively early in the course of his disease, at age 2 years 8 months, patients 8 appears to have a severe phenotype with characteristic facial features, joint contractures, dysostosis multiplex, and developmental delay. A high-resolution metaphase karyotype study showed no abnormality at the IDS locus, Xq27–28.

Currently, Southern analysis has provided the only information available on the nature of mutations in patients with Hunter syndrome. Using a probe pc2S15 containing 85% of the IDS coding region, Wilson and col-

Table 1. Reverse transcription-polymerase chain reaction (RT-PCR) genotype and clinical features of patients with Hunter syndrome. GUS, β-glucuronidase; IDS, iduronate-2-sulfatase

Patient	RT-PCR genotype			Clinical phenotype		
	GUS (kb)	5'-IDS (kb)	3'-IDS (kb)	Age at diagnosis	Psychometric evaluation (age)	Current clinical status
Hunter syndrome, mild						
1	0.9	0.7	1.1	2 years 4 months	115[a] (2 years 11 months)	Age 3 years 5 months, no disability
2	0.9	0.7	1.1	5 years 4 months	129[b] (8 years 6 months)	Age 11 years 8 months, mild joint contractures
3	0.9	0.7	1.1	2 years 8 months	101[a] (3 years 5 months)	Age 6 years 8 months, no disability
Hunter syndrome, indeterminate						
4	0.9	0.7	1.1	1 year 7 months	91[c] (2 years)	Age 2 years 3 months, mild contractures
5	0.9	0.7	1.1	3 months	Blind, not tested	Age 1 year, congenital brain malformation
Hunter syndrome, severe						
6	0.9	0.7	1.1	2 years 3 months	43[a] (6 years 3 months)	Age 8 years 4 months, hyperactive, moderate contractures
7	0.9	0.7	1.1	4 years	65[b] (5 years 10 months)	Age 9 years 3 months, hyperactive, moderate contractures
8	0.9	Absent	Absent	2 years	78[c] (3 years)	Age 3 years, moderate contractures
Controls[d]	0.9	0.7	1.1			

[a] Stanford-Binet Intelligence Scale, 4th edn.
[b] Wechsler Intelligence Scale for Children − Revised
[c] Bayley Scales of Infant Development, Mental Development Index
[d] *n* = 10; 6 males, 4 females
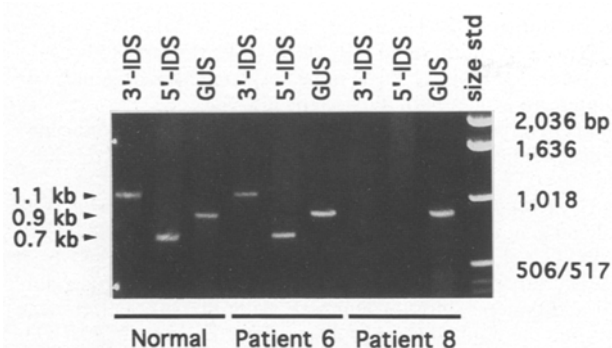
Fig. 2. RT-PCR of 3'-IDS, 5'IDS and GUS products from a normal individual, patient 6 and patient 8. Amplified IDS products from patient 6 were indistinguishable from those of normal individuals. Patient 8 shows no amplification of IDS products despite successful amplification of GUS, a control transcript

leagues (1990, 1991) analyzed 23 unrelated patients by Southern blot; 7 had major structural alterations of the gene of which 2 had deletions of the entire region identified by this probe. The remaining 16 patients had patterns identical to unaffected individuals. Continuing studies using Southern analysis have suggested that approximately one-quarter of patients with Hunter syndrome have partial or total deletions or rearrangements of the IDS gene (Hopwood et al. 1991, Palmieri et al. 1992). However, no information is available as to the presence or absence of IDS mRNA in these patients.

Except in cases where an older relative is affected, prediction of severity of disease is not possible either clinically or with currently available biochemical tests. A test predictive of the clinical phenotype would be of great importance in guiding patient selection and assessment of results with the advent of newer therapeutic modalities including bone marrow transplantation and, potentially, gene therapy. Wilson and colleagues (1990) reported that 2 patients with complete gene deletions on Southern analysis were among the most severely affected in a large British patient population. Palmieri and colleagues (1992) studied 25 additional patients and found that 4 had gene deletions; these patients with deletions also tended to have severe disease. Two possible mechanisms might account for more severe disease in patients with gene deletions.

1. *Contiguous gene defect.* In this model, the mental retardation, which is considered a distinguishing feature of the severe phenotype, actually results from deletion of an adjacent gene. The possibility of involvement of other genes in determining disease severity in MPS II seems unlikely based on the close similarity of the Hunter phenotype with that of Hurler syndrome, a disorder resulting from deficiency of α-L-iduronidase, another enzyme in the pathway of GAG metabolism.

2. *Variable amounts of residual enzyme activity.* This model proposes that severity of disease is determined by residual enzyme activity (Conzelmann and Sandhoff 1983–84). The severe phenotype may result from those

mutations that completely abolish enzyme activity, such as gene deletions or rearrangements, mutations that prevent transcription of a stable mRNA, or mutations introducing early translational stop codons. By contrast, milder forms of the disease may result from mutations giving rise to an enzyme with some residual activity in vivo despite the inability to measure this residual activity in vitro.

The RT-PCR method described here is potentially useful in evaluating these hypotheses and has a number of advantages over the Southern blot technique. Foremost are the relative speed and ease of obtaining results. With this rapid method of analysis, the initial results are available within 24 h of obtaining the blood specimen. This approach should also identify the abnormally short or long products resulting from small mutations causing aberrant splicing, mutations that are not detected by Southern blot technique. The method is potentially automatable. Both approaches, RT-PCR and Southern analysis, are limited in that frameshift mutations, mutations introducing a premature translational stop codon, and other mutations may give rise to a severely affected patient despite the presence of a normal-sized transcript. Further studies need to be undertaken to determine the predictive value of this approach. This RT-PCR method, combined with direct sequencing of PCR products, should also allow for characterization of mutations in the IDS gene.

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