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MUCOPOLYSACCHARIDOSIS IVA: A NOVEL SPLICE ACCEPTOR SITE MUTATION IN INTRON 4 OF THE *N*-ACETYLGALACTOSAMINE-6-SULFATE SULFATASE GENE IN AN AFGHANISTAN GIRL WITH CLASSICAL MORQUIO DISEASE

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Summary We report here a novel splice site mutation in intron 4 of the gene for *N*-acetylgalactosamine-6-sulfate sulfatase (GALNS) in an Afghanistan girl with severe mucopolysaccharidosis IVA (classical Morquio disease). Direct sequencing revealed a homozygous G to A transition in the conserved splice acceptor site in intron 4 (cag<u>G</u>→ca<u>a</u>G: designated IVS 4(−1) G→A) which eliminates 144 nucleotides of exon 5 in her GALNS transcript and introduces an immediate premature termination codon (at Trp 141 of exon 4). The IVS 4(−1) G→A has not been seen in other populations and this is the first report of the molecular basis of classical Morquio disease in an Afghanistan patient.

Key Words mucopolysaccharidosis IVA, splice site mutation, *N*-acetylgalactosamine-6-sulfate sulfatase, Morquio disease

INTRODUCTION

Mucopolysaccharidosis IVA (MPSIVA: Morquio disease) is an autosomal recessive lysosomal storage disorder and is characterized by the lack of activity of *N*-acetylgalactosamine-6-sulfate sulfatase (GALNS: EC 3.1.6.4) (Matalon *et al.*, 1974). Typical clinical manifestations include coxa valga, odontoid hypoplasia, short trunk dwarfism, accumulating undegraded keratan sulfate and chondroitin-6-sulfate in the lysosomes (Morquio, 1929; Maroteaux and Lamy, 1967). On the basis of clinical signs as well as age of onset, three forms severe classical (Morquio,

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1929), intermediate (Glössl *et al.*, 1981) and mild Morquio disease (Orii *et al.*, 1981; Hechit *et al.*, 1984; Beck *et al.*, 1986) can be distinguished. The phenotypic heterogeneity suggests that multiple allelic mutations are responsible for the disease. The analysis of cDNA and genomic DNA structure and organization have made it feasible to study mutations in the GALNS gene (Tomatsu *et al.*, 1991; Nakashima *et al.*, 1994), and over 30 different mutations that underlie MPSIVA have been identified in Japanese, British, Italian, Pakistan and Canadian patients (Fukuda *et al.*, 1992, 1996a, b; Ogawa *et al.*, 1995; Hori *et al.*, 1995; Tomatsu *et al.*, 1994a, b, 1995a, b, c, d, 1996; Cole *et al.*, 1996). Most of the mutations are population specific, supporting a hypothesis that they have developed independently in different ethnic groups. Our purpose is to investigate the molecular basis of MPSIVA as well as to characterize the distribution of mutant alleles and their origins among various populations. Here we report an Afghanistan girl with classical Morquio disease harboring a novel homozygous mutation at the splice acceptor site of intron 4 in the GALNS gene. This is the first documentation of the molecular basis of Morquio disease in the Afghan population.

MATERIALS AND METHODS

At age 7 years the patient whose parents were first cousins was diagnosed as having Morquio disease, based on findings of keratan sulfaturia and deficient activity of GALNS. She was 82 cm tall at 7 years, and showed marked hyperlaxity of wrists, elbows and ankles, and thoracic kyphoscoliosis. There was no facial dysmorphism or hepatosplenomegaly. X-rays revealed platyspondyly. The pathogenic mutation was searched for by genomic PCR as well as RT-PCR, and subsequent SSCP analysis (Ogawa *et al.*, 1995). A pair of primers for RT-PCR were designed as OMF 3: 5′-TTGGACCGGATGGCTGCAGAA-3′ and OMF25: 5′-GCCCGCAAGGGCCAGGCTGGT-3′. The mutation was determined by direct sequencing using an automated sequencer (Perkin Elmer, Applied Biosystems). Since the mutation found in the present study eliminates an *Mva*I restriction site, genomic PCR fragments obtained from the patient and her parents were digested by *Mva*I and vizualized in an 8% acrylamide gel to detect the mutant allele. Haplotypes of the GALNS gene were constructed as described (Rezvi *et al.*, 1996).

RESULTS AND DISCUSSION

An abnormal band was observed only in a PCR-SSCP fragment containing exon 5 and its exon/intron boundary. Direct sequencing revealed a G to A transition in the conserved splice acceptor site in intron 4 (cagG→caaG: designated IVS 4(−1) G→A), the result strongly suggesting that the patient is homozygous for this mutation (Fig. 1). When we compared the mutation site with the wild-type

sequence, the mutant splice site score was reduced from 95.1 to 79.1 (Shapiro and Senapathy, 1987). This mutation is expected to make correct splicing of GALNS mRNA impossible by activating a cryptic acceptor site in exon 5 or by skipping

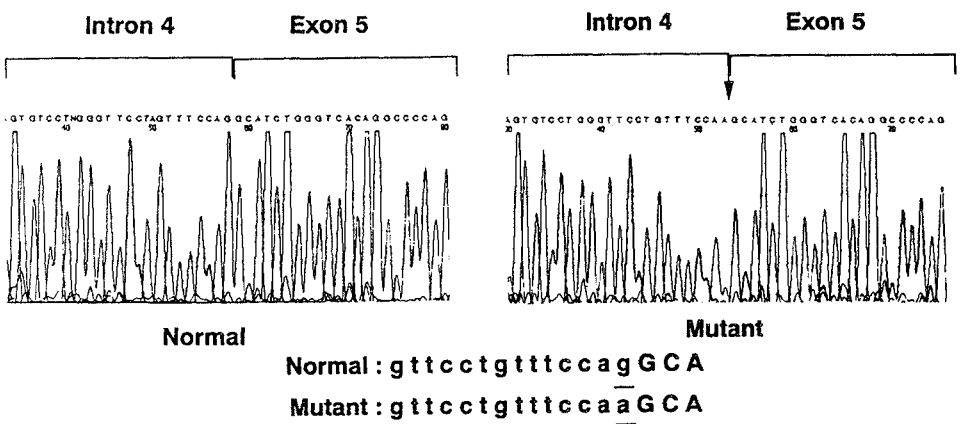

Fig. 1. Nucleotide sequence of splice acceptor site mutation in intron 4. Lower case letters indicate sequence of intron 4 and the upper cases stand for exon 5. The altered nucleotides at the splice junction are underlined. Arrows indicates the mutation in the sequence.

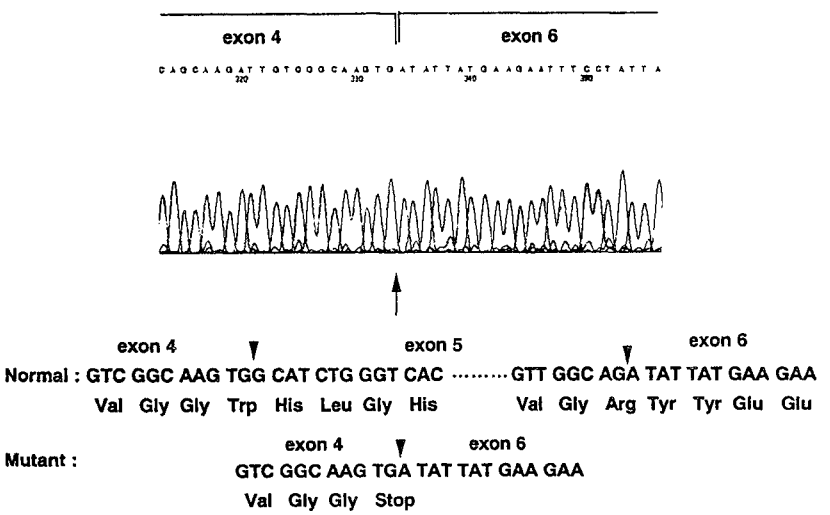

Fig. 2. Sequence of the transcript including skipping of exon 5 and direct splicing of exon 4 to exon 6. Note that pretermination is introduced as a result of exon skipping. Arrows represents the junction of exon 5 skipping. Arrowheads indicates junction of each exon.

of exon 5 and direct splicing of exon 4 to exon 6 in the mutant mRNA. Only a smaller band than expected size was amplified by RT-PCR and 144 nucleotides of exon 5 were completely absent in the GALNS transcript, demonstrating that IVS 4(−1) G→A at the splice acceptor site in intron 4 leads to exon 5 skipping. This mutation introduces an immediate premature termination codon (at Trp 141 of exon 4), which deletes 382 residues at the C-terminal region, most likely resulting in the deficient allele (Fig. 2). Thus, we conclude that the novel IVS 4(−1) G→A mutation is causative for GALNS deficiency in this severely affected patient. Genomic PCR coupled with *Mva*I restriction analysis for her parents revealed that they were heterozygous carriers for the mutation, thus confirming that the patient is homozygous (Fig. 3). The IVS 4(−1) G→A substitution was on haplotype 2, the most frequent haplotype in Caucasian (Rezvi *et al.*, 1996).

Most mutations identified to date are rare, although some of them were common in specific ethnic groups (Tomatsu *et al.*, 1995d, 1996). The IVS 4(−1) G→A is a novel mutation that has not been found in other populations. It is not clear whether IVS 4(−1) G→A is predominant in the Afghan population. However, a screening of mutations in a number of patients in Afghanistan or surrounding areas coupled with haplotype analysis will facilitate more precise

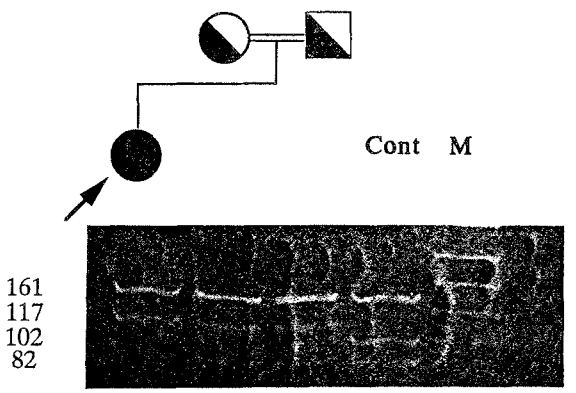

Fig. 3. *Mva*I restriction analysis of family members. Genomic PCR DNA including IVS 4(−1) G→A were subjected to *Mva*I digestion. 117 bp and 102 bp bands stand for mutant and normal allele, respectively. Cont: control subject. M: Size marker pUC13/*Hap*II digest.

detection of carriers and prediction of the development of the disease.

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