Mucopolysaccharidosis type IV: N-Acetylgalactosamine-6-sulfatase mutations in Tunisian patients

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Abstract

Mucopolysaccharidosis type IVA (MPS IVA; OMIM #253000) or Morquio A syndrome is an autosomal recessive inborn error resulting from the deficient activity of the lysosomal enzyme, N-acetylgalactosamine-6-sulfatase (GALNS), and the progressive lysosomal accumulation of sulfated glycosaminoglycans. Clinically, the severe form of this lysosomal storage disease is characterized by characteristic severe bone dysplasia and normal intelligence. To date, a variety of mutations have been associated with the severe MPS IVA phenotype. Here, we report the GALNS mutations in six severe MPS IVA patients from four unrelated Tunisian families. For mutation detection, each of the 14 exons and adjacent intron–exon junctions of the GALNS gene were sequenced after PCR-amplification from genomic DNA. Two novel mutations were identified: a G to A transition in the conserved 5′ donor splice site of intron 1 (GACgt!GACat: designated IVS1+1g→a) and a G to C transversion in codon 66 of exon 2 predicting a glycine to arginine substitution (G66R). The IVS1+1g→a mutation was homozygous in five similarly affected patients from three presumably unrelated families, but haplotype analysis suggested a common ancestor. The affected patient in the fourth family was homozygous for the G66R mutation. These are the first GALNS mutations causing severe MPS IVA disease identified in Tunisia. These molecular findings provide genotype/phenotype correlations, and permit accurate carrier detection, prenatal diagnosis, and counseling for MPS IVA disease in Tunisia where first cousin consanguineous mating remains frequent.

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Introduction

Mucopolysaccharidosis type IVA (MPS IVA; OMIM #253000), also known as Morquio A syndrome, is an autosomal recessive lysosomal storage disease due to the deficient activity of N-acetylgalactosamine-6-sulfatase (GALNS, EC 3.1.6.4) [1]. This enzyme normally hydrolyzes the sulfate ester bonds of the glycosaminoglycans, keratan sulfate, chondroitin-6-sulfate, and galactose-6-sulfate. The enzyme deficiency results in the progressive accumulation of these undegraded substrates leading to a characteristic bone dysplasia. The disease is clinically variable depending on the amount of residual GALNS activity and ranges from a severe spondyloepiphyseal dysplasia including kyphoscoliosis, short trunk dwarfism, coxa valga, corneal clouding, hepatosplenomegaly, and a shortened life span of 20–30 years to an attenuated form with mild skeletal involvement [1,2]. All patients with MPS IVA have normal intelligence. At present, there is no specific therapy for the disease, and treatment is limited to palliative symptomatic procedures to prevent the frequent cervical myelopathy by
occupito-cervical fusion. Efforts to treat MPS IVA by bone marrow transplantation have been of limited success [3], and various experimental strategies are under investigation in cell and mouse model systems [4,5].

The GALNS gene, located on chromosome 16q24.3, contains 14 exons spanning 50 kb (GDB accession ID: 129085) [6,7], and is transcribed into a 1566 bp cDNA, which encodes a 522 residue glycopeptide. The 60 kDa glycopeptide is then processed to 40 and 15 kDa peptides which are linked by disulfide bonds, and the mature human GALNS enzyme is a homodimer [8]. To date, over 100 mutations in the GALNS gene have been identified (Human Gene Mutation Database www.hgmd.org), including 78 missense and nonsense mutations, nine splice site mutations, 15 short and two large deletions, two small insertions, and two complex rearrangements. The allelic heterogeneity presumably correlates with the clinical phenotype [9].

Here, we describe two novel GALNS mutations, the first identified in severe MPS IVA patients from Tunisia. These lesions were homoallelic in the patients from all four families as consanguineous marriages are still frequent in Tunisia [10].

Materials and methods

MPS IVA families

Six patients from four families from Central and Southern Tunisia (Fig. 1) were previously diagnosed by their characteristic clinical findings (Table 1). The patients were all products of consanguineous matings, and there were no known relationships among the families who lived 120–140 km apart. Families 2 and 3 each had two affected children. This study was approved by the Ethics Committees of the respective Tunisian hospitals, and the families gave informed consent.

GALNS activity

The GALNS enzymatic assay was performed as described using the fluorogenic substrate 4-methylumbelliferyl-β-D-galactose-6-sulfate [11]. The GALNS activity was measured in sonicated fresh leukocyte pellets and compared with normal controls. The activity was expressed as nanomoles of substrate cleaved per hour per milligram of leukocyte protein.

GALNS mutation analysis

Genomic DNA was isolated from venous blood by the phenol/chloroform procedure, according to standard protocols as described previously [12]. Each of the 14 exons and flanking intron-exon junctions was amplified by the polymerase chain reaction (PCR) from genomic DNAs isolated from the patients and their parents (GeneAmp, PCR system 9700, Applied Biosystems, Foster City, CA) using previously described primers [13], with the exception of the primers for exon 1, 5'-AGCTGTGTGCTG TGTCTCA-3' (forward) and 5'-CTCGCTCCTCCCTCCATC-3' (reverse). PCR amplifications were carried out in a volume of 50 μl containing 100 ng genomic DNA, 0.2 mmol/L dNTPs, 0.4 pmol of each primer, 1.5 mmol/L MgCl2, 5% DMSO, 50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.8 and 0.5 μl (2.5 U) of Taq polymerase (Promega, Madison, WI). Amplification conditions included an initial 3 min denaturation step at 95 °C, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing for 30 s with the temperature ranging between 55 and 62 °C, and extension for 30 s at 72 °C, followed by a final extension step for 7 min at 72 °C. The PCR products were concentrated and purified from excess primers and dNTPs with the Quiaquick gel-extraction kit (Qiagen, Valencia, CA) and then utilized as templates for direct sequencing with the same PCR primers, in both forward and reverse directions (ABI Prism 3700 Capillary Array Sequencer, PE Biosystems). The ABI Prism TM Big Dye TM Terminator ready reaction kit technology (Perkin-Elmer-Cetus Norwalk, CT) and Amplitaq DNA polymerase (Promega, Madison, WI) were used. Sequence changes were confirmed either by repeat sequencing of the PCR amplicons from the probands and/or their parents and/or by restriction endonuclease analysis on a second PCR product, since the IVS1+1g→a mutation abolishes an Hpy99I restriction site (Biolabs, Saint Quentin Yvelines, France).

Haplotype analyses

For GALNS haplotyping, the mutant and normal alleles were tested for five polymorphisms. These included the following known polymorphisms in: (1) intron 5 (IVS 5 + 134; CCAAGG [allele A] or CCGAGG [allele a] [14]; (2) exon 7 (763 nt from A of the ATG initial codon on the cDNA; GCACGC [allele B] or GCATGC [allele b] [15], (3) exon 11 at cDNA 1232 (GTCC [allele C] or GGCC [allele c]) [16], (4) exon 13 at cDNA 1487 (AAGCCT [allele D] or AGGCCT [allele d]) [14], and (5) exon 14 (CCAG [allele E] or CCGG [allele e] [17]. PCR procedures for haplotype analyses used the sequencing primers except for intron 7 which used 5'-AGGACGACAGTTGTGTCCTCTCTCAT-3' (forward) and 5'-CAC- TCTTCGCTGACACGCGTGGCTGT-3' (reverse) and the annealing temperature was 50 °C.
Results

Clinical features and GALNS activity

The clinical features of each patient and their leukocyte GALNS activities are presented in Table 1. GALNS activity ranged from 0.005 to 0.06 nmol/h/mg of leukocyte protein, values which were <1% of the enzyme activity of normal individuals (normal mean 2.4 ± 1.1 nmol/h/mg leukocyte protein, range 1.2–4.6 nmol/h/mg leukocyte protein, n = 20).

GALNS mutation analysis

Patients 1–5 from Families 1, 2, and 3 (Fig. 2) were all homoallelic for a novel G to A transition in the conserved 5’ splice donor site of GALNS intron 1 (GAC→GAT; designated IVS1+1g→a) (Table 2). The splice site mutation obliterated a Hpy99I restriction enzyme site. PCR amplification of the exon 1 amplicon from genomic DNA and its digestion with Hpy99I resulted in two fragments (123 and 48 bp) in the patients with the splice site mutation, instead of the three fragments (26, 48, and 97 bp) observed in normal individuals.

Patient 6 from central Tunisia was homozygous for a G to C transversion in exon 2 predicting a glycine to arginine missense mutation (G66R). In addition, two previously described intragenic polymorphisms in exons 7 (H236H) and 13 (E477E), as well as a previously unreported sequence variant in exon 12 (P420P) were present in the MPS IVA alleles (Table 2).

Haplotype analyses

Haplotype analyses were performed to investigate the possible relationships between the three families with the splice site mutation. In these families, a single GALNS haplotype was observed (allele ABcde). In contrast, the G66R missense mutation was on a GALNS background with a different haplotype (AbcDe, data not shown).

Discussion

These studies identified the first GALNS mutations in Tunisian patients with the severe MPS IVA phenotype. All six patients from the four presumably unrelated families had GALNS enzymatic activities that were <1% of normal. Thus, homozygosity for the IVS1+1g→a splice site mutation and the G66R missense mutation caused the severe phenotype.

The G66R missense mutation resulted in the replacement of an aliphatic glycine with a larger basic arginine. Homology analyses revealed that glycine-66 is highly conserved among the human and eukaryotic sulfatases (Table 3). The structure of human GALNS was modelled by homology with the crystal structure of human N-acetylgalactosamine-4-sulfatase (4S) and arylsulfatase A (ASA) [9]. Of interest, glycine-66 is a buried residue, and the larger charged arginine could cause misfolding of the nascent polypeptide. This would be consistent with the marked loss of enzymatic activity and the severe phenotype of the homoallelic G66R patient. To date, 15 GALNS missense mutations have been identified that replace a glycine by...
another residue (G23R, G47R, G96C, G96V, G116S, G139S, G155E, G155R, G168R, G247D, G290S, G301C, G309R, G340D, and G421E), and all have been associated with a severe phenotype, except for G23R which is located in a non-conserved region and was associated with a milder phenotype (http://www.hgmd.org) [18].

Among the conserved sequences important for splicing are the essentially invariant gt and at dinucleotides, located at the 5'-donor and 3'-acceptor sites of introns, respectively. Mutations of these sequences result in either the retention of intronic sequence or exon skipping. A splice site mutation also can cause activation of an alternative cryptic splice site in preference to using the legitimate splice site. The gt → at mutation at the 5'-donor splice site of intron 1 presumably caused exon skipping, the loss of exon 2, and an aberrant polypeptide that was misfolded and degraded. This would be consistent with the severe enzyme deficiency and phenotype of the five patients who were homoallelic for this mutation. Of note, this GALNS mutation is the second splicing defect in intron 1 [17], whereas eight other splice site mutations have been identified in other introns (www.hgmd.org). Among these previously reported mutations, the phenotypes of four were reported as severe, while those of the other mutations were not described.

The five patients with the splice site mutations were from three families who lived in three Tunisian cities (Kairouan, Sidi Bouzid, and Sfax) that were over 100 km apart and were not known to be related. Haplotyping of known GALNS polymorphisms revealed that the splice site mutation was on a common background (ABcde). This finding suggested that these mutant alleles were “identical by descent” and were derived from a common ancestor. Further haplotyping is required to determine the frequency of this haplotype in the Tunisian population.

Previous estimates of the incidence of the MPS subtypes indicated that they were higher in Tunisia than in several European countries (13 in 10^5 versus 4.5 in 10^5 live births) [19,20]. The incidence of MPS IVA in Tunisia of ~2.8 in 10^5 live births is also considerably higher than the 1.3 in 10^5 live births.
births in Northern Ireland [21], 0.46 in 10^5 in British Colombia [22], or the 1 in 10^5 live births in Japan [23]. However, complete ascertainment for MPS IVA was unlikely since the Tunisian cohort only included the severe MPS IVA subtype. Several mild or atypical patients may have remained undiagnosed.

Tunisia belongs to the Arab world, characterized by a high rate of cousin marriages, a common traditional practice followed within the same tribe, village, or social unit. First cousin marriages are the most common [10,24]. Such practice brings out rare recessive alleles, thereby increasing the incidence of autosomal recessive disorders. In fact, all the MPS IVA patients studied here are offspring of consanguineous marriages between first degree cousins in families 1, 2, and 4 and second cousins once removed in Family 3 (Fig. 2). This social fact presumably explains the high frequency of MPS IVA in the Tunisian population. Thus, the knowledge of the specific Tunisian MPS IVA mutations will permit accurate prenatal diagnosis and also provide the opportunity to identify carriers for premarital counseling [25].

In summary, two novel GALNS mutations were identified in six severe MPS IVA patients from four Tunisian families. The IVS1^1g->a splice site mutation was detected in three families that share a common GALNS haplotype, suggesting that the mutation was inherited from a common ancestor. The other lesion was a severe missense mutation that presumably resulted in misfolding and degradation of the mutant glycopeptide. The identification of these mutations provides genotype/phenotype correlations for the severe MPS IVA subtypes, accurate carrier detection, and premarital or prenatal diagnosis for Tunisian MPS IVA families.

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References


A. Human Sulfatases

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B. Eukaryotic sulfatases

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<th>Amino acid</th>
<th>GALNS human</th>
<th>GALNS mus musculus</th>
<th>Arylsulfatase A mus musculus</th>
<th>Arylsulfatase B mus musculus</th>
<th>Riken cDNA mus musculus</th>
<th>CGT402 gene product Dro sophila melanogaster</th>
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^ Human glycine 66 and homologous glycines are indicated in bold and boxed. Other highly conserved amino acids are indicated with a gray background [27].
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