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Biochimica et Biophysica Acta 1272 (1995) 129–132

BBA Biochimica et Biophysica Acta

N-acetylgalactosamine-4-sulfatase: identification of four new mutations within the conserved sulfatase region causing mucopolysaccharidosis type VI

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Received 14 April 1995; accepted 14 April 1995

Abstract

Mucopolysaccharidosis type VI (MPS VI; Maroteaux-Lamy syndrome) is the lysosomal storage disorder resulting from the deficient activity of *N*-acetylgalactosamine-4-sulfatase (arylsulfatase B; ASB). MPS VI has been described in man, cats and rats, and several mutations in the ASB gene have been identified in human patients and the animal models. Notably, ASB belongs to a family of sulfatases which are highly conserved, suggesting that they are related evolutionarily and functionally. In this manuscript, four new mutations causing MPS VI are described within the human ASB gene. Each of these mutations occurred in or near the hexapeptide $_{144}$GKWHLG$_{149}$, one of the most highly conserved 'sulfatase' regions. In fact, three of the mutations occurred within the same codon, W146. Thus, these results provide new insights into the molecular lesions causing MPS VI and highlight the importance of this conserved sulfatase region.

Keywords: Mucopolysaccharidosis Type VI; Maroteaux-Lamy syndrome; Sulfatase conservation

1. Introduction

Mucopolysaccharidosis type VI (MPS VI; Maroteaux-Lamy Syndrome) is the lysosomal storage disorder resulting from the deficient activity of *N*-acetylgalactosamine-4-sulfatase (arylsulfatase B; ASB; E.C. 3.1.6.12) and the resultant accumulation of the glycosaminoglycan (GAG), dermatan sulfate. The classic MPS VI phenotype includes course facial features, short stature, dysostosis multiplex, hypoflexible joints, aortic valve dysfunction, hepatosplenomegaly, corneal clouding and normal intelligence [1]. Death usually occurs in the second or third decade of life.

In addition to the human disorder, MPS VI has been described in cats [2] and rats [3]. Recently, the isolation and characterization of the human [4,5], cat [6] and rat [Schuchman et al., unpublished data] cDNAs encoding ASB has provided the molecular tools to investigate the underlying genetic defects in the human disorder and the animal models. To date, fifteen human ASB mutations have been identified in MPS VI patients [8–13]. Unlike other mucopolysaccharidoses, each MPS VI mutation has been private and there is no correlation between the variable phenotype and the molecular lesions.

ASB belongs to a family of sulfatases which hydrolyze sulfate esters present on various natural substrates [4,5]. The N-terminal regions of these sulfatases, in particular arylsulfatase A (ASA; sulfatide sulfatase), ASB, arylsulfatase C (ASC; steroid sulfatase) and *N*-acetylgalactosamine-6-sulfatase (GALNS), exhibit a high degree of evolutionary conservation. In fact, the hexapeptide, GKWHLG, is completely conserved among these sulfatases and believed to participate in the assembly of the 'sulfatase' active site [4,5]. In human ASB, the sequences encoding this hexapeptide are located within exon 2, from codons 144–149 [7]. In this communication, four new mutations in the ASB gene are described in or near the conserved GKWHLG hexapeptide. Most notably, three of these mutations were identified in the same codon, W146.

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SSDI 0925-4439(95)00070-4

2. Materials and methods

2.1. Patient cell lines

The MPS VI patient cell lines were kindly provided by Dr. George Thomas, The Kennedy-Krieger Institute (probands 1 and 2) and Dr. Emmanuel Shapira, Tulane University (proband 3). Cell line GM00538 (proband 4) was obtained from the Human Genetic Mutant Cell Repository, Camden, NJ. Each of the probands was unrelated and had mild (proband 2), intermediate (proband 1) or severe (probands 3 and 4) forms of MPS VI. The diagnosis of MPS VI in the four patients was based on clinical evaluation and confirmed by the finding of markedly deficient ASB activity in cultured cells and/or peripheral mixed lymphoctyes.

2.2. DNA sequencing

To identify mutations in the ASB gene, genomic DNA was isolated by standard techniques [14] and direct solid-phase sequencing of PCR-amplified ASB genomic sequences was performed as previously described [15]. The complete ASB coding region, including the intron/exon boundaries, was amplified in eight fragments [7]. In all cases, the antisense PCR primers were biotinylated at the 5′ end and the PCR products were purified using Dynabeads M-280 according to the manufacturer's instructions (DYNAL; Oslo, Norway). The prewashed Dynabeads were mixed with the PCR products (200 μg each), incubated at 37°C for 40 min, and collected using a Dynal MPC magnet. The samples were washed in 40 μl of 'B&W' buffer (5 mM Tris-HCl [pH 7.5], containing 0.5 mM EDTA and 1 M NaCl), followed by precipitation using the MPC magnet; 30 μl of 0.1 M NaOH was then added for 10 min at room temperature and the samples were washed again with 'B&W' buffer. The immobilized single-stranded DNA was resuspended in 50 μl of TE buffer (10 mM Tris-HCl [pH 8.0] containing 1 mM EDTA) and used for direct dideoxy sequencing. At least two independent amplification reactions were sequenced for each patient to confirm that the putative mutations were not due to PCR artifacts.

3. Results

DNA sequencing of the ASB genomic sequences from probands 1–4 revealed several new mutations. Four of these were within exon 2, at codons 142 and 146 (Fig. 1).

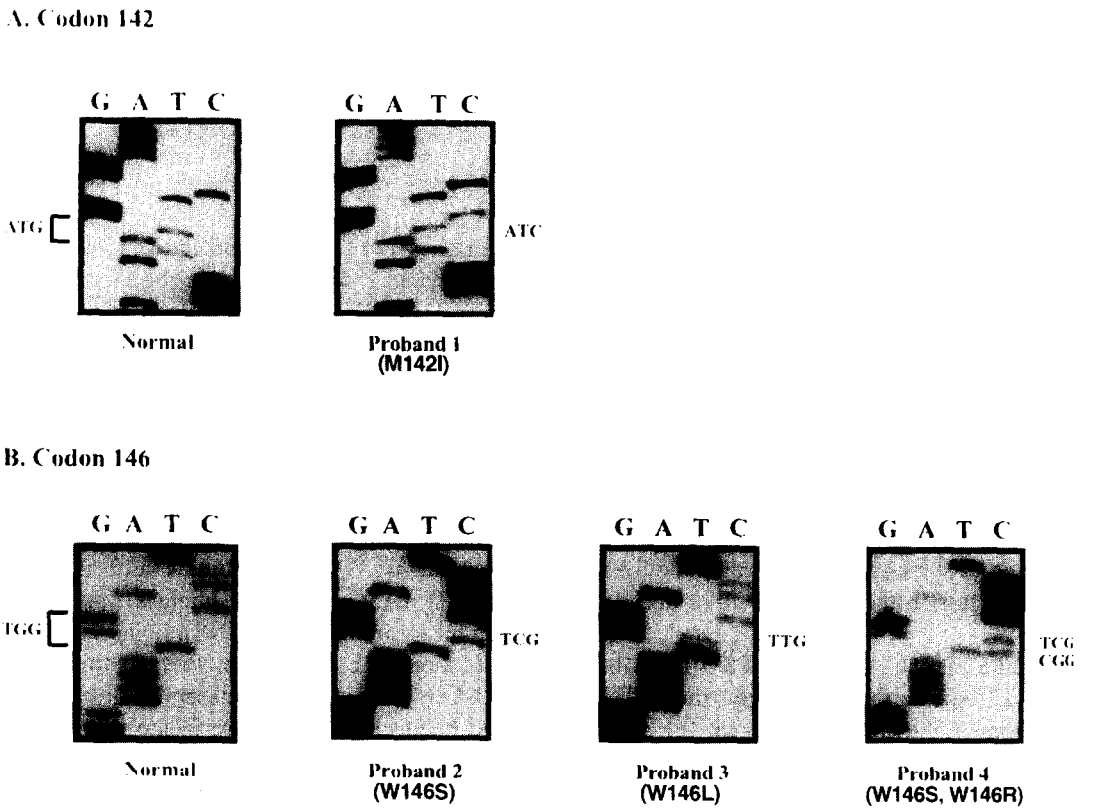

Fig. 1. Identification of the M142I, W146S, W146L and W146R mutations in MPS VI patients. Direct dideoxy DNA sequencing of ASB exon 2 was performed on PCR-amplified genomic DNA obtained from probands 1–4. The normal sequences of codons 142 (ATG; 1A) and 146 (TGG; 1B) are bracketed and the mutated codons are shown to the right of each panel. Probands 1–3 were heteroallelic for the M142I, W146S and W146L mutations, respectively, while proband 4 had two mutations within the same codon (W146S and W146R).

Proband 1 was heteroallelic for a G to C transversion at nucleotide 426 of the full-length ASB cDNA [5], resulting in a methionine to isoleucine substitution at codon 142 (designated M142I; Fig. 1A). Surprisingly, probands 2–4 each contained mutations within codon 146 (Fig. 1B). Proband 2 was heteroallelic for a G to C transversion at nucleotide 437, resulting in a tryptophan to serine substitution (designated W146S). Proband 4, an unrelated MPS VI patient, was heteroallelic for this mutation and a different point mutation within the same codon. The second mutation, designated W146R, resulted from a T to C transition at nucleotide 436 and caused a tryptophan to arginine substitution. Proband 3 also was heteroallelic for a point mutation at nucleotide 436, a G to T transversion resulting in a tryptophan to leucine substitution at codon 146 (W146L). The other ASB alleles in probands 1, 2 and 3 carried mutations that were located outside the conserved hexapeptide region (not shown).

Thus, each of the four mutations described in this manuscript occurred within a small highly conserved region of exon 2. In fact, three mutations occurred within the same codon (W146L, W146R and W146S). Moreover, W146S was found in two unrelated patients (probands 2 and 4), the first example of a non-private mutation in this disorder. Two possible explanations might account for these findings. This region may be a 'genetic hot-spot' which is susceptible to molecular lesions or a 'functional hot-spot' which is critical for ASB structure and/or function. Since none of the mutations occurred at a CpG dinucleotide and all were within a highly conserved peptide sequence, the latter explanation is more likely.

4. Discussion

The hexapeptide GKWHLG has been conserved in sulfatases from bacteria through man (Fig. 2) and the GK dipeptide within this region is conserved among all known

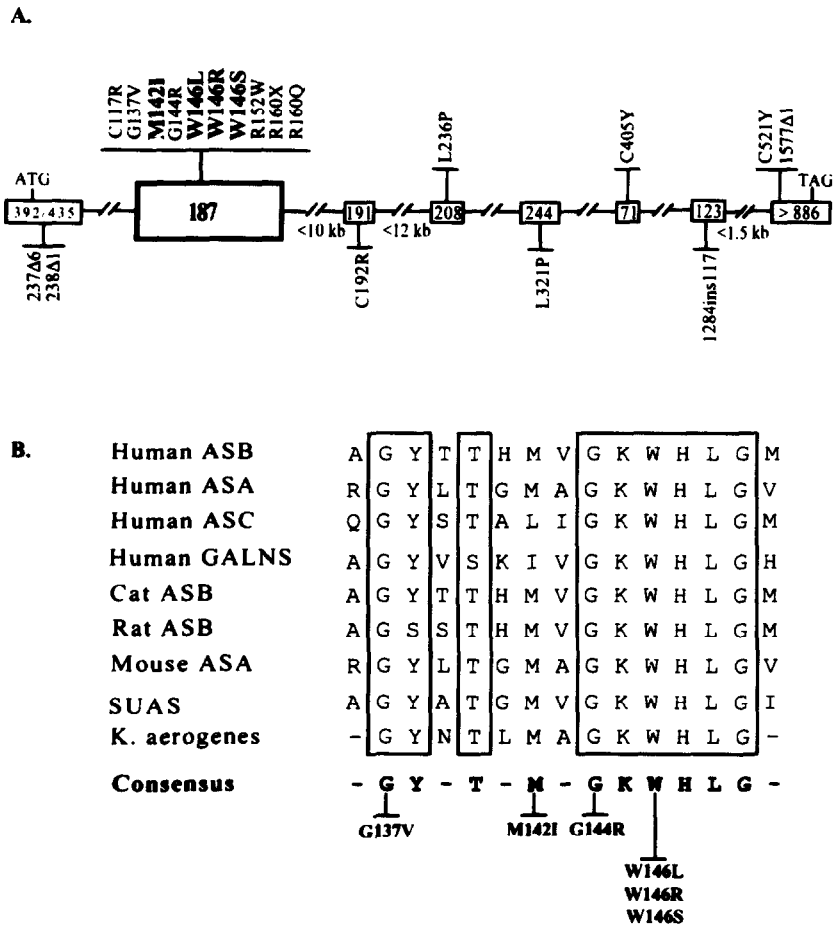

Fig. 2. Mutations in the ASB gene causing MPS VI and conservation of amino acid sequences among different sulfatases. (A) The genomic organization of the human ASB gene [7] and the location of mutations causing MPS VI. Note that of the 19 known mutations, 10 occur within a small region of exon 2. In fact, 9 of these exon 2 mutations are in a 23 amino acid region which includes the hexapeptide GKWHLG. (B) An amino acid comparison of several different sulfatases, including human ASB, ASA, ASC and GALNS, cat and rat ASB, mouse ASA, an arylsulfatase from the sea urchin (designated SUAS) and a bacterial sulfatase from *K. aerogenes*. Note the complete conservation of the hexapeptide sequence among these different sulfatases. The location of the codon 142 and 146 mutations are shown along with the two other previously reported mutations [8–13] within this 15 amino acid region (G137V and G144R).

human sulfatases, including iduronate 2-sulfatase and glucosamine-6-sulfatase [16]. In addition to the three codon 146 mutations described here, a mutation within codon 144 (G144R) of the ASB hexapeptide has been previously reported [12]. Moreover, several other MPS VI mutations (including M142I) are nearby. The high degree of homology within this region and the fact that many of the mutations causing MPS VI, Hunter syndrome (MPS II) and metachromatic leukodystrophy occur in or near it, suggests that it plays a critical role in ASB and other sulfatase activities. For example, in a severe MPS II patient, a mutation in the iduronate-2-sulfatase gene has been identified in codon 135 (K135R), which is located within this conserved hexapeptide [17]. Furthermore, in metachromatic leukodystrophy, a point mutation (G122S) occurs within this region of the arylsulfatase A gene [18]. This glycine codon is homologous to the G144 codon in the ASB gene.

Thus, investigations into the molecular lesions underlying MPS VI and other sulfatase deficiency disorders has provided intriguing insights into this enzyme family. Future studies will include overexpression and purification of these enzymes for structural analysis and site-directed mutagenesis of the conserved regions for biochemical comparison. In addition, new mutations will continue to be identified, providing insights into the the structure and function of these enzymes and the molecular tools to diagnose patients with the deficiency disorders.

Acknowledgements

This work was supported by a research grant (DK 25759) from the National Institutes of Health. Cell lines from probands 1 and 2 were obtained using funds from a NICHD Mental Retardation Research Center Core Grant (HD 24061). C.M.S. was the recipient of a Raymond A. Byran IV Postdoctoral Fellowship from the National Mucopolysaccharidosis Society.

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