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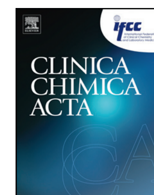

Molecular characteristics of patients with glycosaminoglycan storage disorders in Russia

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A B S T R A C T

Background: The mucopolysaccharidoses (MPSs) are rare genetic disorders caused by mutations in lysosomal enzymes involved in the degradation of glycosaminoglycans (GAGs). In this study, we analyzed a total of 48 patients including MPSI (n = 6), MPSII (n = 18), MPSIIIA (n = 11), MPSIVA (n = 3), and MPSVI (n = 10).
Methods: In MPS patients, urinary GAGs were colorimetrically assayed. Enzyme activity was quantified by colorimetric and fluorimetric assays. To find mutations, all IDUA, IDS, SGSH, GALNS, and ARSB exons and intronic flanks were sequenced. New mutations were functionally assessed by reconstructing mutant alleles with site-directed mutagenesis followed with expression of wild-type and mutant genetic variants in CHO cells, measuring enzymatic activity, and Western blot analysis of protein expression of normal and mutated enzymes in cell lysates.
Results: A total of five novel mutations were found including p.Asn348Lys (IDUA) in MPSI, p.Tyr240Cys (GALNS) in MPSIVA, and three ARSB mutations (p.Gln110*, p.Asn262Lysfs*14, and pArg315*) in MPSVI patients. In case of mutations p.Asn348Lys, p.Asn262Lysfs*14, and p.Gln110*, no mutant protein was detected while activity of the mutant protein was <1% of that of the normal enzyme. For p.Tyr240Cys, a trace of mutant protein was observed with a remnant activity of 3.6% of the wild-type GALNS activity. For pArg315*, a truncated 30-kDa protein that had 7.9% of activity of the normal ARSB was detected.
Conclusions: These data further enrich our knowledge of the genetic background of MPSs.

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1. Introduction

The mucopolysaccharidoses (MPSs) comprise a group of lysosomal storage diseases caused by deficiency of enzymes catalyzing the hydrolysis of glycosaminoglycans (GAGs) and characterized by intra-lysosomal deposits and increased excretion in urine of partially degraded GAGs that in turn results in ultimate dysfunction of organs and tissues across the whole body. GAGs (previously called mucopolysaccharides) are the products of lysosomal degradation of proteoglycans that are the components of the extracellular matrix. Depending on the catabolic pathway, four types of GAGs can be formed including chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate. The step-by-step degradation of GAGs involves a total of 10 enzymes four of which belong to the class of glycosidases, five are sulfatases, and finally one is a non-hydrolytic transferase [1].

Deficiencies of each one of these enzymes lead to seven different MPSs all of which share a series of clinical symptoms such as hepatosplenomegaly, facial dysmorphism, joints mobility, and frequent cardiovascular, visional, and neurological alterations [2]. The continued accumulation of non-degraded GAGs leads to the enlargement of lysosomes. Due to increase in the number of lysosomes, cells expand in their size, an event that causes organomegaly.

Usually, MPSs are recognized *via* analysis of urinary GAGs. However, it is not possible to distinguish between subtypes of MPSs that excrete similar types of GAGs. To perform a definitive analysis, an enzyme-specific assay should be done in patient's blood cells or skin fibroblasts

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in order to detect the enzymatic deficiency. Recently, a tandem mass spectrometry analysis of dried blood spot was introduced for enzymatic assays providing an opportunity for cheap and high-throughput screening of large population datasets [3].

In general, MPSs are autosomal recessive disorders except for MPS II that is X-linked. MPSI results from a deficiency of α-L-iduronidase (EC 3.2.1.76) and displays three major clinical phenotypes such as Hurler (MPS IH; MIM#607014), Hurler–Scheie (MPS IH/S; MIM#607015), and Scheie (MPS IS; MIM#6070016) syndromes. Hurler and Scheie syndromes represent the severe and mild clinical outcomes of MPS I respectively while Hurler–Scheie syndrome represents the intermediate phenotype [4]. The underlying enzymatic defect is common to all of them being caused by alterations in the gene that encodes α-L-iduronidase (*IDUA*) and is located on chromosome 4p16.3 [5]. This enzyme involves in hydrolysis of the terminal α-L-iduronic acid residues from both dermatan sulfate and heparan sulfate [6].

MPSII, also known as Hunter syndrome (MIM#309900), is caused by deficiency of iduronate sulfatase (EC 3.1.6.13), which is encoded by the *IDS* gene (chromosome Xq28) and catalyzes cleavage of sulfate from heparan and dermatan sulfate. Indeed, urine from patients with MPSII contains increased amounts of both these GAGs [7]. Mutations in the *IDS* gene lead to MPSII. In the vicinity to the *IDS* gene (20 kb telomeric), a non-functional pseudogene (*IDS2*; symbol approved by HUGO is *IDSP1*) was identified [8]. The pseudogene contains sequences that are highly identical to exons 2 and 3 and introns 2, 3, and 7 of the *IDS* gene [9]. In up to 20% of MPSII patients, the *IDS2* gene is responsible for genomic rearrangements that involve the *IDS* locus [10].

MPSIII (Sanfilippo syndrome) comprises four diseases caused by mutations in enzymes involved in degradation of heparan sulfate. There are heparan N-sulfatase (MPSIII type A; MIM#252900), α-N-acetylglucosaminidase (MPSIII type B; MIM#252920), acetyl CoA: α-glucosaminide acetyltransferase (MPSIII type C; MIM#252930), and N-acetylglucosamine-6-sulfatase (MPSIII type D; MIM#252940). Clinically, all MPSIII subtypes are characterized by neurological complications that are the most severe in MPSIII type A [11]. MPSIIIA results from by mutations in the gene encoding N-sulfoglucosamine sulfohydrolase, or heparan sulfate sulfatase (*SGSH*; chromosome 17q25.3; EC. 3.10.1.1.1). This enzyme is responsible for removal of N-linked sulfate groups of glucosamine. Sanfilippo syndrome subtype B is caused by defects in the *NAGLU* gene (chromosome 17q21) encoding α-N-acetylglucosaminidase (EC 3.2.1.50). This enzyme catalyzes cleavage of the N-acetylglucosamine residues from the heparan sulfate molecule [12]. The subtype C of MPSIII develops as a consequence of mutations in the *HGSNAT* gene (chromosome 8p11.1) encoding heparan acetyl-CoA: α-glucosaminide N-acetyltransferase (EC 2.3.1.78). This enzyme is involved in acetylation of the glucosamine amino groups that have become exposed after the catalytic action of heparan-N-sulfatase [13]. Finally, type D of MPSIII is caused by deficiency in N-acetylglucosamine-6-sulfatase (EC 3.1.6.14) encoded by the *GNS* gene on chromosome 12q14. This enzyme desulfates 6-sulfated N-acetylglucosamine residues of heparan sulfate as well as of a free monosaccharide [14].

MPS IV, or Morquio's syndrome, is caused by deficiency in two enzymes involved in the degradation of keratan sulfate. The type A of MPSIV (MIM#253000) results from mutations in the *GALNS* gene that is mapped to chromosome 16q24.3 and encodes galactosamine-6-sulfate sulfatase (EC 3.1.6.4), an enzyme that participates in degradation of both chondroitin sulfate and keratan sulfate [15]. The type B of MPSIV (MIM#253010) is caused by alterations in the *GLB1* gene that encodes β-galactosidase and maps to chromosome 3p21.33 [16]. β-galactosidase removes terminal β-linked galactose residues in GM1 ganglioside, glycoproteins, oligosaccharides, and in keratan sulfate.

MPSVI (Maroteaux–Lamy syndrome; MIM#253200) results from deficiency of arylsulfatase B. In lysosome, this enzyme catalyzes removal of C4 sulfate ester group from the N-acetylgalactosamine sugar residue at the non-reducing terminus of dermatan sulfate and chondroitin sulfate [17]. Arylsulfatase B (EC 3.1.6.12) is encoded by the *ARSB* gene (chromosome 5p11-q13) whose mutations lead to MPSVI.

We did not consider other MPS types since we have no patients affected with MPSVII and MPSIX. MPSVII (MIM#253220) caused by deficiency in β-glucuronidase is an extremely rare disease worldwide [18] and, to date, no MPSVII cases found in Russia. According to Krasnopolskaya et al. [19], a total prevalence of lysosomal storage diseases in Moscow region has been estimated to be 1:15,000. In this study, we present data involving genetic analysis of a total of 48 Russian children affected with MPSI, MPSII, MPSIII, MPSIV, and MPSVI. The data presented in this work represent the largest-ever dataset of Russian children genetically analyzed for presence of mutations causing glycosaminoglycan storage disorders.

2. Patients and methods

2.1. Patients

This study was performed according to the ethical principles of the Declaration of Helsinki. The study protocol was approved by the local Institutional Review Ethics Committee. Informed consents were obtained from the parents of all patients. A total of 48 MPS patients (30 males, 18 females) from 43 families were enrolled in the Research Center of Children's Health. Those include 6 MPSI children (2 males, 4 females), 18 MPSII patients (all males), 11 MPSIII patients (5 males, 6 females), 3 MPSIV children (all males), and 10 MPSVI patients (1 male, 9 females). There were 4 MPSVI probands (all females) belonging to two different families, 2 MPSII probands (all males), and 4 MPSIII probands (3 males and female) belonging to two different families while the remaining 38 patients were unrelated.

2.2. Biochemical measurements

The diagnosis was made by quantification of urinary GAGs and subsequently by demonstrating a deficient activity of an appropriate enzyme involved in degradation of GAGs in patients' leukocytes. Multiple sulfatase deficiency was excluded by detection of normal activity of other lysosomal sulfatases. The total GAG concentration in urine samples of MPS patients was measured by a colorimetric assay by measuring absorbance of a complex between GAG and 1,9-dimethylmethylene blue (DMB) [20]. Urinary creatinine concentrations were also measured separately in order to normalize urinary GAG concentrations (mg GAG/mmol creatinine). Chondroitin sulfate (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard control in DMB binding measurements. For each sample, biochemical measurements were performed in triplicate.

Enzymatic activity of IDUA was assessed using a fluorimetric assay and 4-methylumbelliferyl α-L-iduronide (Glycosynth, Ltd., Cheshire, England) as the assay substrate as described previously [21]. IDS activity was quantified with help of the fluorimetric enzyme assay and 4-methylumbelliferyl-α-iduronate 2-sulfate (Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a substrate [22]. Activity of SGSH was quantified in a two-step protocol using a fluorogenic substrate 4-methylumbelliferyl-α-D-sulfoglucosamine (Moscerdam, Oegstgeest, the Netherlands) as per manufacturer's instruction [23]. NAGLU activity was determined using 4-methylumbelliferyl-N-acetyl-α-D-glucosaminide (Sigma-Aldrich) as a fluorescent substrate [24]. HGSNAT activity was detected with help of the fluorescent substrate 4-methylumbelliferyl β-D-glucosaminide (Moscerdam) as described earlier [25]. GNS activity was evaluated using 4-methylumbelliferyl-α-*N*-acetylglucosamine 6-sulfate (Moscerdam) as a substrate [26]. Catalytic activity of GALNS was tested with the fluorogenic substrate 4-methylumbelliferyl-β-D-galactopyranoside-6-sulfate (Moscerdam) [27]. We measured activity of GLB1 using the beta-galactosidase colorimetric kit (Clontech Laboratories, Mountain View, CA, USA) as recommended by the manufacturers' manual. To test the ARSB activity, we

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