Biochemical and molecular analysis of mucopolysaccharidoses in Turkey

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The mucopolysaccharidoses (MPSs) are a family of heritable disorders caused by deficiency of lysosomal enzymes needed to degrade glycosaminoglycans (GAGs). The undegraded or partially degraded GAGs are stored in lysosomes and/or excreted in urine. In our study, 118 patients seen over the past 20 years and suspected to have lysosomal storage disorders (LSDs) were subjected to clinical and biochemical analysis at Hacettepe University Children's Hospital. We analyzed urine and blood samples from 42 patients given a clinical MPS diagnosis. Using urine screening technique, we were able to show that 34 of the 42 patients had MPS condition. Further analysis of eight patients with normal urine MPS patterns revealed four patients as likely to have α-mannosidosis, fucosidosis, sialidosis, and aspartylglucosaminuria (one each). Four patients had normal oligosaccharide patterns. We were able to clearly identify 4 MPS I, 2 MPS II, 5 MPS IIIA, 8 MPS IIIB, 11 MPS IVA, 3 MPS VI, and 1 MPS IIIC patients. These results provided biochemical diagnosis for these 34 patients, and clearly show that Turkey has a higher incidence of MPS IVA, IIIB, and IIIA than of previously suspected MPS types. Molecular analysis of four MPS I patients revealed three polymorphisms which have been previously reported (A314, T388, and A461T). In MPS II patients, mutation analysis identified one previously detected (R172X) and one novel mutation (W109C).

Key words: mucopolysaccharidoses, lysosomal storage diseases, mutations, Turkish population.

Mucopolysaccharidoses (MPSs) are a large group of lysosomal storage disorders (LSDs) resulted from a deficiency in the enzymes required for the catalysis of the stepwise degradation of glycosaminoglycans (GAGs or mucopolysaccharide)[5]. For the degradation of mucopolysaccharides (or sulfated glycosaminoglycans-heparan, dermatan, keratan, and chondroitin sulfates), each step requires specific enzymes, the absence of which results in specific types of MPSs[5]. This group of diseases mainly follows an autosomal recessive inheritance pattern with a few exceptions, namely Fabry's disease and MPS type II, which are inherited as X-linked recessive[5,11].

The prevalence values for individual LSDs clearly define these cases as rare genetic disorders[9]. Their overall incidence is lower than 1:100,000[10-12]. In fact, there are some exceptions that show high incidence among some populations like Tay-Sachs and Gaucher's disease type I in Ashkenazi Jews and Salla disease in the Finnish populations[5].

The diagnosis and the differentiation of specific types among MPS can be made using urinary screening tests and molecular methods[14]. Early detection of MPSs would provide the option for prenatal diagnosis for many families carrying these disorders[20].

In our study, blood and urine samples from 42 clinically suspect patients were analyzed. For this purpose, biochemical and molecular methods were used in order to determine the specific types of MPSs.

Material and Methods

Patients: Over the past 20 years 118 patients from all over Turkey suspected to have LSD were

referred to İhsan Doğramacı Children's Hospital, Department of Pediatric Nutrition and Metabolism, Hacettepe University. Among these patients, 42 were clinically diagnosed with MPS. Blood and urine samples were collected from 38 and 42 patients, respectively.

Analysis of Urine Samples: All urine samples were analyzed first with semiquantitative Alcian blue procedure for mucopolysaccharides to detect the amount of MPS in urine at the laboratories of the Department of Chemical Pathology, Lysosomal Diseases Research Unit, Women's and Children's Hospital, North Adelaide, Australia. Then with high resolution electrophoresis, the migration patterns of MPS were detected. Each migration pattern is specific for each MPS type. Unfortunately, evaluation of the specific migration patterns with this technique only defects MPS type; further enzymatic analysis is required to identify subtypes.

Oligosaccharides Detection in Urine by Thin Layer Chromatography: Oligosaccharides excreted in urine at high levels can be detected by the thin layer chromatography technique[14].

Isolation of Lymphocytes and Analysis of Enzyme Activities: Specific radioactively labelled substrates were used to analyze the specific enzyme activities by high pressure liquid chromatography (HPLC). For this purpose, isolation of lymphocytes was done by Ficol-Hypaque method. The lymphocyte homogenates were prepared by freezing and thawing the cells 4-5 times. Protein determination in these cell homogenates was done according to Lowry et al[8]. For determination of enzyme activity, 50-100 μg of protein are needed.

Molecular Analysis of MPS I and MPS II Patients: In order to screen the mutations in α-L-iduronidase genes of MPS I and iduronate sulfatase genes of MPS II patients, single strand conformation polymorphism (SSCP) method was used. The fragments were then subjected to sequence analysis.

Allele Specific Oligonucleotide (ASO) Analysis: Biochemically diagnosed patients were first subjected to ASO analysis in order to screen the most common mutations as previously described[19].

Results

Over the past 20 years, 118 patients from different regions of Turkey suspected to have LSDs were referred to Hacettepe İhsan Doğramacı Children's Hospital, Department of Nutrition and Metabolism. Among these patients, 42 of them for whom blood and urine samples were available were evaluated for clinical, radiological, and simple biochemical findings. Skeletal deformities (25.4%) and developmental delay or mental retardation (23.7%) were two major presenting symptoms. Coarse faces with large nose and thick lips (87.3%) was noted in the majority of the patients on physical examination. In our study, once an MPS type was considered in a differential diagnosis, a laboratory evaluation was initiated in order to define the diagnosis biochemically. Two types of urinary screening tests were applied to look for the abnormal excretion of either mucopolysaccharides or oligosaccharides. Biochemical diagnosis of the MPS types was confirmed by measuring the specific enzyme activities. The urine screening test, enzyme activity, and the percentage of each MPS type results are shown in Table I. The MPS types of 42 patients according to urine screening tests and enzymatic activity analysis were detected. For all types, the enzyme activity range of the patients was far lower than the normal activity range, which was consistent with the clinical phenotypes. As a result of the urine glycosaminoglycan patterns, 11 patients were diagnosed as MPS IVA, 8 patients were MPS IIIB, 5 patients were MPS IIIA, 3 patients were MPS VI, 2 patients were MPS II, 4 patients were MPS I, and 1 patient was MPS IIIC. Four patients were found to have oligosaccharide patterns consistent with other lysosomal storage diseases (one each): α-mannosidosis, fucosidosis, sialidosis, and aspartylglucosaminuria. Oligosaccharidoses are also a group of LSDs characterized by the defects of glycoprotein degradation due to the deficiency of specific lysosomal enzymes[1]. The results in four patients were not consistent with either MPSs or oligosaccharidoses, and further analysis is needed to search for other types of LSDs.

Mutation screening was done for four MPS I and two MPS II patients. The results of the molecular analysis are shown in Table II. In MPS I patients, three previously reported polymorphisms were detected (A314, T388, and A361T)[18]. Mutation analysis of MPS II patients showed one previously reported (R172X) and one novel mutation (W109C)[7]. For the confirmation of the novel genetic defect as mutation, 100 alleles from unaffected individuals were tested and it was found that it is a disease-causing defect.

Table I. Enzyme Activity, Relative Frequency, and Urine Oligosaccharide Results of the MPS Patients

MPS type	Diagnosed patients	Deficient enzyme	Enzyme activity range (pmol/mg protein/min)	Normal enzyme activity (pmol/mg protein/min)	% of MPS
MPS I	4	α-L-Iduronidase	0.14-5.83	15-34	11.76
MPS II	2	Iduronate sulfatase	0.27-0.69	10.9-88	5.88
MPS IIIA	5	Sulfamidase	0.03-0.22	0.3-4.2	14.71
MPS IIIB	8	α-N-Acetylglucosaminidase	0.02-0.05	0.4-3.4	23.53
MPS IIIC	1	AcetylCoA: α-glucosaminidase-N-acetyl transferase			
MPS IVA	11	N-Acetylgalactosamine-6-sulfase	0.27-2.9	38.5-166	32.35
MPS VI	3	N-Acetylgalactosamine-4-sulfatase	0.04-0.08	5.8-18.9	8.82

Urine oligosaccharides results	
Diagnosed patients	Diagnosis
1	Fucosidosis
1	Sialidosis
1	Aspartylglucosaminidase
1	α-Mannosidosis
4	Normal*

* Normal for oligosaccharide pattern for MPSs but further analysis for other possible LSDs is needed.
MPS: mucopolysaccharidoses.

Table II. Results of the Polymorphism and Mutation Analysis of MPS I and MPS II Patients

Polymorphisms found in MPS I Patients		
Amino acid	Exon	Base change
A314	VII	GCG → GCC
T388	VIII	ACG → ACC
A361T	VIII	GCG → ACG
Mutations found in MPS II patients		
R172X	5	CGA → TGA
W109C*	3	TGG → TGC

* Novel mutation.

Discussion

Mucopolysaccharidoses are inherited diseases showing relevant clinical overlap with other LSDs, and they show a wide variety of clinical manifestations[1,3,11]. Differences in environmental and genetic backgrounds, in part, explain differences in the clinical phenotypes of the patients with the same disease genotype[6]. Due to the extreme phenotypic variability of many of these disorders, usually it is very difficult to discriminate between both types and subtypes without having detailed clinical, biochemical, and molecular data. As can be seen from Table I, enzyme activity ranges are lower than normal for all MPS types, and the enzyme results are consistent with the clinical phenotypes. However, it is very difficult to distinguish the clinical phenotype of the disease by evaluating only the enzyme activity, since there is a wide variety of heterogeneity among clinical phenotypes. Because of this variability, clinical, biochemical, and molecular findings together are very important in the differential diagnosis of MPS types. Analysis of undegraded and accumulated metabolites, demonstrating a profound deficiency of a specific enzyme, and finally the molecular analysis of the gene of interest are the basic approaches used to diagnose these diseases. The analysis of 42 patients' urine and blood samples using these approaches revealed that the most common MPS types in our population are MPS IVA and MPS IIIB, followed by MPS IIIA, MPS I, MPS VI, MPS II, and MPS IIIC, respectively. In their study, Ozand et al.[13] found the most common type in Saudi Arabia to be MPS IVA. It has been shown that the most common MPS types in the Russian population were types II, I, IIIA, and IVA[6]. Krasnopolskaya et al.[6] analyzed 363 patients, and 241 were found to have MPSs. Another research from Greece showed the MPS IIIB type as the most common among the Greek population[10].

Of the 42 clinical and biochemical diagnoses, there were two individuals who had one affected sibling each who were MPS IIIB, and MPS IVA, respectively. In our population, the consanguinity rate is very high (75%), and 37.5% of families have a history of MPS condition. Because of the high consanguinity rate in the population,

heterozygote screening is very important. This will help affected families in genetic counselling, eventually leading to a prenatal diagnosis in pregnancies at risk.

The molecular analysis of the α-L-iduronidase gene of four MPS I patients was done by SSCP/ sequence analysis. The MPS type I has a broad spectrum of clinical presentations, which are severe Hurler syndrome and the milder Scheie syndrome[5,18]. It has been difficult to predict the severity of a disease using only the biochemical techniques[18]. Before analyzing patients with SSCP, screening for common mutations in the iduronidase gene (W402X and Q70X for Hurler; R89Q and 678 7g→a for Scheie) was done using ASO method[18]. None of these mutations was detected in our patients. As a result of the molecular analysis of the four MPS I patients, three polymporphisms were detected in the α-L-iduronidase gene. In three patients, polymorphisms were detected in exons 7 and 8. Two of the polymorphisms in these exons were A314 and T388, respectively. They show no association with any specific mutations, but it is known that these polymorphisms seriously affect enzyme activity. The other polymorphism in exon 8 leads to the change of amino acid alanine[361] to treonine. This polymorphism is uniquely found in association with the R89Q mutation, and potentiates its effect, thus altering the clinical phenotype from mild to intermediate[18]. In our patient, we could not detect this mutation-polymorphism association, and the clinical phenotype was at the milder spectrum of the disease. In one patient with MPS type I we could not detect mutation by SSCP analysis; sequencing was required. In MPS II patients, molecular analysis revealed one previously reported (R172X) and one novel (W109C) mutation[7]. MPS II (Hunter) is an X-linked recessive disorder caused by various lesions in the iduronate sulfatase gene. Two clinical extremes of MPS II, mild and severe, have been recognized[5,7]. In general, the severe form has early onset at two to four years. In contrast, mildly affected patients preserve normal intelligence and survive into late adulthood. Our MPS II patients were both severely affected. The previously detected R172X mutation is the cause of the clinical pathology. It creates a stop codon at arginine residue position 172 and causes a truncated protein, which results in the defective activity. The other mutation is W109C, which causes the change of amino acid tryptophan to cysteine. This change is a nonconservative change of an aromatic tryptophan to polar cysteine and might have a serious effect on the activity of the enzyme (0.31 pmol/mg protein/min) (normal range in Table I). However, since our patient was mildly affected, the residual activity of the mutant enzyme keeps the patient in the mild spectrum of the disease. Since this mutation was not reported previously, the exact effect of the change on enzyme structure and expression remains to be analyzed.

The molecular genetic analysis of each MPS type remains to be done in the near future. Molecular analysis will reveal the exact mutations of the each type and the effects of these mutations on the activity and expression of each enzyme. Molecular genetic analysis of MPS IIIB, IIIA, I, and IVA is under investigation. In MPS IIIA and IIIB patients, novel mutations are found[3,4].

In this study, both biochemical and molecular defects in MPS types were revealed. The definition of more complete genotypes, and more detailed clinical descriptions of the patients will greatly assist the development of molecular analysis for MPS types and the establishment of genotype phenotype relationships. Recently, exciting progress has been made in the treatment of MPSs based on gene therapy, bone marrow transplantation, and enzyme replacement therapies. In this respect, as a result of biochemical and molecular diagnosis, any suspect patient is a potential candidate for new therapeutic strategies and deserves a precise diagnosis. On the other hand, molecular information regarding these families at risk would provide the option for prenatal diagnosis for many more families carrying these disorders.

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