Evaluation and identification of IDUA gene mutations in Turkish patients with mucopolysaccharidosis type I

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1. Introduction
Mucopolysaccharidosis type I (MPS type I) is a rare autosomal recessive disorder resulting from deficiency of α-L-iduronidase (IDUA, EC 3.2.1.76), an important lysosomal enzyme in the catabolism of dermatan sulfate and heparan sulfate. Patients with MPS type I are unable to degrade dermatan and heparan sulfates, resulting in the progressive storage of glycosaminoglycans within the lysosomes.

MPS type I is classified according to the enzymatic activity of IDUA, while affected individuals are further classified into three main groups: Hurler syndrome (MPS I-H; OMIM# 607014), Hurler–Scheie syndrome (MPS I-HS; OMIM# 607015), and Scheie syndrome (MPS I-S; OMIM# 607016) (1). MPS type I occurs in approximately 1/100,000 people worldwide. MPS I-H is the most common, while MPS I-S is a rare form (2,3).

MPS type I is a progressive multisystemic disorder with a wide range of clinical manifestations. These include coarse facial features, hepatosplenomegaly, dysostosis multiplex, severe arthropathy, visual impairment, hearing loss, restrictive lung disease, upper airway obstruction, valvular heart disease, communicating hydrocephalus, mental retardation, and spinal cord compression. The phenotype is usually characterized as Hurler syndrome when the onset of the symptoms occurs before 12 months of age. Survival is then estimated to be around 10 years and mental retardation manifests before the age of 3 years. For Hurler–Scheie syndrome, the onset of the disease is between 1 and 6 years, survival is variable, and mental retardation is absent or mild, but is not present before 3 years of age. Scheie syndrome manifests itself after 5 years of age, survival is normal, and mental retardation is absent (4).

MPS type I is diagnosed by measuring IDUA enzyme activity in urine, leukocytes, and cultured skin fibroblasts. Hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT) can effectively treat
MPS I subtypes. ERT may be used to treat Hurler–Scheie
and Scheie syndromes, while the application of both ERT
and HSCT is pivotal to successful treatment of Hurler
syndrome (5–7). The IDUA gene is approximately 19 kb
in length. It maps to chromosome 4p16.3 and contains 14
exons, producing a transcript of 2.3 kb in length, which
encodes a precursor protein consisting of 653 amino
acids (8). The first 27 amino acids of the protein represent
a signal peptide. To date, 199 different disease-causing
IDUA gene mutations have been reported (9) (http://www.
hgmd.org), with variable distribution across populations.
are the most common mutations worldwide. p.W402X
has a frequency of approximately 50% in northern Europe,
the United Kingdom, North America (10–12), and
Spain (13). However, it appears less frequently in Russia
(4%) (14), Italy (11%) (4,15), and Brazil (20%) (16). The
frequency of p.Q70X is 50% in Russia and Scandinavia; it
appears less often in other countries (14,17). p.G51D is a
common mutation in Italy, with a frequency of 13% (4).
It was reported that p.P533R originated in North Africa
and is common in Mediterranean countries, appearing at
a frequency of 13% in Italy and 10% in Spain (4,14,18).
These findings clearly demonstrate that the distribution
of IDUA gene mutations is varied among populations.
The main purpose of the present study was to identify
IDUA gene mutations in Turkish patients morphologically
(phenotypic) diagnosed with MPS type I and to discuss the
possible effects of detected mutations on IDUA enzyme
function.

2. Materials and methods
The study’s participants were patients from Hacettepe
University, from within the Faculty of Medicine, Metabolism
Unit of the Department of Pediatrics. Approval was obtained
from an ethical review board and the principles outlined in the Declaration of Helsinki
regarding human experimental investigations were
followed. In total, 15 patients were screened for mutations
in the IDUA gene.

Genomic DNA was isolated from peripheral blood
samples (10 mL) using a standard salting-out method.
All IDUA exons were amplified using intronic primers
flanking the exonic nucleotide sequences. Standard
and touchdown PCR analyses were performed in a total volume
of 25 μL (1X PCR buffer, 1.5 mM MgCl₂, 200 μM of each
dNTPs, 50 pmol of each primer, and 0.5 U HotStartTaq
DNA polymerase), using 50 ng of genomic DNA. PCR
products were purified with MinElute 96 UF plate systems
(Qiagen, Hilden, Germany). Samples were sequenced in
both directions using the BigDye Terminator Kit version
3.1 (Applied Biosystems, Foster City, CA, USA) and were
analyzed in an automated DNA sequencer (ABI 3130,
Applied Biosystems). Analysis Software version 5.2 Patch
2 (Applied Biosystems) was used for data evaluation.

One hundred healthy chromosomes were screened
for novel substitutions via DNA sequencing, while
computational programs (ASSEDA and NetGene2) were
used to predict the putative effect of novel mutations.

3. Results
In this study, 15 Turkish patients with MPS type I were
screened for IDUA gene mutations using direct DNA
sequencing. Ten of the screened patients were carriers of
nine different mutations. These included two novel (c.793-
6C>G, c.793-5C>A) splice site substitutions, 7 already-
reported missense (p.M1L, p.A327P, p.P533L), nonsense
(p.Y64X, p.W402X, p.R628X), and splice site (c.494-
1G>A) mutations (Table).

Using the aforementioned programs, we were able to
predict the impact of novel mutations on protein function.
The results indicate that both mutations affect a splice
mechanism between intron 6 and exon 7. Neither novel
mutation was observed in the 100 healthy chromosomes.

Nine of the screened patients were carriers of
heterozygous IDUA gene mutations. Compound
heterozygosity was observed in the other patients.

The c.494-1G>A splice mutation seems common and
was detected in three out of the 10 patients. Additionally,
rare p.Y64X and p.P533L mutations could be detected in
the screened cohort.

DNA sequencing analysis revealed 7 different
previously described polymorphisms (p.A8A, p.A20A,
We were unable to find IDUA gene mutations in the 5
patients diagnosed with MPS-I.

4. Discussion
Screening genetic variations that exhibit variable allelic
distribution across populations facilitates the assessment
of the genetic profile of populations and the development
of appropriate medical strategies to prevent severe
symptoms. Due to the limited sample size in studies of
genetic disorders, the results obtained from many different
studies provide knowledge regarding the disease-associated
mutation spectrum for a given population. In this study,
15 Turkish patients were screened and 9 different IDUA
gene mutations were determined. The mutation spectrum
in the screened cohort was heterogeneous. We could
identify novel c.793-5C>A and c.793-6C>G substitutions,
which have not previously been reported in the Turkish
population. Furthermore, p.A327P and the common
p.W402X mutation was described in the screened cohort,
along with the c.494-1G>A mutation, which was already
indicated as specific to the Turkish population.
Both of the novel mutations were detected in a homozygous state in one of the screened patients manifesting the Hurler–Scheie phenotype. Segregation analysis revealed that healthy family members were carriers of the same variation in a heterozygous fashion. Computational analysis, which was used to predict pathogenicity of these variations, indicated improper splicing between intron 6 and exon 7.

One of the screened patients had a homozygous p.M1L mutation, which causes a skipping of 133 amino acids and leads to a synthesis of an inactive, truncated enzyme (19). Consequently, the next methionine serves as the start codon and the produced enzyme loses the amino acids encoded by exons 1, 2, and 3. In addition, p.M1L leads to a loss of the signal peptide sequence of the protein, which results in the disruption of the transport process into the endoplasmic reticulum and lysosomes (19). This mutation has already been reported in Chinese and Spanish (19,20) patients.

The p.Y64X mutation is known as a rare alteration that generates a premature stop codon, which truncates the protein by 589 amino acids. It has already been reported in the Israeli Arab community (21). We found this mutation to be a compound heterozygous state with p.A327P. Variable frequency was observed for the p.A327P across different populations. It was reported at a frequency of 11% for Central Europe (German and Dutch) (11), 3.5%–6% for England (11,12), 5.6% for Italy (15), 2.2% for Austria (22), and 3.3%–8.3% for Brazil (16,23). It is worth noting that the studies were performed mostly on European Caucasians and that the frequency of this mutation was 3.5% for Turkish MPS-I patients (24). Proline is an amino acid that causes loss of flexibility with its angular bond in the site of localization. Therefore, it may disrupt regular secondary structures (25). A327 is localized in the interior face of the 6th α-helices of the protein. Proline at this site likely affects conformation of the protein and may decrease its stability as well (25).

Two of the screened patients had the homozygous p.W402X mutation, which was identified as a common variation with 45.3% allele frequency in Turkish MPS-I patients (24). The produced enzyme is inactive and lacks 402 amino acids. Studies reported an association between the homozygous version of this mutation and severe Hurler syndrome (12,14). Another mutation associated with a severe phenotype in a homozygous fashion is p.R628X, which was observed in one of the patients. This mutation is localized in exon 14, quite near to the C terminal part of the IDUA protein. A nonsense mutation at this site truncates the C terminus and causes a severe MPS-I phenotype (12,23,26,27).

We observed homozygous c.494-1G>A splice site mutations in 3 patients. Interestingly, this mutation was reported as being specific to Turkey (allele frequency,

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Nucleotide position</th>
<th>Protein</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.1A&gt;C</td>
<td>p.M1L(hom)</td>
<td>Hurler</td>
</tr>
<tr>
<td>2</td>
<td>c.192C&gt;A/ c.1067G&gt;C</td>
<td>p.Y64X/p.A327P</td>
<td>Hurler</td>
</tr>
<tr>
<td>3</td>
<td>*c.494-1G&gt;A</td>
<td>p.R166GfsX27(hom)</td>
<td>Hurler</td>
</tr>
<tr>
<td>4</td>
<td>*c.494-1G&gt;A</td>
<td>p.R166GfsX27(hom)</td>
<td>Hurler</td>
</tr>
<tr>
<td>5</td>
<td>*c.494-1G&gt;A</td>
<td>p.R166GfsX27(hom)</td>
<td>Hurler</td>
</tr>
<tr>
<td>6</td>
<td>*c.793-5C&gt;A(hom)/ *c.793-6C&gt;G(hom)</td>
<td>Splice site</td>
<td>Hurler/Scheie</td>
</tr>
<tr>
<td>7</td>
<td>c.1205G&gt;A</td>
<td>p.W402X(hom)</td>
<td>Hurler</td>
</tr>
<tr>
<td>8</td>
<td>c.1205G&gt;A</td>
<td>p.W402X(hom)</td>
<td>Hurler</td>
</tr>
<tr>
<td>9</td>
<td>c.1598C&gt;T</td>
<td>p.P533L(hom)</td>
<td>Hurler/Scheie</td>
</tr>
<tr>
<td>10</td>
<td>c.1882C&gt;T</td>
<td>p.R628X(hom)</td>
<td>Hurler</td>
</tr>
</tbody>
</table>

The numbering of nucleotide changes is based on cDNA sequence in accordance with the GenBank entries NM_ 000203.3. The amino acid numbers were designed according to the ENST 00000247933. hom: homozygous.

*Intronic mutations.
41%) (20). The mutation occurs in the last nucleotide of intron 4. It causes a frameshift starting from position 166. Following the 27 amino acids, a stop codon is generated. Truncation of the protein by 193 amino acids leads to synthesis of an inactive IDUA enzyme, causing severe expression of the Hurler phenotype (20).

p.P533L is a rare mutation reported only in Russian patients as a compound heterozygous state (14). We detected the same mutation in one of the screened patients in a homozygous fashion. This mutation occurred in the CpG-rich site of the gene and corresponds to the functional importance of the C terminal part of the protein (14). It was reported that mutations occurring in this site, including p.P533R, are associated with severe disease phenotypes (12,23,28–30). The results of protein alignment indicate a similarity between the C terminal part of the IDUA enzyme and the fibronectin III protein, which is important in protein–protein interaction (25,26). The replacement of P533 (such as leucine or arginine residues) causes a steric conflict to form severe or severe-intermediate phenotypes (31).

In addition to the pathogenic mutations, we described 7 known polymorphic variants (p.A8A, p.A20A, p.H33Q, p.R105Q, IVS5-8C>T, p.A314A, p.T410T) in this cohort. Up until now, 37 different nonpathogenic variations have been described (20). No clear information is available regarding their effect on enzyme activity or stability in MPS-I patients. However, it is known that polymorphic variants may contribute to variable IDUA activity in healthy individuals (32) and may be a cause of the phenotypic variability of the disease, in combination with different pathogenic mutations.

Consequently, including novels, we described six mutations that have not been previously reported in the Turkish population. The results of the study indicate the heterogeneous mutation spectrum in this population. Molecular characterization of the IDUA gene facilitates a reliable diagnosis of clinical subtypes, which improves prognostic prediction, provides accurate carrier detection, and contributes to the development of better therapeutic approaches.

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References


