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

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Background/aim: This study aimed to identify *IDUA* gene mutations in Turkish patients morphologically (phenotypic) diagnosed with MPS type I. It also sought to discuss the possible effects of detected mutations on alpha-L-iduronidase enzyme function based on current knowledge.

Materials and methods: Genetic analysis was carried out in 15 patients using direct DNA sequencing. Moreover, segregation analysis was performed among family members to predict the pathogenic effect of novel mutations, and computational programs were used to predict their functional impact.

Results: Nine different mutations (c.494-1G>A, c.793-6C>G, c.793-5C>A, p.M1L, p.Y64X, p.A327P, p.W402X, p.P533L, and p.R628X) were identified. Computational analysis results supported the pathogenicity of novel mutations, suggesting improper splicing. Seven already-known polymorphisms were detected in the screened cohort as well.

Conclusion: Our results revealed heterogeneity in the mutation spectrum of Turkish patients. Six of the mutations, including the novel ones, have never before been reported in the Turkish population. Moreover, 5 patients who were phenotypically diagnosed with MPS type I could not be confirmed by genetic analysis, indicating the importance of the molecular characterization of MPS subtypes.

Key words: IDUA gene, mutation analysis, MPS type I, alpha-L-iduronidase

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

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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. Among them, p.W402X, p.Q70X, p.P533R, and p.G51D 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 $MgCl_2$, 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 homozygous *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, p.H33Q, p.R105Q, p.A314A, p.T410T and IVS5-8C > T). 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, in addition to p.M1L, p.Y64X, p.P533L, and p.R628X, 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.

Table. Mutation patterns for the *IDUA* gene in Turkish patients with MPS type I.

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

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.

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,

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

1. Wang RY, Bodamer OA, Watson MS, Wilcox WR. Lysosomal storage diseases: Diagnostic confirmation and management of presymptomatic individuals. Genet Med 2011; 13: 458–478.
2. Baehner F, Schmiedeskamp C, Krummenauer F, Miebach E, Bajbouj M, Whybra C, Kohlschütter A, Kampmann C, Beck M. Cumulative incidence rates of the mucopolysaccharidoses in Germany. J Inherit Metab Dis 2005; 28: 1011–1017.
3. Murphy A, Flanagan O, Dunne K, Lynch S. High incidence of Cohen syndrome among Irish travelers. Clin Dysmorphol 2007; 16: 257–259.
4. Venturi N, Rovelli A, Parini R, Menni F, Brambillasca F, Bertagnolio F, Uziel G, Gatti R, Filocamo M, Donati MA et al. Molecular analysis of 30 mucopolysaccharidosis type I patients: evaluation of the mutational spectrum in Italian population and identification of 13 novel mutations. Hum Mutat 2002; 20: 231.
5. Cox-Brinkman J, Boelens JJ, Wraith JE, O'Meara A, Veys P, Wijburg FA, Wulffraat N, Wynn RF. Haematopoietic cell transplantation (HCT) in combination with enzyme replacement therapy (ERT) in patients with Hurler syndrome. Bone Marrow Transplant 2006; 38: 17–21.
6. Soni S, Hente M, Breslin N, Hersh J, Whitley C, Cheerva A, Bertolone S. Pre-stem cell transplantation enzyme replacement therapy in Hurler syndrome does not lead to significant antibody formation or delayed recovery of the endogenous enzyme post-transplant: a case report. Pediatr Transplant 2007; 11: 563–567.
7. Wraith JE, Scarpa M, Beck M, Bodamer OA, De Meirleir L, Guffon N, Meldgaard Lund A, Malm G, Van der Ploeg AT, Zeman J. Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy. Eur J Pediatr 2008; 167: 267–277.
8. Scott HS, Anson DS, Orsborn AM. Human α-L-Iduronidase: cDNA isolation and expression. Proc Natl Acad Sci USA 1991; 88: 9695–9699.
9. Stenson PD, Ball ED, Howells K, Phillips AD, Mort M, Cooper DN. The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalised genomics. Hum Genomics 2009; 4: 69–72.
10. Clarke LA, Nelson PV, Warrington CL, Morris CP, Hopwood JJ, Scott HS. Mutation analysis of 19 North American mucopolysaccharidosis type I patients: identification of two additional frequent mutations. Hum Mutat 1994; 3: 275–282.
11. Scott HS, Bunge S, Gal A, Clarke LA, Morris CP, Hopwood JJ. Molecular genetics of mucopolysaccharidosis type I: diagnostic, clinical, and biological implications. Hum Mutat 1995; 6: 288–302.

12. Beesley CE, Meaney CA, Greenland GA, Adams V, Vellodi A, Young EP, Winchester BG. Mutational analysis of 85 mucopolysaccharidosis type I families: frequency of known mutations, identification of 17 novel mutations and in vitro expression of missense mutations. Hum Genet 2001; 109: 503–511.

13. Gort L, Chabas A, Coll MJ. Analysis of five mutations in 20 mucopolysaccharidosis type 1 patients: high prevalence of the W402X mutation. Hum Mutat 1998; 11: 332–333.

14. Voskoboeva EY, Krasnopolskaya X, Mirenburg T, Weber B, Hopwood J. Molecular genetics of mucopolysaccharidosis type I: mutation analysis among the patients of the former Soviet Union. Mol Genet Metab 1998; 65: 174–180.

15. Gatti R, Di Natale P, Villani GR, Filocamo M, Muller V, Guo XH, Nelson PV, Scott HS, Hopwood JJ. Mutations among Italian mucopolysaccharidosis type I patients. J Inherit Metab Dis 1997; 20: 803–806.

16. Matte U, Leistner S, Lima L, Schwartz I, Giugliani R. Unique frequency of known mutations in Brazilian MPS I patients. Am J Med Genet 2000; 90: 108–109.

17. Bunge S, Kleijer WJ, Steglich C, Beck M, Zuther C, Morris CP, Schwinger E, Hopwood JJ, Scott HS, Gal A. Mucopolysaccharidosis type I: identification of 8 novel mutations and determination of the frequency of the two common alpha-L-iduronidase mutations (W402X and Q70X) among European patients. Hum Mol Genet 1994; 3: 861–866.

18. Alif N, Hess K, Straczek J, Sebbar S, Belahsen Y, Mouane N, Abkari A, Nabet P, Gelot MA. Mucopolysaccharidosis type I in Morocco: clinical features and genetic profile. Arch Pediatr 2000; 7: 597–604.

19. Lee-Chen GJ, Wang TR. Mucopolysaccharidosis type I: identification of novel mutations that cause Hurler/Scheie syndrome in Chinese families. J Med Genet 1997; 34: 939–941.

20. Bertola F, Filocamo M, Casati G, Mort M, Rosano C, Tylki-Szymanska A, Tüysüz B, Gabrielli O, Grossi S, Scarpa M et al. IDUA Mutational profiling of a cohort of 102 European patients with mucopolysaccharidosis type I: Identification and characterization of 35 novel α-L-iduronidase (IDUA) alleles. Hum Mutat 2011; 32: E2189–E2210.

21. Bach G, Moskowitz SM, Tieu PT, Matynia A, Neufeld EF. Molecular analysis of Hurler syndrome in Druze and Muslim Arab patients in Israel: multiple allelic mutations of the IDUA gene in a small geographic area. Am J Hum Genet 1993; 53: 330–338.

22. Kroepfl T, Milos I, Paul K, Plecko B, Paschke E. The frequency of common mutations among patients with mucopolysaccharidosis types I, II and IIIA diagnosed in Austria over the last 17 years. Clin Genet 2001; 60: 393–394.

23. Matte U, Yogalingam G, Brooks D, Leistner S, Schwartz I, Lima L, Norato DY, Brum JM, Beesley C, Winchester B et al. Identification and characterization of 13 new mutations in mucopolysaccharidosis type I patients. Mol Genet Metab 2003; 78: 37–43.

24. Barkar V, Yılmaz A, Ergün MA, Ezgü FS, Menevşe A, Hasanoğlu A. Molecular analysis of mucopolysaccharidosis type I in Turkish population. Turkiye Klinikleri J Pediatr Sci 2005; 1: 127–128 (article in Turkish with an abstract in English).

25. Rempel BP, Clarke LA, Withers SG. A homology model for human alpha-l-iduronidase: insights into human disease. Mol Genet Metab 2005; 85: 28–37.

26. Vazna A, Beesley C, Berna L, Stolnaja L, Myskova H, Bouckova M, Vlaskova H, Poupetova H, Zeman J, Magner M et al. Mucopolysaccharidosis type I in 21 Czech and Slovak patients: mutation analysis suggests a functional importance of C-terminus of the IDUA protein. Am J Med Genet 2009; 149: 965–974.

27. Chkioua L, Khedhiri S, Turkia HB, Tcheng R, Froissart R, Chahed H, Ferchichi S, Dridi MFB, Vianey-Saban C, Laradi S et al. Mucopolysaccharidosis type I: molecular characteristics of two novel alpha-L-iduronidase mutations in Tunisian patients. Diagn Pathol 2011; 6: 47.

28. Tieu PT, Bach G, Matynia A, Hwang M, Neufeld EF. Four novel mutations underlying mild or intermediate forms of alpha-L-iduronidase deficiency (MPS IS and MPS IH/S). Hum Mutat 1995; 6: 55–59.

29. Lee-Chen GJ, Lin SP, Tang YF, Chin YW. Mucopolysaccharidosis type I: characterization of novel mutations affecting alpha-L-iduronidase activity. Clin Genet 1999; 56: 66–70.

30. Lee-Chen GJ, Lin SP, Chen IS, Chang JH, Yang CW, Chin YW. Mucopolysaccharidosis type I: identification and characterization of mutations affecting alpha-L-iduronidase activity. J Formos Med Assoc 2002; 101: 425–428.

31. Saito S, Ohno K, Maita N, Sakuraba H. Structural and clinical implications of amino acid substitutions in α-L-iduronidase: Insight into the basis of mucopolysaccharidosis type I. Mol Genet Metab 2014; 111: 107–112.

32. Scott HS, Litjens T, Nelson PV, Thompson PR, Brooks DA, Hopwood JJ, Morris CP. Identification of mutations in the alpha-L-iduronidase gene (IDUA) that cause Hurler and Scheie syndromes. Am J Hum Genet 1993; 53: 973–986.