



Three novel α -L-iduronidase mutations in 10 unrelated Chinese mucopolysaccharidosis type I families

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Abstract

Mucopolysaccharidosis type I (MPS I) arises from a deficiency in the α -L-iduronidase (IDUA) enzyme. Although the clinical spectrum in MPS I patients is continuous, it was possible to recognize 3 phenotypes reflecting the severity of symptoms, viz., the Hurler, Scheie and Hurler/Scheie syndromes. In this study, 10 unrelated Chinese MPS I families (nine Hurler and one Hurler/Scheie) were investigated, and 16 mutant alleles were identified. Three novel mutations in *IDUA* genes, one missense p.R363H (c.1088G > A) and two splice-site mutations (c.1190-1G > A and c.792+1G > T), were found. Notably, 45% (nine out of 20) and 30% (six out of 20) of the mutant alleles in the 10 families studied were c.1190-1G > A and c.792+1G > T, respectively. The novel missense mutation p.R363H was transiently expressed in CHO cells, and showed retention of 2.3% IDUA activity. Neither p.W402X nor p.Q70X associated with the Hurler phenotype, or even p.R89Q associated with the Scheie phenotype, was found in this group. Finally, it was noted that the Chinese MPS I patients proved to be characterized with a unique set of *IDUA* gene mutations, not only entirely different from those encountered among Europeans and Americans, but also apparently not even the same as those found in other Asian countries.

Key words: mucopolysaccharidosis type I, α -L-iduronidase, mutation, polymorphism.

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Mucopolysaccharidosis type I (MPS I, McKusick 252800) is an autosomal recessive disease, caused by a deficiency in the lysosomal enzyme α -L-iduronidase (IDUA, E.C. 3.2.1.76). This leads to the accumulation of partially degraded mucopolysaccharides, thereby giving rise to certain clinical conditions, expressed notably by the Hurler (IH), Scheie (IS) and Hurler/Scheie (IH/S) syndromes (Hopwood and Morris, 1990). The incidence rate of MPS-IH is 1/100,000 to 1/150,000, thus making it, not only the most common among all the forms of MPS, but also the main one in Chinese MPS I patients, as a whole. IH is the most severe, with symptoms developing during the first year of life, as severe mental retardation, skeletal deformities, hepatosplenomegaly and corneal clouding. IS, on the other hand, besides developing at a later period in life, manifests as aortic valve disease, stiff joints, corneal clouding and mild visceral organic problems, albeit no mental retardation. IH/S patients present most of the skeletal problems

associated with IH, but with symptoms developing during the early to mid teens, associated with relatively little mental retardation (Schuchman and Desnick, 1988). It has been proposed that the variable severity of symptoms is an outcome of the different types of pathogenic mutations in the gene itself. Consequently, molecular biological analysis of MPS I exerts an important role in diagnosis. To date, more than 100 pathogenic mutations have been found in the *IDUA* gene (Human Gene Mutation Database, 2010-03-01), and the higher prevalence of several mutations in certain geographic locations, such as p.W402X and p.Q70X among Caucasian patients (Bunge *et al.*, 1994), has been demonstrated. Identification of the specific mutations in affected patients is useful both for prenatal diagnosis, as well as genotype-phenotype correlations, although the mutant type in Chinese mainland MPS-I patients is still unclear. Herein are described the results of mutation analysis of pathogenic mutations of the *IDUA* gene in a group of 10 Han Chinese MPS-I families, with a view to identifying the major mutations specific to China.

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The group of 10 MPS I families under study included nine Hurler and one Hurler/Scheie patients. Primary clini-

cal diagnosis was confirmed biochemically by the demonstration of increased excretion of urinary dermatan sulfate and heparan sulfate in the former, and IDUA activity in isolated leukocytes in the latter. Blood samples were collected, not only from the 10 patients, but also their respective parents. This study was approved by the Ethics Committee of the first Hospital of China Medical University, with full informed consent by the parents. The α -L-iduronidase enzyme activities in leukocytes of the patients were measured using the artificial substrate 4-methylumbelliferyl α -L-iduronide (Sigma). The data of enrolled patients are summarized in Table 1.

Genomic DNA was extracted from venous blood by using a modified version of the ammonium acetate salting-out method. The *IDUA* gene was amplified from genomic DNA covering the entire *IDUA* coding region and exon – intron boundaries. Primer sequences, amplicon length and PCR reaction temperature are listed in Table 2. PCR was carried out in a mixture containing 200 ng of DNA extracted from whole blood, 100 nM of each outside primer, 10 mM of Tris – HCl, pH 8.3, 1.5 mM of KCl, 2 mM of MgCl₂, 100 μ M of each deoxyribonucleotide, and 1 unit of LA *Taq* DNA polymerase (Takara), in a total reaction volume of 50 μ L. PCR conditions were as follows: denaturation at 95 °C, 3 min, followed by 35 cycles at 95 °C, 1 min, 62–68 °C, 1 min, 72 °C, 1 min, and a final elongation at 72 °C, 7 min. Each resulting PCR product was run on an 8% PAGE gel with a 100-bp marker for comparison. Restriction enzyme tests were designed to screen for p.W402X, p.Q70X (Clarke *et al.*, 1994) and p.R89Q (Yamagishi *et al.*, 1996) (Table 3). Negative, heterozygous and homozygous control samples were analyzed together with patient samples. All 14 exons of the *IDUA* gene were amplified and analyzed by means of single strand conformational polymorphism (SSCP) analysis. Electrophoresis was carried out overnight at 4 °C. Those samples

showing alterations were then sequenced. Mutations were confirmed by direct sequencing the reverse strand and restriction analysis.

The p.R363H mutation was introduced into wild-type *IDUA* cDNA which had been cloned into the pSP72 plasmid vector (Promega), by using the QuickChange site-directed mutagenesis kit (Stratagene), according to manufacturer's instructions. Clones containing the mutagenized *IDUA* cDNA constructs were sequenced to ensure that no changes other than the desired mutation had been introduced. Chinese Hamster Ovary (CHO) cells were transfected with either the wild-type or a mutant cDNA construct using lipofectamine (Invitrogen). For each experiment, 2 x 10⁵ cells were seeded 24 h prior to transfection. Each construct was transfected in triplicate using 0.4 μ g DNA and 5 μ L lipofectamine. Transfection complexes were removed after 6 h and replaced with full-growth medium. 24 h after transfection, efficiency was estimated by the number of cells expressing EGFP, as observed through an inverted fluorescent microscope. The cells were then washed twice with phosphate-buffered saline (PBS) and harvested in 1 mL of PBS, whereupon they were sonicated in 50 μ L of sterile water, centrifuged at 2000 rpm for 10 min, and then stored on ice. IDUA enzyme activity was measured with the artificial substrate 4-methylumbelliferyl α -L-iduronide. After incubation for 1 h at 37 °C, the reaction was terminated by way of 1.14 mL of 0.25 M glycine NaOH buffer, pH 10.4. The fluorescence of released 4-methylumbelliferone was measured with an excitation wavelength of 365 nm and an emission wavelength of 450 nm (Young, 1992).

The common mutations, p.W402X and p.Q70X, associated with the Hurler phenotype, and p.R89Q, with the Scheie phenotype, were not found by restriction digestion of PCR products amplified from exon 2 and 9 of the *IDUA* gene, in any of the ten families.

Table 1 - Characteristics of the 10 Han Chinese MPS I patients.

Patient number/sex	Phenotype	Leukocyte IDUA activity (nmol/h/mg protein)	Mutation identified	Major clinical manifestations
1/m	H	0	c.1190-1G > A/?	Severe mental retardation, corneal clouding, skeletal deformities, and hepatosplenomegaly
2/m	H	0	c.1190-1G > A/c.1190-1G > A	Same as above
3/m	H	0	c.1190-1G > A/ c.792+1G > T	Same as above
4/m	H	0	c.792+1G > T/ c.792+1G > T	Same as above
5/m	H	0	c.1190-1G > A/c.1190-1G > A	Same as above
6/m	H	0	c.792+1G > T/?	Same as above
7/m	H	0	c.1190-1G > A/?	Same as above
8/m	H	0	c.792+1G > T/?	Same as above
9/m	H	0	c.1190-1G > A/ c.792+1G > T	Same as above
10/F	H/S	0.18	c.1190-1G > A/ p.R363H	Hepatosplenomegaly, joint stiffness, corneal clouding, and slightly mental delay

As shown in Table 1, the novel mutations found included one p.R363H missense mutation (c.1088G > A) and two splice-site mutations (c.1190-1G > A and c.792+1G > T). None of these were detected in 50 normal persons after direct sequencing of the PCR-amplified fragment containing intron 6, exon 8 and intron 8 of the *IDUA* gene, respectively, thereby implying their not being polymorphisms, but in fact new mutations. The splice-site mutations c.1190-1G > A and c.792+1G > T were further analyzed at the transcript level. The deletion of five exonic nucleotides caused by c.1190-1G > A, resulted in the shift of the splice

acceptor site of intron 8 (r.1189_1195del1190_1194), presumably leading to a frameshift, which started at the 397th amino acid residue and ended at a premature stop codon, 110 amino acid residues downstream (p.D397GfsX110). The insertion of 17 intronic nucleotides caused by c.792+1G > T, resulted in a shift of the intron 6 splice-donor site (r.792_793ins792+1_792+17), presumably leading to a frameshift, which started at the 265th amino acid residue and ended at a premature stop codon, 58 amino acid residues downstream (p.G265LfsX58). Notably, 45% (nine out of 20) and 30% (six out of 20) of the mutant alleles in

Table 2 - PCR primer sequences, amplicon length and PCR reaction temperature.

Exon	Primer sequence	Amplicon length (bp)	Annealing temperature (°C)
1	F: 5'-ACCCAACCCCTCCAC-3'; R: 5'-GCTCCGGTCTCTGAAGCT-3'	398	64
2	F: 5'-GGCTTGAACGTGTGTGTCAGCCGC-3'; R: 5'-GTAAGGGGCTCTGGGACGCCAGA-3'	258	65
3	F: 5'-AGTCCTGCCTGGCTCCTGA-3'; R: 5'-GGCTGGGAGCAGAGCCCACA-3'	450	66
4	F: 5'-ACCTCTCCCTCACCCAG-3'; R: 5'-GCGTGATAGGGGTGCAAC-3'	312	64
5	F: 5'-CATCACCTTGCACCCTCC-3'; R: 5'-CGTCTACACCTGCCCTGG-3'	273	64
6	F: 5'-CCGCTCATCCCCAGGGCAGGTGTA-3'; R: 5'-ACAGCGGCTGAGGGCGCAGAACAC-3'	301	66
7	F: 5'-CGCGCTGACCCTGGTGGTGGTGA-3'; R: 5'-GCCGGGGGCGCGCAGGGCGTT-3'	254	68
8	F: 5'-TTCCTCCCGAGACGGGACAGGCCA-3'; R: 5'-TTCCTCCCGAGACGGGACAGGCCA-3'	350	67
9	F: 5'-TGGGGACTCCTTACCAAGGGGAG-3'; R: 5'-CAGAGCCCCAGCGGGGCCAGAGAC-3'	371	68
10	F: 5'-GGTGACCCTGCGGCTG-3'; R: 5'-TCCTCAGGGTTCTCCAGG-3'	421	61
11	F: 5'-GTGTGGGTGGGAGGTGGAGCGGTG-3'; R: 5'-AGGGAAGGCCTGTGATGGCGTCGG-3'	301	65
12	F: 5'-GCAGGCAAGTGGCAGTCCC-3'; R: 5'-GGCAAGTGGCCGAGTGAC-3'	253	68
13	F: 5'-GGGGCTTGAGGGAATGAG-3'; R: 5'-CCTGACCCAGGCTTCTC-3'	300	62
14	F: 5'-CAGGGCAGTACTGGGTGG-3'; R: 5'-TATATTGCAAAGGGGGTGATG-3'	331	62

F: forward primer; R: reverse primer.

Table 3 - Restriction enzyme tests for 3 known mutations in MPS I patients.

Phenotype	Mutation	Primers	Restriction enzyme	Fragment sizes (bp)
Hurler	p.Q70X (exon 2)	F: 5'-CGCTGCCAGCCATGCTGAGGCTCG-3'; R: 5'-ACACAGGGATGCTCACGGGTGACC-3'	- <i>Sau96I</i>	N:310+99+57+5 M:310+156+5
Hurler	p.W402X (exon 9)	F: 5'-TGGCGGGGCTGGGGACTCCTTACCTA-3'; R: 5'-GCGGGTGTCTGCTCGCTCGCTAGAT-3'	+ <i>BfaI</i>	N:26+248 M:26+110+138
Scheie	p.R89Q (exon 2)	F: 5'-CGCTGCCAGCCATGCTGAGGCTCG-3'; R: 5'-ACACAGGGATGCTCACGGGTGACC-3'	- <i>MspI</i>	N:312+159 M:471

All PCR reactions were carried out with 200 ng genomic DNA.

(N normal; M mutant; +/- gain or loss of restriction enzyme site respectively).

the Chinese MPS I families were c.1190-1G > A and c.792+1G > T, respectively. 4 previously reported polymorphisms, viz., p.R105Q, p.L118, p.A314 and p.A361T, were found. All sequence changes were confirmed by restriction enzyme testing on a second PCR product.

A mutant construct was generated, in order to determine whether the novel missense mutation p.R363H was disease-causing. CHO cells were transfected with either the wild-type or a mutant construct, using lipofectamine. The mutant construct reduced IDUA enzyme activity to 2.1 nmol/h/mg protein, whereas enzyme activity in CHO cells transfected with the wild-type was 90.8 nmol/h/mg protein. p.R363H expression showed retention of 2.3% IDUA activity, thereby implying that this novel mutation is, in fact, pathogenic.

MPS I is caused by mutations of the *IDUA* gene, these including missense and nonsense, splice-site, and insertion or deletion mutations. The Han Chinese ethnic group is the most populous in the world, whereby the significance of specifically identifying the molecular genetical background of MPS I patients in this assemblage. 80% (16 out of 20) of the alleles in the 10 Han Chinese MPS I families studied, were identified. The four unidentified ones are probably situated further within the intronic or promoter regions, not included in this study. The method employed could also be in arrears. The results of screening for known mutations showed that p.W402X and p.Q70X, the most frequent mutations in European patients, did not appear in Han Chinese MPS I patients. Moreover, p.R89Q, the most common mutant *IDUA* allele in Japanese MPS I patients, and other mutations, such as p.Y64X, p.Q310X, p.T366P (Bach *et al.*, 1993), p.P228Q, p.S534X, p.Y618X, c.193delT (Lee *et al.*, 2004) and p.E299X (Ketudat Cairns *et al.*, 2005) in Asian MPS I patients, were also not found in the Han Chinese.

The two missense mutations, p.Q60X and p.D203N, were previously described in a Chinese MPS IS patient (Dou *et al.*, 2007), and p.Q60X alone as a rare *IDUA* allele in MPS I patients from the UK (Beesley *et al.*, 2001), although p.D203N has, as yet, not been identified in patients elsewhere. Furthermore, eight rare *IDUA* alleles were found in MPS I patients from Chinese Taipei. These included two previously reported missense mutations, p.Y343X (Voskoboeva *et al.*, 1998) and p.A79V (Yogalingam *et al.*, 2004), as well as four missense mutations, p.T364M (Lee-Chen and Wang, 1997), p.R619G (Lee-Chen *et al.*, 1999), and p.L346R and p.Q584X (Lee-Chen *et al.*, 2002), one splice-site mutation c.388-3C > G (Teng *et al.*, 2000), and one deletion mutation c.46_57del unique for China. *IDUA* gene mutations identified in Chinese MPS I patients are listed in Table 4. A screening for the known mutation in 35 Chinese MPS I patients revealed that p.W402X, p.Q70X, p.P533R and p.R89Q were not present among 70 alleles of the *IDUA* gene (Yu *et al.*, 2001). Unfortunately, there is a lack of pertinent information regarding the patients' ethnic group in the literature.

Thus, it is shown that Chinese MPS I patients are characterized with a unique set of *IDUA* gene mutations, not only different from those encountered among Europeans and Americans, but also apparently diverse from those present in other Asian populations. Furthermore, p.W402X and p.Q70X were absent from Asian MPS I patients, thereby emphasizing their uniqueness.

Genotype-phenotype correlations have been reported, as, for example, the p.W402X and p.Q70X Hurler mutations. These have been shown to be particularly prevalent (72%) in north European populations, with a high proportion of severely affected patients. Nevertheless, several mutations, such as p.P533R, seemed to correlate to different phenotypes in Italian MPS-I patients (Gatti *et al.*, 1997),

Table 4 - Summary of *IDUA* mutations in Chinese MPS-I patients.

Mutation	Codon change	Location	Associated phenotype	Mutation type	Reference
c.46_57del	-	Exon 1	Hurler	Deletion mutation	Lee-Chen <i>et al.</i> 2002
p.Q60X	CAG-TAG	Exon 2	Scheie	Missense mutation	Dou <i>et al.</i> 2007
p.A79V	GCC-GTC	Exon 2	Hurler/ Scheie	Missense mutation	Lee-Chen <i>et al.</i> 2002
c.388-3C > G	-	Intron 2	Hurler	Splice mutation	Teng <i>et al.</i> 2000
p.D203N	GAT-AAT	Exon 6	Scheie	Missense mutation	Dou <i>et al.</i> 2007
c.792+1G > T*	-	Intron 6	Hurler	Splice mutation	This study
p.Y343X	TAC-TAG	Exon 8	Hurler/ Scheie	Nonsense mutation	Lee-Chen <i>et al.</i> 1997
p.L346R	CTG-CGG	Exon 8	Hurler/ Scheie	Missense mutation	Teng <i>et al.</i> 2000
p.R363H*	CGC-CAC	Exon 8	Hurler/ Scheie	Missense mutation	This study
p.T364M	ACG-ATG	Exon 8	Hurler/ Scheie	Missense mutation	Lee-Chen <i>et al.</i> 1997
c.1190-1G > A*	-	Intron 8	Hurler	Splice mutation	This study
p.Q584X	CAG-TAG	Exon 13	Hurler	Nonsense mutation	Lee-Chen <i>et al.</i> 2002
p.R619G	CGA-GGA	Exon 14	Hurler/ Scheie	Missense mutation	Lee-Chen <i>et al.</i> 1999

*Unique mutations in Han Chinese MPS I families.

while a patient homozygous for p.W402X, and presenting the less severe Scheie phenotype, has also been described (Pereira *et al.*, 2008). On the other hand, missense mutations distributed along the entire length of the gene may be associated with severe phenotypes (Vazna *et al.*, 2009).

Most of the patients were severely affected in the cases under study. The homozygote for both c.1190-1G > A and c.792+1G > T, as well as the heterozygote for c.1190-1G > A and c.792+1G > T, exhibited the Hurler syndrome. Thus, these two mutations might be associated with a severe phenotype, consistent with Terlato's idea that most splice-site mutations result in a severe phenotype, unless in combination with a less severe missense mutation (Terlato and Cox, 2003). c.1190-1G > A and c.792+1G > T were further analyzed at the transcript level, whereupon they proved to be acceptor and donor splice-site mutations, respectively, thus presumably leading to a frameshift. Moreover, these mutations were absent from 50 unrelated controls, thereby implying their probable pathogenicity.

The only Hurler/Scheie syndrome patient was the heterozygote for c.1190-1G > A/p.R363H. The novel p.R363H missense mutation was due to a G to C transversion in codon 363, thereby resulting in the substitution of a histidine by an arginine residue. It is generally accepted that less severe H/S syndrome patients are associated with a milder allele that produces some residual enzyme activity. Thus, as c.1190-1G > A homozygotes possess a severe phenotype, p.R363H can be most likely associated with a mild to intermediate one. p.R363H expression showed retention of 2.3% IDUA activity, thus in accordance with that predicted. The analogous mutation p.R363C (substitution of a cysteine by an arginine residue) has been previously reported (Yogalingam *et al.*, 2004), although no IDUA activity was detected in CHO p.R363C cells. This is possibly due to those conformational changes in IDUA taking place on the basic amino acid, being replaced by neutral cysteine, thereby leading to a loss in enzyme activity.

Identification of *IDUA* mutations in the 10 Chinese MPS I families permits accurate carrier detection and genetic counseling of at-risk relatives, and should facilitate molecular prenatal diagnosis in China. Moreover, thereby genetic clues for the diagnosis and treatment of overseas Chinese MPS I patients might be provided, due to their unique *IDUA* gene mutations. The available treatments for MPS I patients include hematopoietic stem cell transplants and enzyme replacement therapy. Genotype studies assist in the prediction of disease severity in MPS I patients, and could, together with the age of the patient at diagnosis, aid in taking a decision regarding treatment measures. Recently, a further more efficacious treatment for MPS I Hurler patients-hematopoietic stem-cell gene therapy, based on lentiviral vectors and the success with mouse models, completely corrects disease manifestations (Visigalli *et al.*, 2010). Our data provide additional evidence for

the future development of clinically testing this same therapy.

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Internet Resources

Human Gene Mutation Database, <http://www.hgmd.org>.

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