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Genetic analysis of 63 Chinese patients with mucopolysaccharidosis type II: Functional characterization of seven novel IDS variants

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ABSTRACT

Mucopolysaccharidosis type II (MPS II) is an X-linked recessive lysosomal storage disorder resulting from the deficiency of the enzyme iduronate-2-sulfatase (IDS).This study described the molecular characteristics of 63 Chinese children with MPS II and investigated functional characterization of seven novel IDS variants. We analyzed mutations in the IDS gene of 63 children with MPS II. Seven novel mutations were further characterized by transient expression studies. 49 different mutations were identified in the IDS gene including 33 previously reported and 16 novel mutations. The mutation p.R443X and c.1122C > T(p.G374G) may be link to attenuated type. The novel missense mutations were predicted damaging in silico. The bioinformatic structural analysis of the novel missense mutations showed that these amino acid replacements would cause a severe impairment of protein structure and function. In vitro functional analysis of the seven novel mutants, showing a very low IDS activity, clearly demonstrated their pathogenic nature. In western blotting analysis of the IDS protein, the examined mutations showed a similar or slightly lower molecular mass of precursor without mature forms being detected. Our study expands the spectrum of genotype of MPS II, provides new insights into the molecular mechanism of MPS II and helps to the future studies of genotype-phenotype correlations to estimate prognosis and develop new therapeutic approach.

1. Introduction

Mucopolysaccharidosis type II (MPS II; MIM 309900) or Hunter disease, is an X-linked recessive lysosomal storage disorder resulting from the deficiency of the enzyme iduronate-2-sulfatase (IDS) with an incidence of 0.30–0.71 per 100,000 live births [1]. IDS catalyses the stepwise degradation of glycosaminoglycans (GAGs), heparan sulfate (HS) and dermatan sulfate (DS) in lysosomes [2]. Its deficiency results in systemic accumulation of HS and DS. MPS II displays a wide range of clinical expressions including hepatosplenomegaly, short stature, dysostosis multiplex, inguinal hernia, coarse facial features, hearing difficulty, ophthalmic problems, respiratory defects, heart diseases, and occasional neurologic involvement [2]. This disease is generally categorized in two clinical subtypes according to neurological involvement and length of survival: severe and attenuated forms [3]. The severe form is the most frequent and characterized by an early onset of symptoms with progressive neurologic impairment [4,5]. The onset appears between 18 and 36 months and death usually occurs in the mid-teenage years due to a combination of neurological deterioration and cardiorespiratory failure [6,7]. The attenuated form is characterized by preservation of intelligence and survival into adulthood [8,9]. The onset appears between 4 and 8 years but death often occurs between the ages of 20 and 30 years from cardiac or respiratory disease [10].

Analysis of urinary GAGs (HS and DS) is the usual screening test for MPS II. Definitive diagnosis is confirmed by the measurement of IDS enzyme activity in leukocytes, fibroblasts or plasma. It should be noted that the amount of enzyme activity cannot be used to predict the severity of the phenotype [11].

The IDS gene contains 9 exons spread over 24 kb on chromosome Xq28 and encodes a polypeptide of 550 amino acids. A IDS pseudogene, termed IDS2, is located about 25 kb telomeric to the functional gene. Homologous regions shared by IDS and IDS2 include the sequence of exons 2 and 3 as well as introns 2, 3 and 7 leading to large complex genetic rearrangements [12–14]. The synthesized IDS requires post-

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translational modification converting the 76kDA precursor through intermediates into the 55 kDa and 45 kDa mature forms by removal of the signal sequence peptide, glycosylation, phosphorylation, proteolysis [15].

To date, about 600 different IDS gene mutations have been detected ((Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff: IDS Gene: http://www.hgmd.cf.ac.uk). Among these mutations, approximately half of them are missense/nonsense mutations; other mutations include splicing, small deletions, small insertions, small indels, gross deletions, gross insertions, complex rearrangements of the IDS gene.

In this study, we described the molecular characteristics of 63 Chinese patients with MPS II including 16 novel mutations and investigated functional characterization of seven novel IDS variants.

2. Materials and methods

2.1. Patients

A total of 63 patients form unrelated families with MPS II were included in this study. The diagnosis was confirmed by clinical manifestations and measurement of IDS activity at Guangzhou Women and Children's Medical Center from January 2008 to October 2017. As reported, patients who showed neurologic involvement were classified as the severe type, whereas patients whose mental status was normal were classified as the attenuated type. The neurologic involvement was assessed according to the DSM 5 (Diagnostic and Statistical Manual for Mental Disorders) criteria for developmental quotient (DQ of 50–70: mild intellectual disability; DQ 35–50: moderate intellectual disability; DQ of 20–35: severe intellectual disability and DQ < 20: profound intellectual disability) [8]. The patients with moderate to severe intellectual disability and or neurodegeneration were classified as severe type.

Informed consent was obtained from all patients' parents. The study was approved by the Institutional Review Board of Guangzhou Women and Children's Medical Center.

2.2. Biochemical measurements

All the 63 patients in this study had measurements of urinary GAGs concentration and IDS activity in the leukocytes. The concentration of urinary GAGs was measured using the Dimethylene Blue assay in relation to urinary creatinine [16]. The results were compared with the established reference ranges per age group of urinary GAGs per grams of creatinine [17].

The IDS activity was measured on peripheral blood leukocytes using fluorogenic substrate 4-methylumbelliferyl-alpha-iduronide-2-sulfate (4-MU-IDS, Moscerdam substrates, The Netherlands). The assays were performed according to the protocol at the Department of Endocrinology and Metabolism, Guangzhou Women and Children's Medical Center [18]. Normal IDS activity is within the range of 30.0–120.0 nmol/mg protein/4 h [18].

2.3. IDS mutational analysis

Genomic DNA was extracted from peripheral leukocytes of all individual patients using a standard procedure. All exons and exon-intron splice junctions were amplified by PCR (Mastercyclers Pro TM Gradient Thermal Cycler, Eppendorf, Hamberg, Germany). Nested PCR was specifically done for the amplification of exon 3 to avoid coamplification of a homologous region in the IDS pseudogene using two sets of primers (Exon 3 and Exon 3L). The primer sequences are listed in Table S1. PCR products were purified and sent to BGI (Beijing, China) for direct DNA sequence analysis (DNA Analyzer 3730, ABI, USA). Sequences were compared with the reference sequence (NM_000348) using Chromas software (V.2.01, Technelysium Pty Ltd., Tewantin QLD, Australia). The novel mutations in the study were determined by comparing the Human Gene Mutation Database (HGMD) and the National Center for Biotechnology Information (NCBI) database. Genetic variants were searched in the Single Nucleotide Polymorphism Database (dbSNP) and the 1000 Genomes Project. Intronic variants were analyzed with GenSCAN (http://genes.mit.edu/GENSCAN.html) to determine whether the consensus sequence of any splice site was altered. Novel mutations were verified by direct sequencing of the PCR products in 100 unrelated healthy controls and comparing the Exome Aggregation Consortium (ExAC) database along with the NHLBI exome variant database.

2.4. In silico analysis

To predict the effect of amino acid substitutions, we performed in silico analysis using the SIFT/PROVEAN (http://sift.jcvi.org) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2) web software. SIFT Score ranges from 0 to 1. The amino acid substitution is predicted as damaging if the score is ≤ 0.05, and tolerated if the score is > 0.05 (J. Craig Venter Institute, USA). The variant is predicted to be deleterious if the PROVEAN score is ≤ -2.5), and neutral if the score is > -2.5 (J. Craig Venter Institute, USA). Polyphen-2 prediction outcome can be one of “probably damaging”, “possibly damaging”, or “benign”.

2.5. Protein visualization and structural analysis

The effect of the missense mutations on the overall structure of the protein and on its activity was also investigated using the crystal structure of human IDS protein (PDBID 5FQL). Virtual models of IDS mutations and bioinformatics analysis was performed with the PyMOL (TM) Molecular Graphics System (Version 1.5.0.3.)

2.6. Plasmid construct

The wild-type IDS cDNA (GenBank: NC_000023.11) was linked to the enhanced green fluorescent protein (EGFP) reporter gene and cloned into the pcDNA3.1 plasmid (Invitrogen, Carlsbad, California). Seven mutations of IDS gene, including c.242A > G (p.Q81R), c.328A > G (p.R110G), c.457 T > C (p.W153R), c. 1047C > A (p.S349R), c.118_120delCTT (p.L40del), c.1404_1405ins9 (p.468-469ins3) and c.748_749delGCinsA (p.A250Tfsx28) were introduced in the wild-type cDNA fused to EGFP by a Quikchange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA). The primers used for site-directed mutagenesis were listed in Table S2.

2.7. Cell culture and transient transfection

293FT cells were grown in Dulbecco's modified Eagle's medium (Gibco BRL, Grand Island, NJ, USA) supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, NJ, USA), 100 U/mL penicillin and 100 U/mL streptomycin (Hyclone, Logan, UT, USA) at 37 °C in a humidified atmosphere enriched with 5% CO2. 293FT cells were transfected by pcDNA3.1-EGFP vector with wild-type IDS cDNA and seven mutants using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After 72 h transfection, cells were harvested for IDS enzyme assays and Western blotting. All transfections were performed in duplicate in three individual experiments.

2.8. IDS activity assay

The harvested 293FT cells were sonicated in ddH2O and then protein concentrations were determined using Micro BCA Protein Assay Kit (Pierce, Rockford, IL, USA). The enzyme activity assay was performed according to the protocol at the Department of Endocrinology and Metabolism, Guangzhou Women and Children's Medical Center [17].

2.9. Western blotting

The transfected cells were lysed by RIPA Lysis Buffer (Beyotime, Beijing, China) containing 1% PMSF (Beyotime, Beijing, China). Protein (30 μg) was loaded for electrophoresis on 10% SDS-PAGE gels and transferred to PVDF membranes. The membranes were then blocked with 5% non-fat milk in TBS, containing 0.1% Tween 20 for 1.5 h at room temperature, and then incubated with the respective primary antibody IDS and GAPDH (Multi Sciences, Hangzhou, China) at 4 °C overnight. Finally, membranes were incubated with the HRP secondary antibody (Multi Sciences, Hangzhou, China) for 2 h at room temperature. The signals were detected using the ECL western blotting detection reagent (Pierce, Rockford, IL, USA).

2.10. Statistical analysis

The difference between the wild-type enzyme activities and the individual mutant enzyme activities was analyzed by GraphPad Prism 5 (GraphPad Software Inc., CA, USA) using a Student's *t*-test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Clinical features

A total of 63 male patients were recruited from 61 unrelated families in the study. The ages at diagnosis ranged from 6 month to 15 years old. There were four patients in this study who belonged to two sets of families. The initial symptoms included growth retardation, short stature, speech delay or bone dysmorphology, as indicated by X-ray examination. According to the DSM 5 criteria for developmental quotient, the patients with DQ < 70 were classified as the severe type otherwise as the attenuated type. Among 63 patients, the severe type was the most common, accounting for 76.2% (48/63) of cases, while the attenuated type was seen in 23.8% (15/63) of cases.

3.2. Biochemical findings

The affected 63 patients had GAG between 17.2–206.2 g/mol creatinine beyond the average reference values for age. The enzyme activity analyzed in peripheral blood leukocytes from the 63 patients ranged from 0 to 0.1 nmol/mg protein/4 h.

3.3. IDS gene mutation analysis

Sequencing analysis of IDS gene identified 49 different mutations in 63 patients belong to 61 families (Table 1). Of the 49 mutations, 33 (67.3%) have been previously reported and 16 (32.7%) have never been described. Two patients were detected the deletion of exon 3 to exon 6 and exon 1 to exon 3 of the IDS gene respectively both of which have not been documented previously. One patient was detected the deletion of exon 4 to exon 7 of the IDS gene which has been reported previously [19]. Sixteen novel mutations were detected: seven frameshift mutations (p.T118KfsX22, p.P157PfsX5, p.L241FfsX15, p.A250TfsX28, p.P260PfsX77, p.E274EfsX4, p.V304GfsX12), three missense mutations (p.Q81R, p.R110G, p.W153R), two small deletions (p.L40del, p.G394_398delinsV), two gross deletions (EX3_6del, EX1_3del), one nonsense mutation (p.E166X) and one small insertion (p.468_469ins3).

A total of 49 different mutations included 20 (40.8%) missense, 9 (18.4%) frameshift, 6 (12.2%) nonsense, 5 (10.2%) splicing, 4 (8.2%) small deletion, 4 (8.2%) gross deletion mutations, 1 (2.0%) small insertion. While mutations were found in all exons except exon 1, they were concentrated in exon 9 (20.6%), exon 3 (15.9%) and exon 8 (12.7%). None of these mutations were detected in 100 alleles of unrelated healthy controls.

In the attenuated type of MPS II, we identified 8 mutations of the IDS gene in the 15 patients: 7 (46.7%) missense, 4 (26.7%) nonsense, 3 (20.0%) splicing, 1 (6.7%) small deletion mutations. In the severe type of MPS II, the IDS mutations were classified as follows among the 48 patients: 20 (41.7%) missense, 9 (18.8%) frameshift, 8 (16.7%) nonsense, 5 (10.4%) gross deletion (the deletion of exon 1 to exon 3, the deletion of exon 3 to exon 6, the deletion of exon 4 to exon 7 and the deletion of all the nine exons), 3 (6.3%) small deletion, 2 (4.2%) splicing, 1 (2.1%) small insertion. Although most mutations were unique or individualized, the mutation p.R172X and p.R443X occurred fourth; the mutations p.R468W occurred thrice; the mutations p.P86L, p.S142F, p.S333L, c.1122C > T(p.G374G), p.R468Q and EX1_9del occurred twice. These recurring mutations were all previously reported.

3.4. Protein function prediction of novel mutations

The missense mutations (p.Q81R, p.R110G, p.W153R and p.S349R) in the IDS gene were predicted damaging by the SIFT/PROVEAN and Polyphen-2 web software.

3.5. Bioinformatic structural analysis

To investigate the possible consequences of the missense mutations at protein level, we visualized the crystal structure of human IDS (Fig. 1).

p.Q81R: the structural analysis of the mutation predicted that the replacement of a neutral glutamine (Q) with the basic arginine (R), occurring in the proximity of a residue C84 known to be involved in post-translational modification as a key catalytic residue in the active site.

p.R110G: The R110 residue is adjacent to the active site residues and on the solvent-accessible surface of the enzyme. Substitution from the basic arginine (R) to the neutral glycine (G) at this position may cause loss of N-linked glycosylation.

p.W153R: The W153 residue is adjacent to the active site residues and makes structural interactions with the catalytic core. Substitution from the hydrophobic tryptophan to the hydrophilic arginine may influence the function of catalysis.

p.S349R: The S349 made the structure interaction with the K347 residue locating in the catalytic core. This change of the neutral serine (S) to the basic arginine (R) likely resulted in protein misfolding or catalytic inactivation.

3.6. Characterization of the novel sequence variants

To determine the impact of mutants on protein function, we characterized seven mutations: p.Q81R, p.R110G, p.W153R, p.L40del, p.468_469ins3, p.A250TfsX28 and p.S349R. Although the mutation p.S349R was no longer novel since it was reported during the preparation of the present study by cobos et al. [20], was also included in the analysis. Seven mutant vectors were constructed by site-direct mutagenesis in 293FT cells and the IDS activity in extracts of 293FT cells was detected. Using the same conditions, the wild-type, untransfected and 7 mutant proteins were analyzed for comparative purposes. The IDS activity in extracts of 293FT cells transfected with the wild-type IDS was 978.29 ± 68.10 nmol/4 h/mg and that of untransfected cells was 31.51 ± 6.23 nmol/4 h/mg. The seven mutants had very low IDS activity and none of them showed significant activity above the background (Table 2).

IDS protein was analyzed by western blotting (Fig. 2). The wild-type IDS showed a precursor band of 75–78 kDa and two mature bands of 55 kDa (major band) and 45 kDa (faint band). In the seven mutants, the precursor band was similar or lightly lower and the two mature bands were not detected.

Table 1
Summary of IDS mutations in 63 patients with MPS II and their corresponding phenotypes.

Patient	Category	Mutation	Consequence	Location	Phenotype	Status
	Missense					
1		c.212G > A	p.S71N	Exon2	Severe	Reported
2		c.242A > G	p.Q81R	Exon3	Severe	Novel
3		c.257C > T	p.P86L	Exon3	Severe	Reported
4		c.257C > T	p.P86L	Exon3	Severe	Reported
5		c.257C > G	p.P86R	Exon3	Severe	Reported
6		c.262C > T	p.R88C	Exon3	Attenuated	Reported
7		c.263G > C	p.R88H	Exon3	Severe	Reported
8		c.283C > G	p.R95G	Exon3	Severe	Reported
9		c.328A > G	p.R110G	Exon3	Severe	Novel
10		c.425C > T	p.S142F	Exon4	Attenuated	Reported
11		c.425C > T	p.S142F	Exon4	Attenuated	Reported
12		c.457 T > C	p.W153R	Exon4	Severe	Novel
13		c.692C > T	p.P231L	Exon5	Attenuated	Reported
14		c.781C > G	p.P261A	Exon6	Severe	Reported
15		c.998C > T	p.S333L	Exon7	Severe	Reported
16		c.998C > T	p.S333L	Exon7	Severe	Reported
17		c.1001A > G	p.D334G	Exon7	Severe	Reported
18		c.1025A > C	p.H342P	Exon8	Severe	Reported
19		c.1047C > A	p.S349R	Exon8	Severe	Reported
20		c.1048A > C	p.N350H	Exon8	Attenuated	Reported
21		c.1142 T > C	p.L381P	Exon8	Attenuated	Reported
22		c.1402C > T	p.R468W	Exon9	Severe	Reported
23		c.1402C > T	p.R468W	Exon9	Severe	Reported
24		c.1402C > T	p.R468W	Exon9	Severe	Reported
25		c.1403G > A	p.R468Q	Exon9	Severe	Reported
26		c.1403G > A	p.R468Q	Exon9	Severe	Reported
	Nonsense					
27		c.496G > T	p.E166X	Exon4	Severe	Novel
28		c.514C > T	p.R172X	Exon5	Severe	Reported
29		c.514C > T	p.R172X	Exon5	Severe	Reported
30		c.514C > T	p.R172X	Exon5	Severe	Reported
31		c.514C > T	p.R172X	Exon5	Severe	Reported
32		c.702C > A	p.Y234X	Exon5	Severe	Reported
33		c.1075delC	p.L359X	Exon8	Severe	Reported
34		c.1123G > T	p.E375X	Exon8	Severe	Reported
35		c.1327C > T	p.R443X	Exon9	Attenuated	Reported
36		c.1327C > T	p.R443X	Exon9	Attenuated	Reported
37		c.1327C > T	p.R443X	Exon9	Attenuated	Reported
38		c.1327C > T	p.R443X	Exon9	Attenuated	Reported
	Frameshift					
39		c.353_370del17	p.T118KfsX22	Exon3	Severe	Novel
40		c.468_469 intC	p.P157PfsX5	Exon4	Severe	Novel
41		c.720_721 intT	p.L241Ffsx15	Exon6	Severe	Novel
42		c.748_749delGCinsA	p.A250Tfsx28	Exon6	Severe	Novel
43		c.780_793del14	p.P260PfsX77	Exon6	Severe	Novel
44		c.822delA	p.E274EfsX4	Exon6	Severe	Novel
45		c.911delT	p.V304GfsX12	Exon7	Severe	Novel
46		c.1274delC	p. P425Lfs	Exon9	Severe	Reported
47		c.1444_1445intTT	p.L482FfsX1	Exon9	Severe	Reported
	Splicing					
48		c.1122C > T	p.G374G (20 amino acid deletion)	Exon8	Attenuated	Reported
49		c.1122C > T	p.G374G (20 amino acid deletion)	Exon8	Attenuated	Reported
50		c.241-2A > G		Intron2	Severe	Reported
51		c.419-2A > T		Intron3	Severe	Reported
52		c.880-1G > C		Intron6	Attenuated	Reported
53		c.880-8A > G		Intron6	Attenuated	Reported
	Small deletion					
54		c.118_120delCTT	p.L40del	Exon2	Severe	Novel
55		c.121_123delCTC	p.L41del	Exon2	Severe	Reported
56		c.285_287delGAG	p.R96del	Exon3	Attenuated	Reported
57		c.1181_1192 del12	p.G394_398delinsV	Exon9	Severe	Novel
	Small insertion					
58		c.1404_1405int9(TATCCCCGG)	p.468_469int3	Exon9	Severe	Novel
	Gross deletion					
59		EX1_3del		Exon1–3	Severe	Novel
60		EX3_6del		Exon3–6	Severe	Novel
61		EX4_7del		Exon4–7	Severe	Reported
62		EX1_9del		Exon1–9	Severe	Reported
63		EX1_9del		Exon1–9	Severe	Reported

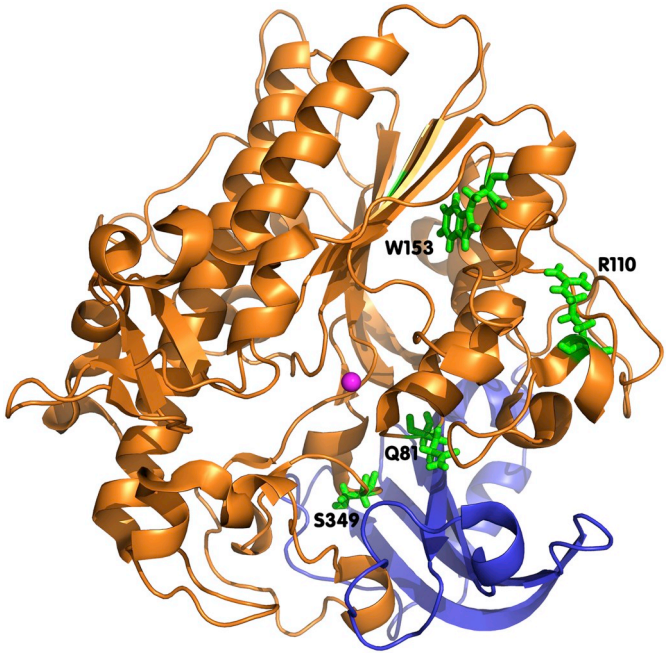

Fig. 1. Location of IDS mutations on the 3D structural model. SD1 and SD2 are drawn as yellow and purple ribbons respectively. Positions of mutations are indicated by the residues represented by green ribbons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Iduronate-2-sulfatase activity in 293FT cells transfected with wild-type and mutant cDNAs.

Transfected vector	IDS activity [a] (nmol/mg protien/4 h)	Total (percentage of wild-type)
Untransfected	31.51 ± 6.23	3.2
Wild-type	978.29 ± 68.10	100.0
242A > G(Q81R)	34.10 ± 3.84	3.5
328A > G(R110G)	30.71 ± 4.13	3.1
457 T > C(W153R)	33.64 ± 9.15	3.4
1047C > A(S349R)	32.85 ± 3.26	3.4
118_120delCTT(L40del)	31.30 ± 4.70	3.2
1404_1405ins9(468_469int3)	34.54 ± 6.38	3.5
748_749delGCinsT(A250Tfsx28)	28.38 ± 2.89	2.9

[a] IDS activity values are the average of three different clones of untransfected or transfected cells.

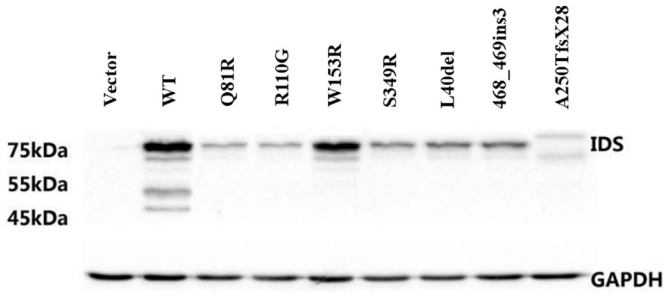

Fig. 2. Western blotting showing the IDS protein expression of vector, wild-type (WT) and the seven novel mutations, respectively. The molecular mass of the premature and mature protein is approximately 75 KDa and 55/45 KDa respectively.

4. Discussion

In this study we reported the clinical and molecular characterization of a group of Chinese patients with MPS II. The group included 48 patients with the severe type and 15 patients with the attenuated type. Our patients manifested more severe phenotypes with neurologic involvement in 76.2% of patients in comparison to 37% described in the literature [10,19,21]. In the 48 patients with the severe type, missense mutations were most frequently observed (20/48, 41.7%). However, the severe type of MPS II was strongly associated with mutations including frameshift, nonsense and gross deletion mutations. In this study, 63.5% of patients have private mutations and novel mutations were detected of a frequency of 32.7%, accordingly with another study from China [22].

The 49 mutations detected in the 63 patients reflected that most of the mutations were private mutations. However, the codon 468 position could be a “hot” spot as five patients showed mutations, with three of them p.R468W and two of them p.R468Q. The guanidinium group of R468 joins in a buried hydrogen bonding network that stabilizes several nearby loops, helical turns and β-strands [23]. Mutation of this residue (R468Q/L/W/G/P) is linked to a range of MPS II phenotypes from mild to severe [23]. Meanwhile, the five patients with the mutations of R468Q/W in our study presented with the severe phenotype.

The correlation between the phenotype and the genotype of MPS II is heterogeneous. In this study, we identified 15 cases of the attenuated type of MPS II including 7 (46.7%) missense, 4 (26.7%) nonsense, 3 (20.0%) splicing, 1 (6.7%) small deletion mutations. Among these mutations, we found an unexpected nonsense mutation p.R443X. This nonsense mutation leaded to the appearance of a premature stop codon, disturbed the synthesis of enzyme protein and influenced the function of the enzyme. This nonsense mutation was first reported in a case of MPS II with skeletal deformities and normal psychomotoric development by Bunge et al. [24]. This nonsense mutation was also reported to exist in the Japanese [25], Korean [26], Chinese [22], and the British [4] populations as the attenuated type of MPS II. According to the crystal structure of IDS, the IDS main chain has been devided into two subdomains, SD1 and SD2, corresponding to fragments resulting from lysosomal proteolytic processing events [23]. The N-terminal SD1 (residues 34–443) comprises the “heavy” 42 kDa chain, and the C-terminal SD2 (residues 455–550) corresponds to the “light” 14 kDa chain. SD1 subdomain contains the catalytic core. The mutation p.R443X would produce a truncated protein with a loss of 107 amino acids in the C-terminus. The mutant IDS protein remains the SD1 subdomain containing the catalytic core, however, it lacks two putative N-linked glycosylation sites at codons 513 and 537 and several nearby loops, helical turns and β-strands, leading to the destabilization of the protein structure and susceptibility to proteolytic cleavage. In other studies, western blot analysis in the presence of related mutations p.E430X, p.E521X, and p.Q531X showed the synthesis of the truncated IDS precursor and some residual enzymatic expression that was markedly decreased compared to the normal protein [15,27,28]. Thus, it was predicted that the p.R443X IDS mutant preserved a very low, residual enzymatic activity. The mutation p.R443X may be link to attenuated type of MPS II.

The patient with the mutation p.R88C was a twelve-year old boy who had normal mental status. This mutation was detected in one Taiwanese patient and two Russian patients who had the severe phenotype [15,27,28]. The residue R88 is highly conserved among sulfatases which makes several structural interactions within the catalytic core. Chang and colleagues reported that COS-7 cells expressing the p.R88C mutant cDNA exhibited trace amounts of IDS activity with 0.5% residual activity whereas western blot analysis showed a similar molecular mass of precursor, with little reduced mature forms being detected [28]. In our study, two patients with the mutation p.S142F had a

attenuated phenotype. This mutation was first found in a Russian patient with a severe phenotype by Chistiakov and colleagues [27]. The structural analysis of the mutation predicted that the replacement of a hydrophilic residue (S) with the hydrophobic residue (F), occurring in the proximity of two residues (K135 and H138) know to be involved in substrate binding, may lead to an impairment of the enzyme activity. In vitro functional analysis of the mutant p.S142F protein, showing a very low enzymic activity, clearly demonstrated its pathogenic nature [27]. Therefore, the genotype-phenotype correlation of these two mutations was not elucidated.

The mutation p.P231L, p.N350H, c.880-1G > C and c.880-8A > G have been reported in patients with the attenuated phenotype as the same with our study [29,30]. The synonymous mutation c.1122C > T (p.G374G) creates a new donor splice site in exon 8 and results in a 20 amino acid deletion of the IDS protein. This mutation always correlated with the attenuated phenotype [29]. The two patients with this mutation in our study also presented with an attenuated phenotype. However, in few studies this mutation was associated with the severe phenotype [22–26].

Forty-eight cases of the severe type of MPS II were identified including 20 (41.7%) missense, 9 (18.8%) frameshift, 8 (16.7%) nonsense, 5 (10.4%) gross deletion (the deletion of exon 1 to exon 3, the deletion of exon 3 to exon 6, the deletion of exon 4 to exon 7 and the deletion of all the nine exons), 3 (6.3%) small deletion, 2 (4.2%) splicing, 1 (2.1%) small insertion. All cases of mutations with frameshift and gross deletion errors in this study indicated the severe phenotype. The novel mutations identified in this study were associated with the severe phenotype. Except the mutation p.R443X, the other nonsense mutations were associated with the severe phenotype.

In this study, 16 novel mutations were identified: seven frameshift mutations (p.T118KfsX22, p.P157PfsX5, p.L241FfsX15, p.A250TfsX28, p.P260PfsX77, p.E274EfsX4, p.V304GfsX12), three missense mutations (p.Q81R, p.R110G, p.W153R), two small deletions (p.L40del, p.G394_398delinsV), two gross deletions (EX3_6del, EX1_3del), one nonsense mutation (p.E166X) and one small insertion (p.468_469int3). All the novel mutations found in this study were associated with the severe phenotype. The frameshift and nonsense mutations would lead to the generation of the premature stop codon and produce shorter transcripts. The gross deletions caused the structure of the enzyme protein was incomplete and influenced the function of the enzyme.

To verify the pathological impact of seven IDS variants on the IDS activity, we carried out in vitro expression experiments among the mutations p.Q81R, p.R110G, p.W153R, p.S349R, p.L40del, p.468_469ins3, p.A250TfsX28. Meanwhile, the mutation p.S349R, although no longer novel since it was reported during the preparation of our study by cobos et al. [19], was also included in the analysis. Transfection of 293FT cells with wild-type IDS cDNA resulted in an 31-fold increase of the enzyme activity compared to the activity of untransfected 293FT cells. The in vitro residual activity of the seven mutants was significantly lower than the activity of the normal protein. Cells expressing the wild-type IDS cDNA had major bands of 75–78 kDa of the precursor forms and 55 kDa and 45 kDa of the mature forms of the enzyme. The examined missense mutations showed a similar or lightly lower molecular mass of precursor, with no mature forms being detected.

5. Conclusions

In conclusion, 49 different mutations were identified in the IDS gene of 63 patients with MPS II including 16 novel mutations. Although most mutations were unique or individualized, the mutation p.R172X and p.R443X occurred fourth; the mutations p.R468W occurred thrice; the mutations p.P86L, p.S142F, p.S333L, p.G374G, p.R468Q and EX1_9del occurred twice. The mutation p.R443X and p.G374G may be link to attenuated type of MPS II. The bioinformatic structural analysis of the possible effect of the novel missense mutations (p.Q81R, p.R110G, p.W153R and p.S349R) on protein structure showed that these amino acid replacements would cause a severe impairment of protein structure and function. In vitro functional analysis of the seven mutants (p.Q81R, p.R110G, p.W153R, p.S349R, p.L40del, p.468_469ins3, p.A250TfsX28), showing a very low IDS activity, clearly demonstrated their pathogenic nature. In western blotting analysis of the IDS protein , the examined mutations showed a similar or slightly lower molecular mass of precursor, with no mature forms being detected. Our results confirmed the remarkable heterogeneity of the mutational spectrum of the IDS gene and demonstrated the genotype-phenotype correlations in patients with MPS II. This study comprises the functional analysis of 7 new sequence variations identified in the IDS gene. The characterization of gene mutation demonstrated their functional consequence on IDS activity and processing. Our study expands the spectrum of genotype of MPS II, provides new insights into the molecular mechanism of MPS II and helps to the future studies of genotype-phenotype correlations to estimate prognosis and develop new therapeutic approaches.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.01.009.

Conflict of interest

The authors declare that they have no conflict of interest.

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