ELSEVIER

Contents lists available at ScienceDirect

Clinica Chimica Acta



journal homepage: www.elsevier.com/locate/cca

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



Wen Zhang¹, Ting Xie¹, Huiying Sheng, Yongxian Shao, Yunting Lin, Minyan Jiang, Aijing Xu, Xueying Su, Zongcai Liu, Xiaoyuan Zhao, Li Liu², Yonglan Huang^{*,2}

Department of Genetics and Endocrinology, Guangzhou Women and Children's Medical Center, Guangzhou, China

ARTICLE INFO	A B S T R A C T		
Keywords: Mucopolysaccharidosis type II Genetic analysis Functional characterization Novel mutation	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 midteenage 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-

https://doi.org/10.1016/j.cca.2019.01.009

Received 12 December 2018; Received in revised form 9 January 2019; Accepted 10 January 2019

Available online 11 January 2019

0009-8981/ © 2019 Elsevier B.V. All rights reserved.

^{*} Corresponding author at: Department of Genetics and Endocrinology, Guangzhou Women and Children's Medical Center, 9 Jinsui Road, Guangzhou, Guangdong 510623, China.

E-mail address: huangylxx321@163.com (Y. Huang).

¹ Wen Zhang and Ting xie contributed equally to this work.

 $^{^2\,{\}rm Co-corresponding}$ author.

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 μ g) 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 Xray 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 frame-(p.T118KfsX22, p.P157PfsX5, p.L241FfsX15, shift mutations 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 \pm 68.10 nmol/4 h/mg and that of untransfected cells was 31.51 \pm 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
1 C.202 > A PATA PATA<		Missense					
- -2020. > G -0018. Land Severe Noed - - - - P864. Land Severe Reported - - - - P864. Land Severe Reported - - - - P874. Land Severe Reported - - - - P873. Land Severe Reported - - - P873. Land Noed Reported - - - P873. Land Noed Reported - - - P873. Lan	1		c 212G > A	n \$71N	Exon2	Severe	Reported
- - - - - - None Noe Noe	2		$c_{242A} > C$	p.091P	Exon2	Severe	Novel
3	2		C.242A > G	p.Qolk	EXUIIS	Severe	Novel
4 C.2000 P. 1 pASC Local Perform Appointed 6 C.2000 P. 1 pASC Local Perform Appointed 7 C.2000 P. 1 pASC Local Severes Reported 8 C.2000 P. 1 pASC Local Severes Reported 9 C.2000 P. 1 pASC Local Severes Reported 11 C.2000 P. 1 pASC PASC PASC PASC PASC 12 C.4500 P. 1 pASC	3		c.25/C > 1	p.P86L	Exon3	Severe	Reported
5 C.257.0 G p.488.1 Bando Averee Magnetic 6 C.366.6 C p.488.1 Bando Averee Magnetic 6 C.366.6 C p.489.6 Bood Averee Magnetic 6 C.388.6 C G p.499.6 Bood Severe Nether 10 C.328.6 C G p.494.7 Bood Avereed Magnetic 11 C.428.7 T G.314.87 Bood Avereed Magnetic 13 C.428.7 T G.314.87 Bood Avereed Reported 14 C.428.7 C G p.433.4 Bood Avereed Reported 15 C.436.7 C p.438.4 Bood Severe Reported 16 C.136.7 C p.438.4 Bood Severe Reported 16 C.136.7 C p.438.4 Bood Avereed Reported 17 C.136.7 C p.438.4 Bood Avereed Reported 14 <td>4</td> <td></td> <td>c.257C > T</td> <td>p.P86L</td> <td>Exon3</td> <td>Severe</td> <td>Reported</td>	4		c.257C > T	p.P86L	Exon3	Severe	Reported
6 	5		c.257C > G	p.P86R	Exon3	Severe	Reported
7	6		c.262C > T	p.R88C	Exon3	Attenuated	Reported
6 C3302 · C C p.0505 Severe Netrone 9 C3202 · C C p.11106 Severe Netrone 10 C425C · T p.5142P Bon-1 Atternanded Reported 11 C425C · T p.5142P Bon-1 Atternanded Reported 11 C425C · T p.5142P Bon-1 Atternanded Reported 12 C426C · T p.5333L Bon-7 Severe Reported 13 C436C · C p.13340P Bon-7 Severe Reported 14 C436C · T p.5337H Bon-7 Severe Reported 14 C436C · T p.5337H Bon-7 Severe Reported 14 C436C · T p.5337H Bon-7 Severe Reported 14 C436C · T p.5436P Bon-7 Severe Reported 14 C436C · T p.5436P Bon-7 Severe Reported 14 C436C · T p.344	7		$c_{263G} > C$	n R88H	Exon3	Severe	Reported
0 - C320. * 0 - p.1100 Doods Severe Novel a 10 - C430. * 17 - p.1407 Doods Severe Novel a 11 - C430. * 7 - p.1407 Doods Severe Novel a 12 - C430. * 7 - p.7331. Doods Severe Novel a 13 - C430. * 0 - p.7331. Doods Severe Reported 14 - C430. * 0 - p.1334. Doods Severe Reported 16 - C400. * 0 - p.1334. Doods Severe Reported 17 - C100. * 0 - p.1354. Doods Severe Reported 18 - C100. * 0 - p.1354. Doods Severe Reported 19 - C100. * 0 - p.1354. Doods Severe Reported 21 - C100. * 7 - p.1456. Doods Severe Reported 22 - C100. * 7 - p.1456. Doods Severe Reported 1400. * 7	, 0		$a_{2}^{2} = c_{2}^{2} = c_{2}^{2}$	p ROEC	Evon2	Severe	Reported
9 C.262 0 p.110 ¹⁰ D008 Severe More 11 C.232 7 p.5139 ¹⁷ Evene Attenuated Reported 12 C.437 7 C p.1539 ¹⁷ Evene Attenuated Reported 13 C.432 7 C p.1531 ¹¹ Even ¹ Attenuated Reported 14 C.392 7 P.3331 ¹¹ Even ² Reported Reported 15 C.1030 ¹⁰ C p.1530 ¹¹ Even ² Reported 18 C.1030 ¹⁰ C p.1540 ¹¹ Even ² Reported 19 C.1030 ¹⁰ C p.1540 ¹¹ Even ² Reported 19 C.1040 ¹⁰ T p.1450 ¹¹ Even ² Reported 19 C.1040 ¹⁰ T p.1450 ¹¹ Even ² Reported 19 C.1040 ¹⁰ T p.1450 ¹¹ Even ² Reported 10 C.1040 ¹⁰ T p.1450	0		0.2030 > 0	p.K95G	EXUIIS	Severe	Reported
10 -c.43C > T p.31427 Exord Attended Reported Attended Reported Attended Reported Control (1997) Attended Reported Reported Control (1997) 13 -c.63C > T p.7331. Exord Attended Reported Control (1997) Reported Control (1997) 14 -c.63C > T p.5333. Exord State Reported Control (1997) Reported Reported Control (1997) Reported Reported Control (1997) 15 -c.60C > T p.5333. Exord State Reported Control (1997) Reported Reported Control (1997) Reported Reported Control (1997) 19 -c.160C > A p.53364 Exord State Reported Control (1997) Reported Reported Control (1997) 20 -c.160C > A p.53364 Exord State Reported Control (1997) Reported Reported Control (1997) Reported Reported Reported Control (1997) 21 -c.160C > T p.154807 Exord State Reported Reported Control (1997) Reported Rep	9		C.328A > G	p.R110G	Exon3	Severe	Novel
11 c.4357 > C p.5142F Exord Attenued Reported 12 c.4377 > C p.1513 Exord Severe Novel 13 c.701 > C p.1521A Exord Novel Reported 14 c.701 > C p.5333. Exord Severe Reported 15 c.1001A > G p.13346 Exord Severe Reported 16 c.1001A > G p.13142P Exord Severe Reported 17 c.1001A > G p.13142P Exord Severe Reported 18 c.1002A > C p.13142P Exord Severe Reported 19 c.1001A > G p.13146P Exord Severe Reported 21 c.1402C > T p.1466W Exord Severe Reported 22 c.1402C > T p.1466W Exord Severe Reported 23 c.1402C > T p.1468W Exord Severe Reported 24 c.1402C > T p.1468W Exord Severe Reported 25 c.1402C > T p.112X Exord Severe Reported 26 c.1402C > T p.112X Exord Severe Rep	10		c.425C > T	p.S142F	Exon4	Attenuated	Reported
12 c.497 T > C µV331. Bond Arrows Severe Nord 13 c.602. C T µV331. Bond Arrows Severe Nord 14 c.701. C O µV331. Bond Arrows Severe Nord Reported 14 c.701. A C O µV334. Bond P Arrows Severe Nord Reported 15 c.101. A C O µV334. Bond P Arrows Severe Nord Reported 18 c.101. A C O µV334. Bond P Arrows Severe Nord Reported 19 c.101. A C O µV3501. Bond P Arrows Severe Nord Reported 20 c.101. A C O µV3501. Bond P Arrows Severe Nord Reported 21 c.101. A C O µV3501. Bond P Arrows Severe Nord Reported 21 c.101. A C O µV3501. Bond P Arrows Severe Nord Reported 22 c.101. A C O µV4480. Bond P Arrows Severe Nord Reported 22 c.101. A C O µV4480. Bond P Arrows Severe Nord Reported 23 c.514. C > T µV172X.	11		c.425C > T	p.S142F	Exon4	Attenuated	Reported
13e692C > Tµ231LEuxofAutsuidedReported14C798C > Tµ3261.0ExoreSevereReported15C.998C > Tµ35331.0ExorSevereReported17C.1001.> CQ.13350.0ExorSevereReported18C.1001.> CQ.13350.0ExorSevereReported19C.1047.0 Cµ3530.0ExorSevereReported20C.14402.0P.1470.0P.14863.0ExorSevereReported21C.14202.0Tµ4663WExorSevereReported22C.14202.0Tµ4663WExorSevereReported23C.14202.0Tµ4663WExorSevereReported24C.14202.0Tµ4663WExorSevereReported25C.14202.0Tµ172XProfoSevereReported26C.1420.0Tµ172XProfoSevereReported27C.1420.0Tµ172XProfoSevereReported28C.1420.0Tµ172XProfoSevereReported29C.1420.0Tµ172XProfoSevereReported20C.1420.0Tµ172XProfoSevereReported29C.1420.0Tµ172XProfoSevereReported20C.1420.0Tµ172XProfoSevereReported21C.1420	12		c.457 T > C	p.W153R	Exon4	Severe	Novel
14	13		c 692C > T	n P2311.	Exon5	Attenuated	Reported
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	14		$c_{791C} > C$	p D261A	Exon6	Soucro	Peported
1010101010101010010010017100101010100	14			p.F201A	EXOID	Severe O	Deported
16 $C OPRC > T$ p.5333.Even CReported17 $C OPRC > T$ p.5333.Even CReported18 $C OPRC > T$ p.5339.Even CReported19 $C OPRC > T$ p.5359.Even CReported21 $C OPRC > T$ p.1448Even CReported22 $C OPRC > T$ p.1458.Even CReported23 $C OPRC > T$ p.1458.Even CReported24 $C OPRC > T$ p.1458.Even CReported25 $C OPRC > T$ p.1458.Even CReported26 $C OPRC > T$ p.1458.Even CReported27 $C OPRC > T$ p.1458.Even CReported28 $C OPRC > T$ p.1458.Even CReported29 $C OPRC > T$ p.1458.Even CReported29 $C OPRC > T$ p.1458.Even CReported29 $C OPRC > T$ p.1127.Even CReported29 $C OPRC > T$ p.1227.Even CReported29 $C OPRC > T$ p.1277.Even CReported29 $C OPRC > T$ p.1277.Even CReported29 $C OPRC > T$ p.1444.Even CReported<	15		c.998C > T	p.8333L	Exon7	Severe	Reported
17 c.1001A > G p.13346 Sonot Severe Reported 19 c.105A > C p.14242 Sonots Severe Reported 19 c.104A > C p.15304R Sonots Atenuated Reported 10 c.104A > C p.15304R Boots Atenuated Reported 10 c.104A > C p.1666W Sovere Reported 23 c.1040C > T p.1666W Sovere Reported 24 c.1040C > T p.1666W Sovere Reported 25 c.1040C > T p.1666W Sovere Reported 26 c.1040C > T p.166X Sovere Reported 27 c.514C > T p.1122X Sovere Reported 28 c.514C > T p.1122X Sovere Reported 29 c.514C > T p.1122X Sovere Reported 21 c.514C > T p.1122X Sovere Reported 22 c.514C > T p.1122X Sovere Reported 23 c.514C > T p.123X Sov	16		c.998C > T	p.S333L	Exon7	Severe	Reported
18 c.1047.0 C A p.1342P Exos S Severe Reported 20 c.1048.4 C A p.1359H Exos S Severe Reported 21 c.1042.5 T A p.1359H Exos S Mermained Reported 22 c.1042.5 T A p.1468W Exos S Severe Reported 23 c.1042.5 T A p.1468W Exos S Severe Reported 24 c.1042.5 T A p.1468W Exos S Severe Reported 25 c.1030.5 A p.1468Q Exos S Severe Reported 26 c.1030.5 A p.1468Q Exos S Severe Reported 27 Severe Reported Severe Reported Severe Reported Severe Reported 29 c.704.5 T p.1127X Exos S Severe Reported 20 c.704.5 T p.1127X Exos S Severe Reported 21 c.704.5 T p.1127X Exos S Severe Reported 23 c.704.5 T p.1127X Exos S Severe Reported 24 c.704.5 C T p.1423X Exos S Severe Need 2137.5 T	17		c.1001A > G	p.D334G	Exon7	Severe	Reported
19 $C 1047C > A$ p S399RPoor p S399RPoor Poor Poor Poor Poor Poor 	18		c.1025A > C	p.H342P	Exon8	Severe	Reported
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	10		c 1047C > A	p \$3498	Exon8	Severe	Reported
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	20		a 1049A > C	- N250U	Exono	Attomusted	Deported
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	20		C.1048A > C	p.N350H	Exono	Attenuated	Reported
22c. 1.402C > Tp. PA68WBonPSevereReported23c. 1.402C > Tp. PA68WBonPSevereReported24c. 1.403C > Ap. PA68QBonPSevereReported25c. 1.403C > Ap. PA68QBonPSevereReported26c. 1.403C > Ap. PA68QBonPSevereReported27C.446C > Tp. PLI6KXBonPSevereReported28C.514C > Tp. PLI2XBonSSevereReported29C.514C > Tp. PLI2XBonSSevereReported30C.514C > Tp. PLI72XBonSSevereReported31C.514C > Tp. PLI72XBonSSevereReported32C.514C > Tp. PLI72XBonSSevereReported33C.1073dDCp. PLI72XBonSSevereReported34C.120C > Tp. PL44XXBonPAttenuandReported35C.127C > Tp. PL44XXBonPAttenuandReported36C.127C > Tp. PL44XXBonPAttenuandReported37C.333.70del17p. T118Kh3Z2BonPAttenuandReported38C.127C > Tp. PL44XXBonPSevereNovel41C.7302 / PL44XPL457KABonPSevereNovel42C.7302 / PL44XPL457KABonPSevereNovel43C.127C > Tp. PL44XXBonPSevere <td>21</td> <td></td> <td>c.1142 T > C</td> <td>p.L381P</td> <td>Exon8</td> <td>Attenuated</td> <td>Reported</td>	21		c.1142 T > C	p.L381P	Exon8	Attenuated	Reported
23	22		c.1402C > T	p.R468W	Exon9	Severe	Reported
24 c1403C > T p.14680 Exon9 Severe Reported 25 c1403C > A p.14680 Exon9 Severe Reported 26 .1403C > A p.14680 Exon9 Severe Reported 27 .408C > T p.1172X Exon5 Severe Reported 28 .514C > T p.1172X Exon5 Severe Reported 29 .514C > T p.1172X Exon5 Severe Reported 29 .514C > T p.1172X Exon5 Severe Reported 21 .514C > T p.1172X Exon5 Severe Reported 21 .514C > T p.1137X Exon8 Severe Reported 22 .514C > T p.1337X Exon8 Severe Reported 23 .6132C > T p.1443X Exon9 Attenuited Reported 23 .6132C > T p.11845X Exon9 Severe Novel 24 .6332C > T	23		c.1402C > T	p.R468W	Exon9	Severe	Reported
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	24		c.1402C > T	p.R468W	Exon9	Severe	Reported
	25		c 1402G > A	p.R. 100 //	Evon	Corroro	Poportad
consensep.14468/0p.14468/0p.8409/0p.8409/0p.8409/0Reported27c.544 C > Tp.116KXExon5SevereReported28c.514 C > Tp.1172XExon5SevereReported29c.514 C > Tp.172XExon5SevereReported31c.514 C > Tp.172XExon5SevereReported32c.702 C > Ap.172XExon5SevereReported33c.702 C > Ap.1359XExon8SevereReported34c.1327 C > Tp.1443XExon9AttenuatedReported35c.1327 C > Tp.1443XExon9AttenuatedReported36c.1327 C > Tp.1443XExon9AttenuatedReported37c.1327 C > Tp.1443XExon9AttenuatedReported38c.1327 C > Tp.1443XExon9AttenuatedReported39c.456,469 infCp.1719KX22Exon1SevereNovel41c.729,7304117p.1219KX52Exon5SevereNovel43c.749,74040CExaAp.12274EX44Exon9SevereNovel44c.729,7304147p.12274EX44Exon9SevereNovel45c.911414p.12274EX44Exon9SevereNovel45c.127404Cp.14267AExon9SevereNovel45c.124414SevereNovelExon9SevereNovel45c.124414 <t< td=""><td>20</td><td></td><td>14000 × A</td><td>p.K400Q</td><td>EXUII9</td><td>Severe</td><td>Reported</td></t<>	20		14000 × A	p.K400Q	EXUII9	Severe	Reported
Presence	26		c.1403G > A	p.R468Q	Exon9	Severe	Reported
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		Nonsense					
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	27	Tonsense	a 406C > T		Evon4	Course	Noval
28 $(z, 5)$ (4, > 1 (b) (1/2AExonisSevereReported29 $(z, 5)$ (4, $< T$ (b) (1/2XExonisSevereReported30 $(z, 5)$ (4, $< T$ (b) (1/2XExonisSevereReported31 $(z, 5)$ (4, $< T$ (b) (1/2XExonisSevereReported32 $(z, 7)$ (2, $< A$ p (2, 2) (2, $< A$ p (2, 2) (2, $< A$ ExonisSevereReported33 $(z, 1)$ (2, 2) (2, $< T$ p (1, 2) (2, $< T$ p (1, 2) (2, $< A$ ExonisSevereReported34 $(z, 1)$ (2, 2) (2, $< T$ p (1, 2) (2, $< T$ p (2, 2) (2, $< T$ <td< td=""><td>27</td><td></td><td>C.490G > 1</td><td>p.EIOOX</td><td>EXOII4</td><td>Severe</td><td>Novel</td></td<>	27		C.490G > 1	p.EIOOX	EXOII4	Severe	Novel
29 $c.514C > T$ $p.11/2X$ Exon5SevereReported30 $c.514C > T$ $p.11/2X$ Exon5SevereReported31 $c.514C > T$ $p.1172X$ Exon5SevereReported32 $c.702C > A$ $p.1234X$ Exon5SevereReported33 $c.1025delC$ $p.1355X$ Exon8SevereReported34 $c.1123C > T$ $p.1434X$ Exon9AttenuatedReported35 $c.1327C > T$ $p.1443X$ Exon9AttenuatedReported36 $c.1327C > T$ $p.1443X$ Exon9AttenuatedReported37 $c.1327C > T$ $p.1443X$ Exon9AttenuatedReported38 $c.1327C > T$ $p.1443X$ Exon9AttenuatedReported41 $c.353.370del17$ $p.11815x12$ Exon4SevereNovel42 $c.648.469$ intC $p.15795x5$ Exon4SevereNovel43 $c.702.721$ intT $p.124115x15$ Exon6SevereNovel44 $c.780.793del14$ $p.220695x77$ Exon6SevereNovel45 $c.1122C > T$ $p.3374G_{12}$ Exon9SevereReported46 $c.1274delC$ $p.143245x11$ Exon8ReportedReported47 $c.144.1445intTT$ $p.143245x11$ Exon8SevereReported47 $c.241.24 > G$ $c.1122C > T$ $p.6374G_{12}$ Exon9SevereReported58 $c.1122C > T$ $p.6374G_{12}$	28		c.514C > T	p.R172X	Exon5	Severe	Reported
30 $c.514C > T$ $p.RJT2X$ Exon5SevereReported31 $c.514C > T$ $p.RJT2X$ Exon5SevereReported32 $c.702C > A$ $p.1234X$ Exon5SevereReported33 $c.1075delC$ $p.1359X$ Exon8SevereReported34 $c.123G > T$ $p.1359X$ Exon9AttenuatedReported35 $c.1327C > T$ $p.R443X$ Exon9AttenuatedReported36 $c.327C > T$ $p.R443X$ Exon9AttenuatedReported37 $c.3327C > T$ $p.R443X$ Exon9AttenuatedReported38 $c.327C > T$ $p.R443X$ Exon9AttenuatedReported39 $c.3237C$ >T $p.R443X$ Exon9AttenuatedReported40 $c.230, 270$ del17 $p.T118K5X22$ Exon3SevereNovel41 $c.2746, 469$ inC $p.1251Fx55$ Exon4SevereNovel42 $c.230, 370$ del17 $p.1241Fx15$ Exon6SevereNovel43 $c.730, 793$ del14 $p.1260Fx77$ Exon6SevereNovel44 $c.730, 793$ del14 $p.1241Fx14$ Exon6SevereReported47 $c.112C > T$ $p.0374G$ Exon8AttenuatedReported47 $c.112C > T$ $p.0374G$ Exon9SevereReported47 $c.112C > T$ $p.0374G$ Exon9SevereReported48 $c.1122 > 4C$ $p.0374G$ Exon9Severe	29		c.514C > T	p.R172X	Exon5	Severe	Reported
31c.514C > Tp.172XExon5SevereReported32c.70Z > Ap.1237XExon5SevereReported33c.1123C > Tp.1357XExon8SevereReported34c.1123C > Tp.1443XExon9AttenuatedReported35c.1327C > Tp.1443XExon9AttenuatedReported36c.1327C > Tp.1443XExon9AttenuatedReported37c.1327C > Tp.1443XExon9AttenuatedReported38c.1327C > Tp.1443XExon9AttenuatedReported39C.353_370del17p.T118K5X2Exon3SevereNovel41c.454_469intCp.1257K5X5Exon4SevereNovel42c.730_71intTp.1241Fx15Exon6SevereNovel43c.730_7342141p.1250Fx577Exon6SevereNovel44c.730_7342141p.1244Fx15Exon6SevereNovel45c.1124C > Tp.13426fx12Exon6SevereNovel46c.1124C > Tp.14326fx11Exon6SevereNovel47c.1124C > Tp.14326fx11Exon6SevereReported48Splicingc.1122C > Tp.6374GExon6SevereReported49c.1122C > Tp.6374GExon6SevereReported50c.1132_1 > Gc.113_1192GGReported51c.114_145intT <td>30</td> <td></td> <td>c.514C > T</td> <td>p.R172X</td> <td>Exon5</td> <td>Severe</td> <td>Reported</td>	30		c.514C > T	p.R172X	Exon5	Severe	Reported
32 $c702C > A$ $pT234X$ Evon5SevereReported33 $c1075deC$ $pL339X$ Exon8SevereReported34 $c1123C > T$ $pR443X$ Exon9AttenuatedReported36 $c1327C > T$ $pR443X$ Exon9AttenuatedReported36 $c1327C > T$ $pR443X$ Exon9AttenuatedReported37 $c1327C > T$ $pR443X$ Exon9AttenuatedReported38 $c327C > T$ $pR443X$ Exon9AttenuatedReported40 $c368,49$ inC $pT118K5A22$ Exon3SevereNovel40 $c468,49$ inC $pT128K5A5$ Exon6SevereNovel41 $c372,721$ inT $pL321K5A5$ Exon6SevereNovel42 $c748,73446GCinsA$ $pL327K5A5$ Exon6SevereNovel43 $c748,73446GCinsA$ $pL327K5A5$ Exon6SevereNovel44 $c682deA$ $p1527K5A5$ Exon6SevereNovel45 $c.9114eT$ $p.1424K5A1$ Exon6SevereNovel46 $c.9114eT$ $p.1424K5A1$ Exon6SevereRopred47 $c.1122C > T$ $p.6374G$ Exon7SevereRopred47 $c.1124c > G$ $c.1122C > T$ $p.6374G$ Exon8AttenuatedRopred47 $c.1124c > G$ $c.1122C > T$ $p.6374G$ Exon8AttenuatedRopred59 $c.241-2A > G$ $c.112.1234eICT$ $p.6346$	31		c.514C > T	p.R172X	Exon5	Severe	Reported
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	32		c702C > A	p V234X	Exon5	Severe	Reported
3-3C.107.deft.p.1337XExonsSevereReported34C.1327C > Tp.18473XExon9AttenuatedReported35C.1327C > Tp.18443XExon9AttenuatedReported36C.1327C > Tp.18443XExon9AttenuatedReported37C.1327C > Tp.18443XExon9AttenuatedReported38C.1327C > Tp.18443XExon9AttenuatedReported39C.533.3704017p.711.18K5472Exon3SevereNovel40C.463.469 InCp.713.77525Exon3SevereNovel41C.720.721.1ntTp.123.77523Exon6SevereNovel42C.746.7494060GnsAp.4250715228Exon6SevereNovel43C.730.793.0114p.12240975377Exon6SevereNovel44C.622.0241Ap.122407534Exon6SevereNovel45C.911.0417p.1432155Exon6SevereNovel46C.1274.461Cp.1432155Exon7SevereNovel47c.1442.143617Tp.1432155Exon7SevereNovel48C.1122C > Tp.6374GExon8AttenuatedReported50c.212.744E1Cp.6374GExon8AttenuatedReported51c.1122C > Tp.6374GExon8SevereReported52c.212.24 > GIntron3SevereReported53c.112.123404CTTp.14041<	32		1075 1-10	p.1254X	Exolig	Severe O	Deported
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	33		c.10/5delC	p.L359X	Exon8	Severe	Reported
35c.1327C > Tp.R443XExon9AttenuatedReported36c.1327C > Tp.R443XExon9AttenuatedReported38c.1327C > Tp.R443XExon9AttenuatedReported38c.333.70del17p.T118KfaX2Exon9AttenuatedReported40c.488,460 intCp.P157KaXExon4SevereNovel41c.730.721 intTp.1241Ffx15Exon6SevereNovel42c.748,749delCinasAp.2250Ffx28Exon6SevereNovel43c.780.739del14p.2250Ffx28Exon6SevereNovel44c.780.739del17p.7250Ffx28Exon6SevereNovel45c.748.749delCinasAp.2250Ffx27Exon6SevereNovel46c.1274delCp.1425fx31Exon9SevereReported47c.1441.145inTTp.443Efx11Exon9SevereReported48c.1122C > Tp.G374GExon8AttenuatedReported50c.241-2A > GIntron6AttenuatedReported51c.1122C > Tp.I40delExon9SevereReported52c.890-6A > GIntron6AttenuatedReported53c.241-2A > GIntron6AttenuatedReported54c.118_1204clCTp.I40delExon9SevereNovel55c.112_123delCCp.I40delExon9SevereNovel56c.118_1192 del12p.I40del <t< td=""><td>34</td><td></td><td>c.1123G > T</td><td>p.E375X</td><td>Exon8</td><td>Severe</td><td>Reported</td></t<>	34		c.1123G > T	p.E375X	Exon8	Severe	Reported
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	35		c.1327C > T	p.R443X	Exon9	Attenuated	Reported
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	36		c.1327C > T	p.R443X	Exon9	Attenuated	Reported
33C.1327C > Tp.R415XExon?RtunatedReportedFrameshift9SevereNovel40c.463.469 intCp.P137P5X5Exon3SevereNovel41c.230.720 IntTp.1241Ffsx15Exon6SevereNovel42c.748.749delGGinsAp.2200FisX77Exon6SevereNovel43c.780.793del14p.1220FfsX14Exon6SevereNovel44c.822delAp.1224ffsX4Exon6SevereNovel45c.011delTp.V304GfsX12Exon7SevereNovel46c.1244delCCp.422LfsExon9SevereReported47c.144_1445intTTp.1482FfsX1Exon9SevereReported48c.1122C > Tp.G374GExon8AttenuatedReported49c.1122C > Tp.G374GExon8AttenuatedReported50c.241-2A > GIntron6AttenuatedReported51c.103 > Cc.118_1204CTTp.140delExon2SevereReported52c.118_1204CTTp.140delExon2SevereNovel54c.118_1204CTTp.140delExon3AttenuatedReported55c.118_1204CTTp.140delExon3SevereNovel56c.280-8A > GExon3SevereNovel57c.118_1192 del12p.394,398delinsVExon3SevereNovel58c.121_	37		$c_{1327C} > T$	p B443X	EvonQ	Attenuated	Reported
33 $C.132/C > 1$ $p.R443X$ $Exony$ $Attenuated$ $Avvel$ 40 $C.453,370dell7$ $p.118/K5X22$ $Exon3$ $Severe$ $Novel$ 41 $C.720,721$ intT $p.1257/F5X5$ $Exon6$ $Severe$ $Novel$ 42 $C.748,749delGCinsA$ $p.22507fsx23$ $Exon6$ $Severe$ $Novel$ 43 $C.748,749delGCinsA$ $p.22507fsx23$ $Exon6$ $Severe$ $Novel$ 44 $C.322delA$ $p.E274fifsA4$ $Exon6$ $Severe$ $Novel$ 45 $C.1124C$ $p.P42516$ $Exon9$ $Severe$ $Reported$ 46 $C.12274delC$ $p.P42516$ $Exon9$ $Severe$ $Reported$ 47 $C.1122C > T$ $p.G374G$ $Exon9$ $Attenuated$ $Reported$ 48 $C.1122C > T$ $p.G374G$ $Exon8$ $Attenuated$ $Reported$ 49 $C.1122C > T$ $C.374G$ $Exon8$ $Attenuated$ $Reported$ 50 $C.41-2A > G$ $Intron6$ $Attenuated$ $Reported$ 51 $C.41-2A > G$ $Intron6$ $Attenuated$ $Reported$ 52 $C.380-8A > G$ $Intron6$ $Evere$ $Novel$ 53 $C.21-12AelCTT$ $p.140del$ $Exon2$ $Severe$ $Novel$ 54 $C.138_122delCTT$ $p.140del$ $Exon3$	37		10070 × T	p.R445X	EXUITS	Attenuated	Reported Demonto 1
FrameshiftVI18K6X22Exon3SevereNovel39	38		c.132/C > 1	p.K443X	Exon9	Attenuated	Reported
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		Frameshift					
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	30		c 353 370del17	n T118KfeY22	Evon3	Severe	Novel
40 $c.468,469$ int. $p.1/3 / P12x1$ ExonfExonfSevereNovel41 $c.720,721$ inf. $p.12x1$ $Exonf$ SevereNovel42 $c.748,793$ delGGins.A $p.250$ fix.28ExonfSevereNovel43 $c.780,793$ dellA $p.P250$ fix.27ExonfSevereNovel44 $c.822$ delA $p.227$ defs.X2ExonfSevereNovel45 $c.011$ delT $p.V30$ defs.X12ExonfSevereNovel46 $c.1274$ delC $p.4482$ fix.1ExonfSevereReported47 $c.1444,1445$ intTT $p.432$ fixExonfSevereReported48 $c.1122$ C > T $p.6374$ GExonfExonfReported49 $c.1122$ C > T $p.6374$ GExonfSevereReported50 $c.441-2A > G$ $(20 amino acid deletion)$ IntronfAttenuatedReported51 $c.41-2A > G$ IntronfAttenuatedReported52 $c.80-8A > G$ IntronfAttenuatedReported53 $c.1181, 120$ delCT $p.140$ delExon2SevereNovel54 $c.1181, 120$ delCT $p.140$ delExon3AttenuatedReported55 $c.1181, 1192$ del12 $p.6394, 398$ delinsVExon3AttenuatedReported56 $c.235, 287$ delGA $p.140$ delExon3SevereNovel57 $c.1181, 1192$ del12 $p.6394, 398$ delinsVExon3SevereNovel5	39		C.555_57 0def17	p.1110KI3X22	EXUIIS	Severe	NOVEL
41c.720,721 intTp.120 ffsx15Exon6SevereNovel42c.780,793del14p.A250Tfsx28Exon6SevereNovel43c.780,793del14p.2207EfsX7Exon6SevereNovel44c.822delAp.2207EfsX7Exon6SevereNovel45c.911delTp.V304GfsX12Exon7SevereNovel46c.1274delCp. V304GfsX12Exon7SevereReported47c.1227delCp. 425LfsExon9SevereReported48c.1122C > Tp.G374GExon8AttenuatedReported49c.1122C > Tp.G374GExon8AttenuatedReported50c.241-2A > GIntron6AttenuatedReported51c.419-2A > TIntron6AttenuatedReported52c.880-8A > GIntron6AttenuatedReported53c.11212delCTTp.140delExon2SevereReported54c.181_120delCTTp.140delExon3AttenuatedReported55c.121_123delCTCp.141delExon3AttenuatedReported56c.285_287delGAGp.966delExon3AttenuatedReported57c.140_1405int9(TATCCCCGG)p.468469int3Exon3AttenuatedReported58EX1_3delEX01-3SevereNovelExon4SevereNovel60EX1_3delEX01-3SevereNovelExon4SevereReported <td>40</td> <td></td> <td>c.468_469 intC</td> <td>p.P157PfsX5</td> <td>Exon4</td> <td>Severe</td> <td>Novel</td>	40		c.468_469 intC	p.P157PfsX5	Exon4	Severe	Novel
42c.748,749delGCinsAp.A250FtsX2Exon6SevereNovel43c.780,793del14p.P260FtsX77Exon6SevereNovel44c.822delAp.P274EfsX4Exon6SevereNovel45c.911delTp.V304GfS122Exon7SevereReported46c.1274delCp.1482FfsX1Exon9SevereReported47c.1444_1445intTTp.I482FfsX1Exon9SevereReported48c.122C > Tp.G374GExon8AttenuatedReported49c.1122C > Tp.G374GExon8AttenuatedReported50c.241-2A > GIntron6SevereReported51c.419-2A > TIntron6SevereReported52c.880-16 > CIntron6AttenuatedReported53c.118_120delCTTp.I40delExon3SevereReported54c.118_120delCTTp.140delExon3SevereReported55c.121_123delCTCp.140delExon3AttenuatedReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.118_1192 del12p.468_69int3Exon3SevereNovel58C.124_13delFX1_3delExon3SevereNovel59Ex1_3delExo1-3SevereNovel60EX1_3delExo1-3SevereNovel61Exo1-4Exo1-3SevereNovel62Exo1-5	41		c.720_721 intT	p.L241Ffsx15	Exon6	Severe	Novel
43c.780.793del14p.P306/FX77Exon6SevereNovel44c.822delAp.2746/KX4Exon7SevereNovel45c.911delTp.V304G/KS12Exon7SevereNovel46c.1274delCp. P.4821/KSExon9SevereReported47c.1444_145intTp.2324/SExon9SevereReported48c.1122C > T0.3374GExon8AttenuatedReported49c.1122C > Tp.G374GExon8AttenuatedReported50c.241-2A > G.201amina acid deletion)Intron3SevereReported51c.419-2A > T.202 amino acid deletion)SevereReported52c.880-6B > GIntron6AttenuatedReported53c.118_120delCTTp.140delExon3SevereNovel54C.118_120delCTTp.140delExon2SevereNovel55c.181_122delCCCp.141delExon3AttenuatedReported56c.121_123delCTCp.140delExon3AttenuatedReported57c.1018_1192 del12p.140delExon3SevereNovel58Gross deletionExon3SevereNovel59FX1_3delp.140delExon3SevereNovel59Gross deletionExon3SevereNovel59Exon3SevereNovelNovel50Exon3SevereNovelNovel50Exon3	42		c.748_749delGCinsA	p.A250Tfsx28	Exon6	Severe	Novel
44c.82delAp.274EfsX4Exon6SevereNovel45c.911deTp.304GfsX12Exon7SevereNovel46c.1274delCp.1482FfsX1Exon9SevereReported47c.1444_1445inTTp.1482FfsX1Exon9SevereReported48c.122C > Tp.6374GExon8AttenuatedReported49c.1122C > Tp.6374GExon8AttenuatedReported50c.241-2A > GIntron3SevereReported51c.149-2A > TJamina acid deletion)Intron3SevereReported52c.80-16 > CIntron3SevereReported53c.80-8A > GIntron6AttenuatedReported54c.118_120deICTTp.140delExon2SevereReported55c.121_123deICTCp.141delExon2SevereReported56c.285_287deIGAGp.896delExon3AttenuatedReported57c.118_1192 de112p.468_469int3Exon9SevereNovel58c.121_123deICTCp.468_469int3Exon9SevereNovel59g.1404_1405in9(TATCCCCGG)p.468_469int3Exon3AttenuatedReported59Ex3_6delExon3-6SevereNovelNovel60EX3_6delExon3-6SevereNovel61EX4_7delExon3-6SevereNovel62EX3_6delExon3-6SevereNovel63 </td <td>43</td> <td></td> <td>c.780 793del14</td> <td>p.P260PfsX77</td> <td>Exon6</td> <td>Severe</td> <td>Novel</td>	43		c.780 793del14	p.P260PfsX77	Exon6	Severe	Novel
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	44		c 822delA	n F274FfsX4	Fron6	Severe	Novel
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	45		a 011delT	p.122/ 1116111	Exon7	Severe	Novel
46c.127/40e1Cp. P425LfsExon9SevereReported47c.1444_1445inTTp. H4282F6X1Exon9SevereReported48c.1122C > Tp. G374GExon8AttenuatedReported20c.1122C > Tp. G374GExon8AttenuatedReported49c.1122C > Tp. G374GExon8AttenuatedReported50c.241-2A > GIntron3SevereReported51c.419-2A > TIntron3SevereReported52c.880-1G > CIntron6AttenuatedReported53c.112_123delCTTp.140delExon2SevereReported54c.118_120delCTTp.140delExon3AttenuatedReported55c.121_123delCTCp.141delExon3SevereNovel56c.181_1192 del12p.6394_398delinsVExon9SevereNovel57c.118_120delCTTp.464_69int3Exon9SevereNovel58c.131_132 del12p.6394_398delinsVExon3-6SevereNovel59Small insertionIntron5SevereNovelNovel50EX3_6delExon3-6SevereNovel60EX3_6delExon3-6SevereNovel61EX4_7delExon4-7SevereReported62EX1_3delExon4-7SevereReported63EX1_9delExon1-9SevereReported64EX1_9delExon4-7	40		1074110	p. v 504015A12	EXUII/	Severe	novei
47c.1444_1445intTTp.L482FfsX1Exon 9SevereReportedSplicing48c.1122C > Tp.G374GExon 8AttenuatedReported49c.1122C > Tp.G374GExon 8AttenuatedReported50c.241-2A > G[20 amino acid deletion]Intron 2SevereReported51c.419-2A > TIntron 3SevereReported52c.880-1G > CIntron 6AttenuatedReported53c.880-6A > GIntron 6AttenuatedReported54c.118_120delCTTp.I40delExon 2SevereNovel55c.121_123delCTCp.P60delExon 2SevereNovel56c.285_287delGAGp.R60delExon 3SevereNovel57c.118_1192 del12p.G394_398delinsVExon 9SevereNovel58Exon 4Exon 4SevereNovelNovel59c.1404_1405int9(TATCCCCGG)p.468_469int3Exon 1-3SevereNovel60EX1,3delExon 4SevereNovelSevereNovel61EX1,3delEX1,3delExon 4SevereNovel62EX3,5delEX0,404SevereReported63EX1,9delExon 4SevereNovel63EX1,9delExon 4SevereReported63EX1,9delExon 4SevereReported63EX1,9delExon 4SevereReported	46		c.1274delC	p. P425Lfs	Exon9	Severe	Reported
$\begin{tabular}{ c c c } & $ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $ $	47		c.1444_1445intTT	p.L482FfsX1	Exon9	Severe	Reported
A8 c.1122C > T p.G374G (20 amino acid deletion) 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 (.419-2A > T Intron3 Severe Reported 51 c.419-2A > T Intron3 Severe Reported 52 c.880-1G > C Intron6 Attenuated Reported 53 c.880-4B > G Intron6 Attenuated Reported 54 c.118_120delCTT p.140del Exon3 Severe Novel 56 c.285_287delGAG p.R96del Exon3 Attenuated Reported 57 c.118_1192 del12 p.6394_398delinsV Exon9 Severe Novel 58 c.1404_1405int9(TATCCCCGG) p.468_469int3 Exon3-3 Severe Novel 59 f.160 EX3_5del P.468_469int3 Exon1-3 Severe Novel 60 EX3_5del EX3_5del Exon-4 Severe <td></td> <td>Calising</td> <td></td> <td></td> <td></td> <td></td> <td></td>		Calising					
48c.1122C > 1p.G374GExon8AttenuatedReported (20 (20 amino acid deletion)49c.1122C > Tp.G374GExon8AttenuatedReported (20 (20 amino acid deletion)50c.241-2A > GIntron2SevereReported51c.419-2A > TIntron3SevereReported52c.880-1G > CIntron6AttenuatedReported53c.880-8A > GIntron6AttenuatedReported54c.118_120delCTTp.L40delExon2SevereReported55c.121_123delCTCp.L40delExon2SevereReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.118_1192 del12p.G34_398delinsVExon9SevereNovel58c.1104_1405in9(TATCCCCGG)p.468_469int3Exon3-6SevereNovel59EX1_3delExon3-6SevereNovelNovel60EX3_6delEX01-3SevereNovelReported61EX1_9delExon3-6SevereReportedReported63EX1_9delExon3-6SevereReportedReported63EX1_9delExon3-9SevereReported63EX1_9delExon3-9SevereReported63EX1_9delExon3-9SevereReported63EX1_9delExon3-9SevereReported64EX1_9delExon3-9SevereReported<	10	opnenig	11000	- 00740	F 0	A 41 - 1 - 1	P 1
49 c.1122C > T p.0374G c.080 Attenuated Reported 50 c.241-2A > G Intron2 Severe Reported 51 c.419-2A > T Intron3 Severe Reported 52 c.419-2A > T Intron3 Severe Reported 53 c.419-2A > T Intron3 Severe Reported 53 c.880-16 > C Intron6 Attenuated Reported 54 c.880-8A - G Intron6 Revere Novel 55 c.118_120deICTT p.140del Exon2 Severe Novel 55 c.121_123deICTC p.141del Exon3 Attenuated Reported 56 c.121_1192 del12 p.896delinsV Exon3 Netenuated Novel 57 c.118_1192 del12 p.468_469int3 Exon9 Severe Novel 58 c.190_114050int9(TATCCCCGG) p.468_469int3 Exon3-6 Severe Novel 59 Exon4 Severe Novel Severe	48		c.1122C > T	p.G374G	Exon8	Attenuated	Reported
49c.1122C > Tp.G374G (20 amino acid deletion)Exon8AttenuatedReported50c.241-2A > GIntron3SevereReported51c.419-2A > TIntron6SevereReported52c.880-1G > CIntron6AttenuatedReported53c.880-8A > GIntron6AttenuatedReported54c.118_120delCTTp.L40delExon2SevereReported55c.121_123delCTCp.L40delExon2SevereReported56c.252_527delCAGp.R96delExon3SevereReported57c.118_1192 del12p.G394_398delinsVExon9SevereNovel58c.104_1405int9(TATCCCCGG)p.468_469int3Exon3SevereNovel59EX1_3delFExon3-6SevereNovel60EX3_6delEX3_6delExon3-6SevereNovel61EX4_7delEX1_9delExon3-6SevereReported62EX1_9delEX1_9delExon3-6SevereNovel63EX1_9delExon3-6SevereReported63EX1_9delExon3-9SevereReported63EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported </td <td></td> <td></td> <td></td> <td>(20 amino acid deletion)</td> <td></td> <td></td> <td></td>				(20 amino acid deletion)			
(20 amino acid deletion)50c.241-2A > GIntron2SevereReported51c.419-2A > TIntron3SevereReported52c.880-1G > CIntron6AttenuatedReported53c.880-8A > GIntron6AttenuatedReported54c.118_120delCTTp.L40delExon2SevereReported55c.121_123delCTCp.L41delExon2SevereReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.1181_1192 delI2p.G394_398delinsVExon9SevereNovel58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovel59EX1_3delFSevereNovelSovereNovel60EX3_6delEX3_6delExon3-6SevereNovel61EX4_7delEX04-7SevereReported63EX1_9delEX1_9delExon1-9SevereReported63EX1_9delEX1_9delExon1-9SevereReported63EX1_9delEX1_9delExon1-9SevereReported	49		c.1122C > T	p.G374G	Exon8	Attenuated	Reported
$\begin{array}{c c c c c c c c c c c c c c c c c c c $				(20 amino acid deletion)			
50CATAPATIONAlt portedIntronzSevereReported51CATAPATIONIntronIntronzSevereReported52C.880-1G > CIntron6AttenuatedReported53C.880-8A > GIntron6AttenuatedReportedSmall deletion54C.118_120delCTTp.L40delExon2SevereReported55C.121_123delCTCp.L41delExon2SevereReported56C.285_287delGAGp.R96delExon3AttenuatedReported57C.1181_1192 del12p.G394_398delinsVExon9SevereNovel58C.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovelGross deletion59EX1_3delExo1-3SevereNovel60EX3_6delEX3_6delExon3-6SevereNovel61EX4_7delExon4-7SevereReported62EX1_9delExo1-9SevereReported63EX1_9delExon1-9SevereReported	50		c 241-2A > G		Intron?	Severe	Reported
51 $c.419-2A > 1$ Intron3SevereReported52 $c.880-1G > C$ Intron6AttenuatedReported53 $c.880-8A > G$ Intron6AttenuatedReportedSmall deletion54 $c.118_120$ delCTT $p.L40$ delExon2SevereNovel55 $c.121_123$ delCTC $p.L41$ delExon2SevereReported56 $c.285_287$ delGAG $p.R96$ delExon3AttenuatedReported57 $c.1181_1192$ del12 $p.G394_398$ delinsVExon9SevereNovel58 $c.1404_1405$ int9(TATCCCCGG) $p.468_469$ int3Exon9SevereNovelSmall insertionEX1_3del $Lxon3-6$ SevereNovel60EX3_6 delEx3_6 delExon3-6SevereNovel61EX3_6 del $Lxon4-7$ SevereNovel6162EX1_9 del $Lxon1-9$ SevereReported63EX1_9 del $Lxon1-9$ SevereReported	50		$C_{241-2A} > 0$			Severe	Reported
52c.880-1G > C c.880-8A > GIntron6AttenuatedReported53c.880-8A > GIntron6AttenuatedReportedSmall deletion54c.118_120delCTTp.L40delExon2SevereNovel55c.121_123delCTCp.L41delExon2SevereReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.1181_1192 del12p.G394_398delinsVExon9SevereNovel58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovelSmall insertion59Exo1-3SevereNovel60EX1_3delExon3-3SevereNovel61EX4_7delExon4-7SevereNovel62EX1_9delExon4-7SevereReported63EX1_9delExon1-9SevereReported	51		c.419-2A > 1		intron3	Severe	Reported
53c.880-8A > GIntron6AttenuatedReportedSmall deletion	52		c.880-1G > C		Intron6	Attenuated	Reported
Small deletionSmall deletionSmall deletion54c.118_120delCTTp.L40delExon2SevereNovel55c.121_123delCTCp.L41delExon2SevereReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.118_11192 del12p.G394_398delinsVExon9SevereNovel58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovel59EX1_3delSevereNovelSevereNovel60EX3_6delExon3-66SevereNovel61EX4_7delExon4-77SevereReported62EX1_9delExon1-90SevereReported63EX1_9delExon1-90SevereReported	53		c.880-8A > G		Intron6	Attenuated	Reported
Sinal deferiorc.118_120delCTTp.L40delExon2SevereNovel55c.121_123delCTCp.L41delExon2SevereReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.118_11192 del12p.G394_398delinsVExon9SevereNovel58c.1404_1405int9(TATCCCGG)p.468_469int3Exon9SevereNovelSevereEX1_3delSevereNovelSevereEX1_3delSevereNovel60EX3_6delExon3-66SevereNovel61EX4_7delExon4-77SevereReported62EX1_9delExon4-79SevereReported63EX1_9delExon4-9SevereReported		Concell of the state of					
54c.118_120delCTTp.L40delExon2SevereNovel55c.121_123delCTCp.L41delExon2SevereReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.1181_1192 del12p.G394_398delinsVExon9SevereNovelSmall insertion58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovelSovereNovel59Exon1-3SevereNovel60EX1_3delExon3-66SevereNovel61EX4_7delExon4-77SevereReported62EX1_9delExo1-90SevereReported63EX1_9delExon1-90SevereReported		Small deletion			_		
55c.121_123delCTCp.L41delExon2SevereReported56c.285_287delGAGp.R96delExon3AttenuatedReported57c.1181_1192 del12p.G394_398delinsVExon9SevereNovelSmall insertion58c.1404_1405int9(TATCCCGG)p.468_469int3Exon9SevereNovel59Exon1-3SevereNovel59EX1_3delExon3-60SevereNovel60EX3_6delEx3_6delExon4-70SevereNovel61EX4_7delExon4-70SevereReported62EX1_9delExon1-90SevereReported63EX1_9delExon1-90SevereReported	54		c.118_120delCTT	p.L40del	Exon2	Severe	Novel
56c.285_287delGAGp.R96delExon3AttenuatedReported57c.1181_1192 del12p.G394_398delinsVExon9SevereNovel58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovel59EX1_3delsevereNovel60EX3_6delExon3-66SevereNovel61EX4_7delExon4-77SevereReported62EX1_9delExo1-90SevereReported63EX1_9delExon1-90SevereReported	55		c.121_123delCTC	p.L41del	Exon2	Severe	Reported
57c.1181_1192 del12p.G394_398delinsVExon9SevereNovelSmall insertion58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovelGross deletion59EX1_3delExon9SevereNovel60EX3_6delExon3-6SevereNovel61EX4,7delExon4-7SevereReported62EX1_9delExo1-9SevereReported63EX1_9delExon1-9SevereReported	56		c.285 287delGAG	p.R96del	Exon3	Attenuated	Reported
Small insertion Exons Exons Severe Novel 58 c.1404_1405int9(TATCCCCGG) p.468_469int3 Exon9 Severe Novel Gross deletion 59 EX1_3del Exon3-6 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	57		c 1181 1192 del12	n G394 398delineV	Evon9	Severe	Novel
Small insertionExon9SevereNovel58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovelGross deletion59Exo1-3SevereNovel60EX3_6delExon3-66SevereNovel61EX4_7delExon4-77SevereReported62EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported			C.1101_11/2 (C112	p.007 [_070definsv	LAUIT	DEVEL	110101
58c.1404_1405int9(TATCCCCGG)p.468_469int3Exon9SevereNovelGross deletion59EX1_3delExon1-3SevereNovel60EX3_6delExon3-66SevereNovel61EX4_7delExon4-77SevereReported62EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported		Small insertion					
Gross deletion 59 EX1_3del EX1_3del Exon3-6 Severe Novel 60 EX3_6del EX4_7del Exon3-6 Severe Reported 61 EX4_7del Exon4-7 Severe Reported 62 EX1_9del EX1_9del Exon1-9 Severe Reported	58		c.1404 1405int9(TATCCCCGG)	p.468 469int3	Exon9	Severe	Novel
Gross deletion59EX1_3delExon1-3SevereNovel60EX3_6delExon3-6SevereNovel61EX4_7delExon4-7SevereReported62EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported				r · · · · = · · · · · · · · · ·			
59EX1_3delExon1-3SevereNovel60EX3_6delExon3-6SevereNovel61EX4_7delExon4-7SevereReported62EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported		Gross deletion					
60EX3.6delExon3-6SevereNovel61EX4.7delExon4-7SevereReported62EX1.9delExon1-9SevereReported63EX1.9delExon1-9SevereReported	59		EX1_3del		Exon1–3	Severe	Novel
61EX4,7delExon4-7SevereReported62EX1,9delExon1-9SevereReported63EX1,9delExon1-9SevereReported	60		EX3 6del		Exon3-6	Severe	Novel
61EXQ.42/dE1Exon4-/SevereReported62EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported	61		EV4 7dol		Ever 4 7	Course	Domonto J
62EX1_9delExon1-9SevereReported63EX1_9delExon1-9SevereReported	01		EA4_/ del		Exon4-/	Severe	Reported
63 EX1_9del Exon1-9 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 Wild-type 242A > G(Q81R) 328A > G(R110G) 457 T > C(W153R) 1047C > A(S349R)	$\begin{array}{r} 31.51 \pm 6.23 \\ 978.29 \pm 68.10 \\ 34.10 \pm 3.84 \\ 30.71 \pm 4.13 \\ 33.64 \pm 9.15 \\ 32.85 \pm 3.26 \end{array}$	3.2 100.0 3.5 3.1 3.4 3.4
118_120delCTT(L40del) 1404_1405ins9(468_469int3) 748_749delGCinsT(A250Tfsx28)	31.30 ± 4.70 34.54 ± 6.38 28.38 ± 2.89	3.2 3.5 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, wildtype (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 Cterminus. 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, al-though 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.

Acknowledgments

This work was supported by Research Grants from Guangdong Science and Technology Department (CN) (2014A020212016) and National Natural Science Foundation of China (CN) (81671119).

References

- R. Martin, M. Beck, C. Eng, R. Giugliani, P. Harmatz, V. Muñoz, J. Muenzer, Recognition and diagnosis of Mucopolysaccharidosis II (Hunter syndrome), Pediatrics 121 (2008) e377–e385.
- [2] E.F. Neufeld, J. Muenzer, The mucopolysaccharidoses, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, et al. (Eds.), The Metabolic and Molecular Basis of Inherited Disease, 8th ed., Vol. III Mc-Graw-Hill; Medical Publishing Division, New York, NY, 2001, pp. 3421–3452.
- [3] J.E. Wraith, M. Beck, R. Giugliani, J. Clarke, R. Martin, J. Muenzer, HOS investigators, initial report from the Hunter outcome survey, Genet. Med. 10 (2008) 508–516.
- [4] E. Vafiadaki, A. Cooper, L.E. Heptinstall, C.E. Hatton, M. Thornley, J.E. Wraith, Mutation analysis in 57 unrelated patients with MPS II (Hunter disease), Arch. Dis. Child. 79 (1998) 237–241.
- [5] S. Al-Sawaf, E. Mayatepek, B. Hoffmann, Neurological findings in Hunter disease: pathology and possible therapeutic effects reviewed, J. Inherit. Metab. Dis. 31 (2008) 473–480.
- [6] L. Dvorakova, H. Vlaskova, A. Sarajlija, D.P. Ramadza, H. Poupetova, E. Hruba, A. Hlavata, V. Bzduch, K. Peskova, G. Storkanova, B. Kecman, M. Djordjevic, I. Baric, K. Fumic, I. Barisic, M. Reboun, J. Kulhanek, J. Zeman, M. Magner, Genotype-phenotype correlation in 44 Czech, Slovak, Croatian and Serbian patients with mucopolysaccharidosis type II, Clin. Genet. 91 (2017) 787–796.
- [7] I.V. Schwartz, M.G. Ribeiro, J.G. Mota, M.B. Toralles, P. Correia, D. Horovitz, E.S. Santos, I.L. Monlleo, A.C. Fett-Conte, R.P. Sobrinho, D.Y. Norato, A.C. Paula, C.A. Kim, A.R. Duarte, R. Boy, E. Valadares, M. De Michelena, P. Mabe, C.D. Martinhago, J.M. Pina-Neto, F. Kok, S. Leistner-Segal, M.G. Burin, R. Giugliani, A clinical study of 77 patients with mucopolysaccharidosis type II, Acta Paediatr. 96 (2007) 63–70.
- [8] M.A. Chiong, D.M. Canson, M.A. Abacan, M.M. Baluyot, C.P. Cordero, C.L. Silao, Clinical, biochemical and molecular characteristics of Filipino patients with mucopolysaccharidosis type II - Hunter syndrome, Orphanet. J. Rare Dis. 12 (2017) 7.
- [9] R. Froissart, I.M. Da Silva, I. Maire, Mucopolysaccharidosis type II: an update on mutation spectrum, Acta Paediatr. 96 (2007) 71–77.
- [10] J.E. Wraith, M. Scarpa, M. Beck, O.A. Bodamer, L. De Meirleir, N. Guffon, A. Meldgaard Lund, G. Malm, A.T. Van der Ploeg, J. Zeman, Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy, Eur. J. Pediatr. 167 (2008) 267–277.
- [11] Y.V. Voznyi, J.L. Keulemans, O.P. van Diggelen, A fluorimetric enzyme assay for the diagnosis of MPS II (Hunter disease), J. Inherit. Metab. Dis. 24 (2001) 675–680.
- [12] M.L. Bondeson, N. Dahl, H. Malmgren, W.J. Kleijer, T. Tönnesen, B.M. Carlberg, U. Pettersson, Inversion of the IDS gene resulting from recombination with IDSrelated sequences is a common cause of the Hunter syndrome, Hum. Mol. Genet. 4 (1995) 615–621.
- [13] M. Rathmann, S. Bunge, C. Steglich, E. Schwinger, A. Gal, Evidence for an iduronate-sulfatase pseudogene near the functional Hunter syndrome gene in Xq27.3-

q28, Hum. Genet. 95 (1995) 34-38.

- [14] K.M. Timms, F. Lu, Y. Shen, C.A. Pierson, D.M. Muzny, Y. Gu, D.L. Nelson, R.A. Gibbs, 130 kb of DNA sequence reveals two new genes and a regional duplication distal to the human iduronate-2-sulfate sulfatase locus, Genome Res. 5 (1995) 71–78.
- [15] K. Sukegawa-Hayasaka, Z. Kato, H. Nakamura, S. Tomatsu, T. Fukao, K. Kuwata, T. Orii, N. Kondo, Effect of Hunter disease (mucopolysaccharidosis type II) mutations on molecular phenotypes of iduronate-2-sulfatase: enzymatic activity, protein processing and structural analysis, J. Inherit. Metab. Dis. 29 (2006) 755–761.
- [16] G. Gray, P. Claridge, L. Jenkinson, A. Green, Quantitation of urinary glycosaminoglycans using dimethylene blue as a screening technique for the diagnosis of mucopolysaccharidoses: an evaluation, Ann. Clin. Biochem. 44 (2007) 360–363.
- [17] J.G. de Jong, R.A. Wevers, R. Liebrand-van Sambeek, Measuring urinary glycosaminoglycans in the presence of protein: an improved screening procedure for mucopolysaccharidoses based on dimethylmethylene blue, Clin. Chem. 38 (1992) 803–807.
- [18] Y.L. Huang, S.Y. Li, X.Y. Zhao, L.P. Fan, W.C. Lin, Z.H. Zhou, J. Cheng, L. Liu, Enzymatic diagnosis and clinical characteristics of 52 children with mucopolysaccharidosis, Zhongguo Dang Dai Er Ke Za Zhi 14 (2012) 510–514.
- [19] R. Froissart, I. Maire, G. Millat, S. Cudry, A.M. Birot, V. Bonnet, O. Bouton, D. Bozon, Identification of iduronate sulfatase gene alterations in 70 unrelated Hunter patients, Clin. Genet. 53 (1998) 362–368.
- [20] P.N. Cobos, C. Steglich, R. Santer, Z. Lukacs, A. Gal, Dried blood spots allow targeted screening to diagnose mucopolysaccharidosis and mucolipidosis, JIMD Rep. 15 (2015) 123–132.
- [21] P. Li, A.B. Bellows, J.N. Thompson, Molecular basis of iduronate-2-sulphatase gene mutations in patients with mucopolysaccharidosis type II (Hunter syndrome), J. Med. Genet. 36 (1999) 21–27.
- [22] H. Zhang, J. Li, X. Zhang, Y. Wang, W. Qiu, J. Ye, L. Han, X. Gao, X. Gu, Analysis of the IDS gene in 38 patients with Hunter syndrome: the c.879G > A (p.Gln293Gln) synonymous variation in a female create exonic splicing, PLoS ONE 6 (2011)

e22951.

- [23] M. Demydchuk, C.H. Hill, A. Zhou, G. Bunkóczi, P.E. Stein, D. Marchesan, J.E. Deane, R.J. Read, Insights into Hunter syndrome from the structure of iduronate -2- sulfatase, Nat. Commun. 8 (2017) 15786.
- [24] S. Bunge, C. Steglich, M. Beck, W. Rosenkranz, E. Schwinger, J.J. Hopwood, A. Gal, Mutation analysis of the iduronate-2-sulfatase gene in patients with mucopolysaccharidosis type II (Hunter syndrome), Hum. Mol. Genet. 1 (1992) 335–339.
- [25] M. Kosuga, R. Mashima, A. Hirakiyama, N. Fuji, T. Kumagai, J.H. Seo, M. Nikaido, S. Saito, K. Ohno, H. Sakuraba, T. Okuyama, Molecular diagnosis of 65 families with mucopolysaccharidosis type II (Hunter syndrome) characterized by 16 novel mutations in the IDS gene: Genetic, pathological, and structural studies on iduronate-2-sulfatase, Mol. Genet. Metab. 118 (2016) 190–197.
- [26] Y.B. Sohn, C.S. Ki, C.H. Kim, A.R. Ko, Y.J. Yook, S.J. Lee, S.J. Kim, S.W. Park, S. Yeau, E.K. Kwon, S.J. Han, E.W. Choi, S.Y. Lee, J.W. Kim, D.W. Jin, Identification of 11 novel mutations in 49 Korean patients with mucopolysaccharidosis type II, Clin. Genet. 81 (2012) 185–190.
- [27] D.A. Chistiakov, L.M. Kuzenkova, K.V. Savosťanov, A.K. Gevorkyan, A.A. Pushkov, A.G. Nikitin, N.D. Vashakmadze, N.V. Zhurkova, T.V. Podkletnova, L.S. Namazova-Baranova, A.A. Baranov, Genetic analysis of 17 children with Hunter syndrome: identification and functional characterization of four novel mutations in the iduronate-2-sulfatase gene, J. Genet. Genomics. 41 (2014) 197–203.
- [28] J.H. Chang, S.P. Lin, S.C. Lin, K.L. Tseng, C.L. Li, C.K. Chuang, G.J. Lee-Chen, Expression studies of mutations underlying Taiwanese Hunter syndrome (mucopolysaccharidosis type II), Hum. Genet. 116 (2005) 160–166.
- [29] L. Gort, A. Chabás, M.J. Coll, Hunter disease in the Spanish population: molecular analysis in 31 families, J. Inherit. Metab. Dis. 21 (1998) 655–661.
- [30] E. Piotrowska, J. Jakóbkiewicz-Banecka, A. Tylki-Szymańska, B. Czartoryska, A. Wegrzyn, G. Wegrzyn, Correlation between severity of mucopolysaccharidoses and combination of the residual enzyme activity and efficiency of glycosaminoglycan synthesis, Acta Paediatr. 98 (2009) 743–749.