Mucopolysaccharidosis type II: Identification of 30 novel mutations among Latin American patients

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ABSTRACT

In this study, 103 unrelated South-American patients with mucopolysaccharidosis type II (MPS II) were investigated aiming at the identification of iduronate-2-sulfatase (*IDS*) disease causing mutations and the possibility of some insights on the genotype–phenotype correlation. The strategy used for genotyping involved the identification of the previously reported inversion/disruption of the *IDS* gene by PCR and screening for other mutations by PCR/SSCP. The exons with altered mobility on SSCP were sequenced, as well as all the exons of patients with no SSCP alteration. By using this strategy, we were able to find the pathogenic mutation in all patients. Alterations such as inversion/disruption and partial/total deletions of the *IDS* gene were found in 20/103 (19%) patients. Small insertions/deletions/indels (<22 bp) and point mutations were identified in 83/103 (88%) patients, including 30 novel mutations; except for a higher frequency of small duplications in relation to small deletions, the frequencies of major and minor alterations found in our sample are in accordance with those described in the literature.

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1. Introduction

Mucopolysaccharidosis type II (MPS II, McKusick 309900) is an X-linked recessively inherited lysosomal storage disorder (LSD), resulting from deficiency of iduronate-2-sulfatase activity (IDS, EC 3.1.6.13). *IDS* is involved in the degradation of glycosaminoglycans

(GAGs) dermatan sulfate and heparan sulfate (DS–HS). Failure to hydrolyze the terminal iduronate-2-sulfate esters in these GAGs results in progressive accumulation of undegraded substrates within the lysosomes and in the clinical manifestations associated to MPS II [1].

The *IDS* gene is located on the Xq27/28 boundary of the long arm of the X-chromosome, comprising 9 exons spanning approximately 24 kb [2]. A pseudogene (*IDS2*), containing sequences homologous to exon II, intron 2, exon III and a chimerical intron 3–intron 7, is located approximately 20 kb far, telomeric to the active *IDS* gene, which makes this region prone to the occurrence of recombination events. Approximately 10–20% of MPS II patients present large

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gene alterations, including rearrangements and total *IDS* gene deletions, while 80–90% of them present small gene alterations [3,4]. According to the Human Gene Mutation Database [5], 479 different mutations in the *IDS* gene have been described until July 2013, most of which being private point mutations (52%) or small deletions (17%).

MPS II shows not only wide allelic heterogeneity, but also wide phenotypical heterogeneity. Patients are usually classified as having the severe, intermediate, or attenuated forms, depending mainly on the degree of mental retardation present [1,4,6]. In general, it seems that patients with the more severe forms are diagnosed earlier, have mental retardation that is evident by the age of 4–6 years, and are dead by the age of 10–15 years, while patients with the attenuated forms present normal intelligence and have a longer life expectancy [7,14]. However, as there is no standardized scoring index of severity for MPS II, this classification is not always reliable, especially in the case of patients diagnosed during childhood [4]. Consequently, studies of the genotype–phenotype correlation are subject to various biases. As simple Mendelian disorders are in fact complex traits [8], it is likely that genotype–phenotype correlation does not exist for several *IDS* gene mutations [4].

In the present study, we have investigated 103 unrelated South-American MPS II patients in order to characterize their genotypes.

2. Material and methods

The Molecular Genetics Laboratory of the Medical Genetics Service of Hospital de Clínicas de Porto Alegre, Brazil, is a reference center for MPS diagnosis and receives samples from all over Brazil and from many Latin American countries. The indication for molecular analysis was based on clinical criteria, abnormal urinary GAG excretion and deficient *IDS* activity in dried blood, plasma, leucocytes or fibroblasts. In all cases, the possibility of multiple sulfatase deficiency was excluded by the measurement of the activity of another sulfatase. Regarding the origin of the 103 patients, 91/103 were from Brazil, 4/103 were from Chile, 2/103 were from Peru, 1/103 was from Paraguay, 2/103 were from Argentina, 1/103 was from Cuba and 2/103 were from Bolivia. DNA samples from 66 mothers and 22 affected brothers/cousins of the probands were also analyzed. This study was approved by the Institutional Review Board and by the National Research Ethics Committee of Brazil (project #100066).

Genomic DNA of patients/relatives was extracted from blood following the salt precipitation method [9] and preserved in a Tris–EDTA 0.1 M solution (Tris–HCl (10 mM, pH 7.5) and EDTA (1 mM, pH 8.0)). Samples were first analyzed through PCR/RFLP for the presence of the inversion/disruption of the *IDS* gene caused by intrachromosomal recombination between intron 7 of the gene and its homologous region on the pseudogene, according Lualdi et al. [10]. Samples with no evidence of the *IDS*–*IDS2* recombination were analyzed by PCR/SSCP throughout the 9 exons and intron–exon boundaries of the *IDS* gene.

The PCR amplifications were performed using 5 µL of buffer concentrated 10× (200 mM Tris HCl (pH 8.0); 500 mM KCl), 0.2 mM dNTPs, 1.5–3.0 mM MgCl₂, 0–6% of DMSO (Sigma), 1 U of Taq DNA Polymerase (Invitrogen), and 20 pm of each primer in a final reaction volume of 50 µL. PCR conditions were as follows: 94 °C for 5 min; 34 cycles of 94 °C for 40 s, 50.5 °C–61 °C for 40 s (based on each exon) and 72 °C for 40 s; and a final extension time of 72 °C for 10 min. The primer sequences used for amplification of all the *IDS* exons and for the identification of the inversion/disruption events have been previously described [11–13]. Exon III was amplified using nested-PCR (amplification with primers 2A and 3B followed by amplification with primers 3A and 3B) [12]. This extra step prevents the amplification of the pseudogene sequence.

Two negative PCRs for one determined exon of the *IDS* gene were considered as indicative of deletion of this exon. In the case of total

deletions, DNA integrity was also tested by simultaneous amplification of the α-1-antitrypsin gene.

SSCP was used to screen samples, using mutation detection enhancement gels (BMA) ranging from 0.7 to 1×. The electrophoresis was carried out at 160 V for 16–24 h at room temperature.

The samples showing mobility shifts on SSCP were sequenced on ABI Prism™ 3500 Genetic Analyzer (Applied Biosystem). Mutations were confirmed on a second PCR product by the sequencing of both strands using the same methodology. In the case of novel missense mutations, 100 control alleles were analyzed in order to confirm the pathogenicity of the identified mutation, and bioinformatics analyses were performed using the Polyphen software. The samples of patients that did not show any alteration on SSCP were submitted to sequencing of the 9 *IDS* exons including exon/intron boundaries, according to the laboratory protocol.

After the identification of mutation in the DNA sample from the index case, family members were tested by restriction endonuclease analysis, PCR (mothers of patients presenting the inversion/disruption), SSCP or sequencing.

3. Results

3.1. Detection of large gene alterations (n: 20/103) (Table 1)

The recombination between the *IDS* gene and pseudogene (*IDS2*), which causes a disruption of the *IDS* gene and an inversion of the intervening region, was found in 13/103 (12%) patients (patients H1–H13). Four patients (H14–H17) appear to have partial deletion of the *IDS* gene, after exon by exon amplification. Other three patients (H18–H20) showed a total deletion of the *IDS* gene and we performed whole-genome array-Comparative Genomic Hybridization (Array-CGH) to delineate the deletion breakpoints and to characterize the deletion extension; the deletion showed in the 3 patients extended from the proximal *IDS* region towards contiguous genes. The results were described previously by our group [14]. All of these patients have neurological impairment.

3.2. Identification of small gene alterations (n: 83/103) (Tables 2 and 3)

Amplification, SSCP analysis, and sequencing of exons I to IX of the *IDS* gene allowed for the identification of a small alteration (<22 bp) in 83/103 patients. The success rate found for SSCP screening was 72%. All the missense point mutations were considered to be causative for the disease since the mutation was not found in 100 alleles of unaffected and unrelated controls and bioinformatic analyses, performed by Polyphen v.2, predicted that these mutations are probably damaging (score > 2), it is with high confidence supposed to affect protein function/structure or possibly damaging (score 1, 5–20) it is supposed to affect protein function or structure (Table 1). The multiple alignment in different species showed that mutations involve a highly conserved gene sequence (Table 2).

We have found 57 different small gene mutations in 83 patients. Data on the novel (n: 30) and recurrent mutations (n: 27) found in our sample are shown in Tables 1 and 2. Only 8 mutations were found in at least two unrelated patients: p.R88C, p.Y103X, p.W109X, p.S333L, c.1122C>T, p.R443X, p.P467L and p.R468W. Intronic mutations were

Table 1
Characterization of large *IDS* gene alterations. Inversion and total/partial deletion (n: 20).

Patient	Large gene alteration	Reference
H1–H13	<i>IDS/IDS2</i> inversion	Present work
H14	Deletion of exons V, VI and VII	Present work
H15	Deletion of exons IV, V, VI, VII and VIII	Present work
H16	Deletion of exons V, VI and VII	Present work
H17	Deletion of exons IV, V, VI, VII, VIII and IX	Present work
H18, H19, H20	Total deletion of <i>IDS</i> gene	[14]

Table 2Characterization of the novel *IDS* gene mutations described in this study. Insertions, duplications, and deletions (n: 7).

Patient	Exon	Mutation (cDNA position) ^a	Conserved region among <i>IDS</i> murine protein [15]		Protein alteration	Comments	
H21	I	c.124_129dupATCATC		Yes	Insertion of two aa (ile)	GG preceding the deleted G	
H22	II	c.155delG		Yes	fs; 7 altered aa; truncated		
H23	VI	c.805_808dupGACA		Yes	fs; 25 altered aa		
H24	Intron VII	IVS7-16_IVS7-9 del gcttttta ins GGTGGGCTCTAGG		Yes	Splice site mutation	The sequence inserted is similar to a close sequence	
H25	VIII	c.1134_1152dupCCCTTACCTCGACCCCTTT	Yes		fs; 5 altered aa; truncated		
H26	IX	c.1300_1306dupGAAGGCA	Not highly		fs; 12 altered aa; truncated	1 patient already described having a deletion in the same region: GAGAAGGCA [16]	
H27	IX	c.1349_1364delATCCGTACCTCCCTGG	No		fs; 5 altered aa; truncated		
Point mutations (n: 23)							
Patient	Exon	Mutation	cDNA position ^a	Sequence change	Conserved codon among human and murine <i>IDS</i> [15]	PSIC profile scores for two amino acid variants [17] ^b	Comments
H28	II	p.D45V	134	GAT to GTT	Yes	2.980	D45N [18]
H29	II	p.D45G	134	GAT to GGT	Yes (catalytic site)	2.530	D45N [18]
H30	II	p.S61Y	180	TCC to TAC	Yes	2.245	No mutation described in this codon yet
H31	II	p.Q80R	239	CAG to CGG	Yes (near catalytic site)	2.306	Splice site mutation; Q80X [18,19]
H32	II	p.Q81Y	240	CAA to TAA	Yes	2.475	No mutation described in this codon yet
H33	III	p.C84Y	250	TGC to TAC	Yes	3.681	C84X [18]
H34	III	p.W109X	325	TGG to TGA	Yes	–	W109K [20]
H35	III	p.W109X	325	TGG to TGA	Yes	–	Same as above
H36	III	p.R95S	409	AGG to AGC	Yes (catalytic site)	2.355	R95T, R95G [12,21]
H37	III	p.H138Y	411	CAC to TAC	Yes (catalytic site)	2.667	H138D [16]
H38	III	c.417G>C	417	CCTGgt to CCTCgt	Yes (catalytic site)	–	Splice site mutation
H39	IV	p.P160H	479	CCT to CAT	Yes	2.775	P160R [22]
H40	IntronV	c.709-2 ^b >T	709-2	ccaagGAA to ccatggaa	Yes	–	Splice site mutation; 709-2A>G [23]
H41	VI	p.Q239X	715	CAG to TAG	Yes	–	No mutation described in this codon yet
H42	VI	p.N265K	793	AAc to AAG	Yes	1.817	N265I [3]
H43	VII	p.L314H	941	CTC to CAC	Yes	2.446	L314P [24]
H44	VII	p.D308H	922	GAT to CAT	Yes	2.530	D308N, D308E [24,25]
H45	VII	p.D334Y	1000	GAT to TAT	Yes	2.980	D334N, D334G [16,26]
H46	VII	p.D334V	1000	GAT to GTT	Yes	2.980	D334G,D334N [16,26]
H47	VIII	c.1007G>T	1007	tagGG to tagTG	Yes	–	Splice site mutation; 1007G>A, 1006G>A [16,23,26]
H48	VIII	p.H342P	1025	CAT to CTT	Yes	3.552	H342Y, H342Q [27,28]
H49	VIII	p.E344K	1031	GAA to AAA	Yes	1.833	No mutation described in this codon yet
H50	IX	p.V503D	1508	GTT to GAT	Yes	2.038	Same as above
H51	IX	p.Y536X	1608	TAT to TAA	Yes	–	Same as above

aa: amino acid; fs: frameshift.

^a cDNA numbering follows [29], beginning with the A of the initiation codon. Gene sequence follows GenBank entry M58342.^b Position specific independent counts (PSIC) using Polyphen program; probably damaging (score > 2), it is with high confidence supposed to affect protein function or structure; possibly damaging (score 1, 5–20), it is supposed to affect protein function or structure.

found in 3/100 (3%) patients, all of which involving the consensus splice donor or acceptor site.

Regarding the type of the 57 different small alterations found, 2/57 (3.5%) were deletions, 2/57 (3.5%) were indels, 4/57 (7.0%) were duplications, and 49/57 (85%) were point mutations. Among point mutations, 7/49 (10.6%) were splice site, 10/49 (21.2%) were nonsense, and 32/49 (68%) were missense mutations.

In 23/103 (22%) patients, the mutation was located in exon IX. Considering only the patients that presented exonic point mutations (n: 75/83), exon IX was the exon with the highest frequency of mutations found (21/75 patients), followed by exons III, VIII and VII (17/75, 13/75, 12/75 patients each) and exons II, 8/75 patients (Tables 2 and 3). Point mutations seemed to be rare in exons V (1 patient each), I and IV (2 patients each).

3.3. Family studies

The analysis performed in 63 available mothers of MPS II patients showed that 48/63 (76%) were carriers. Seven of 10 mothers of patients presenting the inversion/disruption were carriers. Also, all brothers/cousins with MPS II that were studied (n: 22) presented the same mutation identified in the proband.

4. Discussion

This is the largest sample of Latin American MPS II patients reported to date. Our findings regarding the genotype analysis are, in general, in agreement with the literature. We have found great allelic heterogeneity among our patients (57 different mutations in 103 patients), and 30 novel mutations. Only 8 point mutations were recurrent in two or more unrelated patients (p.R88C, p.Y103X p.W109X, p.S333L, c.1122 C>T, p.R443X, p.P467L and p.R468W); of these, only p.P467L did not involve CpG sites.

Discordant with the literature is the relatively higher number of small duplications/insertions in relation to the small deletions that we found (7.0% versus 3.5%). According to the literature, the frequency for small duplications/insertions ranges from 1.5% to 6% and from 10 to 20% for small deletions [24,30,31].

As expected, the exon with the highest frequency of point mutations was exon IX (n: 21 patients). It has been reported that, while the distribution of small rearrangements in the *IDS* gene seems random, point mutations tend to be more frequent in exons III, VIII, VII and IX [32]. In our sample, the number of patients showing point mutations in exons I, IV, and V was almost the same. We found a high frequency of the mutation c.1122C>T, in codon 374. This is a silent mutation that creates an

Table 3

Recurrent *IDS* gene point mutations found in this work and worldwide patients described in the literature.

Patients ^a	Mutation ^b	Origin	Clinical phenotype (literature) ^c
H52 a, b, c	R8X	Brazil	Intermediate: 1 patient [3]; attenuated: 2 patients [18,30]
H53	Y54X	Brazil	Severe: 1 patient [33]
H54	A77D	Brazil	Attenuated [34]
H55	N63D	Brazil	Mild: 1 patient [12]
H56	A85T	Brazil	Severe: 1 patient [26]; intermediate: 2 patients [16,24,25,32,35,36]
H57	P86L	Brazil	[37]
H58	S87N	Brazil	Attenuated: 1 patient [37]
H59,60,61	R88C	Brazil	Severe: 3 patients [32]
H62	R88H	Brazil	Severe: 3 patients [32,33]
H63,64	Y103X	Brazil	Intermediate [38]
H65	T130I	Brazil	Mild: 1 patient [33]
H66	P231L	Brazil	Mild: 1 patient [25]; severe: 1 patient [31]
H67	c.708G > A	Brazil	Severe: 1 patient [28]; intermediate: 1 patient [32]
H68	c.709-2A > G	Paraguay	[23] (no clinical description)
H69	S305P	Brazil	Attenuated: 1 patient [39]
H70,H71,H72,H73	S333L	Brazil	Severe: 5 patients [16,18,26,31,33,40,41]
H74	S333L	Chile	Same as above
H75	H335R	Brazil	Intermediate: 2 patients [16,31]
H76	G336V	Brazil	Severe [42]
H77,H78,H79,H80, H81,H82,H83	c.1122C > T	Brazil	Intermediate: 1 patient [3]; attenuated: 21 patients [11,12,18,21,31,32,37,43,44]
H84	c.1122C > T	Bolivia	Same as above
H85 a, b	Q389X	Brazil	Severe: 2 patients [19,46]
H86	C422Y	Chile	Intermediate: 1 patient [25]
H87,H88,H89, H90,H91	R443X	Brazil	Severe: 4 patients [11,16,21,36]; intermediate: 5 patients [16,32,38,40]; attenuated: 6 patients [3,25,30,31]
H92	R443X	Bolivia	Same as above
H93	P467L	Chile	Severe: 2 patients [16,21]
H94 a, b, H95	P467L	Brazil	Same as above
H96,H97,H98,H99	R468W	Brazil	Severe: 10 patients [12,24,31,21,15,37,44,45]; attenuated: 2 patients [45,47]
H100	R468W	Argentina	Same as above
H101	R468Q	Argentina	Severe: 30 patients [3,12,16,18,24,26,32,38,40,48,49]; intermediate: 1 patient [12]
H102	D478G	Brazil	[45]
H103 a, b, c	L482X	Brazil	Intermediate: 2 patients [26,30]

MDD: delay in motor development; LDD: delay in language development; MR: mental retardation; BP: behavioral problems; NR: neurological regression; NA: not available; see specific reference for the clarification of the criteria used for the classification of clinical severity in each study.

^a Patients described with the same number and different letters (a, b, c,) are relatives.

^b cDNA numbering follows [29], beginning with the A of the initiation codon.

^c Cases when author has not characterized the clinical phenotype or when the clinical phenotype was unknown have not been included in this column.

alternative splice site with loss of 20 *IDS* amino acids and that corresponds to around 45% of the mutations in exon VIII [32]. This was expected, given the ethnic background of our samples and the fact that this is the most frequent mutation of the *IDS* gene found in Spain and Portugal, where more than 25 patients have been reported. All of these patients have an attenuated phenotype, suggesting that the very low percentage of normal transcript was sufficient to avoid a severe phenotype [21,25,30,40]. On its turn, the high frequency of point mutations in exon IX may be explained by the existence of mutational hotspots (codon 468, for example) and by the size of this exon (473 bp, approximately twice the size of the remaining exons).

Regarding the pathogenicity of the novel missense mutations described, it is important to point out that all occur in codons that are conserved among the human and the murine *IDS* [15] and that they were not found in 100 control alleles. Of the 63 mothers tested for mutation

analysis, 19 are obligatory carriers, based on family pedigree. Taking into account only the mothers that are not mandatory carriers 44/63, we identified 46% of carriers and 24% as non-carriers, which is in accordance with the rate of spontaneous mutation proposed by Haldane. Assuming no selection among carriers and non-carriers, and the fact that MPS II is X-linked recessive, it is expected that approximately 1/3 of the cases are secondary to new mutations [50]. In the work of Chase et al. [51], 23% of mothers of patients with MPS II were identified as non-carriers, similar to the expected value (approximately 33%); however, Machill et al. [52], Rathmann et al. [32], Froissart et al. [16] and Bellows and Thompson [53] found a ratio of carrier mothers (approximately 90%) higher than expected (66% approximately).

Although there are no uniform criteria to classify the clinical severity of patients with MPS II, most studies that analyze the correlation between genotype and phenotype in this disease classify patients into the severe (presence of severe or moderate mental retardation), intermediate (absence or presence of mild mental retardation and presence of severe somatic disease) or the attenuated form (absence of mental retardation and presence of attenuated somatic disease). However, these studies usually do not provide a more detailed clinical description of the patients.

For instance, *IDS* gene total deletions and the p.S333L mutation are always reported as being associated with the severe phenotype [4,24,30,31,32], while the c.1122C>T mutation (codon 374) is always reported as being associated with normal intelligence [30]; two of our patients present a severe phenotype. Patients with, p.A85T, p.R443X, p.R468W and p.R468Q mutations, are reported as having the severe or the attenuated form of MPS II. Therefore, some few mutations present correlation with the severe form (total deletions, p.S333L), other few mutations present correlation with the attenuated form (c.1122C>T), while a greater number of mutations are correlated both with the severe form and the attenuated form (p.A85T, p.R443X, p.R468W, p.R468Q). It is important to point out that these generalizations can only be made in relation to the small number of recurrent mutations, since most mutations in the *IDS* gene are private or have been described in a limited number of patients.

The three patients that present a frameshift mutation, clearly appear to have a severe phenotype.

According to our findings, point mutations (including mutations in splice sites) seem to be associated with any of the clinical phenotypes. It is interesting to point out that, different from what was expected, the clinical phenotypes associated with the 7 nonsense mutations found range from the attenuated to the severe form of the disease. The mutations p.Y54X and p.Y536X are clearly associated with the severe and the attenuated phenotype, respectively. Mutation p.Y536X is the nearest to the carboxyl terminus of the *IDS* protein described to date. It may be compared with the recurrent mutation p.Q531X, which has already been described in two patients with the attenuated form of MPS II [16,40]; both mutations lead to the synthesis of a protein missing the C-terminal amino acid *IDS* removed during *IDS* protein maturation and should not prevent the *IDS* processing to the lysosome [4]. The phenotypes found in the patients that present the following recurrent mutations (p.R8X, p.Q389X, p.R443X, and p.L482X), are in agreement with those reported in the literature: The p.R8X phenotype could be explained by the proximity of the mutation to the initiation codon, a context in which nonsense-mediated decay could be circumvented [10], and by the presence of the low-fidelity stop codon, which may result in limited natural read through possibilities [30]. In patients carrying the mutation p.R443X a complete spectrum of phenotypes from attenuated to severe has been described suggesting that the natural read-through could vary between individuals and/or that other factors could influence the phenotype [30]. It seems that the severity of nonsense mutations, in the case of the *IDS* gene, shows a relation with its location within the gene, since mutations located in the amino and carboxy terminal ends tend to be associated with attenuated phenotypes. Interestingly, one of the novel point mutations described in

the carboxy terminal end (p.V503D) is also associated with the attenuated phenotype.

As to the mutations that are being described in this study for the second time in the literature (p.S87N, c.709-2A>G, p.C422Y), there is total discordance as to the phenotype associated to p.S87N: the Brazilian patient clearly presents the severe form of MPS II, while the other patient described [37] presents the attenuated form. In our sample, the phenotype associated with c.709-2A>G is the severe one; however, Lissens et al. [23] did not describe the phenotype of their patient. There is some agreement as to the phenotype associated with p.C422Y: the patient being described herein presents an extremely attenuated form of MPS II, while Gort et al. [25] classified their patient as having the intermediate form. Moreover, we are describing two brothers with the c.708G>A, who apparently have no neurological involvement. Interestingly, this mutation was described in one patient with the intermediate form [32] as well as in other patient with the severe form of MPS II [18]. The clinical phenotypes associated with the other recurrent mutations found (p.A85T, p.S333L, c.1122C>T, p.P467L, p.R468W and p.R468Q) are in agreement with those of the literature.

We believe that the methodology employed in this study is appropriate for both the diagnosis and the identification of mutations in patients with MPS II and possible carriers. The correlation between genotype and phenotype for MPS II exists for a small subset of mutations, being probably influenced by other factors that modulate the residual activity of the *IDS*. Therefore, in the present state of knowledge, the identification of the mutation present in individuals with MPS II has, as main objective, the genetic counseling of families.

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