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Genotypic and bioinformatic evaluation of the alpha-L-iduronidase gene and protein in patients with mucopolysaccharidosis type I from Colombia, Ecuador and Peru

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ABSTRACT

Mucopolysaccharidosis type I (MPSI) is a rare autosomal recessive disorder caused by mutations in the gene encoding the lysosomal enzyme α-L-iduronidase (IDUA), which is instrumental in the hydrolysis of the glycosaminoglycans, dermatan and heparan sulfate. The accumulation of unhydrolyzed glycosaminoglycans leads to pathogenesis in multiple tissue types, especially those of skeletal, nervous, respiratory, cardiovascular, and gastrointestinal origin.

Although molecular diagnostic tools for MPSI have been available since the identification and characterization of the IDUA gene in 1992, Colombia, Ecuador, and Peru have lacked such methodologies. Therefore, the mutational profile of the IDUA gene in these countries has largely been unknown. The goal of this study was to characterize genotypes in 14 patients with MPSI from Colombia, Ecuador, and Peru.

The most common mutation found at a frequency of 42.8% was W402X. Six patients presented with seven novel mutations, a high novel mutational rate in this population (32%). These novel mutations were validated using bioinformatic techniques. A model of the IDUA protein resulting from three of the novel missense mutations (Y625C, P385L, R621L) revealed that these mutations alter accessible surface area values, thereby reducing the accessibility of the enzyme to its substrates.

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This is the first characterization of the mutational profile of the IDUA gene in patients with MPSI in Colombia, Ecuador, and Peru. The findings contribute to our understanding of IDUA gene expression and IDUA enzyme function, and may help facilitate early and improved diagnosis and management for patients with MPSI.

1. Introduction

Mucopolysaccharidosis type I (MPSI) is an autosomal recessive lysosomal storage disorder [1] resulting from the deficiency of the lysosomal enzyme α-L-iduronidase (IDUA,) (EC 3.2.1.76, OMIM ID: 252800) [2], which is required for the hydrolysis of the glycosaminoglycans (GAGs), dermatan and heparan sulfates. Lysosomal accumulation of these GAGs leads to multisystemic pathology involving predominantly the skeletal, respiratory, cardiovascular, gastrointestinal, and nervous systems [1]. Depending on the age of onset, how rapidly the disease progresses, and the presence or absence of neurocognitive involvement, MPS I is broadly categorized into three forms: Hurler syndrome (MPS IH), the most severe, neuropathic form, with onset in infancy; Hurler–Scheie syndrome (MPS I H/S) is intermediate in severity with onset in early childhood and mild to moderate cognitive impairment; and Scheie syndrome (MPS IS), the least severe form of MPS I, with an onset in childhood and no cognitive impairment [1]. MPS I is characterized by coarse facial features, joint stiffness and contractures, short stature, cardiopathy and respiratory problems; without treatment, expected survival in the most severely affected patients with Hurler syndrome is less than 10 years [1,3,4].

Screening for MPS I is done by measurement of urinary GAG levels; a formal diagnosis is based on an enzyme activity assay measuring the levels of IDUA in cultured fibroblasts, peripheral blood leukocytes, or dried blood spots [3,5–7]. Molecular analyses to identify mutations in the IDUA gene enable diagnosis of MPS I by genotyping. However, not all mutations are known, and there are regional variations in mutations [8,9]. Despite these limitations, genotyping is a powerful tool for disease confirmation, prenatal diagnosis [10], screening of newborns [11], and for the decision of the better treatment that should be established (HSCT, ERT or both) based on the phenotype–genotype correlation [3]. To date, more than 100 IDUA mutations have been identified [3,8]. These include deletions, insertions, missense, nonsense, and splice site mutations. The most common are the W402X and Q70X nonsense mutations; 37% of reported mutations are novel [9]. Enzyme assays are not very reliable in detecting heterozygotes, but some biochemical methods have shown positive results [12].

Until recently, Colombia, Ecuador and Peru lacked the molecular diagnostic laboratories to characterize the mutational profile of IDUA in Andean patients with MPS I. The goal of this study was to characterize the genotypes of Colombian, Ecuadorian, and Peruvian patients diagnosed with MPS I using molecular and bioinformatic techniques.

2. Material and methods

2.1. Study population

Patients from Colombia, Ecuador and Peru with a clinical diagnosis of MPS I confirmed by biochemical and genotype analysis were included in this study. The responsible adult for each patient signed an informed consent form agreeing to study participation. These forms were then approved by the ethics committee of the Medical Faculty of the National University of Colombia.

2.2. Experimental phase

Genomic DNA was extracted from blood samples of patients using the Ultra Clean TM DNA Blood Isolation kit (MO BIO Laboratories, Inc., Carlsbad, California, USA). Amplicons for sequencing were generated by polymerase chain reaction (PCR) using oligonucleotides as described previously [13,14]. Oligonucleotide sequences are listed in Table 1. The 5′ UTR and 3′ UTR segments were not evaluated because no known mutations have been identified in these regions to date.

PCR-generated amplicons were purified with a Purelink™ PCR Purification kit (Invitrogen, Carlsbad, California, USA), prepared for sequencing using the Big Dye® Terminator 3.1 sequence kit (Applied Biosystems, Foster City, California, USA), and purified again as described above. Resulting DNA was analyzed with an Applied Biosystems 3500 Genetic Analyzer sequencer (Applied Biosystems, Foster City, California, USA). The sequence analyses were done with Sequencher 5.2 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Quality control of the results was done in the molecular biology laboratory of the Faculty of Medicine of the Sao Paulo University in Brazil.

2.3. Analysis of new mutations

Novel mutations were analyzed with the software Splice site prediction by Neural Network [15], with the software for the annotation and prediction of pathological mutations PMUT [16,17], and with PolyPhen, a bioinformatic tool to use to predict the possible impact of an amino acid substitution on the structure and function of a human protein [18].

3. Results

3.1. Patient profiles

A total of 15 patients were evaluated for enrollment in this study. One patient had enzyme levels indicative of MPS I, but she was a heterozygous carrier. Therefore, this patient was excluded from the study. Of the 14 remaining patients, 12 were diagnosed with Hurler syndrome (85.7%), and two with Hurler–Scheie (14.3%) (Table 2). The average age at diagnosis was 7.8 years; 28.6% of the patients were female. Participation by country included 12 patients from Colombia and one patient each from Ecuador and Peru.

Phenotypic characteristics of the 14 patients are provided in Table 3. All patients had variable degrees of multisystem disease involvement: all presented with joint and bone manifestations, coarse facial features, and hernias, and most also had ocular and respiratory abnormalities, visceromegaly, and short stature. All patients with Hurler syndrome had mental retardation and global developmental delay, while neither of the patients diagnosed with Hurler–Scheie syndrome had neurocognitive manifestations. At the time of this analysis, 93% of patients were receiving enzyme replacement therapy (ERT) with laronidase (Aldurazyme®, Genzyme, a Sanofi company, Cambridge, Massachusetts, USA and BioMarin Pharmaceutical Inc, Novato, California, USA); 14% had consanguineous parents, but no family history of MPS I.

3.2. Mutational genetic analyses

3.2.1. Previously-reported mutations

Of the 14 patients studied, eight had previously-reported mutations, as shown in Table 4. The most common mutation W402X, was found at a frequency of 42.8% in our study population. Other previously-reported mutations found were A327P, E404X, and Arg48del in patients with

Table 1
List of oligonucleotides used in this study.

Exon	Name (strand)	Sequence	Nucleotide
1	IDUA1 (+)	ACCCAACCCCTCCCAC	437–425
	IDUA1 (−)	GCTCCGGTCTCTGAAGCT	834–817
2	IDUA2 (+)	GAACGTGTGTGTCAGCCG	1236–1253
	IDUA2 (−)	GCTCGGAAGACCCCTTGT	1539–1522
3	IDUA3 (+)	TCCCACATGCTCCGTTGT	337–354
	IDUA3 (−)	TCTGAGTCCTTGGATGTCCATTC	609–587
4	IDUA4 (+)	ACCCTCTCCCTCACCCAG	565–582
	IDUA4 (−)	GTTGCACCCCTATCACGC	876–859
5	IDUA5 (+)	CATCACCTTGCACCCTCC	1202–1219
	IDUA5 (−)	CCAGGGCAGGTGTAGACG	1474–1457
6	IDUA6 (+)	GAGGAAGGCAGGAGCAGAG	1422–1440
	IDUA6 (−)	GACCCTGGTGGTGCTGAG	1780–1763
7	IDUA7 (+)	TGCGGCTGGACTACATCT	1465–1483
	IDUA7 (−)	GCAGCATCAGAACCTGCTACT	2112–2092
8	IDUA8 (+)	CCACCTTCCTCCCGAGAC	2010–2027
	IDUA8 (−)	GGAGCGCACTTCCTCCAG	2395–2378
9	IDUA9 (+)	TCCTTCACCAAGGGGAGG	2442–2459
	IDUA9 (−)	CCGAGGCCTGAGTGTCAG	2841–2824
10	IDUA10 (+)	GGTGACCCTGCGGCTG	2722–2737
	IDUA10 (−)	CCTGGAGAACCCTGAGGA	3142–3125
11 and 12	IDUA11/12 (+)	GTGTGGGTGGGAGGTGGA	3055–3072
	IDUA11/12 (−)	CTTCACCCATGCGGTCAC	3520–3503
13	IDUA13 (+)	GGGGCTTGAGGGAATGAG	3721–3738
	IDUA13 (−)	GAGAAGCCTGGGGTCAGG	4020–4003
14	IDUA14 (+)	CAGGGCAGTACTGGGTGG	3978–3995
	IDUA14 (−)	CATCACCCCCTTTGCAATATA	4308–4288

Hurler syndrome, and R89W identified in one of the two patients with Hurler–Scheie syndrome.

3.2.2. Novel mutations and bioinformatic analysis

Our analysis led to the identification of seven novel mutations. Among these, three were missense mutations (Y625C, P385L, R621L), one was a nonsense mutation (Q148X), one was a deletion (Asp298Glu+Ala299del), one was an insertion (1557–1558insC), and one was a splice site mutation (IVS9+1g>t) (Table 5).

Sequence and bioinformatic analysis of the splice site mutation IVS9+1g>t allowed further characterization of this mutation. In patients with MPS I who carry this mutation, the guanine residue at the 5′ donor splice site of intron 9 is replaced by a thymine residue. This results in a defective splice site, and preservation of the entire intron 9, as shown in Fig. 1.

Sequence alignments showed a high level of interspecies conservation of the three missense mutations Y625C, P385L, and R621L (Fig. 2). All three mutated residues were conserved among 5 different species, further confirming the importance of these residues in preserving normal IDUA function.

Table 2
Patient demographics.

Patient	Age	Gender	Phenotype
MPS I 001	8	F	Hurler
MPS I 002	7	F	Hurler
MPS I 003	9	F	Hurler
MPS I 004	2	M	Hurler
MPS I 005	28	M	Hurler–Scheie
MPS I 006	5	M	Hurler
MPS I 007	5	M	Hurler
MPS I 008	7	M	Hurler
MPS I 009	8	M	Hurler
MPS I 010[a]	8	M	Hurler
MPS I 011[a]	10	M	Hurler
MPS I 012	5	M	Hurler–Scheie
MPS I 013	3	F	Hurler
MPS I 014	4	M	Hurler

[a] These patients are siblings.

4. Discussion

This study aimed to characterize the genetic profile of the IDUA gene in patients with MPSI from Colombia, Ecuador and Peru through molecular and bioinformatic techniques. The majority (86%) of patients in our study had a clinical and biochemical diagnosis of MPS I Hurler syndrome, the severe form of the disease, and two patients were diagnosed with MPS I Hurler–Scheie syndrome.

The most common mutation found in our study population was W402X. This mutation was previously described in a Brazilian population [19] with a similar frequency (37%) as reported here. Two other frequently-reported mutations worldwide (Q70X [8,9,20] and P533R [20–22]), were not found in our population.

In 1994, Bunge et al. described a differential pattern of the most common found mutation frequencies: W402x was more frequent than Q70X in west Europe (48% vs. 19%) while Q70X was more frequent than W402X in east Europe, including Scandinavia (62% vs 17%) [9]. Later on, other studies like Voskoboeva et al. found the same data suggesting an east Europe origin for the Q70X mutation. According to the studies in Spain [21], the W402X mutation was found with a frequency of 60% while Q70X was found with a frequency of 10%; showing a clinal east–west frequency reduction of the Q70X, and then when we looked inside our population this mutation was not found. The other probable explanation for the absent Q70X mutation in our Andean population is because the racial mixture in this region was with individuals of Spanish origin and not with east Europe individuals [23].

We identified a total of five mutations (W402X, A327P, E404X, Arg48del, and R89W) in our Andean population which were previously reported [8,9,13,19,24]. Two of these mutations, Arg48del and E404X, occurred in a heterozygous state in a pair of siblings (patient MPSI 010 and MPSI 011). Arg48del was previously reported in an analysis of IDUA mutations in a Brazilian MPS I population [19]; E404X was identified in a patient from Iran with MPS I Hurler syndrome [24]. Another mutation found in one of our patients was A327P, which has been reported frequently in the literature and is associated with a severe MPS I phenotype [13]. Additionally, we identified the R89W mutation in one patient with Hurler–Scheie syndrome. This mutation was previously reported in a German patient with an attenuated Hurler–Scheie phenotype [24]. A previous analysis [25] found that this mutation alters

Table 3
Patient's phenotype characteristics.

	Mental retardation / developmental delay	Coarse facial features	Visual impairment	Respiratory disease	Cardiac disease	Visceromegaly	Hernias	Joint disease	Bone disease	Short stature	Enzyme replacement therapy	Consanguinity	Family history
MPS I 001	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
MPS I 002	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y	N	N
MPS I 003	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
MPS I 004	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	N	N
MPS I 005	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
MPS I 006	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
MPS I 007	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N
MPS I 008	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	N
MPS I 009	Y	Y	Y	N	N	N	Y	Y	Y	N	Y	N	N
MPS I 010	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	Y
MPS I 011	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	Y
MPS I 012	N	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N	N
MPS I 013	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	Y
MPS I 014	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N

Y: yes, N: no.

a key residue required for the catalytic activity of the IDUA enzyme, suggesting that the residual enzyme activity resulting from this mutation is associated with an attenuated phenotype [26].

Importantly, our analyses identified seven novel mutations occurring in 42.8% of our study population; three missense mutations (Y625C, P385L, R621L), one nonsense mutation (Q148X), one deletion (Asp298Glu+Ala229del), one insertion (1557–1558insC), and one splice site mutation (IVS9+1g>t). Further sequence and bioinformatic analysis of the missense mutations revealed that there is a high degree of interspecies conservation of the residue which is mutated in patients with MPS I, suggesting that each of the amino acid changes were pathogenic rather than polymorphic (Fig. 2).

The P385L mutation was found in a heterozygous state in a patient with an R89W mutation and Hurler–Scheie phenotype. The attenuated phenotype is associated with the R89W mutation as well as the presence of some residual IDUA activity [26]. Since R89W is not a severe mutation, we propose that the P385L mutation is associated with a severe phenotype because it replaces a small and cyclic amino acid (proline) with a larger and branched one (leucine), resulting in the disruption of the secondary structure (alpha or beta) of the IDUA protein.

The R621L missense mutation was found in a heterozygous state with mutation W402X in a patient (MPSI 014) with severe phenotype. We believe that this novel mutation is also associated with a severe phenotype because it involves the substitution of a basic and charged amino acid (arginine) with a smaller, polar one (leucine) at a conserved site. Furthermore, a nonsense mutation in the same codon (R621X) has already been described as severe [27].

Another novel mutation was found in combination with W402X. This mutation was Y625C in a patient with a Hurler–Scheie phenotype (MPS I 005). We do not consider this mutation to be severe even though it substitutes a smaller amino acid (cysteine), which is capable of assembling sulfur bridges, with a larger aromatic one (tyrosine).

In addition to missense mutations, we found a nonsense mutation (Q148X) in a heterozygous state in a Hurler patient (MPS I 009). Nonsense mutations generate much shorter (truncated) proteins with no significant residual activity, and generally result in severe phenotypes [8]. This patient also had a 3-amino acid deletion (Asp298Glu+Ala299del), which alters the reading frame of the protein; this type of mutation is considered severe [27].

In another Hurler patient (MPS I 006), we found a homozygous insertion (1557–1558insC); this mutation could be associated with a severe phenotype [8,28]. This type of mutation has been shown to alter the reading frame of the protein [29]. Finally, one Hurler patient (MPS I 008) had a splice site mutation (IVS9+1g>t). Our bioinformatic analyses suggest that this type of mutation results in the loss of the donor splice site, thereby altering mRNA maturation and protein translation by generating new and aberrant reading frames, which constitutes a severe change. Similar findings were reported by Terlato and Cox [8]. In the present study, we found that loss of the 5′ donor splice site results in the loss of the required sequence for attachment of the

Table 4
IDUA mutations found in the 14 patients with MPS I.

Patient	Mutation	Phenotype
MPSI 001	W402X[a]	Hurler
MPSI 002	W402X[a]	Hurler
MPSI 003	W402X[a]	Hurler
MPSI 004	W402X[a]	Hurler
MPSI 005	W402X Y625C[b]	Hurler–Scheie
MPSI 006	1557–1558insC[b]	Hurler
MPSI 007	A327P[a]	Hurler
MPSI 008	IVS9+1g>t[a,b]	Hurler
MPSI 009	Q148X[b] Asp298Glu+Ala299del[b]	Hurler
MPSI 010	E404X Arg48del	Hurler
MPSI 011	E404X Arg48del	Hurler
MPSI 012	R89W P385L[b]	Hurler–Scheie
MPSI 013	W402X[a]	Hurler
MPSI 014	W402X R621L[b]	Hurler

[a] This patient is homozygous for this mutation.
[b] Novel mutations.

Table 5
Novel mutations found in the 14 patients with MPS I.

Mutation	Exon	cDNA Position (ATG = 1)	M747152 cDNA position	Nucleotide alteration	Protein change	Frequency (28 alleles)	Patient origin
Y625C	14	1874A → G	1962A → G	TAC → TGC	Tyr → Cys	1/28 (3.57%)	Colombia (Cundinamarca)
1557–1558insC	11	1557–1558insC	1645–1646insC	FRAMESHIFT	–	2/28 (7.14%)	Colombia (Boyacá)
IVS9+1g>t	Intr9	1402+1G → T	1293+1G → T	MUT. SPLICING	–	2/28 (7.14%)	Ecuador
Q148X	4	442C → T	530C → T	CAG → TAG	Gln → Stop	1/28 (3.57%)	Peru
Asp298Glu+Ala299del	7	894_896delCGA	982_984delCGA	–	Asp → Glu + Ala → del.	1/28 (3.57%)	Peru
P385L	8	1154C → T	1242C → T	CCG → CTG	Pro → Leu	1/28 (3.57%)	Colombia (Cundinamarca)
R621L	14	1862G → T	1950G → T	CGA → CTA	Arg → Leu	1/28 (3.57%)	Colombia (Boyacá)

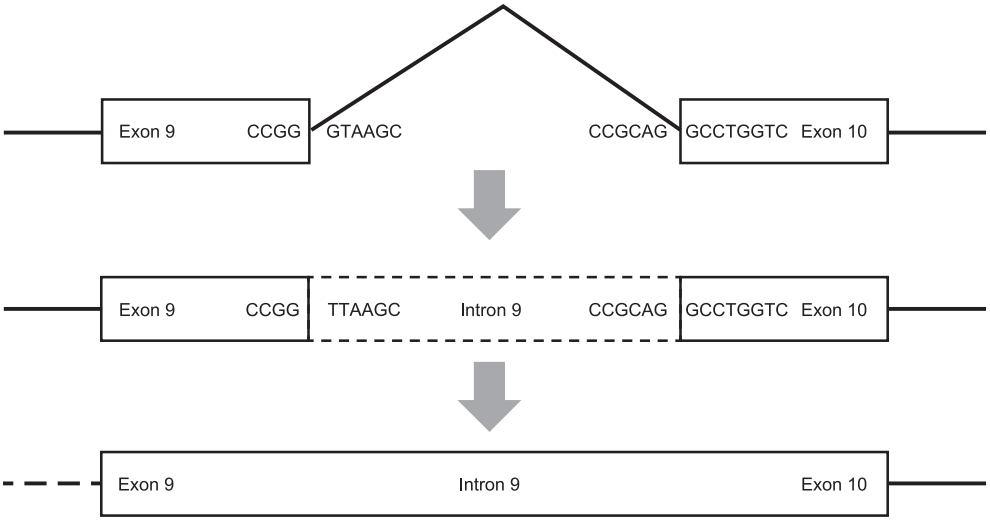

Fig. 1. Model of splice site alteration in the IVS9+1g>t mutation. Model of the IVS9+1g>t mutation in exon 9 of patient MPSI 008. The guanine (G) to thymine (T) substitution at the donor splice site leads to the loss of recognition of this sequence by the spliceosome, and retention of intron 9.

spliceosomal proteins; consequently, intron 9 remains intact and is not excised (Fig. 1).

The exclusion of one patient from this study with low enzyme activity indicative of MPS I, but no confirmatory mutations, implies the importance of both biochemical and molecular analyses in the diagnosis of MPS I. The 6-month-old patient underwent enzyme testing and genotyping because she was the younger sibling of patient MPS 004 who was diagnosed with MPS I (homozygous for the W402X mutation). While her enzyme levels were below the reference range, she was found to be a healthy, heterozygous carrier for the W402X mutation. Clinical follow-ups further confirmed her asymptomatic status; the patient has remained healthy with no signs of MPS I through her 2-year clinical follow-up. Thus, it is important to take such information into account as part of establishing a standard for the diagnosis of MPS I in infants under one year of age who do not have obvious clinical symptoms. Additional support for this notion comes from newborn screening programs in the United States, which have identified apparent pseudodeficiency alleles in the IDUA gene of newborns with low enzyme activity but no clinical manifestations [30]. A recent study by Kingma and colleagues [11] describing an algorithm for the early determination of

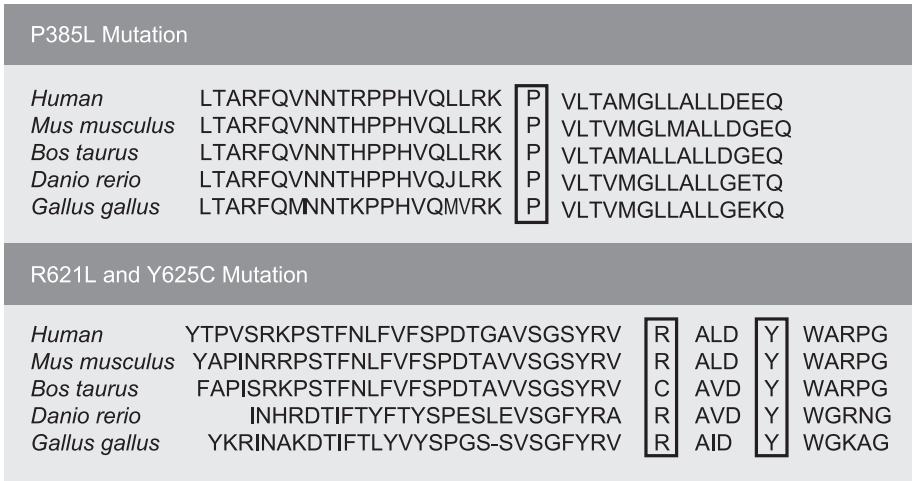

Fig. 2. Sequence alignment of the novel missense mutations among species. Alignment of the IDUA protein sequence in five species. The missense mutations Y625C, P385L and R621L occur in highly conserved sites.

phenotypic severity in patients with MPS I, suggests that IDUA enzyme activity be measured only in newborns in whom the genotype is inconclusive of a phenotypic severity. The presence of early clinical symptoms of MPS I (upper airway obstruction and hernia) is the last step in the algorithm, as these may be difficult to diagnose in newborns [11]. Further data will be required to establish the utility of biochemical analysis (enzyme activity in leukocytes) in this age group, since it can be difficult to diagnose MPS I in children younger than one year of age.

In conclusion, the prevalence of W402X in the Andean population included in our study was similar to the prevalence of this mutation reported in the rest of the world [8]. However, other common mutations (Q70X, P533R, and L490P) were not found and novel mutations were noted with an incidence rate of 42.8%. Finally, the pathogenic potential of these novel mutations will need to be characterized with more robust bioinformatic approaches (docking) and in-vitro methodologies (functional studies).

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