

Molecular Genetics and Metabolism Reports 1 (2014) 401–406



Contents lists available at ScienceDirect

# Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/ molecular-genetics-and-metabolism-reports/



**Short Communication** 

# Identification of 17 novel mutations in 40 Argentinean unrelated families with mucopolysaccharidosis type II (Hunter syndrome)



H. Amartino <sup>a</sup>, R. Ceci <sup>b</sup>, F. Masllorens <sup>c</sup>, A. Gal <sup>d</sup>, C. Arberas <sup>e</sup>, L. Bay <sup>f</sup>, R. Ilari <sup>g</sup>, J. Dipierri <sup>h</sup>, N. Specola <sup>i</sup>, A. Cabrera <sup>j</sup>, P. Rozenfeld <sup>b,\*</sup>

- <sup>a</sup> Hospital Universitario Austral, Pilar, Argentina
- <sup>b</sup> IIFP, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata y CONICET, Argentina
- <sup>c</sup> Hospital Posadas, Buenos Aires, Argentina
- <sup>d</sup> UKE, Hamburgo, Germany
- e Hospital de Niños Dr Ricardo Gutierrez, Buenos Aires, Argentina
- f Hospital Garrahan, Buenos Aires, Argentina
- <sup>g</sup> Policlínico Bancario, Buenos Aires, Argentina
- <sup>h</sup> Universidad Nacional de Jujuy, Argentina
- <sup>i</sup> Hospital de Niños Sor María Ludovica, La Plata, Argentina
- <sup>j</sup> Hospital de Niños VJ Vilela, Rosario, Argentina

# ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 25 August 2014
Accepted 26 August 2014
Available online 18 September 2014

Keywords:
Genetic testing
Genotype-phenotype correlation
Hunter syndrome
Lysosomal storage disorder
Mucopolysaccharidosis type II

## ABSTRACT

Mucopolysaccharidosis type II (MPSII) is an X-linked lysosomal storage disorder caused by deficiency of the enzyme iduronate-2-sulfatase (IDS). The human *IDS* gene is located in chromosome Xq28. This is the first report of genotype and phenotype characterization of 49 Hunter patients from 40 families of Argentina. Thirty different alleles have been identified, and 57% were novel. The frequency of de novo mutations was 10%. Overall, the percentage of private mutations in our series was 75%. © 2014 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license

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## 1. Introduction

Mucopolysaccharidosis type II (MPS II, Hunter disease, MIM 309900) is an X-linked lysosomal storage disorder caused by deficiency of the enzyme iduronate-2-sulfatase (IDS). Clinical manifestations are coarse

<sup>\*</sup> Corresponding author at: 47 y 115, La Plata (1900), Argentina. Tel.: +54 221 4250497; fax: +54 221 4226947. E-mail address: paurozen@biol.unlp.edu.ar (P. Rozenfeld).

**Table 1**List of mutations from 40 families of Argentinean Hunter patients. NA: sample not available.

Family	Number of patients	Type of mutation	Genotype	Protein alteration	Exon	Phenotype	Presence of the mutation in the mother	Reference
1	1	Complete IDS deletion				Severe	NA	
2	1	Complete IDS deletion				Severe	NA	
3	1	Complete IDS deletion				Severe	NA	
4	1	Gene-pseudogene recombination				Severe	NA	
5	1	Gene-pseudogene recombination				Severe	NA	
6	1	Gene-pseudogene recombination				Severe	Y	
7	2	Missense	c.683C > A	p.Pro228Gln	5	Severe	Y	This study
8	4	Missense	c.1403G > A*	p.Arg468Gln	9	Severe	Y	This study, Whitley et al. [7]
9	1	Missense	c.253G > A*	p.Ala85Thr	3	Mild	NA	This study, Li et al. [8]
10	1	Missense	c.1403G > A	p.Arg468Gln	9	Severe	N	This study, Whitley et al. [7]
11	1	Missense	c.1403G > A + c.1394A > T	p.Arg468Gln + p.Gln465Leu	9	Severe	Y	This study
12	1	Missense	c.884A > T	p.Lys295lle	7	Severe	Y	This study
13	1	Missense	c.181 T > C	p.Ser61Pro	2	Mild	Y	This study, Sohn et al [9]
14	1	Missense	c.998C > T	p.Ser333Leu	7	Severe	Y	This study, Flomen et al. [10]
15	1	Missense	c.592G > A	p.Asp198Asn	5	Severe	N	This study
16	1	Missense	c.1033 T > A	p.Trp345Arg	8	Severe	NA	This study
17	1	Missense	c.425C > A	p.Ser142Tyr	4	Severe	Y	This study
18	1	Missense	c.401G > A*	p.Gly134Glu	3	Mild	Y	This study
19	2	Missense	c.359C > A	p.Pro120His	3	Severe	Υ	This study, Hopwood et al. [11]
20	1	Missense	c.935G > A	p.Gly312Asp	7	Mild	Y	This study

21	1	Missense	c.641C > T	p.Thr214Met	5	Severe	NA	This study
22	1	Missense	c.253G > A	p.Ala85Thr	3	Mild	NA	This study, Li et al. [8]
23	1	Missense	c.1403G > A	p.Arg468Gln	9	Severe	NA	This study, Whitley et al. [7]
24	1	Missense	c.1016 T > C	p.Leu339Pro	8	Severe	Y	This study, Guo (2006)
25	1	Missense	c.998C > T	p.Ser333Leu	7	Severe	Y	This study, Flomen et al. [10]
26	1	Missense	c.469C > T	p.Pro157Ser	4	Severe	Y	This study
27	1	Missense	c.1433A > G	p.Asp478Gly	9	Mild	Y	This study, Schröder et al. [12]
28		Missense	c.1393C > T	p.Gln465X	9	Severe	NA	This study, Li et al. [13]
29	4	Nonsense	c.820G > T	p.Glu274X	6	Severe	Y	This study
30	1	Nonsense	c.1327C > T	p.Arg443X	9	Severe	Y	This study, Bunge et al. [14]
31	1	Short deletion	c.908_909delCT	Frameshift	7	Severe	Y	This study
32	1	Short deletion	c.411delT	Frameshift	3	Severe	Y	This study
33	1	Short deletion	c.22_37del16pb	Frameshift	1	Severe	N	This study
34	2	Splicing	c.508-1 g > a	Partial exclusion exon 5	3' de IVS4	Mild	Y	This study
35	1	Splicing	c.1122C > T	G374G. Loss of 20 aa	8	Mild	Y	This study, Rathmann et al. [15]
36	1	Splicing	c.241-5a > t	Exclusion of exon 3	3′ de IVS2	Severe	Y	This study
37	1	Splicing	c.708 + 1 g > a*	Exclusion exon 5	5′de IVS5	Severe	Y	This study
38	1	Splicing	c.1181-1 g > a	Exclusion exon 9	3′ de IVS8	Severe	Y	This study, Popowska et al. [16]
39	1	Splicing	c.1122C > T*	G374G. Loss of 20 aa	8	Mild	Y	This study, Rathmann et al. [15]
40	2	Splicing	c.1122C > T	G374G. Loss of 20 aa	8	Mild	Y	This study, Rathmann et al. [15]

<sup>\*</sup> The polymorphism c.438C > T was present.

facial features, hepatosplenomegaly, dysostosis multiplex, heart disease, obstructive upper respiratory disease, and frequent ear infections. Patients present a spectrum of phenotypes, ranging from severe to attenuated course [1].

The human *IDS* gene is located in chromosome Xq28, spans approximately 24 kb and contains nine exons. A full length cDNA clone of the gene showed an open reading frame of 1650 bp long predicting a protein with 550 aminoacids [2]. An *IDS* pseudogene (*IDSP1*) has been detected 20 kb distal to the telomeric side from the functional gene, whose sequences are highly homologous to exons 2 and 3 and introns 2,3 and 7 of the *IDS* gene [3]. To date, over 500 different mutations associated with MPS II have been identified [Human Gene Mutation Database (HGMD); http://www.hgmd.org/].

We report the results of genotype and phenotype characterization of 49 Hunter patients of 40 families from Argentina.

# 2. Materials and methods

#### 2.1. Patients

Forty nine Hunter patients and their mothers (only 28 of mother samples where available) from 40 families were included in this study. Parents of the patients gave their informed consent previous to the participation in the study. MPS II diagnosis was confirmed by clinical examination and deficient IDS activity (activity of arilsulfatase B was normal in all cases, ruling out multiple sulfatase deficiency). Phenotype of MPSII patients has been classified into attenuated (non-neuronopathic) or severe (neuronopathic) according to the clinical evidence of cognitive or developmental involvement.

# 2.2. IDS genomic DNA analysis

Genomic DNA was extracted from EDTA blood using a QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA, USA). The 9 exons of the *IDS* gene including exon/intron boundaries were amplified by PCR using specific primers as described [3]. Sequencing was carried out in both directions in a MegaBASE 1000 sequencer (Amersham Biosciences, Sunnyvale, CA, USA).

Recombination between *IDS* and *IDSP1* leading to any inversion mutation was analyzed by a PCR amplification assay as described [4].

# 2.3. IDS cDNA analysis

Total cellular RNA was isolated from blood leukocytes by means of a total RNA isolation system (Macherey Nagel, Duren, Germany). The isolated RNA samples were reverse transcribed using a specific primer, according to the method of Jonsson [5] and then amplified in 2 overlapping fragments and sequenced.

#### 3. Results and discussion

Until now, we have found the mutation in all the patients analyzed from Argentina. Thirty different MPS II alleles have been identified in a cohort of 49 Argentinean patients from 40 families: 18 missense (60%), 5 splicing (17%), 3 short deletions (10%), 2 nonsense mutations (7%), 1 complex rearrangement (a gene–pseudogene recombination leading to inversion) (3%) as well as 1 gross gene deletion (3%) (Table 1). We have not detected any insertions.

Of the mutations in this cohort, 17 out of 30 different alleles (57%) were novel (missense: p.Pro228Gln, p.Lys295Ile, p.Asp198Asn, p.Trp345Arg, p.Ser142Tyr, p.Gly134Glu, p.Gly312Asp, p.Thr214Met, p.Gln465Leu, p.Pro157Ser; splicing: c.508-1G > A, c.241-5A > T, c.708 + 1 g > a; nonsense: p.Glu274X; and short deletions: c.908\_909delCT, c.411delT, c.22\_37del16pb).

The effect of novel missense mutations was analyzed by different means. They are all predicted to be probably damaging, as analyzed by the prediction tool PolyPhen, and are localized in conserved aminoacids between human and murine *IDS* but found in patients with both attenuated and severe phenotypes. Glycines 134 and 312 are highly conserved among human sulfatases. Mutations found by us

at these positions introduce negative charged aminoacids. Although the change in the structure of these highly conserved aminoacids is high, both patients display an attenuated phenotype. The mutations p.Pro228Gln and p.Pro157Ser in severe patients result from the replacement of a rigid nonpolar aminoacid by a polar one. The rest of the novel missense mutations are not localized in conserved aminoacids, however the effect in the phenotype is more devastating.

Previously described mutations detected by us were p.Arg468Gln, p.Ala85Thr, p.Ser333Leu, p.Pro120His, p.Ser61Pro, p.Leu339Pro, p.Asp478Gly, p.Gln465X, p.Arg443X, the splice mutations c.1122C > T, c.1181-1 g > a, and the recombination gene–pseudogene.

We found 3 novel splicing mutations. The mutation c.508-1 g > a was found in a patient with an attenuated phenotype. At the molecular level, the mutation produced a predominant alternative transcript and a minor quantity of the correct transcript. The mutant transcript results from the disruption of the  $3^{\prime}$  original splice-site leading to the deletion of exon 5. The presence of the correct mRNA, although in minor quantity, would explain the less severe clinical manifestations observed in this patient. On the other side, the splicing mutations c.241-5a > t and c.708 + 1 g > a, each generate a unique mRNA product, whose sequence corresponds to the complete deletion of exons 3 and 5, respectively, and are associated with a severe phenotype.

In one patient, two different "neighbor" mutations (p.Arg468Gln + p.Gln465Leu) were found. The mother was a carrier of both mutations, but not the grandmother and the 4 maternal aunts. Few other reports described the presence of "neighbor" mutations [6]. The finding of these two close mutations in the patient and his mother but not in the grandmother suggests that they are de novo mutations in a mutation-prone region and reinforces the idea that they arose simultaneously.

Three patients had a complete gene deletion. None of the flanking markers tested were detected, suggesting that the deletion spans more than 20 kb upstream and 7 kb downstream of the *IDS* locus, and includes the *IDSP1* locus. No atypical symptoms of Hunter syndrome were found in these patients, indicating that the deletion did not compromise contiguous genes. The phenotype of these patients is the severe form of the disease. The same phenotype was observed for the three of the patients who exhibited a recombination between gene and pseudogene.

The absence of the probands' mutations in their mother suggests that in the three cases, the mutations occurred de novo (10%). However, the possibility of germinal mosaicism could not be ruled out. The theoretical rate calculated by the Bayesian methods for this condition would be 33%, so the value found in our cohort is much less. In the cohort reviewed by Froissart et al., they found values of de novo mutations close to the expected in any X chromosomal disease in which male reproductive fitness is extremely low [2]. The low value found in our group could be because much of the patients from Argentina were diagnosed because of a previous family history of MPS II, meaning there are still many undiagnosed patients in Argentina. We also found a low rate of the polymorphism in exon 4 c.438C > T, only in 4 families (10%) as compared to the frequency reported in normal controls of around 30% [3].

The percentage of private mutations in our series was 75%, a high proportion as usually seen in other series.

Although genotype–phenotype correlation is difficult to establish, it is worth mentioning that both missense (p.Ser61Pro, p.Ala85Thr, p.Gly134Glu, p.Gly312Asp, p.Asp478Gly) and splice mutations (c.1122C > T, c.508-1G > A) were associated with non-neuronopathic ("attenuated") phenotype. From our series of 30 alleles, 7 (23%) were found associated with mild forms. Three of the mutations associated with the non-neurological phenotype, are new (c.508-1 g > a, p.Gly134Glu, p.Gly312Asp).

This is the first report of mutations of Hunter patients from Argentina. Our results provide further evidence of mutational heterogeneity of the *IDS* gene observed in patients with MPS II.

# Role of the funding source

This work was financially supported by a grant from Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 01070) to PR, however the funding source had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report nor in the decision to submit the article for publication.

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