



Clinical and molecular characteristics of colombian patients with mucopolysaccharidosis IVA, and description of a new galns gene mutation

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ABSTRACT

A study published in 2012 estimated incidence of MPS IVA, in 0.68 cases per 100, 000 live births in Colombia, and according to the Colombian Fund for High-Cost Diseases, in 2014 there were 15 people diagnosed with MPS IV. To enhance the knowledge of the disease in the country, we aimed to characterize clinical and molecular findings in 12 MPS IVA patients. Twelve patients were included in the study, with most patients of female gender ($n = 7$, 58,3%), age range 2 to 28 years, average weight 26 kg (17.6–43 kg), average height 97 cm (92–104 cm), average BMI 27.6 kg/m² (19.92–47.65 kg/m²). Clinical findings were similar to those described in the literature. GALNS gene molecular analysis showed five homozygous missense mutations in exon 11 c.1156C > T or p.R386C, a single nonsense mutation in the heterozygous state c.974G > A p.W325, and heterozygous in exon 9 mutation of exon 3 c.280C > T p.R94C, missense variant reported by Ogawa in 1995 [17]. There was only one patient that presented a homozygous missense mutation in exon 9 c.901G > T p.G301C and four patients showed the heterozygous form. A heterozygous missense mutation in exon 5 c.425A > T p.H142L, which has not been previously reported, was found in a female patient, 2 years 11 months of age. The diagnosis algorithms that include molecular analysis, bioinformatic predictive tools, pharmacogenomics, and proteomics helps to improve the diagnosis, treatment, and prognosis of patients affected by MPS IVA.

1. Introduction

Mucopolysaccharidosis IVA (MPS IVA, Morquio syndrome type A) (OMIM # 253000) is a lysosomal storage disorder caused by a mutation in the GALNS gene located on chromosome 16q24.3 and inherited in an autosomal recessive manner [1]. The disease is characterized by a deficiency of *N*-acetylgalactosamine-6 sulfatase (GALNS), which leads to the accumulation of chondroitin-6-sulfate (C6S) and keratan sulfate (KS) in many tissues and organs [2].

The incidence of the disease in the general population is 1 per 201, 000 live births and ranges from 1 per 76, 320 in Northern Ireland to 1 per 641, 178 live births in Western Australia [3]. In Colombia, a study published in 2012 estimated incidence of MPS IVA, in 0.68 cases per 100, 000 live births [4], and according to the Colombian Fund for High-Cost Diseases, in 2014 there were 15 people diagnosed with MPS IV [5]. There is also a study that shows the findings of pottery from the pre-hispanic period of individuals with apparent features of MPS IVA. According to these results, researchers have suggested the existence of a

founder effect in the country [6].

There are no recent epidemiological studies of MPS IV in Colombia, but since 2015 - as part of public health policy- a routine notification program for orphan diseases has been implemented. The purpose of this program is to generate a database with necessary information about the patients and their diagnosis (clinical, enzymatic and molecular). To provide more considerable knowledge about the incidence, prevalence, mortality, number of cases by geographic area as well as the genotypic and phenotypic expression of autochthonous mutations of orphan diseases [7].

The low expression of GALNS gene encoding the protein Galactosamine 6-sulfate sulphatase involved in the catabolism of chondroitin-6-sulfate (C6S) and keratan sulfate (KS) is responsible for the clinical manifestations of the disease [3]. As C6S and KS are the primary components of proteoglycans in cartilage and bone, the main clinical sign of MPS IVA is skeletal dysplasia, also defined as multiple dysostoses (MD) [3]. Different degrees of bone and joint abnormalities including short stature, short neck and trunk, gait disorders, genu

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valgus, hip dysplasia, joint hyperlaxity, and anomalies in the spinal cord and thorax; result in locomotion deterioration [3].

Non-skeletal manifestations of MPS IVA include respiratory compromise, upper and lower respiratory tract obstruction, respiratory restriction. Cardiac abnormalities include valvular disease and increased heart rate. Nervous system disorders comprehend cervical myelopathy and spinal compression. Eye disorders as corneal turbidity and visual impairment (20/80 or worse); ear or vestibular disorders; dental anomalies, and abdominal abnormalities as hepatomegaly, splenomegaly and hernias are also other signs of the disease [3].

For diagnosis, once physicians have a suspicion of MPS IVA, screening can be performed either by testing for an abnormal elevation of urinary GAG levels and excess of KS or by measuring enzyme activity from a dried blood spot (DBS) [8]. Reduction of GALNS enzyme activity can also be demonstrated in cultured fibroblasts or leukocytes. Alternatively, if suspicion of MPS IVA is strong, physicians may choose to bypass screening tests and request for molecular testing [8].

Molecular analysis, also known as mutation analysis, can be carried out as an additional confirmation of enzyme activity results [8]. The GALNS gene is located on chromosome 16q24.3 [9] contains 14 exons [10] spanning 50 kb, encodes a 522-amino acid protein: *N*-acetylgalactosamine-6-sulfate sulfatase [11], and generates a 1566 nucleotide mRNA [12]. Studies carried out in different population groups have revealed 16 polymorphisms and around 148 mutations of the GALNS gene. The identification of new mutations continues [8, 13], recently Caciotti et al. reported 14 new mutations in GALNS in a study of 37 Italian patients [14]. Because MPS IVA is a recessive disease, both GALNS alleles must contain a pathogenic mutation for a patient to be affected [8]. This extensive allelic heterogeneity of gene mutations gene is consistent with the broad spectrum of clinical phenotypes observed in MPS IVA patients [11].

This study aimed to report information from patients diagnosed with MPS IVA from a geographical zone of Colombia to contribute to the understanding of this disease in the country, despite the country's health system barriers.

2. Methods

This is a descriptive transversal study in which 12 patients that had been diagnosed clinically and enzymatically as MPS IV A were clinically and molecularly characterized. Patients consented to their participation before being enrolled in the research, according to the local regulation (minors' assent was also obtained). The study was approved by the Institutional Review Board - Ethics Committee of Universidad del Valle.

2.1. Clinical analysis

Demographic characteristics were obtained from hospital records as well as anthropometric measurements. Following the recommendations of the *International Guidelines for the Management and Treatment of Morquio A Syndrome* [15], growth charts for patients affected with MPS IVA were used in this study. The classification according to nutritional status was performed by the combination of indicators: weight-age, height-age, and body mass index (BMI-) age. Information regarding urine or blood keratan sulfate was not available for all patients in clinical records.

2.2. Molecular analysis

Molecular characterization was performed by sequence analysis of GALNS gene, the consequences of mutations affecting protein molecular structures and its pathological implications were determined through bioinformatic tools.

Blood samples for measurement of GALNS enzymatic activity were obtained by a digital puncture and fixed to filter-paper; venous blood samples for DNA extraction were collected by standard venipuncture

Table 1
Demographics and characteristics of patients at study entry.

Patient	Gender	Current age	Height (cm)	Weight (kg)
1	M	21	95	25,8
2	M	24	104	26,8
3	F	16	100	23,6
4	F	19	92	19
5	F	23	92	37
6	M	15	104	27,6
7	F	13	95	23
8	M	15	94	17,6
9	F	14	96	19,5
10	F	28	95	43
11	M	13	104	23,4
12	F	2	86	12,5

into vacuum tubes. Samples were prepared by dilution to 100 ng/ul and were sent to Illumina (San Diego, USA) for DNA analysis: OD reading A260 /A280 of 1.80 +/- 0.1.

Bioinformatic research for referential data was performed in databases, and ClinVar tool, The European Bioinformatics Institute the National Center for Biotechnology (NCBI) (EMBL-EBI) was used. For analyzing the amino acid substitutions, the software SIFT prediction, PolyPhen 2 and Taster Mutation were useful as in-silico tools. Based on sequence homology and physical properties of amino acids SIFT predicts whether an amino acid substitution affects the function of the protein, PolyPhen 2 (<http://genetics.bwh.harvard.edu/>) shows exon 5 c.425A > T p. H142L as the cause of damage with a score of 1000, and Taster Mutation (<http://www.mutationtaster.org/>) predicts them as the disease cause with a 0.999 probability.

3. Results

Twelve patients were included in the study, with most patients of female gender ($n = 7$, 58,3%), age range 2 to 28 years, average weight 26 kg (17.6–43 kg), average height 97 cm (92–104 cm), average BMI 27.6 kg/m² (19.92–47.65 kg/m²). Table 1 shows demographics of patients at study entry.

3.1. Clinical results

For the weight/age indicator, one patient was found in risk of obesity, and two in malnutrition risk; for the height/age indicator, all patients were normal; for the BMI indicator, three patients were found to be overweight and two to be severely obese. Growth tables from the Morquio A patients enrolled in the International Morquio Registry, were used to compare BMI results [16].

All patients had genetics, physiatry, ophthalmology, audiology, dentistry and physical therapy assessments. Signs and symptoms showed by all patients were osteoarticular-deformity, short stature and gait alterations.

In five patients (42%) MRI showed vertebral hypoplasia, platyspondyly, kyphosis, and joint hyperlaxity, two patients had spinal canal narrowing at C2 and C3 level with compression and obliteration of anterior and posterior subarachnoid space. 25% percent of patients had long-bone x-ray reports with bilateral genu valgus and hip dysplasia as common findings but at the time of the study, there was no evidence of corrective surgery performed for any of the patients.

Cardiovascular findings included mild mitral regurgitation (one patient) and mitral and aortic sclerosis (one patient). For the 6-min walk test (6MWT) frequent results were maximum limb fatigue (10/10 score in Borg scale) [17] and rested stops at the different time of examination. Regarding pulmonary results, all patients presented moderate to severe restrictive changes without response to bronchodilator with an average oxygen saturation of 94%.

WISC and WAIS test was performed to evaluate intelligence, and all

patients showed average results. For the ophthalmological assessment, all had different degrees of refractive errors (hypermetropia, amblyopia, astigmatism) and two patients presented anterior chamber opacity.

Otorhinolaryngological assessment proved a mild bilateral sensorineural hearing loss for two patients and severe for another one. Abdominal organ enlargement was not detected in clinical or ultrasound examination. Although, the measurement of organ volume by ultrasonography is not commonly done in our country, according to radiologist report organ measures were normal.

3.2. Molecular results

Enzymatic activity (Galactosamine 6-sulfate sulphatase) was altered for all patients (average 0.38 $\mu\text{mol/L/h}$, reference value $\geq 5.3 \mu\text{mol/L/h}$). GALNS gene molecular analysis showed five homozygous missense mutations in exon 11 c.1156C > T or p.R386C, initially reported by Ogawa et al. in 1995 [18] (rs118204437), and characterized by The European Bioinformatics Institute (EMBL-EBI), as a result of multiple observations and genotype-phenotype correlations. This mutation was also found as heterozygous in four patients. Last report in ClinVar in June 2014 by Emory Genetics Laboratory - Pathogenic.

A single nonsense mutation in the heterozygous state was found in one patient c.974G > A p.W325 * HGMD CM105265, reported by Wang in 2010 [19], and heterozygous in exon 9 mutation of exon 3 c.280C > T p.R94C, missense variant reported by Ogawa in 1995 [18].

There was only one patient that presented a homozygous missense mutation in exon 9 c.901G > T p.G301C and four patients showed the heterozygous form. This mutation was reported by Bunge in 1997 [20], (rs118204443), and it was considered pathogenic according to the project “1000 Genomes” [21] and due to multiple observations and genotype-phenotype correlations in The European Bioinformatics Institute (EMBL-EBI). Last evaluation on September 18, 2014, in ClinVar. Table 2 shows molecular results for each patient.

3.3. Clinical and molecular description of missense mutation not previously reported

A heterozygous missense mutation in exon 5 c.425A > T p.H142L, which has not been previously reported, was found in a female patient, 2 years 11 months of age (patient number 12).

At two months of age, the patient was diagnosed with bilateral hip

dysplasia; she underwent radiological follow-up that showed the persistence of dysplasia and signs of dysostosis that led to the suspicion of lysosomal storage disease (mucopolysaccharidosis). At two years 11 months of age, physical examination showed a weight of 12.5 kg (25–50 percentile), a height of 86 cm (50–75 percentile), BMI 16.90 kg/m² (25–50 percentile), coarse facial features, pectus carinatum, umbilical hernia and bilateral genu valgum. Radiographic findings included vertebral hypoplasia and partially altered L4 with discrete dorso-lumbar right convexity scoliosis, bilateral hip dysplasia, and normal thorax.

The activity of the GALNS enzyme was below the normal reference range, 0.06 nmol/mg prot/h (reference values 2–35.9 nmol/mg prot/h). This value was compatible with a diagnosis of MPS IV A. Molecular analysis reported a heterozygous mutation in exon 9 of the GALNS gene (c.901G > T p.Gly301Cys), (rs118204443) as described above.

The new mutation was located in exon 5 of the GALNS gene (c425A > T p.His142Leu), in a region of high nucleotide and amino acid conservation, with average physicochemical differences between the amino acids Histidine and Leucine.

Mutation damage prediction was made using Mutation Taster, and it was found as damaging with a probability of 0.999 (<http://www.mutationtaster.org>), and PolyPhen-2 predictive analysis also identified the mutation as pathogenic with a score of 1000 (<http://genetics.bwh.harvard.edu>).

4. Discussion

MPS IVA is caused by the deficiency of the enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS). The accumulation of chondroitin-6-sulfate (C6S) and KS results in impairment of cartilage and bone development leading to systemic skeletal dysplasia but, compared to other MPS disorders, patients present joint hyperlaxity and a minimal impact on cognitive function. Currently, anthropometric assessment corrected to growth tables allows adequate monitoring and control of metabolic risk. In this study, we reported 12 Colombian patients that show clinical characteristics for MPS IVA as described in the literature. Even though most patients were females ($n = 7$, 58.3%), this finding is due to chance rather than the death of males. Regarding growth charts, it was not possible to retrieve this information as well as data from the head circumference, trunk height, arm length, neck length, and lengths of lower limbs, due to limitations in the local health system. Additionally, we consider that in patient management, it is

Table 2
Molecular findings.

Patient No.	Locus	Nucleotide change	Amino acid change	Reference	Enzymatic activity	Codon	PolyPhen 2	Mutation taster
1	Ex11	c.1156C > T (homo)	p.R386C	Ogawa, 1995 [18]	0.3 $\mu\text{mol/L/h}$	CGT, TGT	0.999	0.999
2	Ex09	c.974G > A (het)	p.W325*	Wang, 2010 [22]	0.7 $\mu\text{mol/L/h}$	Stop Codon	0.999	0.999
	Ex03	c.280C > T (het)	p.R94C	Ogawa, 1995 [18]		TCT, TCC	1.000	0.999
3	Ex11	c.1156C > T (het)	p.R386C	Ogawa, 1995 [18]	0.4 $\mu\text{mol/L/h}$	CGT, TGT	0.999	0.999
	Ex09	c.901G > T (het)	p.G301C	Bunge, 1997 [20]		GGC, TGC	0.999	0.999
4	Ex09	c.901G > T (homo)	p.G301C	Bunge, 1997 [20]	0.3 $\mu\text{mol/L/h}$	GGC, TGC	0.999	0.999
5	Ex11	c.1156C > T (het)	p.R386C	Ogawa, 1995 [18]	0.3 $\mu\text{mol/L/h}$	CGT, TGT	0.999	0.999
	Ex09	c.901G > T (het)	p.G301C	Bunge, 1997 [20]		GGC, TGC	0.999	0.999
6	Ex09	c.901G > T (het)	p.G301C	Bunge, 1997 [20]	0.3 $\mu\text{mol/L/h}$	GGC, TGC	0.999	0.999
	Ex11	c.1156C > T (het)	p.R386C	Ogawa, 1995		CGT, TGT	0.999	0.999
7	Ex09	c.901G > T (het)	p.G301C	Bunge, 1997 [20]	0.3 $\mu\text{mol/L/h}$	GGC, TGC	0.999	0.999
	Ex11	c.1156C > T (het)	p.R386C	Ogawa, 1995 [18]		CGT, TGT	0.999	0.999
8	Ex11	c.1156C > T (homo)	p.R386C	Ogawa, 1995 [18]	0.5 $\mu\text{mol/L/h}$	CGT, TGT	0.999	0.999
9	Ex11	c.1156C > T (homo)	p.R386C	Ogawa, 1995 [18]	0.9 $\mu\text{mol/L/h}$	CGT, TGT	0.999	0.999
10	Ex11	c.1156C > T (homo)	p.R386C	Ogawa, 1995 [18]	0.3 $\mu\text{mol/L/h}$	CGT, TGT	0.999	0.999
11	Ex11	c.1156C > T (homo)	p.R386C	Ogawa, 1995 [18]	0.3 $\mu\text{mol/L/h}$	CGT, TGT	0.999	0.999
12	Ex05	c.425A > T (het)	p.H142L	None	0.3 $\mu\text{mol/L/h}$	GCA, GCT	0.999	0.999
	Ex09	c.901G > T (het)	p.G301C	Bunge, 1997 [20]		GGC, TGC	0.999	0.999

useful to evaluate activities of daily living (ADL) but, in our case, as this was an epidemiological study retrieving information from clinical records and ADL are not routinely asked by treating physicians, this information was not available.

On the other hand, the algorithm for the diagnosis of MPS IVA, developed by working groups at Prague (2011) and in San Diego (2012) [8], includes molecular analysis preceded by GALNS enzymatic activity that can be measured in fibroblasts or leukocytes. The molecular analysis allows the detection of mutations in the GALNS gene, that helps to confirm the diagnosis of MPS IVA, to make genotype-phenotype predictions, to give genetic counseling, and to verify carrier and relatives disease status.

Many mutations are causing MPS IVA, they are heterogeneous, most of them are missense mutations, and the founding effect can significantly alter allele frequencies in the individual GALNS gene populations. We have described in this study five homozygous missense mutations in exon 11 c.1156C > T or p.R386C, a single nonsense mutation in the heterozygous state c.974G > A p.W325, and heterozygous in exon 9 mutation of exon 3 c.280C > T p.R94C, missense variant, all of them previously reported.

Likewise, bioinformatic tools for mutation predictions contribute to the understanding of the disease.

Finally, we would like to emphasize that, as mentioned above, in Colombia there is lack of information regarding MPS IVA, additionally, deficiencies in the country health system do not let to retrieve information regarding patients' characteristics and follow up; these deficiencies include late diagnosis, inadequate management and, change of treating physician. For this reason, even we could not be able to capture all the information about clinical characteristics we would like to; we believe that the add-on value of this study is to provide information about a group of patients with MPS IVA in a specific geographical area and to report one mutation not known previously. We consider that, in orphan diseases, trying to retrieve the most information about patients contributes to general and local disease knowledge.

5. Conclusion

In this study, we reported a mutation in exon 5 c.425A > T p.H142L not described before, as well as the use of PolyPhen 2 and Mutation Taster that defined this mutation as pathogenic. The diagnosis algorithms that include molecular analysis, bioinformatic predictive tools, pharmacogenomics, and proteomics helps to improve the diagnosis, treatment, and prognosis of patients affected by MPS IVA.

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