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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/m^2 (19.92–47.65 kg/m^2). 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 prehispanic 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 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.

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

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/m^2 (19.92–47.65 kg/m^2). 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