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Molecular findings of Colombian patients with type VI mucopolysaccharidosis (Maroteaux–Lamy syndrome)

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ABSTRACT

Introduction: Maroteaux–Lamy syndrome, or mucopolysaccharidosis (MPS) type VI, is an autosomal recessive lysosomal storage disease caused by a deficient activity of the enzyme arylsulfatase B (ARSB), required to degrade dermatan sulfate. The onset and progression of the disease vary, producing a spectrum of clinical presentation. So far, 133 mutations have been reported. The aim of this study is to determine the mutations in the *ARSB* gene that are responsible for this disease in Colombian patients.

Results: Fourteen patients with clinical manifestations and biochemical diagnosis of MPS VI were studied, including two siblings. The 8 exons of the gene were directly sequenced from patients' DNA, and 14 mutations were found. 57% of these mutations had not been previously reported (p.H111P, p.C121R, p.G446S, p.*534W, p.S334I, p.H147P, c.900T>G, and c.1531_1553del) and 43% had been previously reported (p.G144R, p.W322*, p.G302R, p.C447F, p.L128del, and c.1143-1G>C). Of the previously reported mutations, 80% have been associated with severe phenotypes and 20% with intermediate-severe phenotypes. Bioinformatic predictions indicate that the new mutations reported in this paper are also highly deleterious.

Conclusions: Most of the Colombian patients in this study had private mutations.

1. Introduction

Mucopolysaccharidoses are a group of congenital metabolic disorders caused by the deficiency of a specific lysosomal enzyme that affects normal catabolism of glycosaminoglycans (GAGs). Accumulation of GAGs in different organs and tissues leads to the complex signs and symptoms of these multisystemic diseases (Giugliani et al., 2010; Neufeld and Muenzer, 2001). Mucopolysaccharidosis type VI (MPS VI; MIM no. 253200) or Maroteaux–Lamy syndrome is a rare genetic disease with recessive autosomal inheritance caused by deficiency of the N-acetylgalactosamine-4-sulfatase enzyme, also known as arylsulfatase B (ARSB). This enzyme is required to degrade dermatan sulfate and chondroitin sulfate [review in 3]. The disease is characterized by progressive, systemic clinical manifestations that cause significant functional impairment. The rapidly progressive form is typically characterized by a slowing of the growth rate, skeletal and joint deformities, coarse facies, and obstruction and recurrent infections of the upper airway. Later on, patients require wheelchair support or are bed-ridden due to bone deformities, cardiopulmonary disease, blindness, or compression of the spinal canal. Patients with the rapidly progressive disease die in adolescence or by age 20, while individuals with slowly progressive forms have a life expectancy of approximately 40–50 years. Although cognitive impairment is not usually described, physical and functional limitations affect psychomotor development and learning (Giugliani et al., 2007). A global incidence of MPS VI between 1 in 248,000 and 1 in 300,000 live births is estimated (El Dib and Pastores, 2009), but population data indicate that incidence may be higher in Brazil. In a high-risk screening of a Brazilian population with MPS diagnoses, 19% were identified as MPS VI (Giugliani et al., 2007). In the city of Monte Santo, State of Bahia, northeastern Brazil, actual prevalence is estimated to be much higher than the prevalence reported in the literature; a founder effect of the p.H178L mutation has been described in a cluster of patients in this area (Costa-Motta et al., 2011). In 2012, 27 MPS VI cases were identified in Colombia, of which 10 occurred in native groups (Rosselli et al., 2012). Additionally, a series of 20 ceramic artifacts from the Tumaco-La Tolita culture, more than 200 years old, were found. It is possible that these artifacts represent individuals with Maroteaux–Lamy syndrome. The artifacts depict phenotypic characteristics, such as skeletal dysplasia, macrocephaly, coarse features, wide mouths, prominent chests, kyphosis, and scoliosis. These ceramics are likely evidence of this disease occurring in pre-Columbian populations in Colombia (Pachajoa and Rodriguez, 2014). Patients with MPS VI

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have widely variable multisystemic symptoms, with typically chronic and progressive courses, that mainly affect the cardiorespiratory and skeletal systems, corneas, skin, liver, spleen, meninges, and brain (Azevedo et al., 2004). Although the systemic involvement is very similar to the clinical profile of MPS I, intelligence quotient is not affected by MPS VI because there is no accumulation of heparan sulfate, which is predominantly responsible for neurological damage (Neufeld and Muenzer, 2001).

The *ARSB* gene, located on chromosome 5 (5q13-q14), is made up of 8 exons and synthesizes a 2228-bp mRNA that encodes a precursor protein of 533 amino acids (Valayannopoulos et al., 2010; Litjens et al., 1989). As of October 2015, 165 mutations were reported by The Human Gene Mutation Database (HGMD Professional 2015.3) (www.hgmd.cf.ac.uk.) including missense mutations (102), nonsense mutations (9), splice site mutations (18), small deletions (3), small insertions (1), insertion–deletion (indel) mutations, and large deletions (2). In South America, various studies have been conducted on patients with MPS VI, including the first molecular study of South American patients (12 Brazilian and 1 Chilean) by Petry et al. in 2005 that identified 7 new mutations (Petry et al., 2005). Other studies involving South American patients include Karageorgos et al. study in 2007 (Karageorgos et al., 2007) and Garrido et al. study in 2007 (4 Argentinian patients) (Garrido et al., 2007). As mentioned earlier, some studies have been conducted on South American population, but to date, no molecular study has been conducted on Colombian patients.

A correlation between excreted urinary GAGs and phenotype was found by Swiedler et al. in 2005 (Giugliani et al., 2010; Swiedler et al., 2005), but no direct correlation has been established between genotype and phenotype thus far (Giugliani et al., 2010; Litjens et al., 1996). Identification of the genotype may be important for predicting phenotype and, in some MPS cases, making treatment decisions; it is also useful for providing family genetic counseling on reproductive risks, prenatal diagnosis, and prevention of genetic diseases (Giugliani et al., 2010). The aim of this study is to identify the molecular alterations responsible for Maroteaux–Lamy syndrome in Colombian patients.

2. Material and methods

2.1. Patients

Fourteen patients with MPS VI, or Maroteaux–Lamy syndrome, biochemically confirmed by enzyme activity analysis, entered the study after signing an informed consent. All patients came from different areas of Colombia; 50% (7 patients) from Bogotá and 50% (7 patients) from other regions of the country (Cartagena, Ipiales, Funza, and Medellín). In regards to gender distribution, 50% were women and 50% were men. Of the 14 patients, 2 were siblings (patients 4 and 5) and the rest of the patients were not related. This study complied with the provisions established by resolution No. 008430 of 1993 of Colombia Ministry of Health, and it was conducted under the Ethical Principles for Medical Research Involving Humans established by the Declaration of Helsinki. Finally, it was approved by the Ethics Committee of Universidad El Bosque and Pontificia Universidad Javeriana.

2.2. Genomic DNA extraction, polymerase chain reaction (PCR), and sequencing

Genomic DNA was obtained from peripheral blood in EDTA tubes using the salting-out method of extraction (Miller et al., 1988). Each of the 8 exons, including their adjacent intronic regions (approximately 30 bp 5′ and 3′ of each exon), was amplified by PCR. Primers were based on those reported by Garrido et al. (Garrido et al., 2007) and were verified by the Primer 3 program (http://primer3.sourceforge.net). The primer sequences and annealing temperatures are shown in Table 1. The PCR consisted of 40 ng of template DNA, 1.25 U of Taq DNA polymerase (Bioline Ltd., London, UK), 1× buffer, 1 mM $MgCl_2$, 0.08 mM dNTPs, 0.2 mM primers, and 4% DMSO in a final volume of 25 μl. The reactions underwent initial denaturation for 5 min at 95 °C and 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 59–62 °C (depending of the primer) for 30 seconds, elongation at 72 °C for 30 seconds, and a final extension step at 72 °C for 5 min. The PCR products were purified using the EXOSAP-IT® enzymatic column method, and sequencing was performed using an ABI PRISM 3730XL Analyzer® (96 capillary type).

Table 1
Sequence of the primers, size of the product, and the annealing temperature in PCR protocols.

Exon	Primers	Expected product (bp)	Annealing temperature
Exon 1	TTCCTCATTCTATCAGCGGTACAAG GAGAAGCCGCCGGGACCCATAACT	522	59.8 °C
Exon 2	GAAGGCCATTTTATCTGCTTGT TGATTGCACTTGGGTGTGTT	337	59 °C
Exon 3	TAGCCTCGTCACGGGTAATC CAACAATGGCCTTTTCCTACA	382	59.3 °C
Exon 4	GCATAAATCTGAACTGTCTTATCCT GCTAACCGCTCCAATTTGTC	378	63 °C
Exon 5	GGGAATTTAGGGTGGGAAAA TCAGGCTGCTCTTGGAGTTTT	444	59 °C
Exon 6	CTGGCAGGTTTGTTATTTCC AATCAAACCATCTTGGTGGG	236	61.5 °C
Exon 7	CACATTTGCACTCCAGTGTTG CAGGAGGGCAGATAGACTGG	333	61.9 °C
Exon 8	ATGTTTCCACACCCACAACC AAAAGGCCTGAGGTCCAACT	430	62 °C

2.3. Bioinformatic tools

The program Sequencher 5.2.4 (Gene Codes Corporation) was used for sequence analysis. Upon discovery of any discrepancy between the reference sequence (NG_007089.1) and a patient's sequence, a search was conducted at the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/gene/411) to determine whether the variant had been previously reported. If it proved to be a single nucleotide polymorphism (SNP), the allelic frequency of this change was searched in the 1000 Genomes database (http://www.1000genomes.org/). Previous reporting of the mutation was corroborated in the database of specific MPS6 mutations (http://mps6-database.org/) and the human gene mutations database (http://www.hgmd.cf.ac.uk/ac/index.php). If the variant had not been reported, bioinformatic predictions of the defects in the protein variant were performed using the following bioinformatic tools: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) (Adzhubei et al., 2010), MutationTaster (http://www.mutationtaster.org/) (Schwarz et al., 2014), MuStab (http://bioinfo.ggc.org/mustab/) (Teng et al., 2010), SNPs&GO (http://snps-and-go.biocomp.unibo.it/snps-and-go/) (Calabrese et al., 2009), and Provean (http://provean.jcvi.org/index.php) (Choi et al., 2012). Novel mutated amino acids were located on a 3D ARSB protein graphic using modeling of the enzyme arylsulfatase B (1FSU PDB) described by Bond et al. (1997) for Pymol v1.7.4 free accesses software (http://www.pymol.org/) (The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC). Adaptive Poisson–Boltzmann Solver (APBS) and PDB2PQR packages were used to calculate the electrostatic potentials of all the protein atoms (Baker et al., 2001; Dolinsky et al., 2004). The APBS default parameters were set. The thermodynamic stability changes of mutations were computed using the force-field FoldX (http://foldx.crg.es/) (Schymkowitz et al., 2005). The MutationTaster was used to compare the Arylsulfatase B amino acids of different species with mutated proteins, and the conservation scores were calculated by ConSurf (http://consurf.tau.ac.il/) (Ashkenazy et al., 2010).