Identification of a Novel Mutation in the Human ARSB Gene on Chromosome 5q14.1 for Autosomal Recessive Mucopolysaccharidosis Type VI Patients in Southwest Colombia

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Abstract
Mucopolysaccharidosis VI is a rare lysosomal storage hereditary disease, caused by arylsulfatase B enzyme deficiency which leads to the accumulation of harmful amounts of dermatan sulfate.

Methods: A total of 32 MPS VI patients were identified in Colombia, sixteen (45 %) in the Department of Cauca, identified clinically and by enzymatic assay. Two of these individuals belong to the Guambiano Amerindian reservation. DNA extraction and sequencing for the region of the arylsulfatase B by ABI PRISM® 3100 Genetic Analyzer was performed for these two index cases and 22 of their relatives. A principal component analysis of the genetic haplotypes was also performed.

Results: We found a novel single nucleotide change (p.Ser403X) in the ARSB gene, homozygous for the patient and heterozygous for their relatives, and classified as pathogenic. Cases and their relatives shared the same haplotype.

Conclusion: Together with the genealogy analysis, these results suggest a common ancestral allele in the Guambiano Amerindian reservation in the Department of Cauca and a novel mutation for MPS VI.

Keywords
Maroteaux-Lamy syndrome; Mucopolysaccharidosis VI; Novel mutation; Ancestral allele

Abbreviations:
ARSB: Arylsulfatase B; cm: Centimeter; CP: Cephalic Perimeter; DIC: Intercanthal Distance; DIM: Internipple Distance; DNA: Deoxyribonucleic Acid; FEV1: Forced Expiratory Volume in 1 second; FVC: Forced Vital Capacity; G2B2: Gestations and 2 Life Births; GAGs: Glycosaminoglycan; HGVS: Human Genome Variation Society; Kg: Kilogram; MPS: Mucopolysaccharidosis; MPS IV: Mucopolysaccharidosis VI; mol/h: Mole/hour; PEF: Peak Expiratory Flow; SNPs: Single Nucleotide Polymorphisms; SS/ST: Superior Segment / Inferior Segment; TP: Thoracic Perimeter; µg/mg: Micrograms/milligram

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Received: January 25, 2017 Accepted: February 18, 2017 Published: February 25, 2017

Introduction
Mucopolysaccharidosis type VI (MPS VI) or Maroteaux-Lamy syndrome is a rare and severe autosomal recessive disease in humans caused by impaired activity of the lysosomal enzyme hydrolase N-acetylgalactosamine 4-sulfatase or arylsulfatase B [1], resulting from mutations in the ARSB gene (OMIM # 253200) [2]. The enzyme deficiency leads to the accumulation of harmful amounts of ungraded dermatan sulfate inside the lysosomes, causing progressive damage of several tissues. Patients present with a wide clinical spectrum (clinical heterogeneity), ranging from severe to mild forms of disease progression [3]. Severity correlates with the age of onset, organ involvement and disease progression [3-5]. The severe phenotype exhibits a rapidly progressing MPS VI characterized by early onset of symptoms and short life expectancy (20-30 years) [6]; this correlates with certain alleles and urinary glycosaminoglycan (GAGs) levels higher than 100 µg / mg. On the other hand, the mild form is associated with delayed onset of signs and symptoms, and levels of GAGs less than 100 µg / mg [4].

The ARSB gene, located on the long arm of chromosome 5 (5q13.3-q14.1) [7], consists of 8 exons spanning less than 440 kb and encoding for a 539-aminoacid polypeptide and a 37-aminoacid signaling peptide [8]. Translocation across the endoplasmic reticulum membrane occurs through cleavage of the signaling peptide and glycosylation of asparagine residues [7]. The ARSB gene has 3910 intragenic Single Nucleotide Polymorphisms (SNPs), and 68 regulatory elements such as promoters, enhancers and binding sites for transcription factors [9].

Like other mucopolysaccharidosis, MPS VI can be caused by different types of mutations in the ARSB gene such as deletions, insertions, missense, nonsense, as well as mutations in splicing sites (Figure 1).

Such mutations have been identified in patients from North and South America, Europe, Australia and Asia, with clear genotype-phenotype correlations in some cases. The most common mutations identified in patients from North America, South America, Europe and Australasia are c.629A>G, c.944G>A, c.1143-8T>G, c.1143+1G>C and c.1151G>A [10] with a frequency higher than 10 %. Most of these mutations are produced by single nucleotide changes, which lead to loss of transcription. The prevalence of these mutations is mainly reported in Russia, Australia, USA, France, Germany, Portugal, Italy, China, Brazil, Colombia, Chile, Argentina and Spain [1,4,11-21]. New mutations are reported on a regular basis [22,23] and attempts are being made to establish the corresponding phenotype-genotype correlations.

To date, 139 mutations in the ARSB gene have been identified, according to entries in the Human Gene Mutation Data Base and recent publications (Figure 1) [10].

Materials and Methods
A total of 36 MPS VI patients were identified in Colombia, 16 of them (45 %) in the Department of Cauca. Of these 16 patients who were fully classified according to their clinical and enzymatic assay profile (results not available), two belong to a Guambiano Amerindian reservation (municipality of Silvia) (Figure 2).
These two patients were reported as index cases and, together with their 22 relatives (from 2 different families), were put through the following protocol: DNA extraction, amplification, purification and sequencing reaction for the genomic DNA of arylsulfatase B in a capillary electrophoresis instrument ABI PRISM® 3100 Genetic Analyzer. The results of genomic DNA were placed in the format and reading sequence alignment required for the "staden package" [24]. The intragroup and sequence differences were determined by comparison to the reference genome sequence encoding for arylsulfatase B [8]. In addition, a complete and comprehensive analysis of the ARSB gene by sequencing analysis of the 8 exons with the flanking intron-exon was performed.

The main component analysis of the genetic haplotypes was done using the method for SNPs genotyping based on the Sequenom MassARRAY platform [25] and the Haploview software [26].

Results

Clinical phenotype of index cases

F1 index case: A 4-year old male patient coming from the Cacique Alto village in the Guambiano-Cauca reservation, born to a 22-year old woman G2B2 who reported occasional exposure to pesticide sprays during pregnancy, positive fetal movements, cephalic presentation and home birth. Both parents are of indigenous origin. The family history includes treated pulmonary tuberculosis in the maternal grandmother, a speech disorder in a maternal aunt, and three individuals of the same family who died young with a similar undiagnosed clinical picture (Guambiano family F1, Figure 3).

The patient initially presented a giant umbilical hernia, progressive corneal opacity, dorsiolumbar spine deformity (Figure 4.2) and

doi: 10.4172/2327-5790.1000151

varus metatarsal bone (varus 2nd to 5th metatarsals) (Figure 6.2).

Comparative radiological examination of the upper limbs revealed severe mixed, predominantly conductive hearing loss in the right ear and mild sensory hearing loss of the left ear. Radiographic assessments of the cranium showed increased interpeduncular distance of the vertebral bodies, notorious in particular in the lumbar segment; kyphosis of the dorsolumbar junction, L2 retrolisthesis, beak deformity of the lower T12, L1 and L2 angles (Figure 5.1). The radiograph of the rib cage showed bilateral costal spautula deformity (Figure 4.2). The pelvic radiograph showed bilateral coxofemoral subluxation, prominent sciotic spines and generalized osteopenia.

Comparative radiological examination of the upper limbs showed widening of the medullary region of the long bones with cortical thinning, bilateral inferior radio-ulnar diastasis, Madelung’s deformity, metacarpal deformity with proximal metaphyseal thinning, delayed skeletal maturation and phalangeal widening (Figure 6.1). Comparative lower limb films show widening of the medullary regions with cortical thinning, hallux valgus and bilateral varus metatarsal bone (varus 2nd to 5th metatarsals) (Figure 6.2).

Echocardiography showed a dysmorphic mitral valve with mild stenosis and regurgitation, grade II prolapse of the anterior mitral valve, mild aortic stenosis and regurgitation, mild subaortic hypertrophy without a gradient. Spirometry showed a flow/volume curve with diminished FEV1, FVC and PEF (obstructive and restrictive abnormality) with no improvement with inhaled B2. Polysomnography revealed a mixed apnea pattern. Plain computed tomography of the abdomen revealed moderate hepatomegaly and increased pancreatic size, with no additional abnormalities. Cerebral magnetic resonance imaging showed an empty sella syndrome and spinal canal stenosis at the craniocervical junction due to soft tissue thickening posterior to the odontoid process and bilateral inflammatory changes of paranasal and mastoid sinuses. Spinal magnetic resonance imaging showed cervical stenosis with soft tissue involvement of C1-C2 posterior to the odontoid process, with dorsal deformity due to diminished anterior height, and reduced height of the anterior and posterior thirds.

A clinical diagnosis of mucopolysaccharidosis type VI was made on the basis of these findings, while enzymatic activity filter-paper testing for arylsulfatase B showed a value of 2.1 µmol/h (reference value: >5 µmol/h), which was confirmed with a second filter paper sample, and leukocyte testing showed a value of 1.65 µmol/h (reference value: 5.31-21.85 µmol/h). Electrophoresis for MPS showed migration patterns corresponding to dermatan and chondroitin sulfate with diminished mobility. Leukocyte testing revealed a value of 1.65 µmol/h (reference value: >5 µmol/h). Electrophoresis for MPS showed migration patterns corresponding to dermatan and chondroitin sulfate with positive quantification of urinary GAGs. This confirmed the clinical diagnosis, and enzyme replacement therapy was initiated in 2013.

F2 index case: A 5-year old female patient, first-born to a mother of 25 and a father of 26, both from the same indigenous reservation (Guambiano Family 2) (Figure 7).

The initial symptoms appeared at six months of age, with organomegaly, hearing loss, umbilical hernia and heart disease. At 10 months of age, the child developed congestive heart failure, secondary to patent ductus arteriosus, with hemodynamic repercussions, severe mitral regurgitation, tricuspid regurgitation and biventricular dysfunction. This required mitral valve replacement with a biological valve plus surgical closure of the patent ductus arteriosus. A presumptive diagnosis of MPS VI was made at 2 years of age and...
various qualitative acid albumin and azure strip tests were positive. The result of the filter paper test for ARSB enzymatic activity was 2.9 µmol/h (reference value > 5 µmol/h), and it was confirmed by the result of a second filter paper test of 1.2 nmol/h/ml (reference value: 5.3-22 nmol/h/ml). The result for leukocyte arylsulfatase B was 30.38 nmol/mg protein/H (normal range: 115-226), confirming the diagnosis of MPS VI.

The physical examination showed evidence of disproportionate dwarfism, rough facies (Figure 8.1), corneal opacity, macroglossia, short neck, hepatomegaly 4 cm below the right costal margin, umbilical hernia (Figure 8.1), severe delayed psychomotor development, and dysostosis multiplex (Figure 8.2).

A computed axial tomography brain scan taken at 18 months of age showed ventricular dilatation (Figure 9). The echocardiogram showed a normally functioning biological valve, adequate biventricular function and mild left ventricular dilatation. Auditory evoked potentials showed profound neurological compromise of the right ear and profound to severe compromise of the left ear. Polysomnography showed desaturation of up to 79 %, possibly consistent with increased upper airway resistance.

Radiographic assessment showed increased anteroposterior skull diameter with a “J” sellar deformity, dorsolumbar kyphosis, left lumbar scoliosis, lumbarization of the first sacral vertebra, increased interpedicular distance in the dorsolumbar vertebrae, beak deformity of the inferior angle of the vertebral bodies of L2 and L3, with L2 retrolisthesis. The rib cage shows spatula costal deformity.

Films of the upper and lower limbs show widening of the medullary regions with cortical thinning and generalized osteopenia. There is delayed bone maturation, based on carpal bone ossification. In the pelvis, there is bilateral acetabular dysplasia with coxofemoral subluxation. At the gait laboratory, the patient evidenced reduced hip mobility on the sagittal and coronal planes, with bilateral flexion deformity; knee flexion deformity; flat, valgus feet, and high-riding fibular malleoli; crouch gait pattern with very diminished gait speed due to short stride length, as well as signs of adenoid hypertrophy in the pharyngeal cavum with a 90 % reduction of the anteroposterior diameter of the rhinopharynx.

**Molecular test results**

After applying the previously described protocol, we found a single nucleotide change (p.S403X) in the ARSB gene of the two index cases (homozygous) (Figure 11) and 10 of their relatives (heterozygous) (Figure 11, Table 1).

According to the ALAMUT program, version 2 [27], this change found in exon 6 is a transversion that produces a nonsense mutation,
Figure 6.1: Hand radiograph showing wide irregular bones. Second and fifth metacarpals deformity of the with posterior beak.

Figure 6.2: Radiograph of the feet showing bilateral talus varus with bilateral beak deformity of the posterior third of the second and third metatarsals.

Figure 7: F2 Family Pedigree, showing a single index case (case 2) (Program Gen Pro).

Causing a premature stop codon. Following the recommendations of the HGVS program, version 2, this mutation will have the following nomenclature:

DNA Level (c DNA): NM_000046.3:c.1208C>A
DNA Level (genomic): Chr5(GRCh37):g.78135184G>T
Protein Level: p.Ser403X
Pos. 147327 (A/A) TCA>TAA
Mutation: Ser403Term

Guambiano families were studied with 62 SNPs, and a main component analysis of the genetic haplotypes was done using the method for SNPs genotyping based on the Sequenom MassARRAY platform [25], and the Haploview software [26]. The same haplotype (TTAATTT) was found in the probands and their relatives (Figure 12).

Discussion

The reported incidence of MPS VI ranges from 1 in 43,261 live births in Turkey to 1 in 1,505,160 live births in Sweden [28]. To date, there is no reported incidence of MPS VI in Colombia. Nevertheless,
a total of 36 cases have been identified nationwide, of which 16 come from the Southwest, an area of Colombia with Native American ethnicity.

This study reports a novel c.1208C>A mutation in the gene encoding for ARSB in two probands with MPS IV (homozygous) from the Guambiano population (Southwestern Colombia) and 10 carriers (heterozygous) within their families. High inbreeding in this population supports the clinical finding in the reservation, where there is a high frequency of genetic diseases, including MPS VI. This nonsense mutation is caused by a second base C>A substitution of a TCA codon encoding for serine in exon 6, leading to a TAA codon or stop codon (p.S403X). This results in a loss of the last 130 amino acids in the C-terminal end of the protein, which changes from a 533-aminoacid protein to a 402-aminoacid protein (Figure 13).

This shortening of the protein involves almost a complete loss of the lower domain of the enzyme, but leaves unchanged the main domain of the peptide sequence. Therefore, and based on the stability and preservation of the structure observed from that domain in different sulfatases, the three-dimensional structure of the truncated proteins would be expected to be very similar to the wild type in which the peptide fragment corresponding to the lower domain is deleted. This aberrant protein with its main domain preserved would have a 20-amino acid peptide chain in its C-terminal end, and most likely an absence of a secondary structure due to its short size, with an aberrant dissolution and crystalline state. The capacity of the protein to act as a sulfatase enzyme should remain unchanged by the p.S403X mutation, since the domain responsible for the catalytic process is intact. The biological function of the terminal domain remains unclear after the review of the literature [29,30]. However, according to the biological process in which the enzyme is involved, we think this domain is responsible for the molecular recognition of the substrate to be hydrolyzed, and for its solubility and bioavailability in the cell organelles and in the intracellular space.

The nonsense mutation and, therefore, the resulting truncated protein, will result in a higher exposure of the active domain to the intracellular space and, consequently, a considerable loss of selectivity against substrates. Additionally, the protein’s solubility would change leading to a different bioavailability when compared to the wild type.

The clinical phenotype in the two homozygous index cases is explained by the impaired protein function previously described. Since MPS VI is an autosomal recessive disease, when both parents carry the heterozygous mutation, the offspring has a 25 % risk of having the disease. Our family study identified both mothers as carriers and multiple family members as carriers also. The two index cases displayed the classical phenotype for severe MPS VI and are currently under enzyme replacement therapy.
Figure 11: Electropherograms comparing the reference genome with a heterozygous relative and the homozygous proband (novel mutation: c.1208 C>A, p.S403X)

Figure 12: Genotyping with Sequenom MassARRAY.
Table 1: Mutation analysis for MPS VI probands and their relatives.

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<tr>
<th>Patient #</th>
<th>dbSNP</th>
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<th>Protein</th>
<th>Site of mutation</th>
<th>Type of Mutation</th>
<th>Phenotype</th>
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<td>Exon 6</td>
<td>Nonsense</td>
<td>Heterozygous</td>
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<td>Pos. 147327 (A/A)</td>
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</tbody>
</table>

(*) Novel Mutation

Patients 4, 7-11, 16-18 and 22-24 didn’t present any genomic change
Cases 2 and 6 are index cases

Haplotype analysis using intragenic polymorphism analysis was able to probe a common ancestral allele for c.1208C >A in the population of the Guambiano reservation analyzed. The high inbreeding observed among the people of the Guambiano reservation contributed to the development of genetic diseases and of MPS VI in our case. This supports the hypothesis of a common ancestral allele.

Conclusions

Together with the genealogy analysis, these results suggest a common ancestral allele in the Guambiano Amerindian reservation in the Department of Cauca and point to a novel mutation for MPS VI.

Public health policies for the Southwestern region of Colombia must be oriented to health education through genetic counselling, including prevention of new cases in affected families and early diagnosis in the community, by means of neonatal metabolic screening programs and proper management of newly diagnosed cases.

Informed Consent

Informed consent has been obtained from the patients, parents and relatives.

Acknowledgments

We would like to thank the patients and their families for their kind cooperation. We would also like to thank José Manuel Otero Casas, Ph.D., and Centro de Investigación en Química Biolóxica y Materiais Moleculares (CIQUS), Santiago de Compostela University (Spain), for their support in the analysis of biochemical structural changes.

Declaration of Conflicting Interests

The authors declare not having any conflicts of interest.

Funding

This work was supported by Fundación Pública Galega de Medicina Xenómica and by Biomarin Colombia.

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2. Online Mendelian Inheritance in Man, OMIM (TM) MIM Number: 253200.


