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Research Article

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Same Mutation in Two Patients with Mucopolysaccharidosis Type VI (Maroteaux-Lamy Syndrome) Coming from Different Municipalities in the Department of Cauca, Southwestern Colombia

Acosta MA[1]*, Lago RM[2], Barros F[2] and Carracedo AM[2,3]

Abstract

Mucopolysaccharidosis type VI or Maroteaux-Lamy syndrome is a lysosomal disease caused by an enzymatic disorder of N-acetylgalactosamine-4-sulfatase o arylsulfatase B. The diagnosis has generally been accepted with an ASB enzyme activity of <10 % of the lower limit of normal values in cultured fibroblasts or isolated leukocytes in an accredited laboratory with the presence of clinical findings consistent with MPS VI disease. The objective of this study is to make the molecular genetic characterization of two patients coming from two municipalities in the eastern and central areas of the Department, respectively, identified as having the severe clinical form of MPS type VI, and to determine the genotype-phenotype correlation. Genome DNA was isolated and used for the amplification of the 8 exons of the *ARSB* gene and the adjacent intron region (PCR). Sequencing was performed after purification of the amplified product. The same homozygous Cys447Phe mutation was found in the index cases of these two families coming from two different municipalities of the same department. The finding of the same mutation suggests the possibility of an ancestral allele, which would explain the frequency of the Maroteaux-Lamy syndrome in this region of Colombia.

Keywords

Lysosomal diseases; Mucopolysaccharidosis type VI; Matoreaux-Lamy syndrome

Introduction

Lysosomal diseases (LD) result from altered function or synthesis of a lysosomal acid hydrolase or a protein required for normal lysosome biogenesis and functioning. At present, lysosomal diseases are a heterogeneous group comprising close to 50 clinical conditions. Until recently, the only effective healthcare options for the treating physician were accurate diagnosis and genetic counselling, together with symptomatic treatment. However, there has been a significant expansion of therapeutic possibilities over the past ten years, enabling treatment of a growing number of these diseases, with increasingly specific options. Moreover, there is a direct relationship between early initiation of therapeutic measures and good response to treatment, hence the need for timely diagnosis and comprehensive specialized care for the affected patients in specialized centers [1].

In general, the pathogenic processes are well known in their fine details, but the ultimate goal is to prevent, reverse or stop organ and tissue damage using the therapies available [1].

Molecular pathology and clinical manifestations of each lysosomal disorder are specific in each patient. However, the underlying pathophysiology is practically the same in all patients. A gene mutation gives rise to lysosomal function derangement, which in turn results in a cellular pathological process affecting organ structure and function. Symptoms reversibility with the therapy varies depending on the disease, its nature (molecular alteration), the stage of organ damage, and early onset of therapy [1-8].

The Maroteaux-Lamy syndrome or Muccopolysaccharidosis Type VI (MPS VI) is a severe hereditary disease, very rare in humans, caused by a mutation in a single gene encoding for the lysosomal hydrolase N-acetyl-galactosamine-4-sulphatase or arylsulfatase B (*ARSB*). The enzymatic deficiency results in the accumulation of noxious amounts of dermatan sulphate in the lysosomes, giving rise to progressive tissue damage [1,2,9].

Clinical manifestations have range widely from severe, rapidly progressing forms, to mild, very slowly progressing forms [3,5,9-12]. Skeletal dysplasia includes low pathologic stature, dysostosis multiplex and joint stiffness. Cardiac valve disease with diminished pulmonary function, hepatosplenomegaly, sinusitis, recurrent otitis media with secondary hearing loss, sleep apnea, corneal opacity, carpal tunnel syndrome, and inguinal or umbilical hernia, are also found. There usually is no cognitive compromise but some of the most frequent central nervous system findings are cord compression due to cervical vertebral instability, communicating hydrocephalus, optic nerve atrophy and blindness [5,10-12].

Sulfatases, including *ARSB*, are a highly preserved family of genes with a highly homologous sequence. Luchesa et al. have reported on the crystallographic structure of both arylsulfatase A (ARSA) and *ARSB*, showing a similar structure [1,2,9] (Figure 1).

Genetically comprising 208,878 base pairs of chromosome 5, the gene spans nucleotides 7,8073.032 to 7,8281.910. In the *ARSB* gene, more than 3,910 single nucleotide poylimorphisms (intragenic SNPs) have been described. There are 68 regulating elements located in the *ARSB* gene region, including promoters, enhancers and transcription factor binding sites. The amino acid sequence of the *ARSB* protein or N-acetylgalactosamine-4 sulfatase consists of 533 amino acids [2].

Like other MPS, type VI may result from several *ARSB* gene mutations. Those mutations have been identified in patients from North and South America, as well as Europe, Australia and Asia, with clear phenotype-genotype correlations in some cases.

***Corresponding author:** Acosta MA, PhD, Pediatrics Department, Facultad de Medicina, Universidad del Cauca, Carrera 6 No. 13N-50 Popayán-Cauca, Colombia, Tel: 573175282523; Fax: 5728230262; E-mail: morin1924@gmail.com

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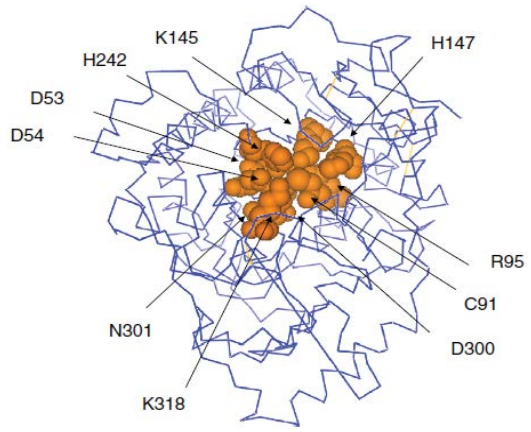

Figure 1: Three-dimensional structure of *ARSB* gene and functionally important aminoacids. The yellow lines represent the disulphide bridges [17].

Mutations include missense, nonsense, splice site, small deletions, small insertions, insertion-deletion (indel), and large deletions. Mutations published recently in a Caucasian population (i.e., Spain and Argentina) [13], involve 9 different mutant alleles, the most frequent mutation in this type of patients (18 %) being a change in the c.1143-IG>C causes an alteration in the splice site, giving rise to few structural changes in the enzyme [1]. Consequently, it is associated with an attenuated phenotype characterized by low levels of urinary glycosaminoglycans (GAGs), late onset, and good survival prognosis [1]. It is important to mention that the ratio GAG/creatinine excreted in urine is higher in infants and children, decreases with age, and remains constant throughout adulthood [14].

According to the Human Gene Mutation Database registry and to recent publications, so far 139 *ARSB* gene mutations have been identified. The mutations most commonly identified in patients from North and South America, Europe and Australasia are: c.629A>G, c.944G>A, c.1143-8T>G, c.1143-1G>C and c.1151G>A. These mutations are found in the patients with a frequency greater than 10 %. Most of them are the result of a single nucleotide change leading to missense. Specifically, these mutations have been reported in certain populations, including those of Russia, Australia, United States, France, Germany, Portugal, Italy, China, Brazil, Chile, Argentina and Spain [4,6-9] (Figures 2 and 3). New mutations [11,15] are published continuously, aiming to stablish the genotype-phenotype correlation.

Mutational screening is essential for the molecular study of single gene hereditary diseases. Maroteaux-Lamy syndrome or MPS type VI are nor the exception. It does not only provide the ability to offer molecular diagnosis but can also identify the need to determine if there are any genotype-phenotype correlations, which would enable prediction of clinical severity and the probability of success with enzyme replacement therapy or other therapies in the individual patient. It will also open the way for the functional analysis of new mutations identified, and for population-based studies (i.e., allele frequencies, origin of the most frequent mutations, scatter, etc.).

Bone marrow transplantation (BMT) was considered the first treatment option for MPS Type VI during the past two decades. However, there are issues associated with this treatment, including the search for a donor, and the high morbidity and mortality. Moreover, patients do not exhibit improvement of their bone disease following transplantation. Over the past decade, numerous studies have reported success with the use of enzyme replacement therapy with recombinant ARBS as the approach to treatment. The use of this therapy in the clinical setting was approved in 2005 [16,17].

Populations in the Southwestern Colombia (department of Cauca) belong to three large ethnic groups: 1) Afro-Colombian (22.19 % of the total population in the department), living mostly on the Pacific coastal region, although there are settlements along the great rivers like the Patía River, and in the valleys and plains of the Northern part of the department. 2) "Mestizo" (mixed race) and "white" populations (56.3 % of the total) living in the central regions. 3) Indigenous or Amerindians (21.5 % of the total population), living mainly in the Tierradentro region. This ethnic group comprises Paez, Guambía and Inga tribes, which are the largest indigenous societies in the country, with approximately 147,000 individual and account for 28.7 % of the total indigenous population nationwide.

The main objective of this study is to characterize, using molecular genetics, two patients identified in Southwestern Colombia with the severe form of MPS type VI, and to determine the genotype-phenotype correlation.

Materials and Methods

Sample and family data collection

Thirty-six (36) patients have been diagnosed to date with MPS VI in Colombia, 16 of them belong to the department of Cauca, accounting for 45 % of the cases recorded in this country [Author data, no published yet]. These 16 patients have been characterized clinically and confirmed by means of enzymatic activity test in leukocytes. Two of these patients, coming from the municipalities of Totoró and Piendamó, were considered index cases for our study (Figure 4) [Author data, no published yet].

Assessment and clinical care were undertaken at the Paediatrics Service of the Hospital Universitario San José in Popayán, Cauca, after having obtained the authorization of the institutional Ethics Committee and the informed consent from the father, mother or guardian of the children.

The protocol described below was used in the two index cases in order to make the molecular characterization of the mutation: peripheral blood sampling through venous puncture in sterile tubes with ethylenediaminetetraacetic acid (EDTA) as anticoagulant, and sample application on FTA cards. DNA was extracted per protocol, using phenol chloroform, and quantified for concentration in the nano-drop equipment. Polymerase chain reaction (PCR) was used for amplification. Specific *ARSB* gene forward and reverse primers were used and, following purification of the amplified product, sequencing was performed for each of the 8 exons of the gene, including the flanking exon-intron regions. A last generation ABI PRISM sequencer was used for the analysis. Differences with respect to the reference gene sequence were identified using the Staden software package.

Results

A single nucleotide change (p.C447F) was found in the two index cases in exon 8 of the *ARSB* gene. The change identified was classified as pathogenic. According to the ALAMUT software, version 2, this change found in exon 8 is classified as a transversion producing a sense change mutation, giving rise to the exchange of one amino acid for another: position 206,029 (T/T) TGT>TTT; mutation p.C447F. The C447 amino acid involved in the mutation is part of the minor domain of the enzyme (Figures 5 and 6).

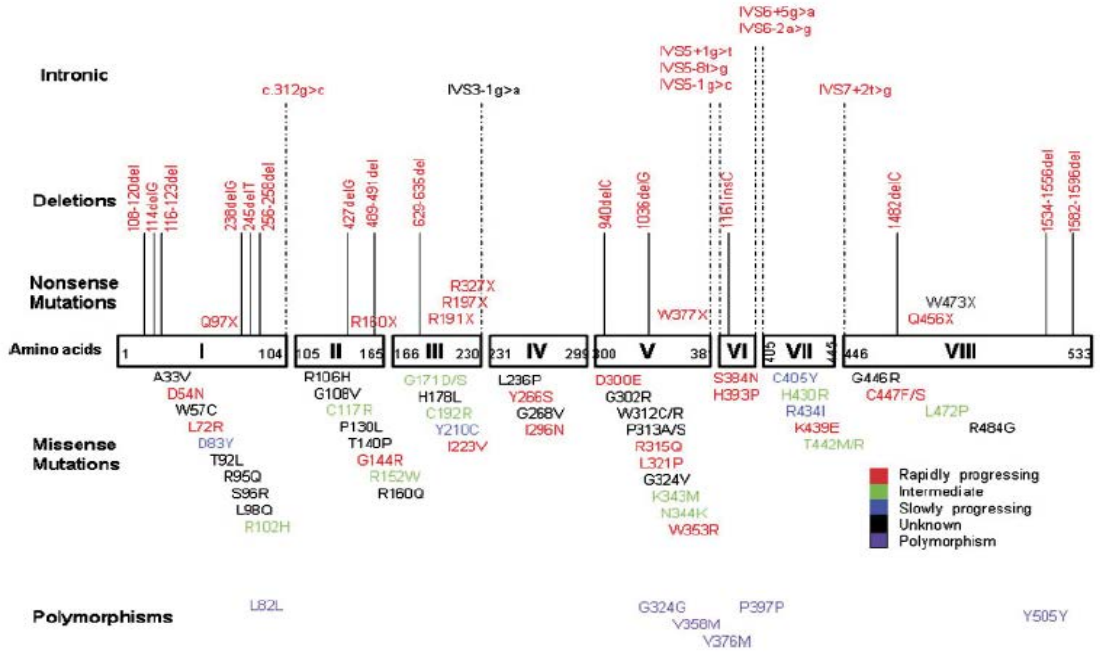

Figure 2: Mutations location in the *ARSB* gene. The exons are represented as rectangles and are numbered with Roman numerals. The prediction of the severity of the mutations is shown in color according to the legend [18].

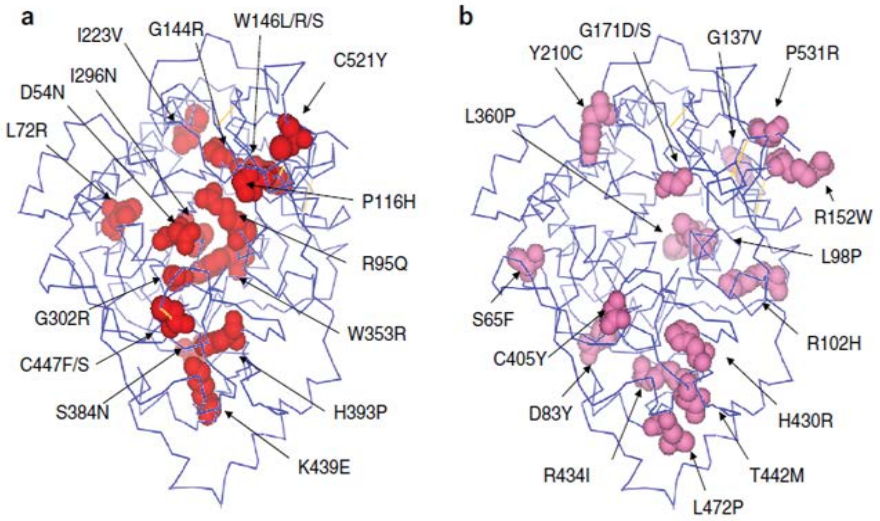

Figure 3: Location of missense mutations on the *ARSB* three-dimensional structure. The arrows indicate the location of the altered amino acids. Amino acids for the severe forms are depicted in red (a) and the amino acids for mild or moderate forms appear in light purple (b) [17].

Discussion

The lateral chain of the C447 amino acid appears as forming a disulphide bridge with the lateral chain of the Cys405 amino acid in the crystallographic structure of the enzyme (PDB code 1FSU). This disulphide bridge, an important secondary structural element, is lost when the p.C477F mutation described takes place, affecting the folding ability of this domain and, consequently, its secondary structure, unlike what happens with the same domain in the native protein. The three-dimensional structure of the major domain (amino acids 1-382) should not be affected by this mutation. Consequently, given that the catalytic activity of the enzyme resides in its major domain, and the biological function of the minor domain appears to be related with molecular recognition of the substrates to be hydrolysed and with enzyme solubility and bioavailability in the different organs, cells and intracellular media, these functions must be severely affected by the mutation as a result of the structural change in the minor domain. This should lead to a loss of selectivity of the enzyme for its substrates as well as to a change in its solubility in the different biological media, resulting in different bioavailability when compared to the native protein.

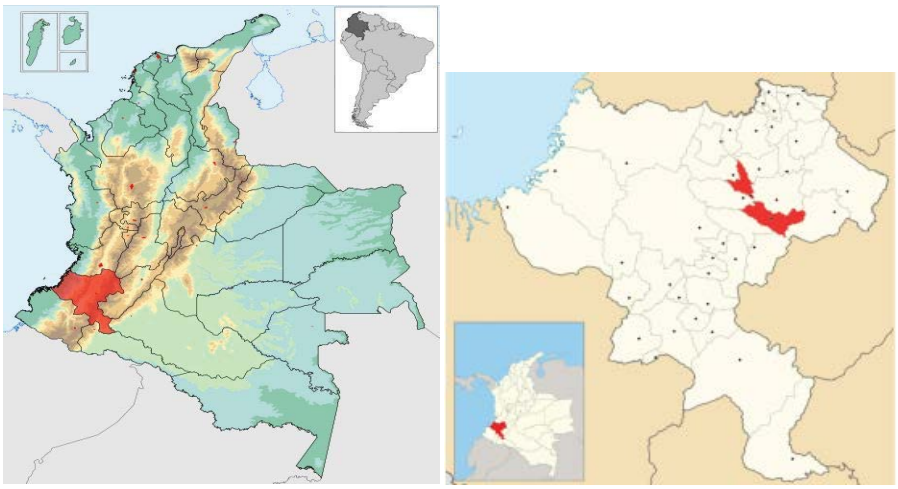

Figure 4: Municipalities of Piendamó and Totoró in the Department of Cauca - Southwestern Colombia.

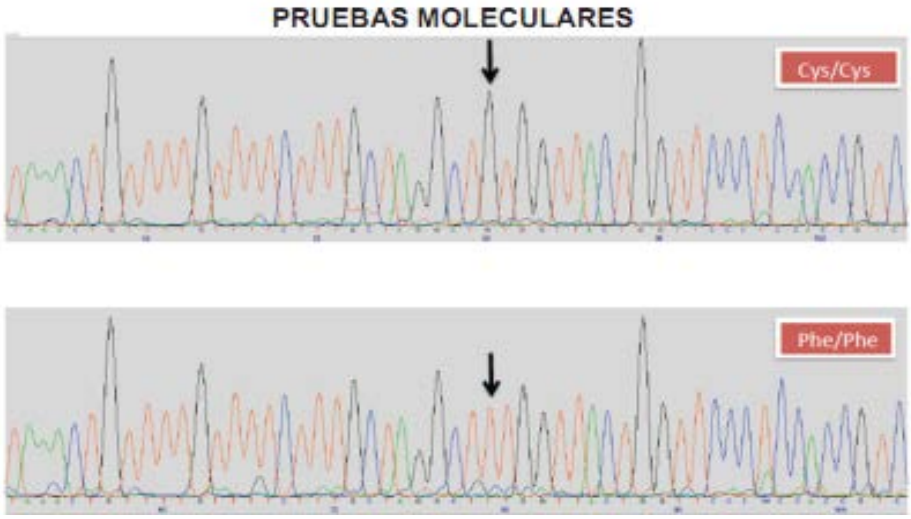

Figure 5: Molecular Test: Electropherogram of the index cases.

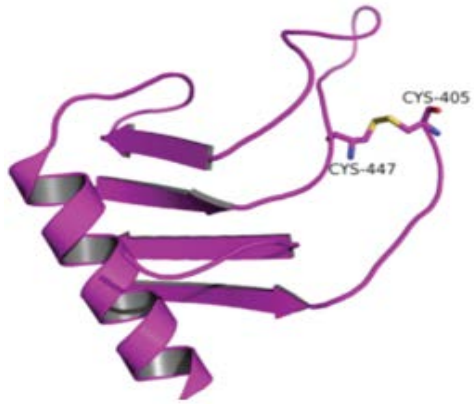

Figure 6: Crystallographic structure of the arylsulfatase minor domain: theoretical structure proposed for sulfatase B with the Cys477Phe mutation described.

Conclusion

The same homozygous p.C447F mutation was found in the index cases of these two families coming from different municipalities in the same department. The finding of the same mutation suggests the possibility of a common ancestral allele, which would explain the unusual frequency of the Maroteaux-Lamy syndrome in the Department of Cauca.

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Author Affiliation

Top

[1]*Pediatrics Department, Facultad de Medicina Universidad del Cauca, Popayán, Colombia*

[2]*Fundación Pública Galega de Medicina Xenómica, Hospital de Santiago de Compostela, Spain*

[3]*Instituto de Medicina Legal, Facultad de Medicina, Universidad de Santiago de Compostela, Spain*

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