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Genetic studies in a cluster of Mucopolysaccharidosis Type VI patients in Northeast Brazil

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ABSTRACT

Mucopolysaccharidosis type VI (MPS VI, Maroteaux–Lamy syndrome) is a lysosomal storage disease caused by deficiency of arylsulphatase B. The incidence of MPS VI is very low, usually less than 1 case for every 1,000,000 newborns. In Northeast Brazil we identified in the county of Monte Santo (52,360 inhabitants) thirteen patients with MPS VI. The aim of this work was to identify the mutation(s) present in these patients and analyze intragenic SNPs to define possible haplotypes. The 13 MPS VI patients were found to be homozygous for the p.H178L mutation. All patients have the same haplotype for the intragenic SNPs. Based on current data, the prevalence of MPS VI in this region is estimated as 1:5,000 newborns. These results, together with pedigree analysis, strongly suggest a founder effect accounting for the high frequency of p.H178L mutation in this area. This reinforces the need of a comprehensive community genetics program for this area.

1. Introduction

Mucopolysaccharidosis type VI (MPS VI; Maroteaux–Lamy syndrome; OMIN 253200) is a lysosomal storage disease caused by deficiency of arylsulphatase (ARSB, N-acetylgalactosamine-4-sulphatase), which is required for the degradation of dermatan and chondroitin sulphates [1].

The isolation and characterization of human ARSB cDNA [2,3] and the partial elucidation of the human ARSB gene structure [4] have made possible the identification of molecular defects in ARSB gene mutant alleles. The gene for human ARSB has 209 kb (genome.ucsc.edu), it is comprised of 8 exons, ranging in size from 71 to 885 bp. The nascent polypeptide has 533 amino acids [5]. Approximately 140 mutations have been described and several polymorphisms have been identified in the gene sequence [6–17].

In Northeast Brazil we identified on the county of Monte Santo (52,360 inhabitants, 350 km far from Salvador, capital of the Bahia state) an increased incidence of several genetic disorders (such as Phenylketonuria and Congenital Hypothyroidism), probably due to relative endogamy and isolation (Fig. 1). Thirteen cases of MPS VI were identified so far in this county, and it is likely that this frequency could be higher, due to patients who are still undiagnosed or died before a diagnosis was established.

To understand the high frequency of this rare syndrome in Monte Santo, the molecular background of these patients was investigated. The aim of this work was to identify the mutation(s) present on the affected families and to define haplotypes using intragenic SNPs.

2. Material and methods

2.1. Patients

Thirteen patients with MPS VI from Monte Santo (Bahia State, Northeast Brazil), being 9 males and 4 females, were diagnosed based on identification of dermatan sulfate in urine and deficiency of ARSB activity in leucocytes. Informed consent was obtained from the patient's parents. We also analyzed 93 patients from the MPS Brazil Network, as explained later.

2.2. Mutation detection

Genomic DNA was obtained from peripheral blood collected in EDTA using the salting-out precipitation technique [18]. The

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Fig. 1. Map of Brazil.

polymerase chain reaction (PCR) was used to amplify exons 1–8 of the ARSB gene. PCR amplification was performed in final volumes of 25 μL with 2 mM $MgCl_2$, 1 mM of each dNTP, 10 pmol of each primer, 1× PCR buffer, 1 U of Taq DNA polymerase (Super-Therm or Invitrogen) and 100–200 ng of genomic DNA. For the amplification of exons 1 to 7, 1.25–2.0 DMSO 100% was added and for exon 1, 2 μL of 5× Q solution (Qiagen) was added. The reactions were subjected to 5 min of initial denaturation at 94 °C, and 30 cycles of denaturation at 94 °C for 15–40 s, annealing at 45–55 °C for 30–60 s and elongation at 72 °C for 40–60 s, with a final extension step at 72 °C for 10 min, exon 1 used elongation and final extension at 68 °C. The primers used to amplify exons 1 and 7 were described by Isbrandt [14], and the primers used to amplify exons 2–6 and 8 were described by Petry [7]. The PCR products were analyzed in 1.5% agarose gel containing ethidium bromide.

After amplification by PCR, the products were subjected to single-strand conformational polymorphism (SSCP) analysis in order to identify alterations in exons 1, 2, 3, 4, 7 and 8. An aliquot of 15 μL PCR products was mixed with 5 μL of loading buffer, the samples were denatured at 95 °C for 5 min and were loaded onto 8% polyacrylamide gels (29:1 acrylamide:bis-acrylamide) for exons 2, 3, 4, 7 and 12% polyacrylamide gels for exons 1 and 8. Exons that showed alterations in the SSCP banding pattern were purified with Exosap® and sequenced with the Big Dye Terminator kit version 3.1 using an ABI 310 automated sequencer (Applied Biosystems). Exons 5 and 6 were directly sequenced due to the high number of polymorphisms. Restriction fragment length polymorphism (RFLP) with *Hsp92II* enzyme was used to confirm the mutation found after sequencing in patients and to study this mutation inheritance in relatives. The carrier status was studied in 21 parents using RFLP.

2.3. Haplotype characterization

Haplotype can be defined as a set of alleles located on the same chromosome segment, which tend to be transmitted in block in genealogy. Therefore, the haplotypes mark chromosome segment, which can be traced through pedigrees and populations, while not disrupted by recombination [19].

We used 10 single nucleotide polymorphisms (SNPs) for haplotype characterization. All SNPs were located in the ARSB gene, being 2 in intronic regions and 8 in exons. PCR amplification was performed as described above. SSCP was the method of choice for IVS3-22t>c, RFLP was used for p.L82L (246G>A) and p.L124L (370C>T) and sequencing for p.G324G (972A>G), p.T356T (1068A>T), p.V358M (1072G>A), p.V376M (1126G>A), IVS5-27a>c, p.S384N (1151G>A) and p.P397P (1191A>G).

3. Results

SSCP analysis of the index case (Fig. 2) showed an altered pattern in exon 3 and the p.H178L (533A>T) mutation was detected in homozygosis after sequencing. This mutation abolishes a restriction site for the *Hsp92II* enzyme. The thirteen patients were screened by RFLP confirming the presence of p.H178L mutation in homozygosis (Table 1). The entire open reading frame of the ARSB gene and all exon–intron boundaries were analyzed by sequencing in the index case to exclude other possible mutations.

After defining the disease causing mutation in this population, we screened 93 patients from the MPS Brazil Network (a partnership involving many services aiming to improve information on MPS and facilitate access to diagnosis in Brazil) in order to check if this mutation was also present in patients from other regions in Brazil. We found 4 heterozygous who are also included in Table 1.

The polymorphisms p.V376M and IVS5-27a>c were present in homozygosis in all patients, and in heterozygosis in all parents and in the four MPS VI patients from the MPS Brazil Network heterozygous for the p.H178L mutation. Polymorphisms p.L82L, p.L124L, IVS3-22t>c, p.G324G, p.T356T, p.V358M, p.S384N and p.P397P were not detected in patients from Monte Santo. Some of these polymorphisms were observed in heterozygosis in parents and in patients screened from the MPS Brazil Network (Table 1). The defined haplotype for individuals carrying the mutation was GCTAAGACGA based on the nucleotide changes caused by each SNP (Fig. 3).

4. Discussion

In this study we identified the p.H178L mutation in a cluster of MPS VI patients from Monte Santo, a county in Bahia state, Northeast Brazil (Fig. 1). According to international studies, the incidence of MPS VI ranges from 1 in 43,261 births in Turkish to 1 in 1,505,160 births in Sweden [20]. An incidence for MPS VI in Brazil has not been published yet, but data from MPS Brasil Network show that MPS VI is not as rare as in other countries (Personal communication).

Monte Santo presents a number of MPS VI cases much higher than that estimated by the literature, over 1 in 5,000 live births; this estimate was made based on individual nuclear families (father, mother and son) once there is a high rate of inbreeding in this region. With this prevalence, MPS VI is considered a disease of great impact on the population of Monte Santo. The high inbreeding in this region justifies the high frequency of several genetic diseases such as MPS VI.

The p.H178L mutation results from A to T transition at nucleotide 533 in exon 3 and causes a histidine to leucine substitution in the protein. We performed an in silico analysis for this mutation using the PolyPhen (*Poly*morphism *Phen*otyping) software, an automatic tool for prediction of possible impact of an amino acid substitution on the structure and function of a human protein, this variant is predicted to be probably damaging with a PSIC (Position Specific Independent Counts) score difference of 3.402.

In 2007, Karageorgos et al. [17] published a study of a mutational analysis in 105 patients, representing about 10% of the world MPS VI population. The sample included 10 patients from Brazil and the p.H178L mutation was found in one patient in heterozygosity. This patient was born in Rio de Janeiro, Southeast Brazil, and also has a brother with MPS VI carrying the same mutation (Table 1 — 16 and

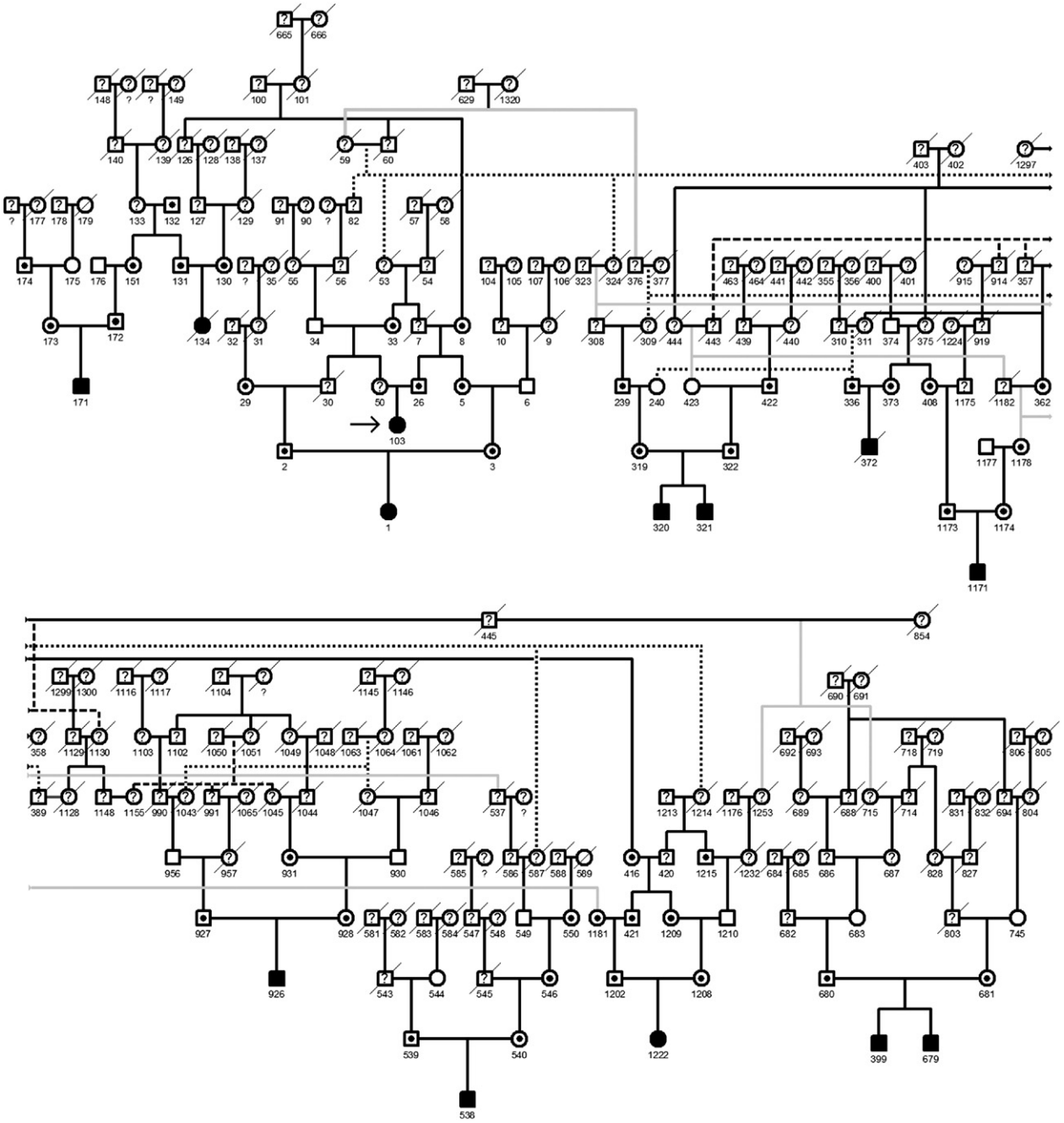

Fig. 2. Extended pedigree showing the relationship among MPS VI patients from Monte Santo/Bahia. Symbols in black indicate patients with MPS VI, symbols with question mark indicate individuals without molecular analysis, White symbols indicate normal subjects for the p.H78L mutation and symbols with a dot in the middle indicate individuals heterozygous for the p.H178L mutation [22].

17). Through screening of this mutation in patients from the MPS Network Brazil, we found two further heterozygous unrelated patients. One from Salvador, capital of Bahia, and one from São Paulo, Southeast Brazil.

To date, 13 polymorphisms have been described in the ARSB gene, being three amino acid substitutions, eight silent transitions and two in intronic regions [13,17,21]. In this study, we analyzed 10 of these polymorphisms and only two were found in the patients from Monte Santo, the p.V376M in exon 5 and the IVS5-27a>c in intron 5, both in homozygosity. The haplotype associated with p.H178L mutation in patients from Monte Santo is shown in Fig. 3. Patients from the MPS Brazil Network, heterozygous for the p.H178L mutation showed these two polymorphisms in heterozygosity, as well as some of the other polymorphisms tested (Table 1).

All patients had typical clinical signs of MPS VI, two patients already died due to disease complications, and ten of the 11 patients started enzyme replacement therapy. Anedoctical reports on early treated patients refer to improved growth pattern and attenuated features, in comparison to the expected outcome on late-treated patients.

Pedigrees of family members showed that all patients have a connection in some generation (Fig. 2). The analysis of haplotypes using intragenic polymorphisms along with the pedigrees analysis corroborates with the hypothesis of a founder effect of this allele in this region.

The presence of three unrelated patients from Salvador (northeast), São Paulo and Rio de Janeiro (Southeast), who were found to be heterozygous for the p.H178L mutation, showing the same common haplotype observed in the Monte Santo county patients, suggests a common Brazilian origin for this mutation.

Table 1
Genotypes of the patients included in the study. 1–13: MPS VI patients from Monte Santo; 14–17: MPS Brazil network patients. Single letters means that nucleotides are in homozygosis.

	1[a]	2[a]	3	4	5	6	7	8	9[b]	10	11	12	13[b]	14	15	16[c]	17[c]
p.H178L (533A>T)	T	T	T	T	T	T	T	T	T	T	T	T	T	T/A	T/A	T/A	T/A
p.L82L (246G>A)	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
p.L124L (370C>T)	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
p.IVS3-22T>C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
p.G324G (972A>G)	A	A	A	A	A	A	A	A	Na	A	A	A	A	A	A	A/G	A/G
p.T356T (1068A>T)	A	A	A	A	A	A	A	A	Na	A	A	A	A	A	A	A	A
p.V358M (1072G>A)	G	G	G	G	G	G	G	G	Na	G	G	G	G	G/A	G	G/A	G/A
p.V376M (1126G>A)	A	A	A	A	A	A	A	A	Na	A	A	A	A	A/G	A/G	A/G	A/G
IVS5-27A>C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C/A	C/A	C/A
p.S384N (1151G>A)	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
p.P397P (1191A>G)	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A

[a, b] and [c]: siblings; Na: Not analyzed.
Bold letters refer to the mutation found in patients from Monte Santo.

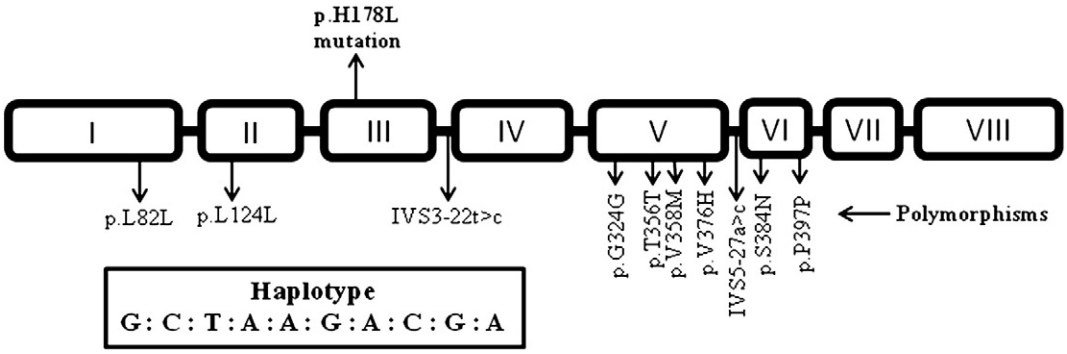

Fig. 3. Location of 10 polymorphisms in the ARSB gene identified in this study. The exons are represented by boxes and numbered by roman numerals.

5. Conclusions

We can conclude that p.H178L mutation, which has so far not been described in other populations, was introduced once in the Monte Santo area and became common on this quite isolated area due to endogamy and consanguinity. The presence of this mutation in homozygosity in all patients who present this autosomal recessive disease supports that a founder effect is a suitable explanation for this finding. Taking into account these results, a comprehensive community genetics program, which could involve education to the public, genetic counseling to the families, newborn screening and appropriate management of the detected cases, should be developed for this area.

Conflict of interest

The authors declare that there are no conflicts of interest.

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