Common N-acetylgalactosamine-6-sulfate sulfatase (GALNS) exon mutations in Brazilian patients with mucopolysaccharidosis IVA (MPS IVA)

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Abstract

Morquio A Syndrome (mucopolysaccharidosis IVA - MPS IVA, OMIM# 253000) is an autosomal recessive inborn error of metabolism caused by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). We investigated five unrelated Brazilian MPS IVA families for mutations in exons 4, 5, 9 and 10 of the GALNS gene. Six out of the 10 mutant alleles were identified. Taken together with a previous study, which included six unrelated families, common mutations among Brazilian patients were p.N164T, p.G116S and p.G301C. Among one hundred control subjects three novel silent mutations were found (p.A107A; GCC → GCT, p.Y108Y; TAC → TAT, p.P357P; CCG → CCA).

Screening starting with exons 4, 5, 9, 10 and 11 may be a good strategy for genotyping of Brazilian patients since these exons include 73% of all mutations identified in the current and previous studies.

Key words: GALNS mutations, GALNS mutation detection, mucopolysaccharidosis IVA.

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Diggelen et al., 1990), where the GALNS activity in leukocytes was less than 1% of normal controls and the arylsulfatase B activity (ARSB) was normal.

Between 2004 and 2005 our Genetics Service diagnosed six Brazilian MPS IVA patients (including two sibs), the clinical data for these patients being summarized in Table 1 (n = 6; patients 1-5) along with Genetics Service data for the seven Brazilian MPS IVA patients reported by Tomatsu et al. (2004a) (n = 7; Patients 6-11). Written informed consent was obtained for each patient, and the study protocol was approved by the Institution Research Board at HCPA.

In our study, we screened GALNS gene exons 4, 5, 9 and 10 because our previous data showed that these were the most frequently mutated exons in Brazilian patients (Tomatsu et al., 2004a). The PCR and single-strand conformation polymorphism (SSCP) analysis conditions for these exons were standardized (Table 2). DNA was isolated from peripheral blood by the ammonium acetate method (Miller et al., 1988) and PCR was carried out with 100 ng of genomic DNA in a total volume of 50 μL on an Eppendorf Personal Thermal Cycler. The reaction include 1X PCR buffer, 0.2 mM dNTPs, 1.5 mM magnesium chloride (MgCl₂), 20 pmol of each primer (forward and reverse) and 1 U of Taq DNA polymerase (Invitrogen), dimethyl sulfoxide (DMSO) being used only for exon 5 at a final concentration of 5.6% (w/v). Primers were designed from the gene sequence and the PCR conditions were 5 min denaturation at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at annealing temperature and 45 s at 72 °C, followed by a final extension of 10 min at 72 °C (Table 2). To identify the GALNS exon alterations the PCR products (Figure 1) were submitted to SSCP analysis, performed with 8 μL of PCR product and 4 μL of SSCP buffer (95% formamide, 20 mM EDTA, 0.005% bromophenol blue, 0.05% xylene cyanol FF) which were mixed, heated to 95 °C for 5 min and chilled on ice before being loaded onto 8 or 12% (w/v) non-denaturing polyacrylamide gel and subjected to electrophoresis using 1X TBE buffer. The SSCP conditions for exons 4, 5, 9 and 10 are given in Table 2. DNA bands on SSCP gels were visualized after silver nitrate staining (Orita et al., 1989). Fragments with an altered pattern were re-amplified, purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) and sequenced on an ABI 310 sequencer (Applied - Byosystems) using the BigDye Terminator kit version 3.1.

Control PCR reactions, without DNA, were done in each reaction. The products from PCR reactions were checked on a 1.5% (w/v) TBEagarose gel containing ethidium bromide.

Table 1 - Clinical and genetic data for Brazilian patients with mucopolysaccharidosis (MPS) IVA. Patients 1 to 5 were analyzed by us for exons 4, 5, 9 and 10 of the N-acetylgalactosamine-6-sulfate sulfatase (GALNS) gene exon. Patients 6 to 11 correspond to patients 17, 16, 18, 19, 15 and 14 investigated by Tomatsu et al. (2004a)† and were analyzed regarding all the GALNS gene exons. Patients 3a and 3b, and 10a and 10b were sibs. The standard deviations (-SD) were as compared to age-matched normal controls (data from the National Center for Health Statistics at http://www.cdc.gov/nchs) and represent the number of standard deviations by which the patients are smaller or lighter than the controls, hence the negative values. A dash (-) indicates that data was not available, ND = not detected.

<table>
<thead>
<tr>
<th>Patient and gender</th>
<th>Consanguinity and origin in Brazil</th>
<th>Age at onset of symptoms; age at last evaluation</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Walking without support</th>
<th>GALNS genotype *mutations exclusive to Brazilian patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F</td>
<td>No, South</td>
<td>12 months; 10 years 2 months</td>
<td>105 (-5.4SD)</td>
<td>28.1 (-0.9SD)</td>
<td>No</td>
<td>p.G301C / p.G301C</td>
</tr>
<tr>
<td>2 F</td>
<td>Yes, Southeast</td>
<td>&lt; 6 months; 9 years 10 months</td>
<td>107 (-4.4SD)</td>
<td>18.7 (+2.6SD)</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>3a F (sib with 3b)</td>
<td>Yes, Northeast</td>
<td>12 months; 6 years 5 months</td>
<td>97.5 (-3.8SD)</td>
<td>17.3 (-1.4SD)</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>3b F (sib with 3a)</td>
<td>Yes, Northeast</td>
<td>12 months; 7 years 7 months</td>
<td>100 (-4.1SD)</td>
<td>19.6 (-1.4SD)</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>4 M</td>
<td>No, Southeast</td>
<td>48 months; 11 years</td>
<td>103 (-6.0SD)</td>
<td>20 (-2.8SD)</td>
<td>Yes</td>
<td>ND</td>
</tr>
<tr>
<td>5 M</td>
<td>Yes, South</td>
<td>24 months; 22 years 1 month</td>
<td>-</td>
<td>36 (-1.7SD)</td>
<td>No</td>
<td>p.N164T*/p.N164T*</td>
</tr>
<tr>
<td>Tomatsu et al., 2004a†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 F</td>
<td>No, South</td>
<td>&lt; 6 months; -</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>p.G139S / p.N164T*</td>
</tr>
<tr>
<td>7 F</td>
<td>No, South</td>
<td>36 months; 16 years 1 month</td>
<td>104 (-8.7SD)</td>
<td>22.5 (-4.3SD)</td>
<td>Yes</td>
<td>p.R386C / ND</td>
</tr>
<tr>
<td>8 M</td>
<td>Yes, South</td>
<td>24 months; 11 years 5 months</td>
<td>113 (-4.7SD)</td>
<td>28.5 (-1.5SD)</td>
<td>No</td>
<td>p.N164T*/p.N164T*</td>
</tr>
<tr>
<td>9 M</td>
<td>No, South</td>
<td>36 months; 15 years 6 months</td>
<td>103 (-8.9SD)</td>
<td>22.0 (-4.0SD)</td>
<td>Yes</td>
<td>p.G116S / p.G116S</td>
</tr>
<tr>
<td>10a M (sib with 10b)</td>
<td>No, Northeast</td>
<td>- ; 2 years</td>
<td>82.5 (-1.0SD)</td>
<td>11.0 (-1.3SD)</td>
<td>Yes</td>
<td>p.L307P*/p.S341R*</td>
</tr>
<tr>
<td>10b M (sib with 10a)</td>
<td>No, Northeast</td>
<td>18 months; 6 years</td>
<td>97 (-3.9SD)</td>
<td>15.0 (-2.3SD)</td>
<td>Yes</td>
<td>p.L307P*/p.S341R*</td>
</tr>
<tr>
<td>11 M</td>
<td>Yes, South</td>
<td>12 months; 7 years 8 months</td>
<td>99.5 (-4.9SD)</td>
<td>21.0 (-1.2SD)</td>
<td>Yes</td>
<td>p.G116S / p.G116S</td>
</tr>
</tbody>
</table>

We used SSCP analysis to screen 100 Brazilian control individuals (anonymous blood donors from Southern Brazil) in the same way as the patients. Analysis of 200 chromosomes in the general population is a methodology used for assessing the frequency of disease-causing recessive variants (Cotton and Scriver, 1998).

Missense mutations in the \textit{GALNS} gene (p.N164T and p.G301C) were detected in three of the five unrelated patients studied (Patients 1 to 5 in Table 1). These mutations were not detected in the control sample of 100 subjects.

Molecular analyses for MPS IVA have been performed previously in various ethnic populations. However, with the exception of our previous study (Tomatsu \textit{et al.}, 2004a), there are no other data on Brazilian patients. The two missense mutations (p.N164T and p.G301C) that we found in the present study account for \~70\% of the mutant alleles so far investigated in Brazilian patients.

We estimated the frequency of the different mutations detected in 11 unrelated patients from our Genetics Service, \textit{i.e.} the five described for the first time in our present paper and the six reported by Tomatsu \textit{et al.} (2004a). We detected 17 out of the 22 mutated alleles (77.3\%), but three homozygous patients were from consanguineous marriages so we corrected these values by one allele per consanguineous marriage and estimated the relative frequency of the detected mutations among 14 mutated alleles (Table 3).

Up to now, 12 different missense mutations have been found in Brazilian patients (Tomatsu \textit{et al.}, 2005) and, indeed, four of these mutations (p.G116S, p.N164T, p.L307P, p.S341R) were first described in Brazilian patients and, except for p.G116S which has also been found in a non-Brazilian patient, may be confined to the Brazilian population (Tomatsu \textit{et al.}, 2004a and 2005). The frequency of mutations in exons 4, 5, 9 and 10 in Brazilian patients (around 70\% of mutant alleles) is higher than the 40\% in the compiled data (Tomatsu \textit{et al.}, 2005). In our previous study we confirmed the allelic heterogeneity observed in this disorder where 46.1\% of the mutations reported were private or occurred at a low frequency (Tomatsu \textit{et al.}, 2004a and Tomatsu \textit{et al.}, 2005).

The p.G116S, p.N164T, and p.G301C mutations could be explained as either “true recurrent mutations” or “common founder mutations” since they were observed among unrelated Brazilian patients. The other two common mutations, p.G139S and p.R386C, are considered as “true

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**Table 2** - Polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) analysis conditions.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primers F = forward, R = reverse</th>
<th>Annealing temperature (°C)</th>
<th>Fragment size (bp)</th>
<th>Polyacrylamide gel concentration (% w/v)</th>
<th>Voltage (V)</th>
<th>Running time (h)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>F: GCTTCTCGGGGTCTCCTCG</td>
<td>61</td>
<td>193</td>
<td>12</td>
<td>230</td>
<td>2</td>
<td>18-24</td>
</tr>
<tr>
<td></td>
<td>R: GTGGATGGAGCCAGGACGCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F: GTGGGTCCTCGAAGTGTCC</td>
<td>60</td>
<td>212</td>
<td>12</td>
<td>200</td>
<td>3.5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>R: CGTGGAGGGGGAGAGGGGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F: CTGGTCCAGTGCGCCTGAC</td>
<td>61</td>
<td>176</td>
<td>12</td>
<td>50</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>R: CTACTGGCCCCGGCAGACGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F: CAGAGTGGCCTGACCGGTG</td>
<td>57</td>
<td>231</td>
<td>8</td>
<td>250</td>
<td>2</td>
<td>18-24</td>
</tr>
<tr>
<td></td>
<td>R: CTCTGGGCTTCACTACTTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3** - Type and frequency of N-acetylgalactosamine-6-sulfate sulfatase (\textit{GALNS}) mutations in Brazilian patients with mucopolysaccharidosis IVA (MPS IVA), all presenting with a severe phenotype. The mutations were detected in the present study and by Tomatsu \textit{et al.} (2004a). Of the 17 mutations identified in unrelated patients, Only 14 alleles were used because in three homozgyous individuals the alleles were identical by descent (consanguineous parents).

<table>
<thead>
<tr>
<th>Nucleotide change**</th>
<th>Codon alteration</th>
<th>Exon</th>
<th>Amino-acid change</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>445G → A</td>
<td>GGC → AGC</td>
<td>4</td>
<td>p.G116S</td>
<td>17.6</td>
</tr>
<tr>
<td>514G → A</td>
<td>GGC → AGC</td>
<td>4</td>
<td>p.G139S</td>
<td>5.9</td>
</tr>
<tr>
<td>590A → C</td>
<td>AAC → ACC</td>
<td>5</td>
<td>p.N164T</td>
<td>17.6</td>
</tr>
<tr>
<td>1000G → T</td>
<td>GCC → TGC</td>
<td>9</td>
<td>p.G301C</td>
<td>23.5</td>
</tr>
<tr>
<td>1019T → C</td>
<td>CTG → CCG</td>
<td>9</td>
<td>p.L307P</td>
<td>5.9</td>
</tr>
<tr>
<td>1122C → A</td>
<td>AGC → AGA</td>
<td>10</td>
<td>p.S341R</td>
<td>5.9</td>
</tr>
<tr>
<td>1255C → T</td>
<td>CGT → TGT</td>
<td>11</td>
<td>p.R386C</td>
<td>5.9</td>
</tr>
</tbody>
</table>


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**Figure 1** - PCR products on 1.5\% (w/v) TBE-agarose gel: lanes 1 and 2, exon 4; lanes 3 and 4, exon 5; lane 5, 50 pb DNA ladder; lanes 6 and 7, exon 9; and lanes 8 and 9, exon 10.
recurrent mutations” since they have been found in other ethnic populations (Tomatsu et al., 2005). Haplotype analysis is needed to confirm these data.

In a recent study on the mutation spectrum of the GALNS gene, it was shown that the three most frequent mutations, p.R386C, p.G301C and p.I113F account for only 20% of the identified mutant alleles (Tomatsu et al., 2005). These data suggest that genotyping for MPS IVA patients should be done for each ethnic population since it seems that the majority of mutations are sporadic or unique to each ethnic group.

The frequency of p.R386C, the most common mutation in most populations studied, may have been underestimated in our study because the new patients were not analyzed for exon 11 where this mutation is present. Among six Brazilian patients who had this exon analyzed this mutation was found only in one allele (Tomatsu et al., 2004a). Further studies are needed to clarify the situation regarding this mutation. The p.G301C mutation was very common in Colombian MPS IVA patients where it accounted for 70% of the mutant alleles investigated, and it was found also in Italian, French, British, Portuguese, and Moroccan patients (Tomatsu et al., 2005), while the p.G139S was described in 1.3% of the alleles of Irish, Argentine and North American patients from the USA (Tomatsu et al., 2004a).

None of the mutations observed in our Brazilian patients (p.G116S, p.N164T, p.L307P and p.S341R) were found in our control group of 100 individuals. There were 16 nonpathogenic variants of the GALNS gene with a single nucleotide change in the data compiled by Tomatsu et al. (2005). In the control group of this study, three novel silent nucleotide changes were found, p.A107A (GCC → GCT), p.Y108Y (TAC → TAT), p.P357P (CCG → CCA), but none of the previously reported silent mutations.

Analyzing the number of all the described mutations in the GALNS exons and the expected number of mutations based on exon size, we observed that mutations are not randomly distributed along the gene (p < 0.01). Our data predict that exons 5, 10 and 11 are hot spots for mutations because they were present at frequencies above the expected frequencies, this increase being 83% for exon 5, 42% for exon 10 and 56% for exon 11. Mutations in exons 4, 5, 9 and 10 accounted for almost 40% of all mutations so far identified. In Brazilian patients, the mutation frequency in these four exons was higher, as 13 out of 14 presumably independently mutated alleles (92.9%) are located in these two exons, as detected by the present study and by Tomatsu et al., (2004a) (Table 3). These data suggest that for Brazilian patients it may be a good strategy to start analyzing exons 4, 5, 9 and 10. Moreover, exon 11 should also be analyzed since it posess the more frequent mutation (p.R386C) in other populations.

In respect to phenotype, 68.2% of all the complied mutations are associated with the severe phenotype, 21% with the attenuated phenotype and 10.8% have not been defined (Terzioglu et al., 2002; Tomatsu et al., 2005). In our patients, onset of signs and symptoms occurred around 20 months of age (SD ± 13.4 months), in accordance with the literature that reports an onset age of around two years of age and final diagnosis at about three years of age (Northover et al., 1996). The standard deviations (‒SD) shown in Table 1 were as compared to age-matched normal controls (data from the National Center for Health Statistics) and represent the number of standard deviations by which the patients are smaller or lighter than the controls, hence the negative values. The age of the Brazilian patients at examination ranged from 2 years to 21 years and 3 months and, compared with data from the age-matched normal controls from the National Center for Health Statistics, height was in the range 82.5 cm to about 113 cm (‒1 to about ‒8.9 standard deviations (SD), i.e. less than the controls) and weight was 11 to about 36 kg (‒0.9 to about ‒4.3 SD, i.e. less than the controls). Since all the patients, except patient 10a, had stopped, or nearly stopped, growing, they all presented a severe phenotype with a final height under 125 cm and, accordingly, all the mutations listed here were associated with the severe phenotype.

In this study, PCR and SSCP conditions were established for exons 4, 5, 9 and 10 of GALNS gene where the majority of mutations were reported for Brazilian MPS IVA patients. Using this strategy, we detected six out of 10 mutated alleles in five patients with Morquio A Syndrome. Common mutations among Brazilian patients are p.N164T, p.G116S, and p.G301C.

Acknowledgments

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References


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