Clinical, enzymatic, and molecular characterisation of a Portuguese family with a chronic form of $G_{M2}$-gangliosidosis B1 variant

M G Ribeiro, T Sonin, R A Pinto, A Fontes, H Ribeiro, E Pinto, M M Palmeira, M C Sá Miranda

Abstract

Mutations in the hexosaminidase A gene (HEXA) causing the B1 variant of $G_{M2}$-gangliosidosis result in the presence of a mutant enzyme protein with a catalytically defective $\alpha$ subunit. A rare and pan-ethnically distributed mutation, transition G533A (Arg178His), is known to be a common allele among Portuguese patients with the subacute phenotype. We now report the presence of an Arg178His allele in three Portuguese sibs with a chronic form of the disease, who carry the transition G755A (Arg252His) on the second allele. This novel mutation is the first B1 allele to be associated with an adult phenotype.

Key words: $\beta$-hexosaminidase; HEXA mutations; Tay-Sachs disease.

The pedigree of a Portuguese family studied in the present report is depicted in fig 1. Table 1 summarises the clinical characteristics of the three affected sibs. The molecular defects were shown by PCR–SSCP analysis (fig 2) followed by direct sequencing (fig 3). The patients were found to be compound heterozygotes for the mutations G533A and G755A. The genotype of the other family members was identified by testing both mutations by PCR–SSCP analysis (fig 2). The mutation G755A was confirmed by RFLP analysis with restriction enzyme NlaIII (fig 4). The mobility shifts corresponding to novel mutation G755A were not seen on SSCP analysis of 100 normal $\alpha$ chain genes (data not shown). Table 2 summarises the clinical, molecular, and biochemical data and compares it with previously published data on Portuguese B1 patients.\(^{13}\) In leucocytes from patients homozygous for the Arg178His allele, the Hex A activity against 4MU-GlcNAc corresponded to about 50% of total hexosaminidase activity. In compound heterozygotes for this mutation, this activity seems to be dependent on the nature of the mutation present in the second allele. If the mutation produces no mature $\alpha$ subunit, such as in the classical infantile form of Tay-Sachs disease, the enzymatic activity will be that generated by one Arg178His allele; therefore it must correspond to one half of the activity in
Table 1  Clinical summary of the affected sibs with B1 variant

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset (y)</th>
<th>First symptom</th>
<th>Language retardation</th>
<th>Speech loss</th>
<th>Loss of ability to walk</th>
<th>Pyramidal signs</th>
<th>EEG</th>
<th>Present age (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-2</td>
<td>11</td>
<td>Behavioural alterations</td>
<td>After 11 years</td>
<td>25 years</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-6</td>
<td>5</td>
<td>Behavioural alterations</td>
<td>5-6 years</td>
<td>25 years</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-9</td>
<td>7</td>
<td>Behavioural alterations</td>
<td>7-8 years</td>
<td>25 years</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2  Clinical, biochemical, and molecular data on Portuguese B1 variant patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical phenotype</th>
<th>Genotype</th>
<th>Hexosaminidase activity in leucocytes*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4MU-GlcNAc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total Hex</td>
</tr>
<tr>
<td>II-2</td>
<td>Chronic</td>
<td>Arg178His/Arg252His</td>
<td>873</td>
</tr>
<tr>
<td>II-6</td>
<td>Chronic</td>
<td>Arg178His/Arg252His</td>
<td>785</td>
</tr>
<tr>
<td>II-9</td>
<td>Chronic</td>
<td>Arg178His/Arg252His</td>
<td>1086</td>
</tr>
<tr>
<td>n=10</td>
<td>Subacute (juvenile onset)</td>
<td>Arg178His/Arg178His</td>
<td>1045 (610-1394) 61-0 (43-0-66-0)</td>
</tr>
<tr>
<td>n=1</td>
<td>Normal</td>
<td>Arg178His/Arg252His</td>
<td>1300</td>
</tr>
<tr>
<td>I-2</td>
<td>Arg252His heterozygous</td>
<td>Arg178His</td>
<td>2133 (1598-2985) 59-454-3-63-3</td>
</tr>
<tr>
<td>II-1</td>
<td>II-4, II-5, II-8</td>
<td>Arg178His heterozygous</td>
<td>1846 (1540-2266) 75-0</td>
</tr>
<tr>
<td>n=10</td>
<td>Normal</td>
<td>Tay-Sachs disease carriers</td>
<td>1396 (1070-1714) 55-7</td>
</tr>
<tr>
<td>n=44</td>
<td>Normal</td>
<td>Normal</td>
<td>1580 (967-2294) 85-0</td>
</tr>
</tbody>
</table>

*The hexosaminidase activity was determined as previously described. **Total Hex, hexosaminidase activity (nmol/h/mg protein) determined against the neutral synthetic substrate; % Hex A, activity of the main peak eluted during NaCl gradient expressed as % of total 4MU-GlcNAc recovered activity after ion-exchange chromatography on DEAE-cellulose; Hex A, hexosaminidase A activity (nmol/h/mg protein) determined against the sulphated synthetic substrate which was further purified as previously described. ***Enzymatic data published in a previous report.****

Figure 3  Sequence analysis of PCR samples. The segment of genomic DNA with an altered SSCP pattern was subjected to asymmetrical amplification, using the temperature profile of asymmetrical PCR. The asymmetrical product was further purified and directly sequenced. The relevant portion of the sequence is shown. E5, exon 5 (antisense strand); E7, exon 7 (sense strand); G, control; P, patient.

homozogous patients. However, if the mutation is compatible with the synthesis of a mature protein with the enzymological characteristics of B1 variant, with residual activity against the synthetic unsulphated substrate, the Hex A activity is expected to be correspondingly higher. The Arg252His substitution, resulting from the mutation G755A, occurs at conserved amino acids in human β chain,14 the α and β chain from mouse,15 and the hexosaminidase polypeptide from Dictyostelium discoideum.16

The clinical phenotype of patients with the substitution Arg178His and Arg252His in respective alleles is less severe that than observed for Arg178His homozygotes. Assuming that the clinical phenotype depends on enzymatic residual activity against ganglioside GM2 in vivo,17 it can be predicted that the Hex A generated by the Arg252His allele shows a higher intralysosomal residual activity than that generated by the Arg178His allele. Therefore it is likely that patients who have at least one Arg252His allele would have a chronic type, irrespective of the nature of the mutation present in the other allele.

The majority of B1 mutations described in published reports occurred at codon 178, in which arginine is replaced by histidine,18 cysteine,19 or leucine.7 The identification of another mutation also in one arginine codon points to the role of this positively charged amino acid in the catalytic domain of the α subunit. On the other hand, Arg252 is 74 amino acids from Arg178 in the coding sequence. Although the nature of the replaced amino acid is similar they might have different effects on the disruption of the tertiary structure of the catalytic domain of the protein.
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Figure 4 Confirmation of G755A mutation by restriction analysis of amplification product with NlaIII (5′ CATG-3′). About 100–200 ng of the amplified fragment encompassing exon 7 was digested overnight at 37°C with 15 units of NlaIII and the products analysed by electrophoresis on 3% Nusieve 3:1 agarose gel. The DNA from the wild type allele will not be hydrolysed by NlaIII and the digestion yields a fragment of 221 bp. The mutant allele will be cleaved into two fragments of 133 bp and 88 bp. DNA from the subjects who are heterozygous for the mutation will yield both intact and digested fragments. Lane 1, pBR322/Mpi digested; lane 2, control; lane 3, subject II-2; lane 4, subject II-6; lane 5, subject II-9.

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