Significance of two point mutations present in each \textit{HEXB} allele of patients with adult $G_{M2}$ gangliosidosis (Sandhoff disease)

Homozygosity for the Ile$^{207} \rightarrow$ Val substitution is not associated with a clinical or biochemical phenotype

Isabelle Redonnet-Vernhet $^a$, Don J. Mahuran $^b$, Robert Salvayre $^a$, Frédéric Dubas $^c$, Thierry Levade $^{a, *}$

\textit{a} Laboratoire de Biochimie Médicale, ‘Maladies Métaboliques’, C/IF INSERM 9206, Institut Louis Bagnard, Bât. L3, CHU Rangueil, 1 Avenue Jean Poulhès, F-31054 Toulouse Cédex, France

$^b$ Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada

$^c$ Service de Neurologie, CHU d’Angers, Angers, France

Received 17 April 1996; revised 12 July 1996; accepted 19 July 1996

Abstract

The molecular defects in the \textit{HEXB} gene encoding the common $\beta$-subunit of lysosomal $\beta$-hexosaminidase A ( $\beta$-Hex A, $\alpha\beta$) and $\beta$-Hex B ( $\beta\beta$) were investigated in a Portuguese family affected with late onset Sandhoff disease ($G_{M2}$-gangliosidosis variant 0). This family comprised two unaffected daughters and three affected sibs who developed at about age 17 cerebellar ataxia and mental deficiency. Their parents were consanguineous and clinically asymptomatic. There was no detectable $\beta$-Hex B activity and a profound reduction in the activity of $\beta$-Hex A in the leukocytes and transformed lymphoid cell lines from the affected sibs. The expected intermediate values were observed in the parents as well as in one daughter and her children. Western analysis revealed the presence of reduced, but detectable amounts of mature $\beta$-chain protein in cell lysates from the probands and intermediate levels in the parents. Nucleotide sequencing of amplified, reverse-transcribed $\beta$-chain mRNA demonstrated the presence of two single point mutations: an A$^{619} \rightarrow$ G transition in exon 5 (Ile$^{207} \rightarrow$ Val), and a G$^{1514} \rightarrow$ A transition in exon 13 (Arg$^{505} \rightarrow$ Gln). Both of these two mutations have been previously linked to the adult form of Sandhoff disease in compound heterozygote patients. All three affected sibs were found to be homoallelic for both mutations. Interestingly, while the mother was heterozygous for each mutation, the father was homozygote for the A$^{619} \rightarrow$ G substitution and heterozygote for the G$^{1514} \rightarrow$ A transition. Since the father is homozygote for the A$^{619} \rightarrow$ G mutation but expresses a biochemical phenotype consistent with a carrier of Sandhoff disease and is clinically asymptomatic, this substitution is likely a neutral mutation. We confirmed this hypothesis by finding this transition present in 4 of 30 alleles from normal individuals. We conclude that homozygosity for the G$^{1514} \rightarrow$ A mutation is exclusively responsible for the adult form of Sandhoff disease in this family, and that the A$^{619} \rightarrow$ G substitution is not a deleterious mutation but rather a common \textit{HEXB} polymorphism.

Keywords: Sandhoff disease; $\beta$-hexosaminidase; Lysosomal enzyme; $G_{M2}$ ganglioside

1. Introduction

Lysosomal $\beta$-hexosaminidases ( $\beta$-Hex; EC 3.2.1.52) are widely distributed hydrolyses involved in the degradation of glycolipids, glycoprotein-derived oligosaccharides and glycosaminoglycans [1]. $\beta$-Hex consists of two major isoenzymes, Hex A which is composed of an $\alpha$- and $\beta$-subunit, and Hex B composed of two $\beta$ subunits. Although there are distinct active sites in each subunit,
dimerization is required in order for either to become functional. The active site of \( \alpha \)-subunit catalyzes the hydrolysis of \( G_{M2} \) ganglioside; however, in vivo the presence of the \( \beta \)-subunit in the Hex A heterodimer and the small \( G_{M2} \) activator protein is also required for this function. As well the \( \alpha \)-active site is responsible for the hydrolysis of other negatively charged natural and artificial substrates, e.g. 4-MUGS. Unlike \( G_{M2} \) ganglioside, the latter water-soluble compounds are also substrates for homodimeric Hex S (\( \alpha \alpha \)) which is an unstable isozyme found in small amount in samples from patients who are unable to synthesize a functional \( \beta \)-subunit. On the other hand, the \( \beta \)-subunit cleaves a variety of neutral, water-soluble substrates including the common substrate 4-MUGlNAc.

Deleterious mutations in the \( HEXB \) gene, located on chromosome 5, which encodes the common \( \beta \)-subunit, result in Sandhoff disease (\( G_{M2} \) gangliosidosis variant 0; MIM # 268800) [1,2]. Thus, Sandhoff disease is characterized by deficient activities of both Hex A and Hex B, leading to tissue accumulation of \( G_{M2} \) ganglioside and other glycolipids, as well as urinary excretion of oligosaccharides.

Based on the age of onset of clinical symptoms, different clinical phenotypes can be classified into infantile, juvenile and adult forms of Sandhoff disease. Whereas the classical infantile form is a rapidly fatal neurological disorder, late-onset forms are compatible with a long survival. The severity of Sandhoff disease seems to correlate with the residual activity against \( G_{M2} \) ganglioside [3]. The nature of the molecular defects underlying the heterogeneity and pathophysiology of Sandhoff disease is still poorly known. In particular, only a few mutations have been characterized in patients affected with late-onset forms of the disease. To date these have been either mutations that affect mRNA splicing or substitution mutations, all of which allow the formation of low levels of Hex A activity [4–11]. In addition, most of the patients have been found to be compound heterozygotes (the second allele being sometimes null, e.g. a common partial gene deletion [12] or not determined). Homoallelism has been described in only one patient [8].

In this study, we report the identification of the molecular alterations present in a family comprising three patients affected with the adult form of Sandhoff disease. This family was selected for genetic studies because the patients’ parents were consanguineous, so that homoallelism would be expected in the affected subjects, thus permitting a genotype/phenotype correlation to be made.

### 2. Subjects and methods

#### 2.1. Family history

Patients (II-3, II-4 and II-5, see pedigree in Fig. 1) were three of five siblings of a consanguineous Portuguese family (the grandfathers of the parents were first cousins).

All presented at 17–18 yr of age with a progressive spino-cerebellar syndrome with a slight mental deficiency. The oldest one (patient II-3) was severely affected with loss of motor autonomy, static tremor, horizontal nystagmus, upper limb amytrophy and mental deficiency. His brother (patient II-4) had walking difficulties, no amytrophy but frequent spontaneous fasciculations. Although brain MR imaging showed a clear cerebellar atrophy, the patient presented a moderate cerebellar syndrome. The affected sister (patient II-5) also had a cerebellar syndrome and a limited mental efficiency. Thin-layer chromatographic analysis of the urine of patients II-3, II-4 and II-5 did not demonstrate abnormal excretion of oligosaccharides.

Repeated clinical examination of the patients’ parents (I-1 and I-2) and sisters (II-1 and II-2) showed that they were unaffected.

#### 2.2. Isolation of leukocytes and cell culture

Leukocytes from heparinized blood were isolated using Plasmagel™ (Roger Bellon Lab., Paris, France) [13]. Lymphoid cell lines were established by Epstein-Barr virus-transformation of blood B lymphocytes obtained from control individuals and from our patients [14]. Lymphoid cells were grown in a humidified 5% CO\(_2\) atmosphere at 37°C in RPMI 1640 medium (Gibco BRL, Cergy-Pontoise, France) supplemented with L-glutamine (2 mmol/1), penicillin (100 U/ml), streptomycin (100 \( \mu \)g/ml) and heat-inactivated fetal calf serum (10%) as previously reported [15–17]. Cells were harvested by low-speed centrifugation and washed 3 times with PBS. Cell pellets were kept frozen at −70°C until use.

#### 2.3. Enzyme studies

Total \( \beta \)-Hex activity was determined in plasma and in extracts of peripheral blood leukocytes and Epstein-Barr virus-transformed lymphoid cells with 4-MUGlNAc as substrate [17]. Hexosaminidase isoenzymes were assayed by the thermodenaturation assay method [18]. Hex A activity was also specifically determined using 4-MUGS as substrate [19].

#### 2.4. Western blot analysis

The proteins in samples of cell lysates (30 \( \mu \)g each) were separated by SDS-PAGE and analyzed utilizing the Amersham ECL system as previously described [7]. A rabbit anti-human Hex B IgG preparation was used as the primary antibody.

#### 2.5. PCR and sequencing

Genomic DNA was prepared from leukocyte and lymphoid cell pellets [20]; total cellular RNA was extracted.
from lymphoid cells by a guanidinium isothiocyanate derived procedure using RNA-B™ (Bioprobe Systems, Montreuil, France) according to the manufacturer’s instructions. Genomic DNA and cDNA were amplified by PCR using different pairs of primers as described [5,6,8,9]. Amplified fragments were sequenced directly, by fluorescent dye terminator cycle sequencing (Applied Biosystems) according to the manufacturer’s instructions, and analysed on an automated DNA sequencer (Applied Biosystems).

2.6. Ava I restriction fragment length polymorphism (RFLP) studies

PCR products obtained with primers 5'-CTTGTCTATGGGGAGAATAT-Y (sense) and 5'-TCAGTCTGTCATAGGCGTCA-3' (antisense) were digested with directly added 20 U of AvaI (Gibco BRL Life Technologies, Eragny, France) and incubated for 2 hours at 37°C. Digestion products were analysed on a 10% polyacrylamide (29:1) non denaturing gel after 2 hours under constant current (25 mA).

2.7. PCR-mediated site-directed mutagenesis (PSM)

PCR was performed on cDNA using the following oligonucleotide primers: 5'-GCTGAATTCCAGGCTAAAACCCA-3' (sense) and 5'-GCAGATAATGTCGTGGATGTATGAA-3' (antisense). Amplified fragments were digested for 2 hours with 20 U of Hinfl (Gibco BRL Life Technologies) and electrophoresed on 10% polyacrylamide (29:1) for 2 hours at 45 mA.

3. Results

As illustrated in Fig. 1 and Table 1, our patients with adult Sandhoff, i.e. the two clinically affected brothers (patients II-3 and II-4) and their affected sister (patient II-5) exhibited clearly reduced levels of total β-hexosaminidase activity in plasma, peripheral blood leukocytes and Epstein-Barr virus-transformed lymphoid cells. The determination of the β-Hex B activity in cells from all three symptomatic patients showed a severe deficiency of this isoenzyme (Table 1), indicating their homozygote status for GM2 gangliosidosis variant 0 (Sandhoff disease). This conclusion was further corroborated by the finding of a considerably decreased β-Hex A activity in leukocytes and lymphoid cells (Table 1), as well as in plasma (data

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Leukocytes</th>
<th>Lymphoid cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Hex (U b)</td>
<td>Hex B (% of total)</td>
</tr>
<tr>
<td>I-1</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>I-2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>II-1</td>
<td>1101</td>
<td>48</td>
</tr>
<tr>
<td>II-2</td>
<td>755</td>
<td>27</td>
</tr>
<tr>
<td>II-3</td>
<td>123</td>
<td>0</td>
</tr>
<tr>
<td>II-4</td>
<td>155</td>
<td>0</td>
</tr>
<tr>
<td>II-5</td>
<td>104</td>
<td>0</td>
</tr>
<tr>
<td>III-1</td>
<td>1584</td>
<td>43</td>
</tr>
<tr>
<td>III-2</td>
<td>1436</td>
<td>49</td>
</tr>
<tr>
<td>III-3</td>
<td>629</td>
<td>27</td>
</tr>
<tr>
<td>III-4</td>
<td>500</td>
<td>26</td>
</tr>
<tr>
<td>Controls</td>
<td>1205 ± 200</td>
<td>54 ± 8</td>
</tr>
</tbody>
</table>

Total β-hexosaminidase activity was assayed with 4-MU-GlcNAc as substrate; Hex B was determined with the same substrate after thermodenaturation [18]. Hex A activity was assayed with 4-MUGS as substrate. For each patient, the activity of another lysosomal enzyme (β-galactosidase or β-glycuronidase) was simultaneously measured and found to be normal. Each determination was performed at least in duplicate and, whenever possible, on different cell batches.

a These values also include Hex S activity

b nmol of methylumbelliferone released per h and per mg of cell protein.
Fig. 2. Western blot of lymphoid cell lysates. The amount of total lysate protein loaded from each cell line was 30 μg. Extracts were from the members of our adult Sandhoff family, two different controls (N) and one patient with infantile Sandhoff disease homozygous for a deletion mutation (SD). The position of the mature, lysosomal β subunit (28 kDa) is indicated. Not shown. Intermediate values were found in their parents and in one sister (II-2) and her children (III-3 and III-4), consistent with a heterozygote status (Fig. 1 and Table 1). Normal levels of total β-Hex, β-Hex A and β-Hex B activities were observed in the other sister (II-1) and her children (III-1 and III-2), which were suggestive of a non-carrier status.

The relative steady state level of mature, lysosomal, β-subunit of Hex B protein in lymphoid cells from the members of our family was estimated by Western blot analysis. Fig. 2 demonstrates that the amount of mature β-protein contained in cell lysates from our probands (II-3, II-5) was greatly reduced, but clearly detectable, when compared with similar amounts of total protein from normal cell lysates. In contrast, as previously observed, β-protein was undetectable in a cell lysate from an infantile Sandhoff patient [7]. Fig. 2 also shows that cells from the older sister (II-1) with normal levels of Hex activities also contained a normal amount of mature β-subunits, and that cells from the obligate carrier-parents had about 50% of the control levels, again consistent with the enzyme activity measurements.

To identify the molecular basis of the β-Hex B defect in this family, direct nucleotide sequencing was performed on overlapping PCR-fragments from reverse-transcribed β-mRNA and/or genomic DNA from lymphoid cells of the probands (patients II-3, II-4 and II-5). For each patient, different PCR products from separate extractions of lymphoid cells were analyzed. The different overlapping fragments encompassing the whole cDNA of the HEXB gene were shown to be amplified by PCR and of the expected size. Thus, there were likely no large deletions or insertions present. We then determined the nucleotide sequence of all the amplified fragments. Two single alterations were detected in double-dose in the probands. The first was an A to G transition which occurred at position 619 of exon 5 of the HEXB gene (see Ref. [21] for numbering) (Fig. 3, patient II-3), which caused an Ile207 to Val amino acid substitution. The second alteration was a G to A substitution at position 1514 of exon 13. This would result in an Arg505 to Gln substitution in the protein (Fig. 3, patient II-3). These two single base substitutions were found in PCR fragments amplified from both the cDNA and genomic DNA of patients II-3, and also II-4 and II-5 (data not shown). The remainder of the coding sequence was completely normal.

The observation that the G1514 → A transition destroys an AvaI restriction site was used to screen the entire family for this mutation. The digestion was carried out on both cDNA (data not shown) and genomic DNA (Fig. 4) in order to determine if there was also a mRNA-negative allele present. With genomic DNA, patients II-3, II-4 and II-5 were shown to be homozygotes for the G1514 → A transition (Fig. 4). A clear heterozygote pattern was found.
for both their parents (I-1 and I-2), thus eliminating the possibility of deleted chromosomal DNA at the mutant locus. The two other siblings appeared either not to carry the mutant allele (subject II-1) or to be heterozygote for it (subject II-2).

Unlike the G$^{1514} \rightarrow$ A transition, the A$^{619} \rightarrow$ G mutation neither creates nor destroys a restriction enzyme site. Therefore, we used two methods to confirm its existence. We first used the PSM technique. It is a non isotopic technique allowing rapid detection of point mutations [22].

The 3' end of the reverse primer included a single base mismatch designed to alter the sequence of the amplified product. PSM gives rise to products carrying a novel restriction site for the enzyme Hinfl. This restriction site is associated with the wild-type allele but not with the mutant allele. Using this methodology, patients II-3, II-4 and II-5 again appeared to be homozygotes for the A$^{619} \rightarrow$ G mutation (Fig. 5). The mother (I-2) and one asymptomatic daughter (II-2) were shown to be heterozygotes, with only one digested allele. Unexpectedly, the healthy father (I-1) was found to be heterozygote for this mutation (Fig. 5).

Furthermore, the clinically as well as biochemically normal daughter (II-1), who did not carry the G$^{1514} \rightarrow$ A transition, was found to be heterozygote for the A$^{619} \rightarrow$ G mutation. Identical genotypes could also be assigned to these individuals from the results obtained using another method, i.e., allele-specific oligonucleotide hybridization, employing digoxigenin-labelled oligonucleotides (data not shown).

These surprising data were further confirmed by sequencing genomic DNA extracted from blood leukocytes and/or lymphoid cells derived from the father. A homozygote pattern for the A$^{619} \rightarrow$ G mutation was observed in the father, identical to those found in the probands (data not shown). Similar results were obtained on the genomic DNA extracted from a newly established lymphoid cell line. A heterozygote pattern was found in subject II-1 (data not shown). The inheritance of the two molecular alterations of HEXB gene we identified in our adult Sandhoff family is summarized in Fig. 6.

4. Discussion

This study reports a Portuguese family with an adult form of Sandhoff disease associated with two point mutations. Both of these alterations have previously been documented in separate reports of two patients with late-onset forms of Sandhoff disease. One adult Sandhoff patient was heterozygous for the G$^{1514} \rightarrow$ A mutation and a partial HEXB gene deletion [9]. The other was also a compound heterozygote for the A$^{619} \rightarrow$ G mutation and a missense mutation at codon 456 encoding a Tyr $\rightarrow$ Ser substitution. The β-chains carrying the latter substitution are trapped in the endoplasmic reticulum and degraded [6].

Although our Sandhoff patients had two previously described mutations, our observations are unique for two reasons. First, the two point mutations were present in the

![Fig. 6. Summary of inheritance of mutant alleles and clinical presentation in the present Sandhoff family. For each individual, the left and right parts of the symbol correspond to the two different HEXB alleles.](image-url)
same allele and our three clinically affected patients were shown to be homoallelic for the two defects. Secondly, we demonstrate that the father (aged 60), who still remains asymptomatic, is homozygous for the A\textsuperscript{619} \rightarrow G transition, which raises the question of its physiological significance.

The conclusion that both the A\textsuperscript{619} \rightarrow G and the G\textsuperscript{1514} \rightarrow A substitutions are carried by the same allele is based on nucleotide sequencing of both cDNA and genomic DNA from the probands, and sequencing. AvaI digestion and PSM of DNA from the patients' parents. All of which indicated the mother's carrier status for both mutations. The fact that our probands are homoallelic was not unexpected since their parents are consanguineous.

Much more surprising was the observation that the father, who is heterozygote for the G\textsuperscript{1514} \rightarrow A substitution, is also homozygote for the A\textsuperscript{619} \rightarrow G transition. This finding was confirmed by nucleotide sequencing (by both manual and automated methodologies) of the patients' father's cDNA and genomic DNA, as well as PSM and allele-specific oligonucleotide hybridization. The presence in the father of two mutated sequences for exon 5 might be caused either by homozygosity for this mutation, or by a heterozygosity for a partial gene deletion. However, the latter possibility was excluded because two species of mRNA encoding the \(\beta\)-chain were detected, i.e., the G\textsuperscript{1514} \rightarrow A mutant and the normal alleles were found after reverse-transcription of both parents' mRNA. Moreover, the homozygosity of the father is corroborated by the genotype we determined for one of the asymptomatic daughters, II-1 (see Fig. 6). This daughter was shown not to carry the G\textsuperscript{1514} \rightarrow A mutation, but to be heterozygote for the A\textsuperscript{619} \rightarrow G transition. Such a genotype can be explained only by inheritance of a normal allele from her mother and an allele carrying only the A\textsuperscript{619} \rightarrow G mutation from the father.

Comparing our data to previously published observations [6,9,10] suggest that a reevaluation of the physiological consequences of these HEXB mutations is necessary. The G\textsuperscript{1514} \rightarrow A mutation was shown to be responsible for a labile \(\beta\)-Hex B as demonstrated by expression in COS cells [9]. This defect was reported to be likely responsible for a late-onset form of G\textsubscript{M2} gangliosidosis, since it was found in combination with a null allele [9]. This conclusion is in accordance with our findings that only our three patients who are homoallelic for this mutation express an adult phenotype. Moreover, intermediate levels of Hex B and Hex A activities were found only in the individuals heterozygote for the G\textsuperscript{1514} \rightarrow A mutation (see Table 1 and Fig. 6). Additionally, our data suggest that the G\textsuperscript{1514} \rightarrow A allele produces very little heat-labile mutant Hex B activity (1–2%) in patient cells, as opposed to being highly expressed in transiently transfected COS cells [9]. This conclusion is based on the similar levels of Hex B that were obtained for any of our unaffected patients regardless of genotype (see Table 1) by using either the common substrate assay with (Hex B) and without (Hex A, B and S) heat denaturation (in this assay, any heat-labile Hex B as well as Hex S would be scored as Hex A) or by calculating the ratio of activities produced from the common (Hex B, A and S) versus the \(\alpha\)-specific (Hex A and S) substrates, i.e. units of 4-MUGS/4-MUG-GlcNAc. As well the levels of \(\beta\)-Hex activity (Table 1) in the patients’ cells correlates with the levels of mature \(\beta\)-CRM as seen in Western blot analysis (Fig. 2). Thus, most of the mutant Arg\textsuperscript{505} \rightarrow Gln \(\beta\) chains is likely unable to exit the endoplasmic reticulum. Similar clinical (adult form of Tay-Sachs disease) and biochemical phenotypes have been reported to result from a HEXA gene mutation. This mutation, a Gly\textsuperscript{269} \rightarrow Ser substitution, results in a significant level of heat-labile Hex A in transfected COS cells [23], but only low levels of expression (4–6% of normal) in patient cells [24].

Our study brings into question the previous conclusion regarding the impact of the A\textsuperscript{619} \rightarrow G mutation. Banerjee and coworkers [6] first suggested that the A\textsuperscript{619} \rightarrow G transition results in impaired G\textsubscript{M2} activator protein binding. Then, the same authors proposed that the A\textsuperscript{619} \rightarrow G mutation leads to slow self-association of the mutant \(\beta\)-chains, which results in the inability to produce active Hex B only when the \(\beta\)-chain is expressed at low concentration by a second mutant HEXB allele [10].

Our observation and that of Banerjee and colleagues share the following point: patients who are heterozygotes for the A\textsuperscript{619} \rightarrow G mutation (i.e., the patient’s father in the Banerjee’s report and our subject II-1) are asymptomatic and express normal levels of Hex B activity and \(\beta\)-CRM. Therefore, the possibility that this mutation has a deleterious effect on the active site of \(\beta\)-Hex can be excluded.

When the A\textsuperscript{619} \rightarrow G allele is expressed in the homozygous form, two pathophysiological hypotheses can be considered. First, as suggested by Banerjee and coworkers [10], even in the homozygous form, the A\textsuperscript{619} \rightarrow G allele might still produce a functional Hex B if the mutation in the other allele does not reduce the effective \(\beta\)-chain concentration below some critical level. Indeed, the G\textsuperscript{1514} \rightarrow A transition (present in one of individual I-1 alleles along with A\textsuperscript{619} \rightarrow G) has not been reported to affect the concentration of \(\beta\)-chains in transfected COS cells, but to result in a thermo-labile Hex B [9]. However, as previously discussed our data indicate that in patients’ cells the \(\beta\)-chains carrying this Arg\textsuperscript{505} \rightarrow Gln substitution are retained and rapidly degraded, just as the \(\beta\)-chains carrying the Tyr\textsuperscript{456} \rightarrow Ser substitution were reported to do by Banerjee et al. [6]. Thus, the situation in the homozygous (A\textsuperscript{619} \rightarrow G, Ile\textsuperscript{207} \rightarrow Val) father, also heterozygous for G\textsuperscript{1514} \rightarrow A (Arg\textsuperscript{505} \rightarrow Gln), is very similar to the patient described by Banerjee et al. (heterozygous for both A\textsuperscript{619} \rightarrow G (Ile\textsuperscript{207} \rightarrow Val) and A\textsuperscript{1367} \rightarrow C (Tyr\textsuperscript{456} \rightarrow Ser)), and based on their hypothesis, the majority of mutant \(\beta\)-chains would not be expected to self-associate and exit the endoplasmic reticulum. However, our data clearly indicate that neither \(\beta\)-Hex B activity nor mature, lysosomal \(\beta\)-CRM is lower...
than would be expected for carriers of Sandhoff disease in cells from either parent, only one of whom (the father I-1) is homozygote for the A619→G mutation.

From the above data, we concluded that the most likely possibility is that the A619→G mutation represents a neutral substitution mutation. Two other lines of research conclusively proved this hypothesis. Firstly, an extensive review of the literature revealed a recent report that identified the mother of a patient with infantile Sandhoff disease as being a compound heterozygote for the A619→G mutation and for a novel 50 kb deletion [25]. Because the focus of this report was on characterizing the novel 50 kb deletion, the authors did not recognize this substitution as being identical to the one previously reported to cause adult Sandhoff disease by Banerjee et al. Thus, the importance of their observation in this respect was not reported. According to Banerjee’s hypothesis [6,10], this woman should have been affected as severely as their Sandhoff patient because of the presence of a second totally non-functional mutant HEXB allele. However, this woman was asymptomatic [25]. In addition, we inquired into the levels of β-Hex activity in white blood cells of this woman which were not reported by Zhang et al. Whereas they were slightly outside the lower range for carriers (still far higher than for affected patients), levels in serum were within the expected range (G.H. Thomas, personal communication), suggesting little or no effect of the A619→G mutation. Coupled with the activity and Western blot data we report for our patients’ family, this demonstrates that the A619→G has little or no effect on the functions or levels of the Hex isoenzymes regardless of the identity of the second HEXB allele, and that it has no similarity to any of the Tay-Sachs pseudo-deficiency mutations described in the HEXA gene [1]. Secondly, we investigated 15 normal adult individuals and found this allele to be present in the heterozygous form in two, and homozygous form in one subject (data not shown). Thus, the A619→G substitution is clearly a neutral mutation which is also a common HEXB polymorphism, and the G1514→A transition appears solely responsible for the late-onset form of Sandhoff disease.

Acknowledgements

The authors thank Drs. L. Larget-Piet and H. Puissant (Unité de Génétique, CHU d’Angers, France) for providing some clinical and enzymatic data. We acknowledge the technical assistance of M. J. Bonnafé and M. J. Nonnon, J. P. Basile, M. A. Berges and A. L. Burgat (Laboratoire de Biochimie ‘Maladies Métaboliques’, Toulouse, France), M. Bouisson (INSERM U. 151, Toulouse, France), and A. Leung (Research Institute, Hospital for Sick Children, Toronto, Canada) for hexosaminidase assays, oligosaccharide screening, cell culture, sequencing, and Western blotting, respectively. We also thank Dr. M. Potier for critical reading of the manuscript and Dr. E. Bieth for helpful suggestions concerning PSM. This study was supported by grants from INSERM (CIF 9206) and the association ‘Vaincre les Maladies Lysosomales’.

References