Molecular characterization of type 3 (neuronopathic) Gaucher disease in Thai patients

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Abstract

Gaucher disease is an autosomal recessive lysosomal storage disorder due to deficiency of the lysosomal enzyme glucocerebrosidase. Three clinical phenotypes, type 1, nonneuronopathic; and types 2 and 3, acute and subacute neuronopathic are recognized. The incidence of Gaucher disease in the Thai population is unknown, but likely under-diagnosed. We performed molecular analysis in four patients, from three sibships, with type 3 Gaucher disease. Four mutant glucocerebrosidase (GBA) alleles were identified including two novel splice site mutations, IVS6-1G→C and IVS9-3C→G; both are predicted to result in truncated protein products, p.F255fsX256, and p.K464fsX487 and p.S463fsX480, respectively. One patient, homozygous for the L444P point mutation, had a “Norbottnian-like” phenotype, with more severe visceral involvement, kyphosis, barreled chest, and no neurological involvement other than supranuclear gaze palsy. These molecular studies of neuronopathic Gaucher disease will provide additional genotype–phenotype correlation particularly in non-Caucasian population.

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Introduction

Gaucher disease is an autosomal recessive lysosomal storage disorder caused by deficiency of the lysosomal enzyme glucocerebrosidase, which is encoded by acid-beta glucosidase (GBA) gene located on 1q21. A 96% identical GBA pseudogene located 16 kb downstream [1,2] is regularly transcribed at a high level in several tissues, including leukocyte and skin fibroblast, yet is translationally silent [3].

Gaucher disease is divided into 3 subtypes. Gaucher disease type 1 or nonneuronopathic Gaucher disease, and neuronopathic Gaucher disease, which includes Gaucher disease types 2 and 3. Gaucher disease type 2 causes severe neurological involvement in infancy, with death in early childhood. Gaucher disease type 3 has a clinical severity between type 1 and 2 Gaucher disease and is further divided into 3a, 3b, and 3c. Patients with type 3a have mild hepatosplenomegaly and earlier development of neurologic symptoms including myoclonic seizures, strabismus, and supranuclear gaze palsy. Type 3b is the phenotype observed in the northern Swedish region of Norrbotten, characterized by massive hepatosplenomegaly, supranuclear gaze paresis, progressive skeletal abnormalities including kyphoscoliosis and barreled chest, with a later onset of myoclonic seizures and scanning speech, and diminished intelligence [4]. If untreated, type 3b usually leads to death during the 2nd–3rd
decade of life. Type 3c is associated with mitral and aortic valve calcification [4].

Of the over 236 mutations identified in the GBA gene, most are missense, with recombinant alleles being the second most common type of mutation (22%) [5]. There appears to be little genotype–phenotype correlation other than the association of N370S alleles with the nonneuronopathic variant and the occurrence of neuronopathic disease in individuals homozygous for the L444P [4,6]. However, recent data revealed that occurrence of neuronopathic disease in individuals homozygous for L444P mutation was clearly associated with nonneuronopathic Gaucher disease in Taiwan homozygosity for L444P mutation was clearly associated with nonneuronopathic Gaucher disease in Taiwan.[7]. Nonneuronopathic or type 1 Gaucher disease is the most common form of Gaucher disease among Caucasians, yet neuronopathic type 2 or 3 Gaucher disease is predominant among non-Caucasian populations [4,6,7,8].

In the present study, we performed whole gene sequencing and mRNA analysis in four patients from three unrelated families with type 3 Gaucher disease. Hopefully this will allow for additional genotype–phenotype correlation.

Materials and methods

Leukocyte glucocerebrosidase activity was measured by a fluorometric assay as previously described using a mix of 4-methyumbelliferyl β-D-glucoside as substrate and sodium taurocholate as detergent [9]. Genomic DNA and RNA were isolated from peripheral blood with the QIAamp DNA Mini Kit (QIAGEN) and the QIAamp RNA Blood Mini Kit (QIAGEN), respectively. Sequencing of all 11 exons of the GBA gene was performed. This allowed us to detect all mutant alleles present, while genetic variants of the pseudogene were excluded by the use of long-template PCR, which can differentiate the GBA gene from its pseudogene. RNA was reverse transcribed (RT) into single-stranded complementary DNA (cDNA) using SuperScript III Reverse Transcriptase (Invitrogen). Primer sequences for gDNA amplification are available as supplementary data. Primer sequences for RT-PCR of the selected mRNA fragments are as follows: F6, 5'-GTCCCCGTTTCACTCCTTG-3' and R8, 5'-TG-TACTGCATCCCTGATCC-3'; F9, 5'-CTGGAAACCTTGACCC-TGAAC-3' and R11, 5'-CGTGTGAGCTGACTCTGT-3'. One hundred chromosomes from unrelated Thai individuals are analyzed for mutations identified using restriction analysis or PCR sequencing (Table 1). Reference sequences used in the present study are NT_079484 and NM_000157. Nucleotide sequences for RT-PCR of the selected mRNA fragments are as follows: F6, 5'-GTCCCCGTTTCACTCCTTG-3' and R8, 5'-TG-TACTGCATCCCTGATCC-3'; F9, 5'-CTGGAAACCTTGACCC-TGAAC-3' and R11, 5'-CGTGTGAGCTGACTCTGT-3'.

Table 1: Clinical and biochemical phenotypes and genotypes of four patients with type 3 Gaucher disease

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at diagnosis (year)</th>
<th>Sex</th>
<th>Clinical presentation</th>
<th>Physical examination</th>
<th>Hemoglobin (g/dL)</th>
<th>Platelet count (/mm³)</th>
<th>Glucocerebrosidase activity (nmol/h/mg protein)⁴</th>
<th>Mutation at site of missense⁴</th>
<th>Screening in the controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1'b</td>
<td>3.8</td>
<td>M</td>
<td>Myoclonic seizures, developmental regression, failure to thrive</td>
<td>Relative macrocephaly, hepatosplenomegaly, supranuclear gaze palsy</td>
<td>8.4</td>
<td>80,000</td>
<td>0.57</td>
<td>c.762-1G=C (IVS6-1G&gt;C)/c.1589-3C&gt;G (IVS9-3C&gt;G)</td>
<td>MacI digest sequencing</td>
</tr>
<tr>
<td>2'b</td>
<td>0.65</td>
<td>F</td>
<td>Asymptomatic</td>
<td>Normal</td>
<td>ND</td>
<td>ND</td>
<td>0.54</td>
<td>c.762-1G=C (IVS6-1G&gt;C)/c.1589-3C&gt;G (IVS9-3C&gt;G)</td>
<td>Sequencing</td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>M</td>
<td>Myoclonic seizures, gait abnormality</td>
<td>Relative macrocephaly, hepatosplenomegaly, supranuclear gaze palsy</td>
<td>10.5</td>
<td>71,000</td>
<td>0.32</td>
<td>c.754T&gt;A (F213I) homoygous</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.2</td>
<td>M</td>
<td>Abdominal distension, anemia, strabismus</td>
<td>Massive hepatosplenomegaly, barrel chest, kyphoscoliosis, supranuclear gaze palsy</td>
<td>6.2</td>
<td>65,000</td>
<td>0.32</td>
<td>c.1448T&gt;C (L444P) homoygous</td>
<td>HpaI digest</td>
</tr>
</tbody>
</table>

ND. not done.

⁴ Control range 3.52–7.63 nmol/h/mg protein.

b Siblings.

c p.F252I.

d p.L483P.

Results

The clinical information, including leukocyte glucocerebrosidase activity and mutation results, is reported in Table 1. All patients were from non-consanguineous Thai families with the exception of patient 3 whose parents were Thai–Muslim first cousins. Patients 1, 2, and 3 had a devastating neurological regression. Patient 4 had Norbottnian-like features, including barrel chest and kyphoscoliosis (Fig. 1). This patient was...
initially diagnosed of having type 1 Gaucher disease, at 1 year and 2 months when neurological manifestation had not occurred. At the time of this report, Patient 4 is 2.6 years old, and his neurological symptom is limited to supranuclear gaze palsy without other neurological progression or seizures. Two novel splice site mutations, c.762-1G
\textsuperscript{N}C (IVS6-1G
\textsuperscript{N}C) and c.1389-3C
\textsuperscript{N}G (IVS9-3C
\textsuperscript{N}G), were identified in Patients 1 and 2 (Figs. 2 and 3). As seen in Fig. 3, the IVS6-1G
\textsuperscript{N}C mutation results in exon 7 skipping, which leads to a truncated peptide with 255 amino acids (p.F255fsX256). The IVS9-3C
\textsuperscript{N}G mutation leads to activation of two cryptic acceptor splice sites residing in exon 10, the first at nucleotide 4 and the second at nucleotide 25 from the 5′ end of exon 10, respectively. This mutation therefore yielded two distinct types of truncated peptides consisting of 486 amino acids (p.K464fsX487) and 479 amino acids (p.S463fsX480). Homozygous mutations of c.754T
\textsuperscript{N}A, p.F252I of exon 6 (F213I in the mature protein) and c.1448T
\textsuperscript{N}C, p.L444P in exon 10) were found in Patients 3 and 4, respectively. None of these mutations was detected in the controls.

Discussion

Our center has around 4400 pediatric admissions per year. The four patients were referred over a 7-year period, during 2000–2006. Only one of the four (Patient 4) had a correct diagnosis before the referral. The incidence of Gaucher disease in Thailand is not known, but is thought to be rare with only 25 Gaucher disease patients reported over 32 years, between 1966 and 1998, by a few centers combined [8]. Nevertheless, we believe that the incidence may be significantly underestimated due to the difficulty in diagnosis, the lack of laboratory confirmation in the past, and the lack of available treatment in the country; therefore a number of cases may not reach proper referral and definitive diagnosis. Of the 20 cases whose clinical data were available, 70% (14/20) had neuronopathic, type 2 or 3, Gaucher disease [8]. Together with those reported in Taiwanese, Chinese, and Japanese populations, neuronopathic Gaucher disease seems more common in East Asian than in Caucasian populations, in which Gaucher disease type 1 is the most common [7,11–13]. There is no correlation between the residual glucocerebrosidase activity determined in vitro and clinical severity or type of Gaucher disease [4,14]. While N370S, a genotype exclusively associated with nonneuronopathic Gaucher disease, is the most common variant detected among Caucasians and Ashkenazi Jews, the L444P mutation, which is associated with either type 2 or 3 neuronopathic Gaucher disease, and is the most common genotype identified among non-Jewish patients, accounting for 37% of the total positive alleles [7,11,13].

The L444P mutation has been shown to be associated with a neuronopathic phenotype in various populations, including Swedish, Pole, Ashkenazi Jewish, and other Caucasian populations, while it was clearly associated with nonneuronopathic Gaucher disease in Taiwanese–Chinese [7]. These data strongly suggest a modifying gene which could also be ethnic-related genetic diversity. Among Taiwanese, L444P homozygote is the most prevalent (52.6%) mutation identified followed by the recombinant allele, RecNciI, (23.7%) [7]. It is interesting to see that Patient 4, who is homozygous for the L444P mutation, has a Norbottnian-like phenotype.

The two novel splice-site mutations result in frame-shifts leading to truncated protein products. Individuals carrying mutations where both alleles result in truncated protein or null alleles may have an association with neuronopathic Gaucher disease. The F213I, previously reported in types 1, 2, and 3 Gaucher disease, thought to be a rare allele in Caucasian populations, was recently described as the second most common allele among Chinese [11,14,15]. Given that the Thai and Chinese are ethnically more closely related to each other than to Caucasians, L444P and F213I may also be common mutant alleles in the Thai population, but this awaits confirmation with a larger number of patients.

In conclusion, we have demonstrated two novel splice mutations and two known missense mutations in four patients...
with neuronopathic Gaucher disease. These data support the previous observation of genotype–phenotype correlation between F213I and L444P and neuronopathic Gaucher disease across various populations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcmd.2007.06.015.

References


Fig. 2. Genomic DNA analysis of Patients 1 and 2. (a and b) Sequenograms of Patient 1 and a control. (c) 1.5% agarose gel electrophoresis of RT-PCR product amplified with exonic primer for the c.762-1G>C (IVS6-1G>C) (forward primer F6 residing in exon 6 and reverse primer R8 residing in exon 8) and c.1389-3C>G (IVS9-3C>G) (F9 and R11 primers) mutations. mRNA transcripts of the GBA pseudogene were unintentionally amplified because the F6 and R8 exonic cDNA primers used were not exclusively specific to GBA transcripts, but also could bind to the highly homology pseudogene transcripts, which are normally produced by the GBA pseudogene. Bands A, B, b, C, and the triplet of D–D1–D2 were cut, gel-purified, and sequenced. Band A, 1413 bp: exons 6, 7, and 8, and introns 6 and 7 of the pseudogene. (B) 582 bp: wildtype transcript containing exons 6, 7, and 8, seen in Patients 1 and 2 and both parents. (b) 344 bp: aberrant transcript of IVS6-1G>C, containing only exons 6 and 8, without exon 7 identified in Patients 1 and 2, and their father. (C) 855 bp: exons 9, 10, 11, and intron 9 and 10. (D) 422 bp: cDNA amplicon of exons 9, 10, and 11 identified in Patients 1 and 2 and both parents. (D1–D2) Aberrant transcripts containing exons 9, 10, and 11 with deletion of the first 4 bp (D1) and the first 25 bp (D2) of exon 10, seen in Patients 1 and 2 and their mother. Band N represents nonspecific product. (b) Sequenograms of cDNA from wildtype and mutant transcripts of IVS6-1G>C. Note that the IVS6-1G>C mutation leads to exon 7 skipping. (c) Sequenograms of cDNA from wildtype and 2 species of mutant transcripts of IVS9-3C>G. The IVS9-3C>G mutation results in activation of two cryptic acceptor splice sites residing in exon 10, the first at nucleotide 4 and the second at nucleotide 25 from the 5' end of exon 10, leading to mRNA with deletions of the first 4 bp and the first 25 bp of exon 10, respectively. (d) Schematic diagram of missplicing that occurs as a result of IVS6-1G>C and IVS9-3C>G.

Fig. 3. mRNA (cDNA) analysis of Patient 1. (a) 1.2% agarose gel electrophoresis of RT-PCR product amplified with exonic primer for the c.762-1G>C (IVS6-1G>C) (forward primer F6 residing in exon 6 and reverse primer R8 residing in exon 8) and c.1389-3C>G (IVS9-3C>G) (F9 and R11 primers) mutations. mRNA transcripts of the GBA pseudogene were unintentionally amplified because the F6 and R8 exonic cDNA primers used were not exclusively specific to GBA transcripts, but also could bind to the highly homology pseudogene transcripts, which are normally produced by the GBA pseudogene. Bands A, B, b, C, and the triplet of D–D1–D2 were cut, gel-purified, and sequenced. Band A, 1413 bp: exons 6, 7, and 8, and introns 6 and 7 of the pseudogene. (B) 582 bp: wildtype transcript containing exons 6, 7, and 8, seen in Patients 1 and 2 and both parents. (b) 344 bp: aberrant transcript of IVS6-1G>C, containing only exons 6 and 8, without exon 7 identified in Patients 1 and 2, and their father. (C) 855 bp: exons 9, 10, 11, and intron 9 and 10. (D) 422 bp: cDNA amplicon of exons 9, 10, and 11 identified in Patients 1 and 2 and both parents. (D1–D2) Aberrant transcripts containing exons 9, 10, and 11 with deletion of the first 4 bp (D1) and the first 25 bp (D2) of exon 10, seen in Patients 1 and 2 and their mother. Band N represents nonspecific product. (b) Sequenograms of cDNA from wildtype and mutant transcripts of IVS6-1G>C. Note that the IVS6-1G>C mutation leads to exon 7 skipping. (c) Sequenograms of cDNA from wildtype and 2 species of mutant transcripts of IVS9-3C>G. The IVS9-3C>G mutation results in activation of two cryptic acceptor splice sites residing in exon 10, the first at nucleotide 4 and the second at nucleotide 25 from the 5' end of exon 10, leading to mRNA with deletions of the first 4 bp and the first 25 bp of exon 10, respectively. (d) Schematic diagram of missplicing that occurs as a result of IVS6-1G>C and IVS9-3C>G.

