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Detection of 12 new mutations in Gaucher disease Brazilian patients

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Abstract

Gaucher disease is the most frequent lysosome storage disease and presents an autosomal recessive mode of inheritance. It is caused by mutations at the *GBA* gene leading to deficient activity of the glucocerebrosidase enzyme. This report describes 12 new mutations [c.38A>G (K-27R), c.220G>A (G35S), c.448G>A (E111K), IVS4+1G>A, c.746C>T (A210V), c.776A>G (Y220C), c.793delC (Q226_fs4X), c.1102C>T (R329C), c.1300C>T (R395C), c.1309G>A (V398I), c.1324-1326delATT (delI403) and c.1583T>C (I489T)] and 4 novel silent alterations [c.342C>T (F75), c.528C>T (D137), c.1011C>T (D298) and c.1092G>A (G325)] detected among 40 unrelated Brazilian type 1 Gaucher disease patients by a combination of RFLP, dHPLC and DNA sequencing procedures. The R329C mutation, previously described in a Parkinson's disease patient (A. Lwin, E. Orvisky, O. Goker-Alpan, M.E. LaMarca, E. Sidransky. Glucocerebrosidase mutations in subjects with Parkinsonism. Mol. Genet. Metab. 81 (2004) 70–73), is described here for the first time in a Gaucher disease patient. Several genotype–phenotype correlations could be established, contributing significantly to the panel of reported mutations and conferring predictive value to their detection.

Keywords: Gaucher disease; dHPLC; *GBA* gene

Introduction

Gaucher disease is the most frequent lysosomal storage disease and a paradigm in medical genetics due to the existence of an effective enzyme replacement therapy. Clinically, Gaucher disease is a multisystem disease with variable expression, generally characterized by hepatosplenomegaly, anemia and thrombocytopenia. Bone and pulmonary disease may also be present. The presence and severity of neurologic symptoms define the three types of Gaucher disease [1]. Approximately 95% of patients present type 1 Gaucher disease (MIM# 230800), without neurologic involvement and with onset at childhood or adulthood. Neuronopathic variants are type 2 Gaucher disease (MIM# 230900), the more severe form, characterized by early onset and survival of up to 2 years of age, and type 3 Gaucher disease (MIM# 231000) presenting infantile or juvenile onset and a less ominous course with survival into adulthood.

Gaucher disease is inherited as an autosomal recessive trait and is caused by a deficient activity of the acid β-glucosidase enzyme, also known as glucocerebrosidase (EC3. 2. 1. 45). This enzyme is encoded by the *GBA* gene located at 1q21. This gene is distributed in 11 exons and presents a highly homologous pseudogene, located 16 kb downstream [2]. There is an extensive genotype–phenotype correlation observed among Gaucher disease patients, determining the three variants of Gaucher disease. However, this correlation is not absolute and there is a considerable clinical heterogeneity, even among monozygotic twins [3]. In affected children, the eventual identification of certain genotypes may help to predict the probable future onset of a neuronopathic variant, impacting also in enzyme replacement dose [4].

Generally, Gaucher disease causing mutations are characterized as mild, severe or null alleles [5]. The presence of a mild mutation, even in combination with a severe or null allele, warrants a type 1 phenotype, precluding neuronopathic symptoms due to a high residual enzyme activity. The combination of two severe mutations or a severe mutation and a null allele is associated to the neuronopathic variants.

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Homozygosity for null alleles has never been detected in Gaucher disease patients, probably because it is incompatible with life.

To date, approximately 240 Gaucher disease causing mutations have been described [5]. The most frequent are [c.1226A>G;N370S] and [c.1448T>C;L444P], herein called N370S and L444P, a mild and a severe mutation, respectively. Together, these two mutations represented 60 to 75% of Gaucher disease causing alleles in most populations. Detection of these mutations by a restriction fragment length polymorphism (RFLP) procedure is adopted by most screening laboratories. Other mutations have been detected by several other more sensitive methods. Here we describe *GBA* gene screening among 40 Brazilian Gaucher disease patients using denaturing high performance liquid chromatography (dHPLC) followed by DNA sequencing. We describe 12 new mutations, 4 new probable polymorphisms and several novel genotype–phenotype correlations.

Materials and methods

The 40 unrelated patients analyzed had a clinical diagnosis of type 1 Gaucher disease established by their physicians and confirmed by biochemical analysis disclosing reduced acid β-glucosidase activity. These patients were initially tested for most frequent Gaucher disease causing mutations (N370S, G377S and L444P) by a RFLP procedure in a previous analysis but one or both of the alleles remained unidentified [6]. Participants signed informed consent forms, and the project was approved by the local ethics committee.

The complete *GBA* gene screening was performed by dHPLC analysis of PCR products, followed by DNA sequencing of the altered amplicons. The 11 *GBA* exons were subdivided in 10 different amplicons, one for each exon, with the exception of exons 10 and 11 that were analyzed together. Initially, 4 regions were pre-amplified by PCR with primers annealing exclusively at *GBA*, excluding the pseudogene. This is critical to prevent detecting pseudogene sequence variations when searching for *GBA* mutations. The exons were then amplified in a second PCR reaction, using 3 μl of the first PCR product as template. The strategy for each amplicon analysis, including the primers utilized and the dHPLC temperature, is shown in Table 1. The PCR programs are described in Table 2.

Briefly, each of the pre-amplifications (exons 1 to 4; 5 and 6; 7; 8 to 11) was performed using 3 μl of genomic DNA extracted from saliva according to a previously established protocol [7]. The reactions had a 50 μl final volume, containing 200 μM of each of the four dNTPs, 1× buffer, 0.4 μM of the primers described in Table 1, and 4 units of Taq polymerase (Amersham Biosciences, Sunnyvale, CA). The second PCR contained 3 μl of the product of the corresponding pre-amplification and the same composition of the pre-amplifications. The product of the second PCR was submitted to electrophoresis in 2% agarose gel and visualized under UV light.

The PCR product was then denatured at 95°C for 5 min and slowly cooled to room temperature for heteroduplex formation,

Table 1
The amplicons amplified by PCR showing the *primers* utilized, the fragment size and the dHPLC analysis temperature

Amplified region	Forward (F) and reverse (R) primers 5′–3′	Amplicon size (bp)	dHPLC analysis temperature
Exon 1 to 4[a]	F: CCTTTAGAAATATGGCTGTG R: CAGAATGGGCAGAGTGAGAT	1824	–
Exon 1	F: CTGTGTCATGTGACGCTCCT R: TCATTAAATTCCAGTGCCAGG	285	55°C
Exon 2	F: TGCCCAGGAGAGTAGTTGAG R: ACTGGAAGGCTACCAAAGGA	230	61°C
Exon 3	F: CATCAGACCTCACTCTGCTTG R: TGACACCATTTACCTCTAGGA	292	62°C
Exon 4	F: TTCCCGCTGGGTACTGATAC R: TGGCTCTATGTCATCTTGTCC	226	59°C
Exon 5 to 6[a]	F: CGAACTCCTGACCTCGTGAT R: AGCCTGGGTGACAGAGAGAG	1144	–
Exon 5	F: CTCCTATTGACTCGGACTACCA R: TTTCTCAACCCCCAGACATC	236	57.5°C and 61°C
Exon 6	F: TTCCAACTCTGGGTGCTTCT R: CAGTCCTGATCCCACATCCT	250	61°C and 63°C
Exon 7[a]	F: CTCGGCTTCCCAAAGTGCTG R: ATAGTTGGGTAGAGAAATCG	482	–
Exon 7	F: TTACAGTGTGAGCCACCACA R: TGGATGCTGGATTTGAAGGT	366	61°C and 62°C
Exon 8 to 11[a]	F: ACAAATTAGCTGGGTGTGGC R: TAAGCTCACACTGGCCCTGC	1877	–
Exon 8	F: TGTGCAAGGTCCAGGATCAG R: GAGGTCTGCTTTGCAGGAAG	301	61°C
Exon 9	F: CCCAGTGTTGAGCCTTTGTC R: AAGCCATCCGATGTAGGAGA	271	58°C and 61°C
Exon 10 to 11	F: CGAGGGACTCTGACCATCTG R: CCTTTAATGCCCAGGCTGA	398	57°C and 62°C

[a] Pre-amplifications.