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Use of a multiplex ligation-dependent probe amplification method for the detection of deletions/duplications in the *GBA1* gene in Gaucher disease patients

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ABSTRACT

Gaucher disease (GD) is caused by the deficient activity of β-glucocerebrosidase due to pathogenic mutations in the *GBA1*. This gene has a pseudogene (*GBAP*) with 96% of sequence homology. Recombination (Rec) events in the *GBA1* seem to be facilitated by an increased degree of homology and proximity to the *GBAP*. The objectives of this study were to validate the P338-X1 GBA kit (MRC-Holland) for Multiplex Ligation-dependent Probe Amplification (MLPA) and to detect larger deletions/duplications present in *GBA1* in GD patients from Brazil. Thirty-three unrelated Brazilian GD patients, previously genotyped by the Sanger method (both pathogenic alleles identified = 29 patients, only one allele identified = 3 patients, no pathogenic alleles identified = 1 patient), were evaluated by the MLPA assay. MLPA was compatible with the previous results obtained by Sanger sequencing and identified an additional allele (a heterozygous deletion in intron 7 in one patient with only one mutation identified by Sanger). Our data suggest that, although larger deletions/duplications do not appear to be frequent in GD, the P338-X1 GBA kit for MLPA appears to be a good method for *GBA1* analysis. Additional investigations should be performed in order to characterize the remaining four uncharacterized alleles of our sample.

1. Introduction

Gaucher disease (GD) is one of the most common lysosomal storage disorders. It is inherited as an autosomal recessive disorder and is caused by deficient activity of the β-glucocerebrosidase enzyme due to pathogenic mutations in the *GBA1* gene. The function of this enzyme is to catalyze the hydrolysis of the glycolipid glucocerebroside to ceramide and glucose [1]. Enzyme deficiency leads to the accumulation of non-degraded substrate in tissues, especially in the cells of the reticulo-endothelial system, resulting in the formation of Gaucher cells, which will harm the regular function of certain organs [2]. GD frequency is estimated to be around 1 in 40,000–60,000 individuals in the general population and may affect 1 in 850 in the Ashkenazi Jewish population [3].

The disorder is divided into three types, based on the absence (type I) or presence and severity (types II and III) of primary involvement of the central nervous system (CNS) [1]. Type I, or nonneuronopathic GD, is by far the most common type, representing more than 90% of cases, and it includes patients with great variability in the progression and severity of the disease. Type II, or acute neuronopathic GD, is the rarest and the most severe form of the disease; these patients generally die before 2 years of age. Type III, or chronic neuronopathic GD, is the intermediate form of the disease because it commits the functions of CNS more slowly and gradually than type II; these patients survive until 20–30 years of age [1,4].

The clinical features associated with GD include hepatosplenomegaly, anemia, thrombocytopenia, bone and lungs problems. The laboratory diagnosis of GD is based on the measurement of β-glucocerebrosidase in leukocytes and/or fibroblasts. Analysis of the *GBA1* gene is

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Table 1
Genotypes and phenotypes of Gaucher patients included in the present study.

Genotype by Sanger	MLPA results	Number of patients (33)	GD Type
N370S/N370S	Normal	1	I
G377S/G377S	Normal	1	I
N370S/R163*	Normal	1	I
N370S/L444P + A456P	Low signal exon 10	1	I
N370S/L444P	Low signal exon 10	7	I
N370S/Rec*Nci*I	Low signal exon 10	11	I
N370S/L444R	Low signal exon 10	2	I
N370S/?	Normal	2	I
L444P/Rec*Nci*I	Low signal exon 10	2	II
Rec*Nci*I/?	Low signal intron 7 and exon 10	1	II
L444P + E326K/H311R	Low signal exon 10	1	II
L444P/L444P	Low signal exon 10	2	III
No mutations found	Normal	1	I

Location of mutations –R163* (exon 6); E236K and H311R (exon 8); N370S and G377S (exon 9); L444P, L444R, A456P and Rec*Nci*I (exon 10).

complementary to the biochemical diagnosis [5]. Often, the genotype-phenotype correlation is very difficult to establish because there is a wide phenotypic variability among patients with GD. Defining the patient's genotype is extremely important as it can help in the definition of the prognosis, the severity, and rate of progression of the clinical manifestations of the disease. The presence of at least one N370S allele prevents the development of neurological manifestations and confers type I disease, whereas the presence of the L444P allele in homozygosity is highly associated with CNS involvement [6].

The *GBA1* gene is located on chromosome 1q21 and spans 7.6 kb of genomic DNA divided into 11 exons. A highly homologous 5.7 kb pseudogene (*GBAP*) is located 16 kb downstream, with the same organization of exons [7]. The *GBAP* is transcribed, but it does not produce a functional protein [8]. *GBA1* and *GBAP* share more than 96% exonic sequence homology, enhancing the likelihood of homologous recombination. Recombination events between *GBA1* and *GBAP* have been identified, resulting from gene conversion, fusion or duplication and are responsible for several different identified mutant alleles [9]. To date, over 400 different pathogenic *GBA1* mutations have been reported. These include missense mutations, nonsense mutations, small insertions or deletions that lead to either frameshifts or in-frame alterations, splice junction mutations and complex alleles carrying two or more mutations *in cis* (http://www.hgmd.cf.ac.uk, September 2016; [3]).

Our aims in the present study were to validate the P338-X1 GBA kit (MRC-Holland) for MLPA and to detect large deletions and/or duplications in *GBA1* in GD patients from Southern Brazil.

2. Materials and methods

The study was approved by the institutional review board of Hospital de Clínicas de Porto Alegre, Brazil.

2.1. Patients

DNA samples from 33 unrelated patients with GD (type I = 27; type II = 4; type III = 2) were analyzed. The diagnosis of GD was established by the demonstration of deficient β-glucocerebrosidase activity in leukocytes and/or fibroblasts. *GBA1* gene was previously analyzed by Sanger sequencing in all patients (both mutations identified = 29 patients, only one mutation = 3, no identified mutation = 1; no pathogenic alleles identified = 5/66; point mutation alleles = 47/66; Rec alleles = 14/66) (Table 1). The location of the mutations found is as follow: R163* (exon 6); E236K and H311R (exon 8); N370S and G377S (exon 9); L444P, L444R, A456P and Rec*Nci*I (exon 10).

2.2. Multiplex ligation-dependent probe amplification assay

The MLPA kit (P338-X1 GBA kit, MRC Holland) for the *GBA1* gene contains one probe for each of the following regions of *GBA1*: 5'UTR, exons 3, 4, 6, 8, 9, 10, and intron 7; it is used to determine gene or exon deletion/duplication (Fig. 1). The probes do not cover the entire length of the corresponding regions. The assay conditions and reactions were performed according to the manufacturer's recommendations [MRC Holland, Amsterdam, The Netherlands (www.mlpa.com)]. The amplified products were analyzed using the ABI 3500 equipment (Thermo Fisher Scientific). Data results were analyzed using Coffalyser software for MLPA [MRC Holland, Amsterdam, The Netherlands (https://coffalyser.wordpress.com/)]. Subjects with wild-type genotype were included as controls in all reactions.

3. Results

Table 1 shows the results of the Sanger sequencing and MLPA. No evidence for deletion/duplication was found.

Of 66 alleles, 13 (19.7%) were found to have L444P, and 2 (3.03%), L444R, confirming our sequencing results. However, we were unable to distinguish if the reduced signal was due to the presence of Rec*Nci*I (exon 10), L444P + A456P (exon 10) or L444P + E326K (exon 10). Only one patient presented a heterozygous deletion in intron 7; according to the sequencing, this patient is also heterozygous for Rec*Nci*I (Fig. 2d). These results were compatible with the previous analysis by Sanger sequencing.

After MLPA analysis, 4 uncharacterized alleles from 4 GD patients still remain.

4. Discussion

This is the first analysis of the *GBA1* gene by the MLPA technique in Brazilian patients with GD. Genotyping of GD patients is usually made by the Sanger sequencing. The identification of both mutations in the *GBA1* can provide useful information regarding the prognosis of the patients, and it is essential for carrier diagnosis. However, large deletions and duplications that span entire exons are not detected by sequencing techniques, and additional investigation, such as MLPA, should be performed.

Restrictive factors of the MLPA kit are as follows: a) probes reach only part of the gene's coding region; and, b) not all coding regions are covered due to *GBAP* homology and do not cover the full extension of each exon, which may not detect some variation of the gene structure in the uncovered regions. This method is used for relative quantification of 40 different DNA sequences in a single reaction performance, and it only requires a thermocycler, a capillary electrophoresis equipment, and a concentration of 20 ng of human DNA. Among the different applications of this technique are: detection of deletions and duplications in exons of a variety of human genes; trisomy detection, such as Down

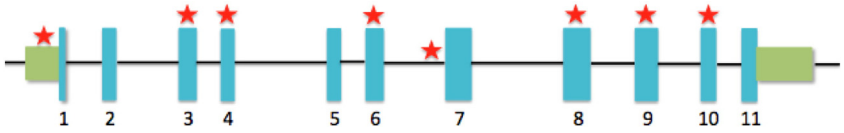

Fig. 1. Schematic representation of probes used in the present study. The MLPA kit contains one probe for each of the following regions of *GBA1*: 5'UTR, exons 3, 4, 6, 8, 9, 10, and intron 7. Each probe is represented by a red star in the corresponding region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)