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False-Positive Results Using a Gaucher Diagnostic Kit - RecTL and N370S

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Abstract

The Pronto[®] Gaucher kit and Pronto[®] Gaucher RecTL Amplification Mix, marketed to identify mutations in the gene for glucocerebrosidase, are widely used for the diagnosis of Gaucher disease. Subjects genotyped using this kit have been reported with an allele including both the common N370S mutation and RecTL, a previously described Gaucher mutation arising from recombination between the glucocerebrosidase gene and pseudogene. Using direct sequencing and Real-Time PCR, we show that the RecTL, N370S allele is a false positive result, demonstrating a deficiency of the kits.

Keywords

Gaucher disease; glucocerebrosidase; molecular diagnostic kit; recombinant allele; Parkinson disease; allele specific oligonucleotides

Introduction

Mutations in the glucocerebrosidase gene (*GBA*) result in Gaucher disease (GD), the most common of the lipidoses. This autosomal recessive storage disorder is characterized by the deficiency of the lysosomal enzyme glucocerebrosidase, leading to the accumulation of the lipid glucocerebroside in the lysosomes of the cells and presents with hepatosplenomegaly, anemia, thrombocytopenia, bone involvement, and neurological symptoms (type 2 and 3)¹. The disorder is encountered with an increased frequency among Ashkenazi Jews, where the carrier frequency is approximately 1 in 15². Almost 300 mutations in *GBA* have been identified in patients with Gaucher disease including point mutations, insertions, deletions, frame-shift changes, splice site alterations and recombinant alleles⁴, but among Ashkenazi Jewish patients six mutations (N370S, L444P, c.84dupG, IVS2+1G>A, V394L, and D409H) account for more than 90% of mutant alleles^{2–6}. Recent studies have reported parkinsonism in patients with Gaucher disease and several studies have demonstrated an increased frequency of *GBA* mutations among patients with Parkinson disease⁷

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With the advent of the polymerase chain reaction as a tool for molecular diagnosis, different molecular diagnostic kits have appeared on the market targeting specific disease populations. Diagnostic kits for detection of *GBA* mutations allowed for facile detection of carriers and patients in a susceptible population. The Pronto[®] Gaucher kits (Pronto Diagnostics, Rehovot, Israel), available as Pronto[®] Gaucher (Cat. No. 9900), Pronto[®] Gaucher Screen (Cat. No. 9900/1), and Pronto[®] Gaucher RecTL Amplification Mix (Cat. No. 9914) were developed to screen for glucocerebrosidase gene mutations based on previous studies⁶. These kits allow for detection of point mutations in the DNA sequence, based on PCR-ELISA allele specific oligonucleotides (ASO) as previously described⁸. These diagnostic kits have been widely popular in Europe and many hospitals and medical centers utilize it for carrier screening and prenatal diagnosis. These kits look for the presence or absence of eight *GBA* mutations, N370S, L444P, c.84dup G, IVS2+1G>A, R496H, V394L, D409H and a recombinant allele resulting from recombination of *GBA* with the glucocerebrosidase pseudogene, RecTL.^{3,5}

It has come to our attention that the Pronto[®] Gaucher kit with Pronto[®] Gaucher RecTL Amplification Mix produces false-positive results for RecTL in some subjects carrying the N370S allele. Genotypes including RecTL, N370S/+ (implying N370S and RecTL are in *cis*) and N370S/RecTL (N370S and RecTL are in *trans*) have been reported using this kit⁹. Generally, the N370S allele is reported to cause a ‘mild’ phenotype, especially if present in the homozygous form. The allele RecTL, however, involves a genetic recombination between the glucocerebrosidase gene and pseudogene that introduces a deletion of 55bp and mutation D409H in exon 9, as well as mutations L444P, A456P, and V460V in exon 10. This allele is associated with a ‘severe’ or lethal phenotype when encountered in the homozygous form^{10, 11}.

After the introduction of this kit, reports appeared describing patients with a RecTL, N370S/+ allele, first recognized in the Israeli Ashkenazi Jewish population⁶. In a recent study⁹, the Pronto[®] Gaucher kit and the Pronto[®] Gaucher RecTL Amplification Mix were used to screen for *GBA* mutations in 420 Ashkenazi Jewish patients with Parkinson disease, and, to our surprise, the RecTL, N370S/+ allele was identified in five patients with Parkinson disease. This allele has never been identified in other cohorts of patients with Parkinson disease⁷. Because we had already noted some inconsistencies in genotypes provided with the Pronto Gaucher kit, we re-evaluated DNA samples from these subjects.

Methods

Sequencing of eight genomic DNA samples from previously genotyped using the Pronto[®] Gaucher kit was performed using a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) as previously described¹⁰ and data was analyzed using Sequencing Analysis v.5.1.1 and SeqScape v.2.1.1 (ABI). All exons and flanking intronic regions of *GBA* were sequenced. We additionally sequenced the intronic regions of *GBA*, which have a high prevalence of polymorphisms, to confirm the amplification of both alleles. In N370S/N370S samples found to be homozygous at all polymorphic sites, the amplification of the entire 6.5kb *GBA* gene was performed to ascertain the status of the 55bp deletion or any other deletion within the gene resulted from a recombination event between *GBA* gene and its pseudogene. Taq Man probes and primers were designed to further evaluate the 55bp region, which is deleted in the RecTL allele. *GBA* probe was labeled with FAM, and was co-amplified with VIC labeled β -globin probe, used as an internal control. All experiments were done on the StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA) as Quantitative C_T experiments. DNA samples from patients previously found to have the 55bp deletion alone, or as part of a duplication or fusion allele, were used as controls.

Results

Sequencing of *GBA* in six patients previously found to carry the RecTL, N370S/+ genotype demonstrated that RecTL was falsely identified, and that in fact, each had a genotype of N370S/+. Two subjects believed to have genotype N370S/RecTL were found to have N370S/N370S, confirmed by the long-template amplification and sequencing of the 6.5 kb *GBA* gene (Table 1). The Real-time analysis also did not indicate the presence of RecTL, as none of the samples carried the 55bp deletion (Figure 1).

Discussion

In conclusion, we found no evidence for a recombinant allele in patients reported to have “RecTL alleles” based on the Pronto® Gaucher kits. However, this “RecTL allele” was detected more frequently in the series of patients with PD carrying a N370S allele as compared to N370S carriers found among young controls⁹. This might be explored further, and could have resulted from an alteration in the *GBA* pseudogene and not in the gene in these subjects.

Diagnostic kits like the Pronto® Gaucher kit are widely used for genotypic analyses. However the *GBA* locus is complex, with a highly homologous pseudogene sequence only 16kb downstream and recombination between the gene and pseudogene are known to lead to different recombinant alleles¹². In this communication we demonstrate some of the pitfalls of a facile screening technique and demonstrate that, at times, genotyping using conventional sequencing is required for accurate mutation analysis. When a suspicion of an unlikely genotype arises, it is highly recommended to pursue more precise mutation detection methods like direct sequencing. According to provided data, we believe that all patients previously identified with RecTL using the Proto® Gaucher kit should be re-evaluated.

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Figure 1.

RT-PCR evaluation of patients with Gaucher disease and a control (WT: normal control; P1, P2: patients reported to have “RecTL/N370S”; “P3, P4: patients reported to have “RecTL, N370S/+”; P5: patient with a duplication in exon 9 (which leads to a duplication of the 55bp region); P6: patient with a fusion in exon 9 (which leads to a deletion of the 55bp region). (Relative quantization of <0.5 considered as deletion, 0.75–1.25 normal and >2.0 as duplication.)

Table 1Genotypes of patients using Pronto[®] Gaucher kit and conventional sequencing

Patient	Results using the Pronto [®] Gaucher Kit	Sequencing results
P1	RecTL/N370S	N370S/N370S
P2	RecTL/N370S	N370S/N370S
P3	RecTL, N370S/+	N370S/WT
P4	RecTL, N370S/+	N370S/WT
P5	RecTL, N370S/+	N370S/WT
P6	RecTL, N370S/+	N370S/WT
P7	RecTL, N370S/+	N370S/WT
P8	RecTL, N370S/+	N370S/WT