A new missense mutation in glucocerebrosidase exon 9 of a non-Jewish Caucasian type 1 Gaucher disease patient

Francis Y.M. Choy*, Chao Wei, Derek A. Applegarth¹ and Siu-Li Yong²

Centre for Environmental Health, Department of Biology, University of Victoria, PO Box 1700, Victoria, British Columbia V8W 2Y2, ¹Department of Pediatrics, University of British Columbia and Biochemical Diseases Laboratory, B.C. Children’s Hospital and ²Department of Medical Genetics, B.C. Children’s Hospital, University of British Columbia, Vancouver, British Columbia, Canada

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Gaucher disease is the most prevalent inherited sphingolipidosis (1). It results from deficient glucocerebrosidase activity and is transmitted as an autosomal recessive trait (2). Three clinical forms of Gaucher disease have been described: Type 1, non-neuronopathic; type 2, acute neuronopathic; and type 3, subacute neuronopathic (1).

The gene frequency of Gaucher disease in the Jewish population was elevated and estimated to be between 0.035 to 0.040 (3). Four ‘public’ mutations were reported to account for about 96% of the Gaucher disease-producing alleles in Jewish patients (3). These are an A→G transition at cDNA nt 1226 that results in 370Asn→370Ser (4), a G insertion at cDNA nt 84 (5), a T→C transition at cDNA nt 1448 that results in 444Leu→444Pro (6), and a splicing (g→a) mutation in intron 2 at genomic nt 1067 (5, 7). Among non-Jewish patients, it was reported (8) that mutations 1448 and 1226 account for more than 50% of the total mutated alleles. However, more than 25% of the total mutations among non-Jewish patients remain unidentified (3, 8, 9). In this report, we describe a new missense mutation in a non-Jewish Caucasian type 1 Gaucher patient. A relatively simple procedure which utilizes the polymerase chain reaction (PCR) method and restriction fragment length polymorphism (RFLP) analysis for its detection is also described.

The patient is of French–Finnish and French–Ukrainian descent. She was investigated at age 6 1/2 years for unexplained splenomegaly. There was no history of bone pain or neurological problems. The diagnosis of Gaucher disease was raised. Bone marrow aspirate and fibroblast culture for glucocerebrosidase activity assay confirmed the clinical diagnosis of Gaucher disease. She was readmitted at age 13 1/2 years because of hypersplenism and left flank pain. The spleen was large and hard, filling the whole left abdomen. She underwent a splenectomy which reversed her hematological complications. Histology on her spleen confirmed the diagnosis of Gaucher disease. At the present age of 21 years, she was asymptomatic and there was no clinical evidence of hepatic or neurological involvement.

Fibroblasts were cultured and harvested for β-glucosidase activity assays as previously described (10). Genomic DNA was isolated from harvested fibroblasts using the TurboGen™ Genomic DNA Isolation Kit [Invitrogen Corporation, San Diego, CA]. The genomic DNA samples were amplified by the PCR method with Taq polymerase (Bethesda Research Laboratory, Gaithersburg, MD), using a method (11) modified from Saiki et al. (12). In order to selectively amplify the glucocerebrosidase structural gene and not the pseudogene which shares more than 96% sequence similarity with the structural gene (13), primers with nucleotide sequences deleted in the pseudogene were synthesized and used for the PCR amplification ([14], Table 1).

Primers A and B flank the anterior part of exon 9 where mutation 1226 is located. The posterior part of exon 9 (where mutation 1366 is located), exon 10 and exon 11 are flanked by primers C and D. Poly-A mRNA was isolated from cultured fibroblasts using the Micro-FastTrack™ mRNA Isolation Kit (Invitrogen Corporation). The cDNA of the glucocerebrosidase gene was synthesized by reversed transcription of fibroblast poly-A mRNAs using the cDNA Cycle™ Kit (Invitrogen Corporation), and amplified by the PCR method as previously described (14). The nucleotide sequences of the primers for the first strand cDNA synthesis (primer D) and PCR amplification (primers D and E) are shown in Table 1. These primers are designed to cover the full length cDNA of glucocerebrosidase (14). Sequence analysis was performed using the dideoxyribonucleotide chain termination method by Sanger et al. (13) and the fmol™DNA Sequencing System (Promega Corporation, Madison, WI). The PCR mismatch method and XhoI RFLP analysis (9) were used to detect mutation 1226. For mutation 1366, genomic DNA amplified by the PCR method using primers C and F (Table 1) was subjected to NeoI RFLP analysis.

XhoI RFLP analysis showed that mutation 1226 is present in the heterozygous form in the patient. This finding was confirmed by sequence analysis. The presence of an A→G transition in the heterozygous form was noted at cDNA nt 1226. In view of this finding and the report that mutation 1226 is present only in type 1 Gaucher disease (16), the patient was diagnosed as having the non-neuronopathic type 1 form.

The mutation in the other Gaucher allele was also identified during sequence analysis. As shown in Fig. 1, there was a T→G transversion in the heterozygous form at cDNA nt 1366, resulting in 417Phe→417Val of glucocerebrosidase. A computer search for

![Figure 1. Sequence analysis of PCR-amplified glucocerebrosidase exons 9–11 from the control and Gaucher patient. A T→G missense mutation in the heterozygous form at cDNA nucleotide position 1366 of the patient was noted.](https://academic.oup.com/hmg/article-abstract/3/5/821/616526/A-new-missense-mutation-in-glucocerebrosidase-exon)
endonuclease cleavage sites showed that the mutation has created a new Ncol site. The presence of mutation 1366 was subsequently confirmed by Ncol RFLP analysis. As shown in Fig. 2, two bands of 226 and 592 b.p. were found in the control because of a naturally occurring Ncol cleavage site at genomic nt 6146. In the patient, the 1366 mutation creates a second Ncol site which results in the cleavage of the band of 226 b.p. to two bands of 61 and 165 b.p. Since both the 226 b.p. and 165 b.p. bands are present (lane 2, Fig. 2), it demonstrates that mutation 1366 is present in the heterozygous form.

In order to rule out the possibility of multiple point mutations that may be present among some Gaucher patients (17,18), we performed sequence analysis of the full length cDNA of the glucocerebrosidase gene. We detected the presence of a heterozygous silent mutation at cDNA nt 84 (G—T transversion) at the degenerate position of the codon for glycine, and a heterozygous base substitution at cDNA nucleotide position 1601 (A—G transition) that results in 495His—495Arg. The later finding is of interest because it was previously reported that this may be a cloning artifact (19) or a neutral mutation among normal individuals (20). In this present study, we performed direct sequence analysis on PCR-amplified glucocerebrosidase cDNA synthesized by reverse transcription. This procedure by-passed the gene cloning step and eliminated the possibility of error arising from gene cloning. The possibility of error resulting from Taq polymerase is also unlikely since consistent reproducible findings were noted using cDNA samples that were PCR-amplified in separate experiments. It will be interesting to screen for the presence of this base substitution at cDNA 1601 among normal individuals and Gaucher patients to confirm this observation and estimate its frequency in the population.

Sequence analysis of the rest of the glucocerebrosidase cDNA showed that the DNA base sequences are identical to that of the published sequences from normal individuals (13).

The 1226 mutation is the most common mutation in Gaucher disease (5). It was postulated that homozygous 1226 mutation will result in mild clinical presentation, delayed onset of symptoms and good prognosis (5,16,21). However, when it is present in the heterozygous form with either the 1448 mutation, exon 2 insertion mutation or intron 2 splicing mutation, the clinical severity scores assigned to the patients are considerably higher (5). The clinical course of Gaucher disease in our patient with the 1226/1366 genotype so far has been a mild one. The splenectomy performed ten years ago on the patient has apparently reversed the hematological complications and there has been no history of bone pain, bruising and no evidence of hepatic or neurological involvement. As she is asymptomatic, further investigations such as MRI of the bones, liver biopsy or bone marrow examination have not been done. It will be interesting to monitor the clinical course of our patient and observe the effect of this 1226/1366 genotype on the clinical expressivity of Gaucher disease.

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REFERENCES