Am. J. Hum. Genet. 49:1378–1380, 1991

A New Glucocerebrosidase-Gene Missense Mutation Responsible for Neuronopathic Gaucher Disease in Japanese Patients

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Summary

We have identified a new T-to-A single-base substitution at nucleotide 3548 (in the genomic sequence) in exon 6 in the glucocerebrosidase gene from a patient with Gaucher disease type 3. This mutation caused a substitution of isoleucine for phenylalanine at amino acid residue 213 (of 497 residues in the mature protein). By in vitro expression study in cultured mammalian cells, this mutation resulted in deficient activity of glucocerebrosidase. By allele-specific oligonucleotide hybridization of selectively PCR-amplified DNA from eight unrelated Japanese Gaucher disease patients, this mutant allele was observed in other neuronopathic Japanese Gaucher disease patients, in moderately frequent occurrence (three of six neuronopathic patients). This observation suggests that this allele was one of several alleles which were related to the development of neurological manifestations of Gaucher disease.

Gaucher disease is the most prevalent lysosomal storage disease. It is caused by an inherited deficiency of lysosomal glucocerebrosidase (E.C.3.2.1.45). Gaucher disease has been classified into three major phenotypes, according to clinical symptoms. Although all three subtypes of Gaucher disease are due to the deficiency of the same lysosomal enzyme, clinical heterogeneity exists even within the same clinical subtype. Recently, the marked genetic heterogeneity of Gaucher disease has been reported (for a review, see Latham et al. 1990). Although some exceptions exist (Dahl et al. 1990; Masuno et al. 1990), the existence of a strong relationship between genotypes and clinical phenotype has been reported (Tsuji et al. 1987; Zimran et al. 1989; Theophilus et al. 1989*b*; Firon et al. 1990; Kolodny et al. 1990).

The patient with Gaucher disease type 3 who was investigated in the present study was a 5-year-old Japanese female who was found to have hepatosplenomegaly at the age of 1 year and was diagnosed as having Gaucher disease on the basis of both the identification of Gaucher cells in bone marrow aspiration and the deficient activity of acid β-glucosidase in white blood cells (Mutoh et al. 1988). At the age of 4 years she had generalized convulsions and developed pyramidal tract signs, ataxia, myoclonic seizure, and oculo-motor abnormalities. Her parents were not consanguineous. Cloning and sequencing of exons and of exon-intron boundaries of the glucocerebrosidase gene from this patient revealed a new T-to-A substitution at nucleotide 3548 in exon 6, causing a substitution of isoleucine for phenylalanine at amino acid residue 213 (of 497 residues in the mature protein). This mutant sequence is normally found in the corresponding region of the pseudogene (fig. 1). This mutation did not represent a complex allele that had multiple pseudogene pattern mutations (Hong et al. 1990; Latham et al. 1990; Zimran et al. 1990), because the other pseudogene-pattern mutations were not found in the cloned DNA. Previous studies have revealed that other mutations in the pseudogene can be found in the active gene (Tsuji et al. 1987; Theophilus et al. 1989*a*). The ethnic diversity of the patients having such pseudogene-pattern missense mutations in their active gene indicates that multiple independent genetic

Received April 24, 1991; final revision received August 8, 1991.

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0002-9297/91/4906-0026$02.00

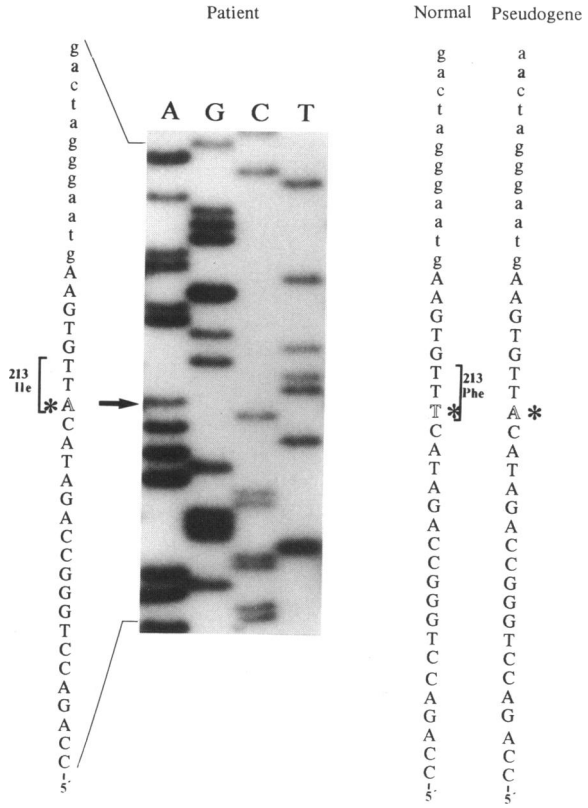

Figure 1 T-to-A single-base substitution in exon 6 of glucocerebrosidase gene from patient with Gaucher disease type 3 (patient 5). The sequences of the normal glucocerebrosidase gene and of the pseudogene are shown on the right. An asterisk (*) indicates the position of the mutation. Cloning of the glucocerebrosidase gene was performed according to the method described by Tsuji et al. (1987). The genomic fragment of the glucocerebrosidase gene was synthesized by PCR amplification, and positive clones were screened by plaque hybridization using this genomic fragment as a probe. The clones containing the active gene were selected by selective amplification and were subcloned into plasmid pUC119. Sequencing of exons and of the exon-intron boundary was performed by the dideoxy-termination method using a Sequenase kit (U.S. Biochemical, Cleveland) with specific oligonucleotide primers.

events such as gene conversion or homologous recombination are responsible for Gaucher disease. The expression plasmid that had the mutant cDNA introduced this mutation was transfected into COS-1 cells. 4MU-β-glucosidase activity of COS-1 cells failed to increase as efficiently as did the normal cDNA (table 1). These data strongly suggest that this allele was responsible for Gaucher disease. The presence of this mutant allele in this patient was confirmed by allele-specific hybridization of selectively PCR-amplified DNA fragments. (Seven additional Japanese patients with Gaucher disease were also examined.) This pa-

Table 1

Transient Expression of Glucocerebrosidase Activity in Transfected COS-1 Cells

Construct	Mean ± SE 4MU-β-Glucosidase Activity (nmol/mg protein/h)	Increase
Mock.....	63.8 ± 2.9	
pcDGC-N6.....	200.5 ± 14.0	214%
pcDGC-M2.....	89.2 ± 3.4	40%

NOTE.—The mutated cDNA constructed by oligonucleotide-directed mutagenesis (GC-M2) and normal cDNA (GC-N6) were subcloned into the *Eco*RI site of mammalian expression vector pcD. COS-1 cells were transfected, with either the mutant plasmid (pcDGC-M2) or with normal plasmid (pcDGC-N6)(10 μg/25-cm² flask), in triplicate by the $CaPO_4$ method. After 60 h, cells were harvested and assayed for 4MU-β-glucosidase activity (Jonsson et al. 1987).

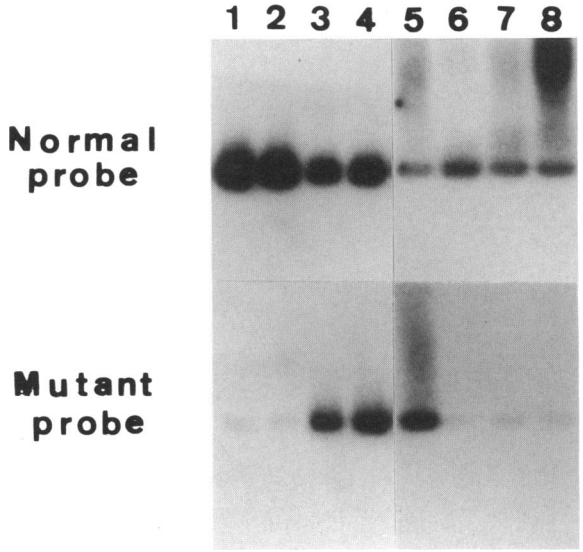

Figure 2 Screening of 213 Phe-to-Ile mutation allele in Japanese Gaucher disease patients by allele-specific oligonucleotide hybridization of the PCR-amplified DNA fragments. Lane numbers correspond to patient numbers: patients 7 and 8, type 1 (6 and 7 years old, respectively); patients 2–4 and 6, type 2 (ages 1–3 years); and patients 1 and 5, type 3 (7 and 5 years old, respectively). The primers used for selective amplification by PCR were 5′-GACTGGCAAGTGATAAGC-3′ (sense) and 5′-GGTTACAGTGAGTGAAGA-3′ (antisense). To prevent the amplification of the pseudogene, we designed the antisense primer in the sequence which was deleted in the pseudogene (Horowitz et al. 1989). Allele-specific oligonucleotide probes for this T-to-A substitution were 5′-GCCAGATACTTTGTGAAGT-3′ for the normal sequence and 5′-GCCAGATACATTGTGAAGT-3′ for the mutant sequence.

tient was heterozygous for this new mutant allele (fig. 2, lane 5). Furthermore, this mutant allele was found in other patients (two Gaucher disease type 2 patients) in a heteroallelic state, especially only in the neuronopathic type (fig. 2, lanes 3 and 4). Since these patients are unrelated, this observation suggests a moderate incidence of this new mutation among Japanese patients with Gaucher disease. It also suggests that this mutation is related to the development of neurological manifestations. The 444 Leu-to-Pro mutation (at nucleotide 6433, T to C) was frequently found in all subtypes of these Japanese Gaucher disease patients in heteroallelic state (data not shown). As a result, seven of eight patients were compound heterozygotes. These findings suggest that marked genetic heterogeneity exists in Japanese patients with Gaucher disease.

Acknowledgments

We thank Drs. Kihei Maekawa (Department of Pediatrics, The Jikei University School of Medicine) and Susumu Sakurai and Hitoshi Suzuki (Division of Molecular Genetics, Institute of Medical Science, The Jikei University School of Medicine) for their helpful discussion and encouragement. We thank Drs. Toya Ohashi and John A. Barranger (Department of Human Genetics, Pittsburgh University) for providing the glucocerebrosidase cDNA clone and pCD expression vector and for helpful discussion. We thank Dr. Hiroatsu Hojo (Shizuoka Children's Hospital, Shizuoka) for referral of the patient. This investigation was supported in part by a grant from the Ministry of Health and Welfare (Japan).

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