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MUTATION IN BRIEF

Two Novel (1098insA and Y313H) and One Rare (R359Q) Mutations Detected in Exon 8 of the β-Glucocerebrosidase Gene in Gaucher's Disease Patients

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INTRODUCTION

Gaucher's disease is the most prevalent sphingolipid storage disorder in humans, affecting approximately 80,000 individuals worldwide (Brady et al., 1994). It is caused by a recessively inherited deficiency of the enzyme β-glucocerebrosidase (E.C. 3.2.1.45). Expression studies have shown that some residues in exons 5, 8, 9, and 10 of the gene are critical for catalytic activity (Grabowski, 1993; Grace et al., 1994). Consequently, it is not surprising that more than 50% of the disease-causing mutations have been located in the region of exons 8 to 10 (Beutler et al., 1993; Horowitz and Zimran, 1994).

Forty-three Spanish Gaucher's disease patients were previously screened for eight mutations (Cormand et al., 1995). This initial screening allowed the identification of mutations in 63 out of 86 chromosomes. In the present study, we focused on the search for new exon 8 mutations, using a non-isotopic single-strand conformation polymorphism analysis of PCR amplified DNA fragments (PCR-SSCP), in the remaining 23 Gaucher alleles.

MATERIALS AND METHODS

Patients

Forty-three unrelated Spanish Gaucher's disease patients, with different clinical subtypes, were screened for the most common mutations. Thirty-five of these individuals, including those presenting the mutations reported here, were previously described (Cormand et al., 1995).

DNA Amplification, SSCP Analysis, and Sequencing

Genomic DNA was isolated from harvested skin fibroblasts, peripheral blood leukocytes, or spleen, using standard methods (Miller et al., 1988; Sambrook et al., 1989). The primers used for PCR amplification of exon 8, which do not amplify the highly homologous pseudogene, were the following: 5′-TGTGCAAGGTCCAGGATCAG-3′ (sense primer) and 5′-TTTGCAGGAAGGGAGACTGG-3′ (antisense primer). The size of the amplified fragment was 292 bp. Fifty microlitres of PCR amplification mixture (containing 100 ng of genomic DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 20 pmol of each primer, and 1 U of Dynazyme DNA polymerase—Finnzymes Oy—in the recommended buffer) was subjected to 35 cycles of 94°C for 40 sec and 55°C for 30 sec, in a two-step PCR protocol.

Single-strand conformation polymorphism analysis (Orita et al., 1989) was performed on PCR amplified DNA fragments (PCR-SSCP) as previously described (Bayés et al., 1995). Four SSCP conditions, using different acrylamide and glycerol concentrations in the gel and various running temperatures, were tested.

PCR products from exon 8, which gave aberrant SSCP patterns, were subsequently purified by Wizard™ PCR Preps (Promega, Madison WI) and cloned into pUC18 using the SureClone™ Ligation Kit (Pharmacia, Gaithersburg, MD). In each case, 10 to 15 clones were sequenced by the

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dideoxy chain-termination method using the T7 Sequencing™ Kit (Pharmacia). Direct sequencing from the purified PCR product was also performed, using the Sequenase™ Version 2.0 DNA Sequencing Kit (USB: Cleveland, Ohio).

Genomic sequence numbering is according to Horowitz et al. (1989). cDNA numbers start from the first ATG.

RESULTS AND DISCUSSION

Here we describe two novel mutations in exon 8 of the glucocerebrosidase gene, an insertion (1098insA) and a missense mutation (Y313H), in 2 unrelated Spanish patients with Gaucher's disease type 1 (patients 1 and 2). One rare mutation, R359Q, was also found in exon 8 of another type 1 patient (patient 3). These 3 patients correspond to individuals I.11, I.6, and I.15 respectively, in Cormand et al. (1995).

Genomic DNA was amplified by PCR and analyzed by SSCP. The aberrant exon 8 SSCP patterns, corresponding to the new mutations described here, are shown in Figure 1.

An adenine insertion at position 5315 in the genomic sequence (cDNA position 1098) was detected in patient 1 (figure reviewed but not shown). This 1098insA mutation causes a frameshift leading to a premature stop codon after 68 amino acids. The predicted product is a truncated enzyme of 395 amino acids that lacks the catalytic domain, suggesting a role in the etiology of the disease. Analysis of other family members, by SSCP and sequencing, revealed that patient 1 had inherited the 1098insA mutation from her mother (of French origin), and the N370S allele from her father. An unaffected sister, heterozygous for the N370S mutation, does not bear the adenine insertion in the other chromosome (see Fig. 1a).

Another novel mutation, a T-to-C transition at genomic nucleotide 5270 (cDNA position 1054), was found in patient 2 (figure reviewed but not shown). This mutation (Y313H) predicts a substitution of a basic amino acid (histidine) in the mutant for an uncharged polar amino acid (tyrosine) in the wild-type allele. In this case, the affected member inherited the mutation Y313H from his mother, while his father was heterozygous for the N370S mutation (see Fig. 1b).

Patient 3 was found to be heterozygous for a G-to-A transition (not shown) at genomic position 5409 (cDNA 1193), which results in a predicted arginine-to-glutamine substitution at residue 359 (R359Q). This mutation, which results in the replacement of a basic by an uncharged polar amino acid, had previously been reported in only 1 Japanese patient (Kawame et al., 1992).

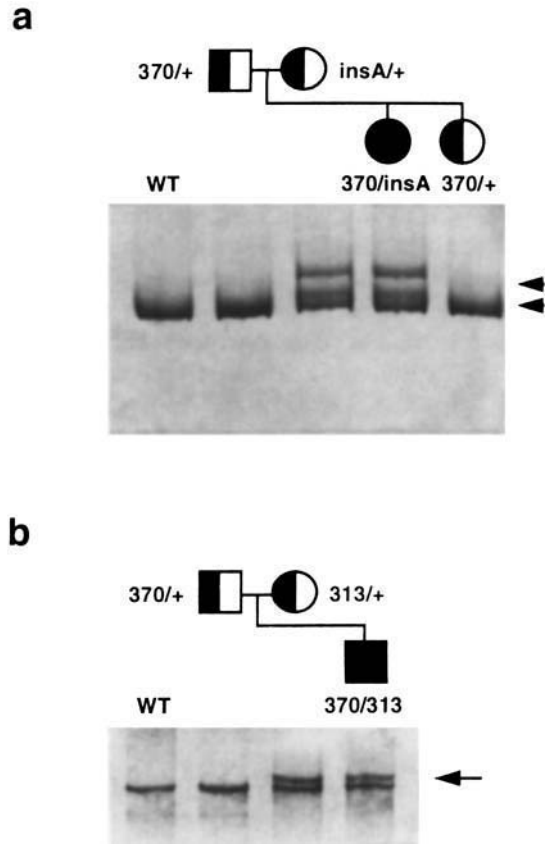

FIGURE 1. **Pedigrees, genotypes, and SSCP-heteroduplex analyses of exon 8 in families of (a) patient 1 (mutation 1098insA) and (b) patient 2 (mutation Y313H). Both patients are also heterozygous for mutation N370S. SSCP patterns are aligned below the individuals in the pedigree to which they relate. A sample from a normal individual (WT) was added for comparison. The *arrow* indicates the aberrant single-stranded DNA band and the *arrowheads* show the aberrant heteroduplexes.**

The screening of 50 unaffected individuals failed to detect these mutations, either by SSCP or by restriction analyses (mutations Y313H and R359Q can be detected by *Kpn*I and *Taq*I digestion, respectively).

The three mutations described here are rare, since they were detected only once in our series of 43 Gaucher's disease patients, two of them had never been reported before, and the other had been reported only once.

Mutation 1098insA is the second insertional mutation described to date in the β-glucocerebrosidase gene. Insertional mutations causing human genetic disease are rare, accounting for less than 10% of the published single-gene defects

(Cooper and Schmidtke, 1993). Most of them consist of the addition of one or a few base pairs, which could have arisen from an endogenous replication-associated mechanism of mutagenesis (Cooper and Krawczak, 1991).

Several facts suggest that the two amino acid substitutions identified in patients 2 and 3, respectively (Y313H and R359Q), are indeed disease-causing mutations. First, they are not found in the non-affected population; second, the two mutations are located in an exon which is believed to be involved in the catalytic activity of the enzyme; third, Tyr313 and Arg359 are conserved residues in the β-glucocerebrosidase of human and mouse (O'Neill et al., 1989), arguing in favor of their relevant role in the function of the enzyme. Besides, these substitutions cause a change in the amino acid charge, which may have a significant effect on protein structure and/or function. Furthermore, when the secondary structures for the normal and mutant proteins are predicted by the method of Chou and Fasman (1978), substantial modifications are clearly shown: Y313H mutation disrupts a predicted β-sheet and R359Q mutation destroys a turn linking an α-helix and a β-sheet (data not shown).

The rest of the glucocerebrosidase gene of these three patients was exhaustively analyzed to rule out the presence of other mutations. Genomic DNA was amplified by PCR in 14 overlapping fragments which covered all the coding region of the gene. Four different SSCP conditions were tested for each fragment. Additional abnormal SSCP patterns were only detected in exon 9. The aberrant bands were identical in all three patients and correspond to the N370S mutation, for which these patients had been previously shown to be heterozygous (Cormand et al., 1995).

Straightforward conclusions on the phenotypic implications of these mutations are difficult to draw as they are in heterozygosity with the mild N370S mutation. Only their presence in homozygosity, or as a compound heterozygote with a severe mutation would provide useful information about their clinical implications. Functional and structural characterization of the mutant products should provide further insights about their role in the Gaucher's disease phenotype and would lead to a better understanding of the genotype/phenotype correlation in this clinically heterogeneous disease.

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