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Mutation in the sphingolipid activator protein 2 in a patient with a variant of Gaucher disease

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The lysosomal degradation of glucosylceramide requires the hydrolase, glucosylceramide-β-glucosidase and a sphingolipid activator protein (Gaucher factor, SAP-2, saposin C). Genetic defects in either of these lysosomal proteins cause phenotypically similar disorders in man, the Gaucher disease. SAP-2 originates from a gene which generates a mRNA that codes for four homologous proteins. In a patient with an immunologically proven SAP-2 deficiency a $G^{1154} \rightarrow T$ transversion (counted from A of the initiation codon ATG) was found in the mRNA of the SAP-2 precursor which results in the substitution of Phe for Cys^{385} in the mature SAP-2. The rest of the coding sequence remained entirely normal.

SAP-2 deficiency; Gaucher disease

1. INTRODUCTION

The physiological degradation of glycosphingolipids with short oligosaccharide chains is catalyzed by lysosomal exohydrolases in the presence of small, heat-stable lysosomal glycoproteins, so called sphingolipid activator proteins (SAPs) [1]. Their physiological significance is demonstrated by fatal lipid storage diseases caused by deficiencies of these activator proteins [1,2]. So far, human diseases have been described with defects in three different activator proteins: AB variant of GM2 gangliosidosis caused by a deficiency of the GM2 activator protein (SAP-3) [3], a variant of metachromatic leukodystrophy due to a defect in SAP-1 [4], a variant of Gaucher disease with an immunologically proven deficiency of SAP-2 [5] and a patient with pleiotrophic lipid accumulation and the immunologically demonstrated absence of two activator proteins, SAP-1 and SAP-2 [6].

SAP-1 and SAP-2 are derived from the same gene that generates an mRNA coding for four homologous proteins [7–9]. SAP-1 is the second and SAP-2 the third domain of the four homologous proteins generated from the precursor protein.

Up to now three different mutations in the coding region of the SAP-1 domain have been identified in patients with atypical forms of metachromatic leukodystrophy [10–13].

Abbreviations: SAP, sphingolipid activator protei :; PCR, polymerase chain reaction; Glucosyl-ceramide-β-glucosidase, β-D-glucosyl-*N*-acylsphingosine glucohydrolase (EC 3.2.1.45)

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In this report we describe for the first time a mutation in the coding region of the SAP-2 domain (saposin C) in a patient with an atypical form of Gaucher's disease that was described by Christomanou et al. [5].

2. MATERIALS AND METHODS

2.1. *RNA isolation and characterization*

Total RNA was isolated from cultured skin fibroblasts by using the guanidine isothiocyanate/cesium chloride procedure [14]. Poly(A) RNA was selected using Type 7 oligo(dT) cellulose (Pharmacia).

Northern transfer of electrophoresed mRNA onto nitrocellulose membrane was done according to Ausubel et al. [15].

2.2. *Isolation of genomic DNA*

Genomic DNA was isolated from cultured fibroblasts according to a method of Ausubel et al. [15].

2.3. *PCR amplification of cDNA and genomic DNA*

First strand cDNA was synthesized from total RNA by specific oligonucleotide primers, using the kit purchased from Promega according to the manufacturer's instructions. The entire protein coding sequence including 33 bases of 5′-untranslated region and 77 bases of 3′-untranslated region (nucleotides −33 to 1661) was amplified by overlapping PCR [16], using a series of synthetic 18–21-mer oligonucleotide primers (Fig. 1). The genomic fragment containing the mutation [1017–1201 (numbering from the A of the ATG initiation codon)] was amplified by PCR based on the known exon/intron structure of the gene [17].

2.4. *Direct sequencing of the DNA*

The amplified DNA was purified by centrifuge-driven dialysis on a Centricon 30 microconcentrator (Amicon). Direct sequencing of the PCR-products was done by employing a Sequenase kit (United States Biochemical Co.) and the method of Wong et al. [18]. A total of 9 synthetic 18–21-mer oligonucleotide sequencing primers were used (Fig. 1). Sequencing of both strands was done for confirmation.

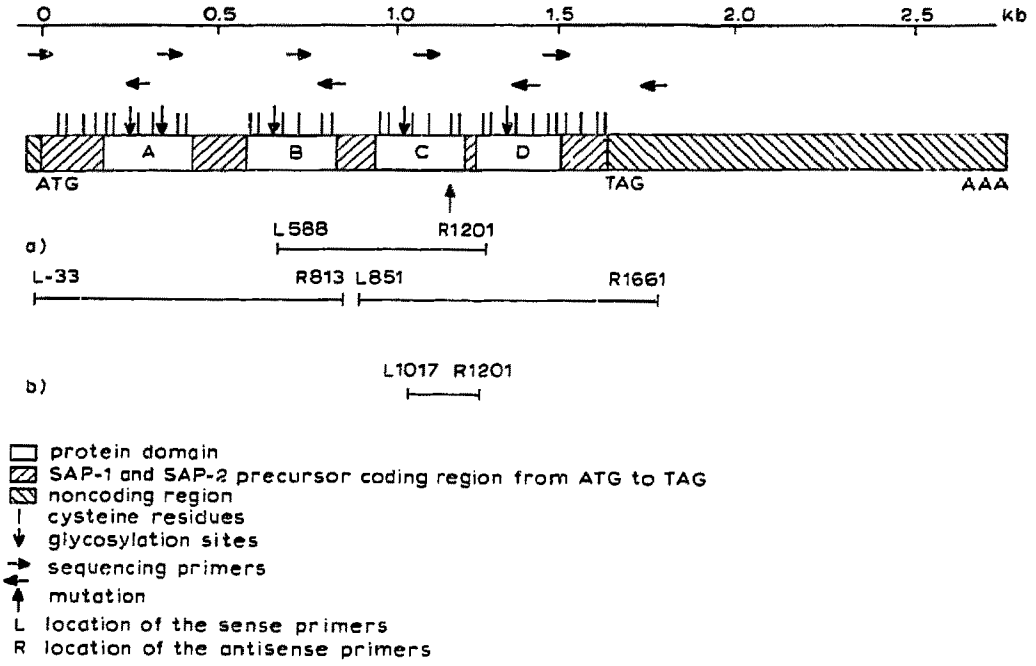

Fig. 1. Organization of the full length cDNA of the precursor protein for SAP-1 and SAP-2 and location of the PCR and sequencing primers. The four white regions represent the four protein domains (A = saposin A, B = SAP-1 (saposin B), C = SAP-2 (saposin C), D = component C (saposin D)). The primer combinations used for amplification are shown above the bars representing the PCR-products of cDNA (a) and genomic DNA (b).

3. RESULTS AND DISCUSSION

We report on a Caucasian female patient with an atypical form of Gaucher's disease who suffered from a severe glucosylceramide accumulation in the spleen without having a deficiency of glucosylceramide-β-glucosidase [19]. Immunological studies of Christomanou et al. [5] gave evidence for the lack of the glucosylceramide activator protein (Gaucher factor, SAP-2).

A preliminary Northern blotting experiment on the poly(A) RNA fraction showed presence of the SAP-2 precursor mRNA of apparently normal size in the patient's cultivated fibroblasts, indicating the feasibility of approaching the mutation from cDNA analysis (data not shown). The entire protein coding sequence of the

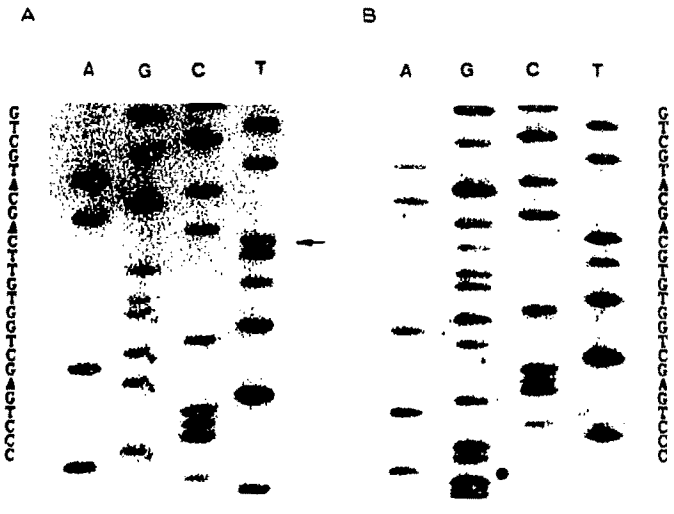

Fig. 2. Sequence of a portion of the SAP-2 cDNA coding strand. Direct sequence data of PCR products were obtained (A) from the patient's mRNA (the G → T mutation is indicated by an arrow) and (B) from a control mRNA.

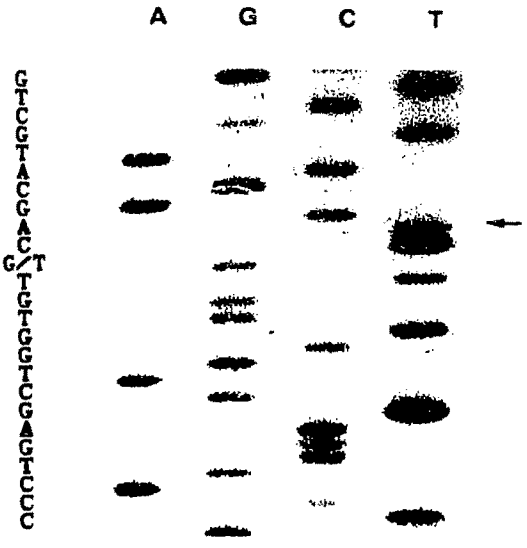

Fig. 3. Part of the genomic sequence of the patient's SAP-2 precursor. There are two bands (G and T) at the same level of the autoradiogram indicating that the index case is heterozygous for the mutation.

patient's cDNA was normal except for a single nucleotide change at position 1154 (numbering from A of the ATG initiation codon) from the normal G to T (Fig. 2). This transversion was observed in 6 batches of cDNA, each prepared by amplification of different batches of cellular RNA. The transversion results in a substitution of Phe for normal Cys in the mature SAP-2. Amplification and sequencing of the genomic DNA around the mutation, however, yielded the mutated sequence as well as the normal sequence (Fig. 3). These findings suggest that the patient is a compound heterozygote with the identified mutation in one allele and another as yet unidentified mutation in the other allele, which might be of an mRNA negative type.

Each of the four homologous proteins generated from the SAP-2 precursor contains six cysteine residues at strictly conserved positions [7–9]. The exchange of the fifth Cys residue in the mutant SAP-2 by Phe may result in a failure to form normal disulphide bonds. The resultant disruption of the normal three-dimensional structure is likely to make the mutant SAP-2 (Gaucher factor) unstable, consistent with the lack of cross-reactive material in the patient's tissues [5].

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