Hum Genet (2005) 117: 275–277
DOI 10.1007/s00439-005-1288-x

SHORT REPORT

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A mutation within the saposin D domain in a Gaucher disease patient with normal glucocerebrosidase activity

Received: 23 December 2004 / Accepted: 25 January 2005 / Published online: 23 April 2005

Abstract Only two Gaucher disease (GD) patients bearing mutations in the prosaposin gene (*PSAP*), and not in the glucocerebrosidase gene (*GBA*), have been reported. In both cases, one mutant allele remained unidentified. We report here the identification of the second mutation in one of these patients, being the first complete genotype described so far in a SAP-C-deficient GD patient. This mutation, p.Q430X, is the first one reported in the saposin D domain and probably produces a null allele by nonsense mediated mRNA decay.

Gaucher disease (GD, MIM 230800, 230900, 231000) occurs mainly as a result of mutations in the glucocerebrosidase gene (*GBA*). Only very rarely it is due to a deficiency in the GBA activator protein SAP-C. The prosaposin gene (*PSAP*) is translated as a polypeptide of 524 amino acids, which is processed into four saposins, SAP-A, B, C, and D (for a review see Qi and Grabowski 2001)

In the two SAP-C-deficient GD patients reported to date (Christomanou et al. 1986, 1989), only one mutant allele was identified. The first patient bore mutation p.C382F (Schnabel et al. 1991) and the second presented a different change in the same codon, p.C382G (Rafi et al. 1999), within the SAP-C domain. In the present work we describe the identification of the second mutant allele in the last patient. The clinical and pathological data of this patient, who meets the clinical criteria of GD but has normal acid β-glucosidase activity, were previously described (Christomanou et al. 1989; Pampols et al. 1999).

The patient inherited mutation p.C382G from his father. The maternal mutation was expected to be also in the SAP-C domain to be consistent with a GD phenotype. However, after analysing DNA from the patient's mother (since no sample from the patient was available) no change was identified upon sequencing exons 10 and 11, encoding the SAP-C domain, and the promoter region of the *PSAP* gene (GenBank AF057307). Thus, a complete sequencing of the whole coding region was undertaken. Fifteen PCR fragments, covering all the exons and the intron/exon boundaries were amplified and sequenced. Primers for a three-step PCR reaction (annealing temperatures 59–60°C) are described in Table 1.

This approach allowed the identification of the second mutation, p.Q430X, in the patient (Fig.1a, b), which was confirmed by mismatch PCR (see primers in Table 1) and *Rsa*I restriction analysis (Fig.1c), and was not found in 100 control chromosomes. It corresponded to a C to T transition at position 1288 of the cDNA (GenBank M32221, with nucleotide 7 of that sequence, the A of the ATG initiation codon, corresponding to +1), giving rise to a premature stop codon in exon 12. This exon corresponds to the SAP-D domain, in which no mutation has previously been reported. A mutation in this domain is not expected to cause GD. However, the presence of that premature stop codon could trigger degradation of the whole mRNA by nonsense-mediated decay (NMD), since it fulfils the requirements for this process (Nagy and Maquat 1998). Although no sample was available for an experimental confirmation, the NMD hypothesis is consistent with the previous observation that *PSAP* RNA levels in the mother's fibroblasts were reduced by 50%, as shown by Northern blot analysis (Rafi et al. 1999).

In the search for mutations, two new polymorphic variants were identified. The first was the change −130T/

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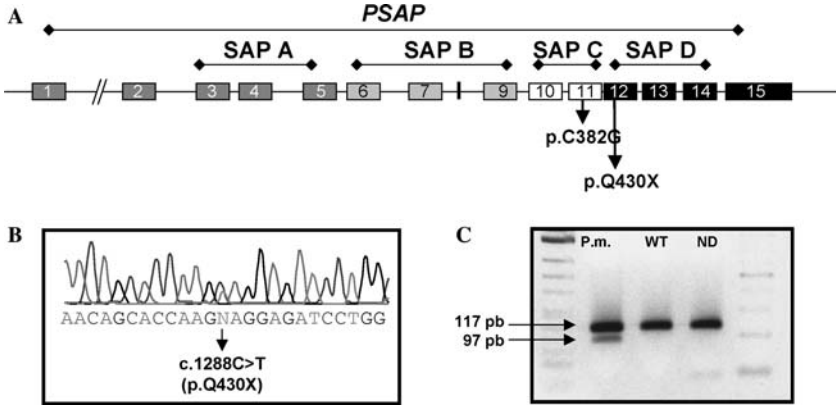
Table 1 Primers used in the analysis of the *PSAP* gene

	Primer	Sequence (5′ → 3′)
Promoter region 1	PSAP p1 F	TTTAAGCAATTTCTGGCC
	PSAP p1 R	ACATAGAATAAGTAGTCGTG
Promoter region 1	PSAP p2 F	ACTGAGCCCTGTACCCTGTT
	PSAP p2 R	ATAGTGGGAGGAGTGAGGAA
Exon 1	PSAP exo1 F	GGGCTTTTCTTTTATGACCTT
	PSAP exo1 R	GACGCTGCGAGGGTCAAATCCT
Exon 2	PSAP exo2 F	CTGGGGAAATAAGTCAGGTCG
	PSAP exo2 R	CTGAGCCTCCATCTCCTCTG
Exon 3	PSAP exo3 F	AGTCACACCTCTTCCCTC
	PSAP exo3 R	TATACGGCTCATATACCCTAA
Exon 4	PSAP exo4 F	GCTGTTTTCCAGGCTTGGTT
	PSAP exo4 R	TTACATTCCTTCAGCAGTCCG
Exon 5	PSAP exo5 F	AGGGACTAATTCAGAGGCACT
	PSAP exo5 R	GCCCCAGTTTAAGAACCAC
Exon 6	PSAP exo6 F	ATTTGAGAGCCTGTAAAGCAT
	PSAP exo6 R	CCTACTCCAGCCTCCACA
Exon 7	PSAP exo7 F	GGCCCAGAGCAGACATT
	PSAP exo7 R	GCCCAATTCAGCACTCTAAG
Exon 8	PSAP exo8 F	AGAGCATTTCCCCTGAACCT
	PSAP exo8 R	AGCCCTCCCCAGCCTAT
Exon 9	PSAP exo9 F	GAGAGGGAGGTAGCCTTGAC
	PSAP exo9 R	GGGAACCGAAAGAAACAAGT
Exon 10	PSAP exo10 F	CAAGACCAAGGGCTGTGAGT
	PSAP exo10 R	CACTGGGACATTCAGGCTCG
Exon 11	PSAP exo11 F	TCCGGCCTCCCGTCTTCTCA
	PSAP exo11 R	ACCATCCTCTCCCGCACCAC
Exon 12	PSAP exo12 F	CATGCTGCACCTCTGCTCT
	PSAP exo12 R	CAAAATGTACCCAGCCTTG
Exon 13	PSAP exo13 F	GGAGCTCTCAGGGAACAGTG
	PSAP exo13 R	GCCAAGTCTCAGAGCAACCT
Exon 14	PSAP exo14 F	TGTTCTGAAAGAGCGTGGTG
	PSAP exo14 R	CTGGGTTCCCATTAAAGCAG
Exon 15	PSAP exo15 F	CTGGGTCTTCAGCATCTGGT
	PSAP exo15 R	GTGGGGGAGCCCTATTTTTA
Mismatch p.Q430X	M-exon12 F	GTTCACGTGACTCAGCCAA
	M-exon12 R	AAGAGCAGCCAGGATCTCGT

Fig. 1 a Schematic representation of the *PSAP* gene, which encodes four different saposins. Mutations present in the patient, p.C382G and p.Q430X, and the gene regions coding for the whole prosaposin (*PSAP*) protein and for the four saposins are indicated. **b** Sequence analysis of exon 12 PCR product from the mother of the patient, showing the presence of a C to T transition at cDNA position 1288, which leads to a change from a glutamine (amino acid 430) to a stop codon. **c** Detection of the c.1288C > T mutation by *Rsa*I restriction analysis of the exon 12 PCR product. *P.m.* mother of the patient, *WT* wild-type control, *ND* non-digested PCR product

C (nt 682, GenBank AF057307) within the promoter region. The allele frequencies in 42 chromosomes from unrelated Spanish individuals were T: 90.5%, C: 9.5%. The second polymorphism was identified at position +5 in intron 12 (c.1350 + 5G > A). The frequencies in 48 chromosomes were 15 and 85% for the A and G alleles, respectively. The normal splicing was not affected by this change (data not shown).



Acknowledgements We thank R. Rycroft for revising the English. This research was supported by CICYT (SAF 2000–0200 and SAF 2003–00386) and FIS (Redes Temáticas, G03/054 REDEMETH).

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