Hum Genet (1997) 100 : 75–79

ORIGINAL INVESTIGATION

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Genetic fine localization of the β-glucocerebrosidase (*GBA*) and prosaposin (*PSAP*) genes: implications for Gaucher disease

Received: 22 October 1996 / Accepted: 4 February 1997

Abstract Mutations in the glucocerebrosidase (*GBA*) and prosaposin (*PSAP*) genes are responsible for Gaucher disease, the most prevalent sphingolipidosis. Somatic cell hybrid analysis and in situ hybridization experiments have localized the *GBA* gene to 1q21 and the *PSAP* gene to 10q21-q22. We performed pairwise and multi-point linkage analyses between the two genes and several highly polymorphic markers from the Généthon human linkage map. Our results show that six markers cosegregate with the *GBA* gene (Z_{max} = 8.73 at θ = 0.00 for marker D1S2714) and define a 3.2-cM interval between D1S305 and D1S2624 as the most probable location for the gene. Three of these markers (D1S2777, D1S303, and D1S2140), as well as the gene encoding pyruvate kinase (*PKLR*), are contained in a single YAC clone together with the *GBA* gene. A new polymorphism was identified within the *PSAP* gene (C16045T) and used for linkage studies. The multi-point analysis places the gene in a 9.8-cM interval between D10S1688 and D10S607. The fine localization of these genes provides a useful tool for cosegregation analysis, indirect molecular diagnosis, and population genetic studies.

Introduction

Gaucher disease (GD) is the most prevalent sphingolipidosis (McKusick 230800). It is inherited in an autosomal recessive manner and it is caused by a deficiency in the lysosomal hydrolase β-glucocerebrosidase or, very rarely, by a deficiency in its activator protein, saposin C.

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The glucocerebrosidase gene (*GBA*) is 7 kb long and contains 11 exons. It was assigned to chromosome 1 (1p11-qter) using mouse-human somatic cell hybrids (Shafit-Zagardo et al. 1981). The region was further narrowed to 1q21-q31 by Chinese hamster-human cell hybrid analysis (Barneveld et al. 1983; Ginns et al. 1985) and to 1q21 by in situ hybridization (Ginns et al. 1985). These data are in contradiction with previous studies which placed the *GBA* locus to 1q42-qter (Devine 1982; Devine et al. 1982).

Twelve polymorphisms have been identified in the *GBA* gene (Beutler et al. 1992). They are all in linkage disequilibrium, producing only two major haplotypes.

The liver-type pyruvate kinase gene (*PKLR*), involved in non-spherocytic hemolytic anemia, also resides on 1q21 (Satoh et al. 1988). A polymorphic site on this gene (Kanno et al. 1992) is in linkage disequilibrium with the *GBA* polymorphisms (Glenn et al. 1994). The maximum probable genetic distance between these two loci was calculated as 0.2 cM based on linkage disequilibrium data between this *PKLR* polymorphism and the three most common GD mutations at the *GBA* gene among Ashkenazi Jews (Glenn et al. 1994).

Saposin C is encoded by the prosaposin (*PSAP*) gene. It was mapped to chromosome 10 by somatic cell hybrid analysis (Inui et al. 1985) and to 10q21-q22 by in situ hybridization (Kao et al. 1987).

In this paper we report the fine mapping of the genes coding for β-glucocerebrosidase and prosaposin with respect to markers from the Généthon human linkage map (Dib et al. 1996). This localization may allow the use of highly informative markers closely linked to the *GBA* and *PSAP* genes for indirect molecular diagnosis and for population genetic studies.

Materials and methods

Families

Families 102, 884, 1331, 1332, 1347, 1362, 1413, and 1416 from the CEPH panel were used in this study.

Analyses of DNA polymorphisms

The GBA gene

The A6144G polymorphism in intron 9 of the *GBA* gene was detected by *Hha*I digestion of a PCR-amplified product. The following primers were used to amplify a 787-bp DNA fragment: sense primer, 5′-CCAATTGGGTGCGTAACTTT-3′ and antisense primer, 5′-TCACTGGCGACGCCACAGGTAGGTGTGAATGGAGTA-3′. Fifteen microliters of PCR amplification mixture [containing 20 ng of genomic DNA, 10 pmol of each primer, 0.2 mM dNTPs, and 1 U Dynazyme polymerase (Finnzymes) in the recommended buffer] was subjected to 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 2 min. The PCR products were digested with *Hha*I according to the manufacturer's recommendations and eletrophoresed in a 1.5% agarose gel. Genomic numbering is according to Horowitz et al. (1989).

The PSAP gene

The C16045T polymorphism was detected by PCR amplification and SSCP analysis of a DNA fragment spanning nucleotides 15 873–16 090, according to the genomic sequence in Rorman et al. (1992). The PCR was performed using the following primers: 5′-CAAGACCAAGGGCTGTGAGT-3′ (sense) and 5′-CACTGGG-ACATTCAGGCTCG-3′ (antisense). The composition of the reaction mixture was identical to the one described above, but the PCR program consisted of 35 cycles of denaturation at 94°C for 40 s with a single annealing/extension step at 55°C for 30 s. The resulting 218-bp DNA fragments were subjected to SSCP analysis: 4 μl of the PCR product was mixed with 6 μl of 95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue, 20 mM EDTA solution. The samples were then denatured by incubation at 80°C for 3 min and placed on ice. Electrophoresis was carried out using a 18 × 24 cm non-denaturing acrylamide:bisacrylamide (29:1) gel for 16 h at 250 V at room temperature. The DNA bands were developed by silver staining as previously described (Cormand et al. 1997). The polymorphism was characterized by direct PCR sequencing using the fluorescent dideoxy cycle method (ABI 373A; Perkin Elmer Cetus).

Linkage analysis

Two-point and multi-point linkage analyses were performed using the MLINK and CMAP programs from the LINKAGE package, version 5.1 (Lathrop et al. 1984).

Pulsed field gel electrophoresis (PFGE)

Chromosomes from yeast cells containing different YACs were embedded in agarose plugs (Anand et al. 1989) and electrophoresed in a 1.2% agarose gel in a PFGE apparatus (Pharmacia Biotech) using switch times of 35 s at 200 V for 18 h in 0.5 × TBE at 13°C. The DNA was then transferred to a nylon membrane (Amersham).

Probe preparation and hybridization conditions

Total RNA was prepared from human cultured fibroblasts by the Ultraspec RNA isolation system (Biotecx). Reverse transcription was done using the Time Saver cDNA synthesis kit (Pharmacia Biotech) with an antisense primer at the 3′ end of the *GBA* mRNA (5′-CTCTTTAGTCACAGACAGCG-3′). The full coding region of the cDNA was PCR-amplified in a single 1836-bp fragment using the reverse transcription primer and a sense primer at the 5′ end of the cDNA (5′-GCCGGAATTACTTGCAGGGC-3′). The PCR reaction was performed with the Expand Long Template PCR system (Boehringer Mannheim). The PCR product was subcloned into pUC18 and sequenced.

The *GBA* and *Alu* probes were labelled with α-[^{32}P]dATP using the random priming procedure. Southern blot hybridization was carried out at high stringency following a standard protocol. The membrane was exposed to Hyperfilm-MP (Amersham) for 3 days.

Yeast DNA isolation and PCR amplification

Yeast DNA was prepared according to Kaiser et al. (1994). Anonymous markers belong to the MapPairs set (Research Genetics) and were amplified by PCR from YAC DNA following the manufacturer's recommendations. The presence of the *PKLR* gene was tested according to Lenzner et al. (1994) and that of the *GBA* gene as described above.

Results

Genetic mapping of the *GBA* gene

The diallelic *Hha*I polymorphism, one of the 12 known variants of the *GBA* gene, was used to localize the gene with respect to markers from 1q21 included in the last version of the Généthon human linkage map (Dib et al. 1996). Three of the eight CEPH families used in this study (1362, 1413, and 1416) were informative for the *GBA* polymorphism with a total of 50 informative meioses. Several recombination events localized the *GBA* gene to a 3.2-cM interval between markers D1S305 and D1S2624. Two-point linkage analysis showed that six markers within this region cosegregate with the *GBA* gene (Z_{max} = 8.73 at θ = 0.00 for marker D1S2714) (Table 1). Four of these markers (D1S2777, D1S2721, D1S2714, and D1S303) were mapped to a single point in the Généthon map. We mapped the other two markers, D1S1153 and D1S2140, with respect to the fixed Généthon map, with maximum multi-point lod scores of 21.4 and 38.5, respectively, at θ = 0.0 from D1S2721. Multi-point analysis was performed between *GBA* and several markers at 1q21, confirming the D1S305-D1S2624 interval as the most probable location for *GBA* (Z_{max} = 11.45) (Fig. 1a).

Physical mapping of the *GBA* gene

Several YACs from the CEPH library (625c8, 709c5, 712g7, 742b9, 742f10, 778h2, 791a7, 808b7, 876b11, and 887h8), reported to contain at least one of the markers D1S2777, D1S2721, D1S2714 or D1S303 (Chumakov et al. 1995), were analyzed by PFGE and Southern blotting using an *Alu* sequence and the *GBA* cDNA as probes. As shown in Fig. 2, *Alu* hybridization allowed detection of all the YACs, while only YAC 887h8 hybridized with the *GBA* probe. Although this YAC has been sized as 340 kb according to CEPH data, our results indicate a greater length (over 450 kb). YAC 887h8 appears as two bands, possibly due to instability, both of which hybridize with the *GBA* probe. PCR amplification from YAC DNA demonstrated that this clone also contained the *PKLR* gene and confirmed the presence of the *GBA* gene and the anonymous markers D1S303, D1S2777, and D1S2140.

Table 1 Genetic linkage of the glucocerebrosidase (*GBA*) and prosaposin (*PSAP*) genes with markers at 1q21 and 10q21-q22, respectively

	θ (θf = θm)							Z_{max}	θ_{max}
	0.00	0.01	0.05	0.1	0.2	0.3	0.4		
GBA									
D1S305	– ∞	8.96	8.91	8.24	6.47	4.40	2.08	9.08	0.023
D1S2777	6.32	6.23	5.83	5.31	4.19	2.91	1.44	6.32	0
D1S2721	8.43	8.30	7.78	7.10	5.62	3.94	2.00	8.43	0
D1S2714	8.73	8.58	7.96	7.15	5.44	3.60	1.66	8.73	0
D1S303	8.13	8.00	7.48	6.80	5.32	3.64	1.72	8.13	0
D1S1153	5.12	5.02	4.63	4.13	3.09	2.00	0.89	5.12	0
D1S2140	8.73	8.57	7.93	7.10	5.35	3.45	1.42	8.73	0
D1S2624	– ∞	8.36	8.35	7.73	6.07	4.11	1.92	8.49	0.025
PSAP									
D10S1688	– ∞	0.41	1.39	1.47	1.03	0.45	0.08	1.48	0.08
D10S1650	4.82	4.70	4.25	3.67	2.51	1.38	0.39	4.82	0
D10S1694	4.52	4.41	3.99	3.46	2.37	1.31	0.38	4.52	0
D10S556	5.12	5.00	4.51	3.88	2.64	1.44	0.40	5.12	0
D10S569	5.12	5.00	4.51	3.88	2.64	1.44	0.40	5.12	0
D10S605	5.12	5.00	4.51	3.88	2.64	1.44	0.40	5.12	0
D10S607	– ∞	2.41	2.67	2.42	1.63	0.79	0.16	2.68	0.04

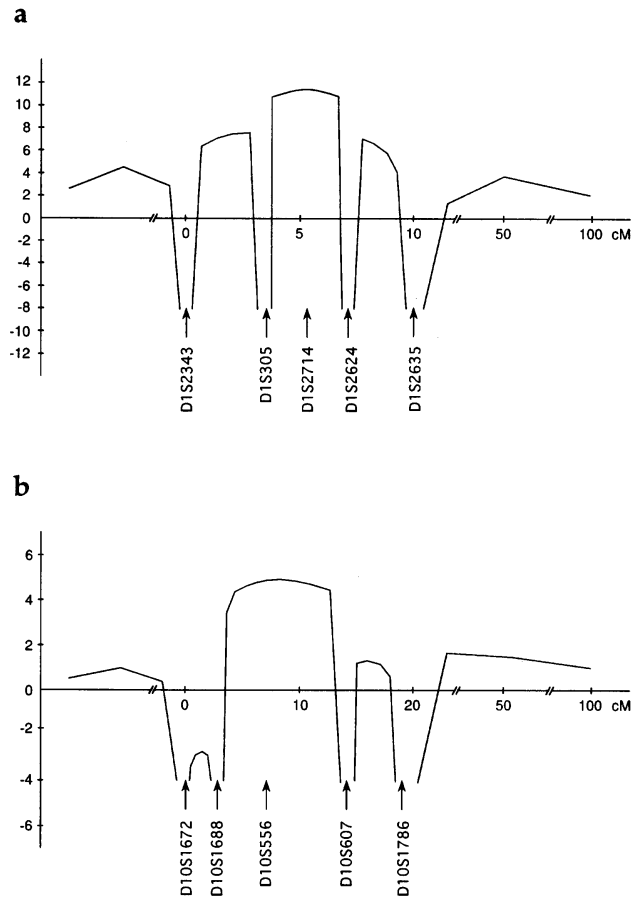

Fig. 1 Multi-point linkage analysis of the glucocerebrosidase (*GBA*) gene (**a**) and the prosaposin (*PSAP*) gene (**b**) with microsatellite markers from 1q21 and 10q21-q22, respectively. Likelihood estimates are given in $\log_{10}$ on the *y*-axis. Genetic distances (cM) are shown on the *x*-axis. Zero is defined at locus D1S2343 for *GBA* and at locus D10S1672 for *PSAP*

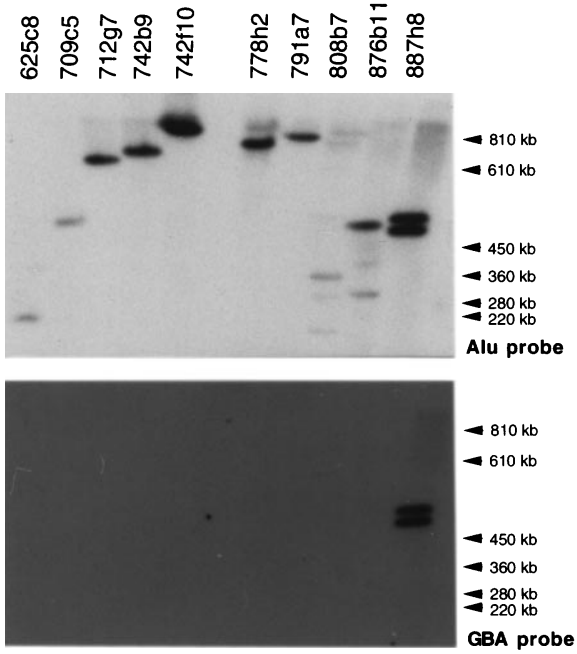

Fig. 2 Pulsed-field gel electrophoresis and Southern blot analysis of several YACs from the 1q21 region containing at least one of the markers D1S2777, D1S2721, D1S2714 or D1S303. The same filter was hybridized with an *Alu* probe and with the glucocerebrosidase cDNA. All YAC clones are recognized by the *Alu* probe, but only YAC 887h8 shows hybridization with the *GBA* cDNA

Characterization of a polymorphism in the *PSAP* gene

In an attempt to identify a polymorphism within the *PSAP* gene, several genomic fragments were amplified using DNA samples from unrelated individuals, and subjected to SSCP analysis. Different SSCP patterns were observed

when analyzing the genomic region spanning nucleotides 15 873–16 090. Sequence analysis revealed a C to T transition at intronic nucleotide 16 045 (data not shown). The 16045T allele was present in only 9 out of 110 chromosomes analyzed (8.2%). The corresponding heterozygosity and PIC values were 0.150 and 0.139, respectively.

Genetic mapping of the *PSAP* gene

The C16045T intronic polymorphism within the *PSAP* gene described above was used to localize the gene with respect to markers at 10q21-q22 from the Généthon human linkage map. Only one of the eight CEPH families used in this study was informative for this polymorphism. Both parents of family 102 were heterozygous, allowing the analysis of 28 informative meioses. Table 1 shows the results of the two-point linkage analysis between the gene and markers from 10q. A maximum two-point lod score of 5.12 at $\theta = 0.00$ was obtained with markers D10S556, D10S569, and D10S605. Two recombination events define a 9.8-cM interval between D10S1688 and D10S607. This was confirmed by multi-point analysis, as shown in Fig. 1b.

Discussion

The fine localization of genes is part of a general effort to build a complete human genome map, but it also has important practical implications. The identification of highly informative polymorphic markers located inside or very close to disease genes provides useful tools for cosegregation analysis, indirect molecular diagnosis, and population genetic studies.

The gene encoding β-glucocerebrosidase, responsible for most cases of GD, was previously assigned to chromosome 1q21 by in situ hybridization (Ginns et al. 1985). We used one of the intragenic polymorphisms to localize the *GBA* gene in relation to markers from the Généthon human linkage map (Dib et al. 1996). No recombination was found between six markers (D1S2777, D1S2721, D1S2714, D1S303, D1S1153, and D1S2140) and the *GBA* gene in 50 informative meioses analyzed. In addition, three of them, D1S2777, D1S303, and D1S2140, are present in a YAC clone which was shown to contain the *GBA* gene. Interestingly, the *PKLR* gene is also present in this YAC.

These data may be particularly useful for reliable indirect diagnosis of GD. GD is highly prevalent among Ashkenazi Jews, a population in which direct mutational diagnosis is feasible because only four mutations account for 96% of the mutated alleles (Beutler 1992). However, in most non-Jewish populations studied so far, about a third of the mutant alleles correspond to a variety of rare mutations. In Spain, for example, two mutations, N370S and L444P, account for 70% of the GD alleles (Cormand et al. 1995) but the remaining 30% includes more than 20 different mutations (B. Cormand et al., manuscript in preparation). This implies that about 50% of the patients bear at least one uncommon mutation. In these cases, cosegregation analysis in the families is indicated for prenatal molecular diagnosis. The low information content of the 12 *GBA* polymorphisms due to the presence of intragenic linkage disequilibrium (Beutler et al. 1992) makes the use of the markers described here a better choice for this kind of analysis. From these data, we propose the use of the dinucleotide repeats D1S2777 and D1S303, the tetranucleotide repeat D1S2140, and a trinucleotide repeat within the *PKLR* gene (Lenzner et al. 1994) as the best markers for indirect molecular diagnosis of the disease. The use of the flanking markers D1S305 (centromeric) and D1S2624 (telomeric) would eliminate the risk of diagnosis errors due to an improbable recombination event.

The fine mapping of the *GBA* gene may also be very useful for population genetic studies. Thus, questions such as the putative unique origin of common GD-causing mutations (e.g., N370S) could be addressed using extended haplotype analysis.

The *PSAP* gene, encoding prosaposin, the precursor for the four sphingolipid activator proteins SAP-A, SAP-B, SAP-C, and SAP-D, has been mapped to 10q21-q22 by in situ hybridization (Kao et al. 1987). We have identified the first polymorphism within the *PSAP* gene (C16045T), which allowed its genetic mapping. Linkage analysis located the gene to a 9.8-cM interval between markers D10S1688 and D10S607. This localization may be helpful for the study of the involvement of the *PSAP* gene not only in GD but also in other lysosomal disorders such as metachromatic leukodystrophy.

Acknowledgements The authors thank Mònica Bayés for technical assistance. The *Alu* probe was kindly provided by Rebeca Valero and Gemma Marfany and the YACs by the Centre d'Etude du Polymorphisme Humain (CEPH). We are also grateful to Robin Rycroft for revising the manuscript. Bru Cormand is a recipient of a fellowship from the CIRIT (Generalitat de Catalunya). This work was partially supported by CICYT (SAF93-0479-C02-01).

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