Hum Genet (1992) 90:402–406

human genetics

The intron 7 donor splice site transition: a second Tay-Sachs disease mutation in French Canada

Peter Hechtman[1,2], **Bernard Boulay**[1], **Marc De Braekeleer**[3], **Eve Andermann**[4], **Serge Melançon**[5], **Jean Larochelle**[6], **Claude Prevost**[6], **Feige Kaplan**[1]

[1]Montreal Children's Hospital Research Institute, McGill University, 2300 Tupper Street Montreal, Quebec H3H 1P3, Canada
[2]Biology Department Centre for Human Genetics, McGill University, Montreal, Canada
[3]Department de Science Humaine, Université de Quebec, Chicoutimi, Quebec, Canada
[4]Department of Neurogenetics, Montreal Neurological Hospital, Montreal, Quebec, Canada
[5]Department de Genetique Humaine, Hôpital St-Justine, Montreal, Quebec, Canada
[6]Hôpital de Chicoutimi, Chicoutimi, Quebec, Canada

Received: 13 January 1992 / Revised: 12 May 1992

Abstract. Mutations at the hexosaminidase A (HEXA) gene which cause Tay-Sachs disease (TSD) have elevated frequency in the Ashkenazi Jewish and French-Canadian populations. We report a novel TSD allele in the French-Canadian population associated with the infantile form of the disease. The mutation, a G→A transition at the +1 position of intron 7, abolishes the donor splice site. Cultured human fibroblasts from a compound heterozygote for this transition (and for a deletion mutation) produce no detectable HEXA mRNA. The intron 7 +1 mutation occurs in the base adjacent to the site of the adult-onset TSD mutation (G805A). In both mutations a restriction site for the endonuclease *Eco*RII is abolished. Unambiguous diagnosis, therefore, requires allele-specific oligonucleotide hybridization to distinguish between these two mutant alleles. The intron 7 +1 mutation has been detected in three unrelated families. Obligate heterozygotes for the intron 7 +1 mutation were born in the Saguenay-Lac-St-Jean region of Quebec. The most recent ancestors common to obligate carriers of this mutation were from the Charlevoix region of the province of Quebec. This mutation thus has a different geographic centre of diffusion and is probably less common than the exon 1 deletion TSD mutation in French Canadians. Neither mutation has been detected in France, the ancestral homeland of French Canada.

Introduction

Tay-Sachs disease (TSD) is an autosomal recessive neurodegenerative disorder characterized by massive storage of GM_2 ganglioside in the neurons of the cerebral cortex. Mutations causing TSD are alleles at the HEXA gene locus, which encodes the α-subunit of the enzyme hexosaminidase A (Hex A; E.C.3.2.1.52). Mutant HEXA alleles are relatively frequent in two population groups: Ashkenazi Jews and French Canadians. Three mutations, a 4-bp (base pair) insertion in exon 11 (Myerowitz and Costigan 1988), a transition at the intron 12 splice junction (Ohno and Suzuki 1988; Arpaia et al. 1988; Myerowitz 1988), and the G805A transition associated with adult or chronic GM_2 gangliosidosis (Navon et al. 1990; Paw et al. 1989) account for over 90% of TSD alleles (Grebner and Tomczak 1991; Paw et al. 1990; Triggs-Raine et al. 1990) in the Ashkenazi Jewish population.

A 7.6-kb deletion removing exon 1 and flanking sequence (Myerowitz and Hogikyan 1986, 1987) occurs uniquely among French Canadians and is the most frequent TSD mutation in this population (Hechtman et al. 1990). The center of diffusion for the French-Canadian deletion (FCD) mutation is the Gaspé-Bas St-Laurent region of Quebec, which is on the south shore of the St. Lawrence River (Fig. 1). The carrier frequency for this gene in the two largest cities of this region, Rimouski, Quebec and Edmunston, New Brunswick has been estimated to be 5%–7% (Andermann et al. 1977). Candidate founders for this mutation have been identified among the first generation of French settlers born in Canada (De Braekeleer et al. 1992). We report the characterization of a second novel TSD mutation, which is unique to French Canada. The new mutation is less frequent than the FCD and has a different center of diffusion.

Materials and methods

Families

Families D and R have been previously reported. Family D corresponds to families 8 and 9 in (Hechtman et al. 1990) and to family

Correspondence to: P. Hechtman, Montreal Children's Hospital Research Institute, McGill University, 2300 Tupper Street, Montreal, Quebec H3H 1P3, Canada

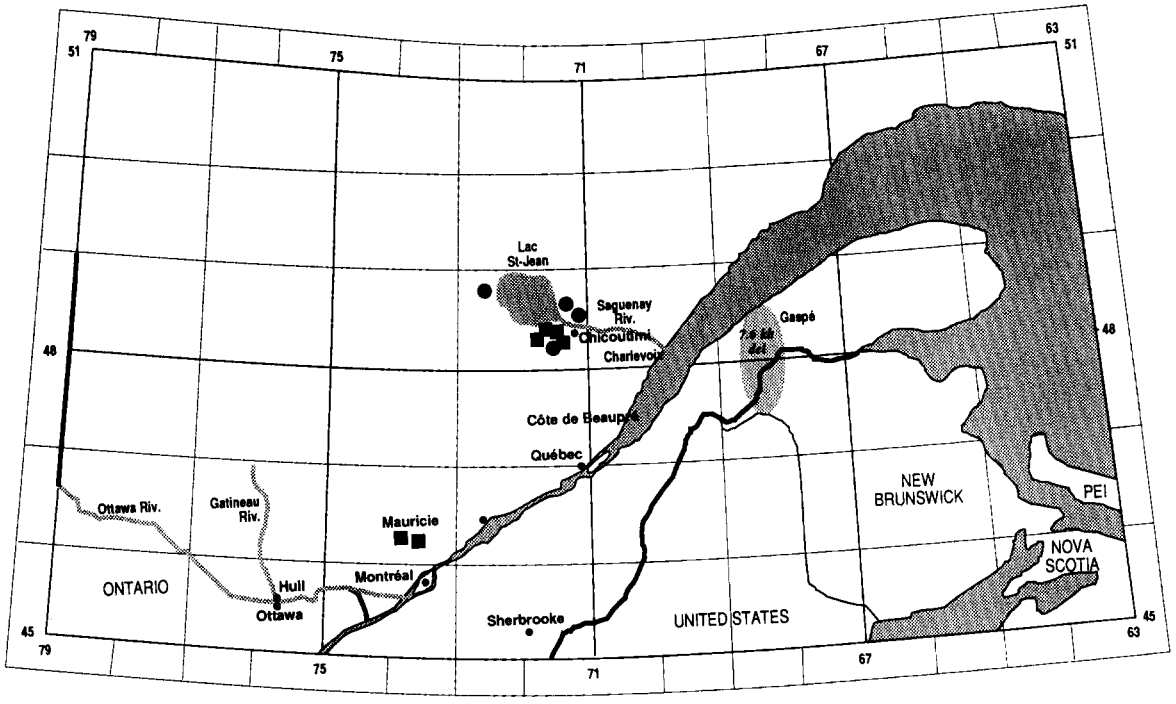

Fig. 1. Distribution of Tay-Sachs disease mutations in the Province of Quebec. *Shaded region* indicates concentration of carriers of the 7.6-kb HEXA deletion mutation. *Circles* heterozygous carriers of intron 7 +1 mutation; *squares*, compound heterozygous probands. Location of symbols indicates birthplaces of individuals

FCG 4 in (Andermann et al. 1977). Family R corresponds to family 12 in (Hechtman et al. 1990). The affected children in these families were previously determined to be heterozygous for the FCD and an unknown allele. Family L (Fig. 2) was referred from Hôpital de Chicoutimi (Chicoutimi, Quebec) based on clinical diagnosis of Tay-Sachs disease, which was confirmed by the finding of less than 5% Hex A in the proband's serum.

DNA amplification

Primer pairs and conditions used to amplify exons of the HEXA gene were as described (Triggs-Raine et al. 1991). For amplifications of DNA for single-strand conformation polymorphism (SSCP) analysis, the concentration of dATP in reaction tubes was reduced to 0.1 m*M* and 10 µCi of α^{32}P dATP (NEN, Boston, Mass., 3000 Ci/mmol) was added as radioactive tracer.

SSCP analysis

SSCP analysis was performed essentially according to Orita et al. (1988) as modified by Triggs-Raine et al. (1991). Electrophoresis was carried out at room temperature for 16h at 15 mA.

Subcloning and sequencing of polymerase chain reaction (PCR) products

Subclones of mutant PCR products were generated by blunt-end ligation of phosphorylated PCR product into dephosphorylated *Eco*RV-digested pBS(−) (Stratagene, San Diego, Calif.). Clones were propagated in *Escherichia coli* DH5αF'IQ. Cloning procedures were according to Maniatis et al. (1982). Nine subclones

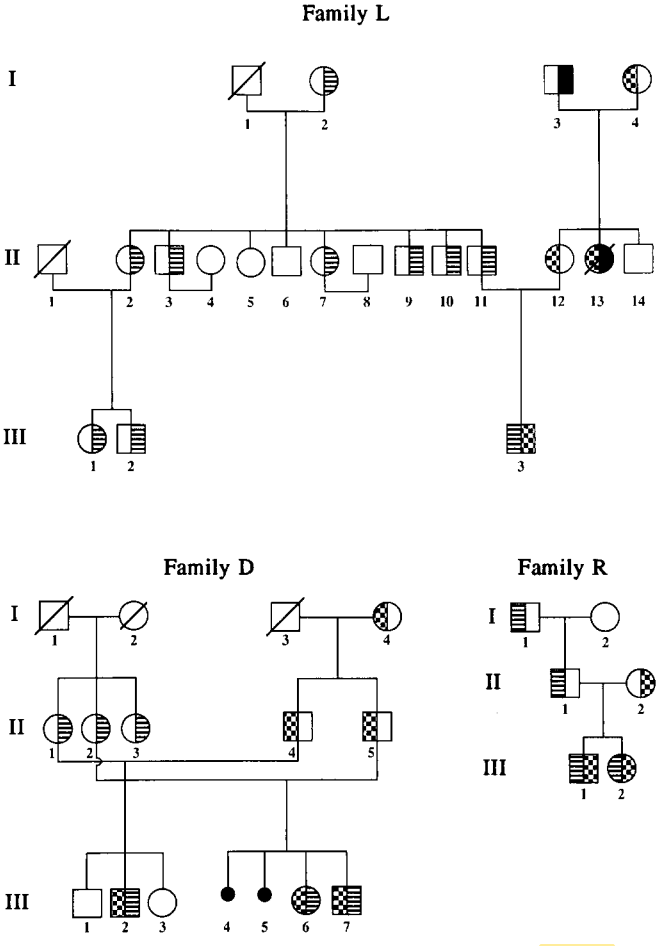

Fig. 2. Pedigree of families with intron 7 +1 mutation. *Striped* carrier of intron 7 +1 mutation; *checked* carrier of 7.6-kb deletion mutation; *solid* carrier of 4-bp insertion mutation. (Family D after Andermann et al. 1977)

were sequenced (four mutant, five normal) for the intron 7 mutation. Sequencing was carried out on individual clones or directly on PCR products using α^{35}S dATP (subclones) or ^{32}P end-labeled oligonucleotides (direct) with a Sequenase kit.

Direct detection of IVS7 mutation

The intron 7 +1, G→A mutation, as well as the adult-onset TSD mutation (G805A) were detected in an amplified fragment containing exon 7 and flanking sequences by the loss of a restriction site for the enzyme *Eco*RII. A 10-µl aliquot of PCR product was digested with 5 units *Eco*RII and subjected to electrophoresis on 8% polyacrylamide at 100 V for 5 h. For unambiguous detection of the intron 7 mutation, allele-specific oligonucleotide hybridization was carried out using ^{32}P end-labeled normal (TGGGGACCAGGTAAGAATG) and mutant (TGGGGACCAGATAAGAATG) probes.

Detection of other TSD alleles

The detection of the French-Canadian deletion mutation was performed according to Kaplan et al. (1991) and the exon 11 4-bp insertion mutation was detected according to Triggs-Raine and Gravel (1990).

mRNA expression

The effect of the mutation on mRNA expression was assessed by reverse transcriptase-PCR amplification (Ferre and Garduno 1989) of a (total) RNA-containing fraction prepared from cultured skin fibroblasts of individual R-III-2 as well as from control fibroblasts. HEXA mRNA expression was monitored using total RNA as a template for RT-PCR amplification with primers (sense) TACAACCCTGTCACCCACAT and (antisense) CTGGATGTAGAAGGACTCCA. These primers amplify a 393-bp fragment from normal mRNA starting at nucleotide (nt) 679. The two RNA preparations were normalized for mRNA content by amplification of prolidase (PEPD locus) sequences. Amplification conditions were as in Trop et al. (1992).

Genealogical reconstruction

Procedures used for reconstruction of genealogies of obligate heterozygotes, source materials for genealogical data bases, and software used for analysis of pedigrees were essentially the same as reported previously (De Braekeleer et al. 1992).

Frequency of the intron 7 +1 allele

Genotypes were ascertained using DNA samples, prepared from peripheral blood leukocytes, which were obtained from 92 individuals living in the Saguenay-Lac-St-Jean area. Of these individuals, all from the city of Chicoutimi, 50 were randomly ascertained as controls for other studies and the remaining 42, who lived throughout the Saguenay region, were unrelated obligate carriers for the cystic fibrosis gene.

Results

Identification of the mutation

PCR amplification of all HEXA exons and flanking sequences followed by SSCP analysis (data not shown) of amplification products produced a pattern of bands that was identical for normal control DNA and for individual II-2 in the D family (obligate heterozygote) and individual III-2 in the R family (affected proband) except for band patterns of exon 7-containing fragments.

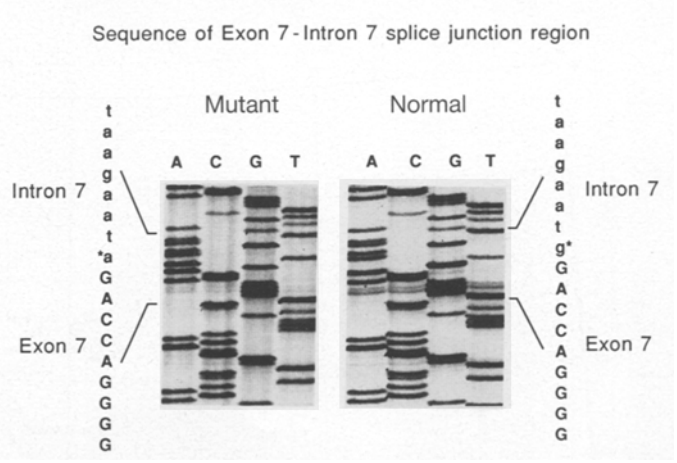

Fig. 3. Sequence of cloned exon 7-intron 7 splice junction region. *Upper case* exon sequences. *lower case* intron sequences

The sequences of exon 7-containing genomic fragments obtained from individuall III-2 in family R are shown in Fig. 3. The mutation, a G→A transition at the first position of intron 7, abolishes the universal donor splice site GT.

Effect on gene expression

Expression of mutant HEXA mRNA was assessed in cultured skin fibroblasts obtained from R-III-2 since the other mutant HEXA allele in this patient, the FCD (Hechtman et al. 1990), is not compatible with the expression of HEXA mRNA. No cDNA fragments were detected when an RNA-containing fraction from patients' cells was used as a template for RT-PCR, whereas the control cell RNA directed the amplification of a single fragment of 393 bp. Primers for amplification of prolidase (PEPD gene) produced cDNA fragments of comparable intensity when RNA preparations from both normal and TSD cell lines were used as templates, indicating that mRNA prepared from patients' cell had not undergone degradation.

Detection of the mutation in genomic DNA

The intron 7 +1 mutation occurs in the nucleotide adjacent to the last base in exon 7, which is the site of the adult-onset G805A TSD mutation. Both mutations result in the loss of a cleavage site for the restriction endonuclease *Eco*RII. A 221-bp product amplified from normal genomic DNA gives digestion fragments of 153, 44, 16, and 8 bp. In the mutant allele a 52-bp band replaces the 44-bp fragment (Fig. 4). Digestion of genomic DNA from two clinically affected individuals known to be heterozygous for the adult TSD mutation gives the identical pattern of restriction fragments. The two mutations can be unequivocally distinguished using allele-specific oligonucleotide hybridization as shown in Fig. 4. In family L an affected individual (II-13) had been diagnosed clinically. PCR amplification and heteroduplex analysis

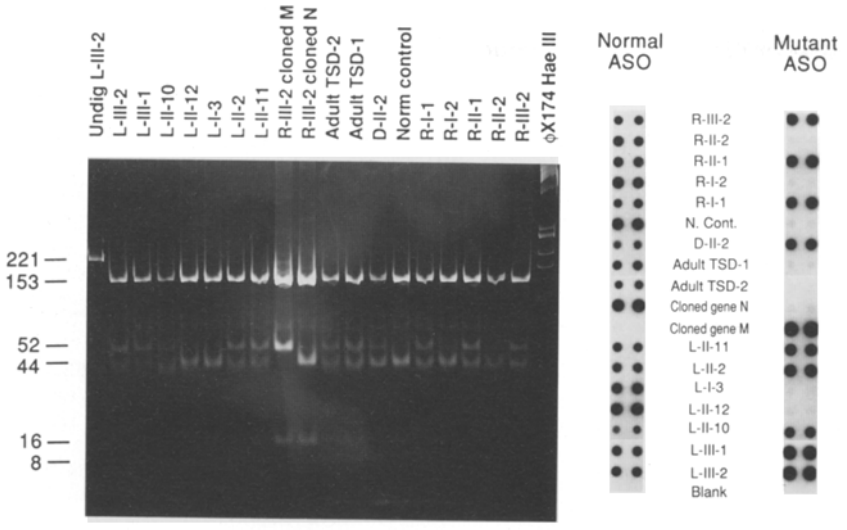

Fig. 4. Diagnosis of intron 7 +1 mutation by *Eco*RII digestion *(left)* and by allele-specific oligonucleotide hybridization *(right)*. *Letters L, D,* and *R* and *numerical symbols* refer to individuals in pedigrees shown in Fig. 2

of exon 11 sequences in DNA obtained from her father (L-I-3) showed that the exon 11 4-bp insert TSD allele also occurs in this family. This individual is the second such (unrelated) obligate heterozygote we have identified in French Canada (Hechtman et al. 1990).

Origin and regional frequency of the intron 7 +1 TSD mutation

Among 92 unrelated individuals from the Saguenay-Lac-St-Jean area one heterozygote was detected using dot hybridization. Genealogical reconstruction performed on the four obligate heterozygotes for the intron 7 +1 mutation identified 82 common ancestors. Among these, 39 were born in Quebec (14 on Côte de Beaupré, 13 in Charlevoix, and 12 in Quebec City). None were born in Saguenay-Lac-St-Jean. The remaining 43 common ancestors were born in Europe with 14/43 coming from the French province of Perche. This proportion is considerably higher than the relative contribution of Perche to the total number of settlers of Quebec during the period of the French regime (217/8500) (Charbonneau and Robert 1987).

Discussion

A novel HEXA mutation has been identified in a population in which the frequency of Tay-Sachs disease approaches that found among Ashkenazi Jews. In neither population can the spread of TSD alleles be attributed to the effect of a single founder since heteroallelism is found in both groups. The most frequent TSD mutation in French Canada, the 7.6-kb deletion accounts for about 75% of independently segregating alleles (inherited from individuals not more closely related than second degree cousins) and has a center of diffusion on the south shore of the St. Lawrence River. This allele has not been detected among 50 independently segregating TSD chromosomes in France (Akli et la. 1991). By contrast, the exon 11 4-bp insertion mutation has been shown to occur in two French-speaking North American populations (Hechtman et al. 1990; McDowell et al. 1991) probably introduced by different non-Jewish founders (P. Hechtman, unpublished).

The intron 7 +1 mutant allele is a second instance of a TSD mutation unique to French Canada that also has not yet been detected in France (Akli et al. 1991). It is unlikely that the founder came from France since in seventeenth century rural France whole families did not, as a rule, emigrate to New France and thus carrier sibs who remained in Europe would have passed the gene on to numerous descendants. Such a prediction was born out for the 10-kb deletion hypercholesterolemia mutation (Jomphe et la. 1988; De Braekeleer et al. 1988) and for the M1V allele at the PAH locus (De Braekeleer et al. 1990) both of which are found in Saguenay-Lac-St-Jean and in France. Although a high proportion of ancestors common to the heterozygotes for the intron 7 +1 TSD allele were from Perche this ancestry is also shared by many individuals from the Saguenay-Lac-St-Jean region who are unselected for any particular genotype (De Braekeleer et al. 1991).

The Saguenay-Lac-St-Jean region is a geographic isolate. The birthplaces and dates of the common ancestors correlate with known patterns of settlement in Quebec. Quebec City served as the port of entry for most immigrants in the early years of the French regime. Agricultural settlements moved downriver initially to Côte de Beaupré and later to Charlevoix and to Bas St-Laurent. The Charlevoix region was the source of 75% of the emigrants to Saguenay-Lac-St-Jean in the initial phase of white settlement (1838–1870: Gauvreau and Bourque 1988). It is, therefore, most likely that the founder was from the Charlevoix area since (1) no common ancestors came from Saguenay-Lac-St-Jean itself, (2) the most recent common ancestors are all from Charlevoix, and (3) the mutation has not been detected in any other region of Quebec. In the twentieth century industrial development in Saguenay-Lac-St-Jean brought immigrants from other regions of Quebec including the south shore of the St. Lawrence River. Some of these carried the deletion

allele accounting for the occurrence in this region of Tay-Sachs disease patients who are compound heterozygotes.

Acknowledgements. This work was supported from a grant from the Medical Research Council of Canada and the National Centres of Excellence.

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