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Different Mutations in Ashkenazi Jewish and Non-Jewish French Canadians with Tay-Sachs Disease

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Tay-Sachs disease patients of Ashkenazi Jewish and non-Jewish French Canadian origin are affected with a clinically identical form of this inherited disease. Both have a similar gene frequency for the disorder, which is tenfold higher than that found in the general population. Unlike other patients with the disease, who often display variation at the clinical or biochemical level, the absence of such differences between these two groups has prompted the idea that they may harbor the same mutation. In this report, a complementary DNA clone coding for the α chain of human β-hexosaminidase has been used to analyze the genetic lesions in the α-chain locus of two patients with Tay-Sachs disease from each of these groups. On the basis of DNA hybridization analyses, the α-chain gene of the Ashkenazi patients appears intact while the α-chain gene of French Canadian patients has a 5′ deletion of approximately 5 to 8 kilobases.

TAY-SACHS DISEASE IS AN INHERITED disorder caused by mutation in the α chain of β-hexosaminidase A, a lysosomal enzyme composed of two polypeptides designated the α and β chains (*1, 2*). Deficiency of β-hexosaminidase A results in storage of its major substrate, G_{M2} ganglioside. Progressive accumulation of substrate leads to the characteristic neurodegenerative changes in patients with Tay-Sachs disease. The disease is heterogeneous (*1–4*), displaying a wide range of severity and age of onset. An early onset and fatal form of the disorder referred to as "classic" Tay-Sachs disease has a tenfold higher gene frequency among Ashkenazi Jews than the general population. A less publicized group having a carrier frequency equal to that of Ashkenazi Jews is a population of non-Jewish French Canadians located in eastern Quebec (*5*). In terms of age-of-onset, clinical course, and biochemical parameters (*6*), French Canadian patients are indistinguishable from Ashkenazi patients. Identity of mutation has been proposed as one possible explanation for the similarities between these two groups at risk for classic Tay-Sachs disease (*5*). We have recently observed that polyadenylated [poly(A)$^+$] RNA preparations from five Ashkenazi (*7*) and two non-Jewish French Canadian (*8*) Tay-Sachs patients lacked detectable α-chain message when analyzed by Northern blotting, with complementary DNA (cDNA) encoding the α chain of human β-hexosaminidase. By means of a cDNA coding for the entire α-chain polypeptide (*9*), we have now analyzed and compared the α chains of Ashkenazi Jewish and French Canadian patients at the DNA level.

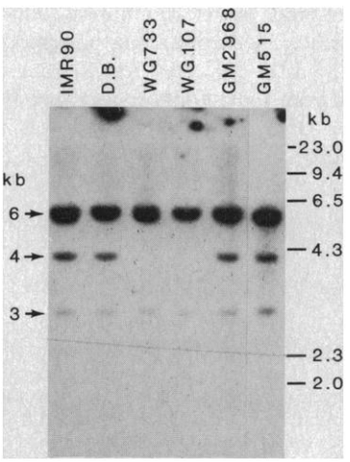

Fig. 1. Southern blot analysis of DNA from normal individuals and from patients with Tay-Sachs disease of French Canadian and Ashkenazi origin. Normal (IMR90) and Ashkenazi Tay-Sachs (GM515, GM2968) fibroblast cultures were obtained from the Human Genetic Cell Repository, Institute for Medical Research, Camden, NJ. The cells from non-Jewish French Canadian Tay-Sachs patients (WG107, WG733) were obtained from the Repository for Human Mutant Cell Strains, Montreal, Canada. DNA was isolated from these cultures (*11*) as well as from whole blood leukocytes (*12*) of a normal non-Canadian Ashkenazi individual (D.B.) who was not a heterozygote carrier for Tay-Sachs disease. Genomic DNA of normals and mutants (7 μg) was digested to completion with Eco RI, fractionated on a 1% agarose gel and transferred to GeneScreen Plus (New England Nuclear) in 10× standard saline citrate. Transferred DNA was then hybridized at 42°C in the presence of formamide (*11*) to the insert of pβHα-5, a cDNA clone that contains the entire coding sequence for the α chain of human β-hexosaminidase (*9*) and that had been labeled with ^{32}P to a specific activity of 2×10^9 count/min per microgram of DNA by the random primer method (*13*). Blots were washed as suggested by the manufacturer and exposed for 36 hours to x-ray film by means of a Cronex Hi Plus intensifying screen.

DNA was isolated from cultured fibroblasts of two non-Jewish French Canadian patients and two Ashkenazi patients. DNA for controls was obtained either from cultured cells or from blood leukocytes of normal subjects. Each sample was digested with Eco RI and analyzed by Southern blotting with a ^{32}P-labeled cDNA probe (pβHα-5) containing the entire coding sequence for the α chain of human β-hexosaminidase (*9*). An identical restriction pattern was obtained for the normal controls and Ashkenazi Tay-Sachs samples; these consisted of three DNA fragments with approximate sizes of 6, 4, and 3 kilobases (kb) (Fig. 1). However, the 4-kb DNA fragment was absent from the restriction pattern of both French Canadian Tay-Sachs samples. (A re-

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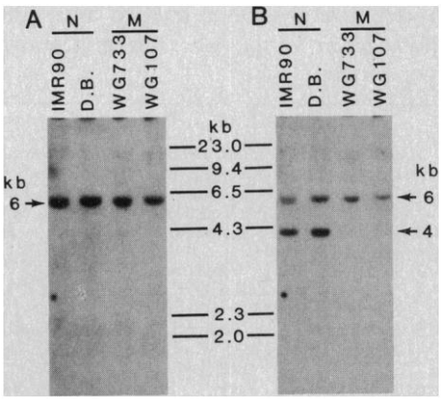

Fig. 2. Location of the deletion in the α-chain gene of β-hexosaminidase in DNA from French Canadian Tay-Sachs patients. Normal (IMR90, D.B.) and non-Jewish French Canadian Tay-Sachs (WG107, WG733) DNA (10 μg per sample) was digested with Eco RI and fractionated on a 1% agarose gel. After transfer to GeneScreen Plus the samples were hybridized with a ^{32}P-labeled (10^9 count/min per microgram of DNA) α-chain cDNA fragment (312 bp) derived from the 3′ terminus of pβHα-5 by restriction enzyme digestion (Hinc II → Pst I) and electrophoretic isolation onto DEAE paper (*14*) (A). The blot was washed and exposed to x-ray film for 48 hours. The same blot was then rehybridized with a ^{32}P-labeled (10^9 count/min per microgram of DNA) α-chain cDNA fragment (356 bp) derived from the 5′ terminus of pβHα-5 (Pst I → Stu I), washed, and exposed to x-ray film for 48 hours (B). N, normal; M, mutant.

striction pattern similar to normal controls was also observed with Ashkenazi samples digested with Pst I, Hind III, or Kpn I.) These results indicate that the DNA from Ashkenazi patients did not show major α-chain gene alterations detectable by Southern blotting, whereas DNA from the two Canadian patients displayed similar deletions of a portion of the α-chain gene.

We determined the region deleted in the α-chain gene of the samples from French Canadian Tay-Sachs patients by analyzing a Southern blot of Eco RI–digested normal and mutant DNA's that had been hybridized sequentially with 3′ and 5′ terminal α-chain cDNA probes prepared from pβHα-5. The 3′ terminal cDNA probe [312 base pairs (bp)] hybridized to the 6-kb genomic DNA fragment in all the samples (Fig. 2A), identifying this fragment as the 3′ end of the α-chain gene and demonstrating that the 3′ end was intact in the French Canadian mutants. Rehybridization of this same blot with a 5′ terminal α-chain probe (356 bp) elicited a band in the normal samples corresponding to the 4-kb DNA fragment, but did not produce a signal in the samples from French Canadian patients (Fig. 2B). These results mapped the 5′ terminus of the α-chain gene to the 4-kb fragment in Eco I digests and indicated that a 5′ terminal region of this gene was deleted in French Canadian Tay-Sachs cell strains WG107 and WG733. This conclusion is supported by the results of an identical experiment performed with Stu I–digested normal and mutant DNA samples. Hybridization with the 5′ terminal α-chain cDNA probe failed to produce a signal in the mutants but did so in the normal (0.6 kb), while the 3′ terminal probe produced a signal (4.6 kb) in both types of DNA samples.

We estimated the size of the α-chain gene deletion in the two French Canadian Tay-Sachs patients by hybridizing normal and mutant DNA with probes obtained from normal α-chain genomic clones. After Southern transfer, normal and mutant DNA samples that had been digested with Eco RI were hybridized with a ^{32}P-labeled genomic probe (300 bp). This probe mapped 700 bp upstream from the 5′ end of the 5′ terminal α-chain cDNA probe and hybridized with DNA in the normal samples but did not hybridize with DNA in the French Canadian samples (Fig. 3A). This result suggests that the mutants lack DNA sequences functioning in the initiation of α-chain gene transcription as well as 5′ exon material, since the 5′ terminal cDNA probe was obtained from a cDNA clone judged to be full length or no more than 50 to 100 bp short (*9*). Further support for this possibility is the observation of promoterlike sequences approximately 100 bp upstream from the 5′ end of the 5′ terminal α-chain cDNA probe in a genomic clone of the normal α-chain gene (*10*). The extent of the deletion beyond 700 bp upstream from the 5′ end of the α-chain cDNA is as yet unknown.

To delimit the 3′ end of the deletion, normal and mutant DNA samples that had been digested with Eco RI and transferred to nylon membranes were probed with two portions of intron from the α-chain gene. One sequence (600 bp), mapping 4.7 kb downstream from the 5′ end of the 5′ terminal cDNA probe, hybridized with the normal samples (9.5-kb band) but yielded no signal in the mutants (Fig. 3B). The second sequence (300 bp), mapping 7.6 kb downstream from the 5′ end of the 5′ terminal cDNA probe, hybridized to both normal (a 9.5-kb band) and mutant (a 23-kb band) DNA samples (Fig. 3C). (Hybridization of this probe to DNA fragments of different sizes in the normals and mutants results from alterations in the Eco RI restriction pattern of the mutant as a consequence of the deletion.) These results allowed us to determine that the α-chain deletion in French Canadian Tay-Sachs patients extended approximately between 5.3 and 7.6 kb downstream from the 5′ terminus of the α-chain cDNA, assuming a minimum requirement of a 50-bp hybridization for production of a signal.

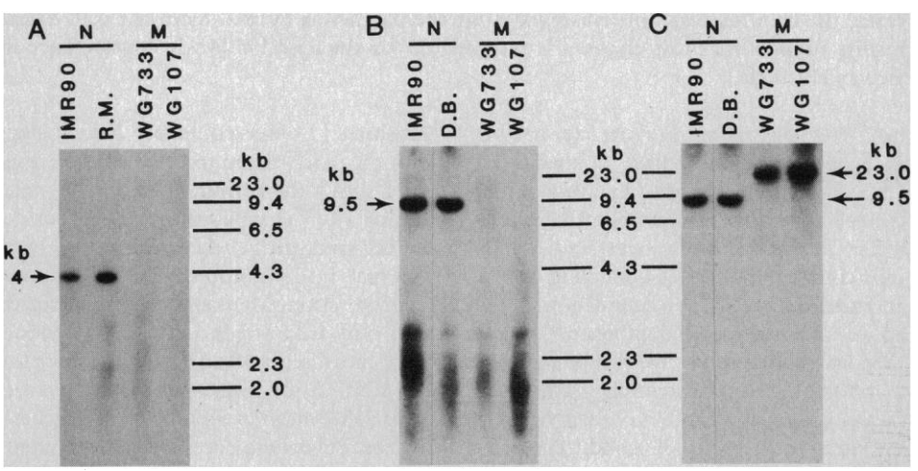

Fig. 3. Extent of the 5′ terminal α-chain gene deletion in French Canadian Tay-Sachs patients. Normal [IMR90, R.M. (an Ashkenazi Jewish non-Canadian subject who was not a heterozygote carrier for Tay-Sachs disease) and D.B.] and French Canadian Tay-Sachs (WG733, WG107) DNA (10 μg per sample) was digested with Eco RI, fractionated on a 1% agarose gel, transferred to GeneScreen Plus, and processed as described (Fig. 1) except that the blots displayed in (B) and (C) were obtained by transfer in 0.4*M* NaOH and 0.6*M* NaCl (*15*). (A) Hybridization with a 300-bp DNA sequence obtained from an α-chain genomic clone and mapping 700 bp upstream from the 5′ end of the 5′ terminal cDNA probe. (B) Hybridization with a 600-bp α-chain intron sequence mapping 4.7 kb downstream from the 5′ end of the 5′ terminal α-chain cDNA probe. (C) Hybridization with a 300-bp α-chain intron sequence mapping 7.6 kb downstream from the 5′ end of the 5′ terminal cDNA probe. Exposure was for 48 hours. N, normal; M, mutant.

The identical clinical picture and heterozygote carrier frequency found among these two populations, one defined by geography, the other by ethnicity, has led to the suggestion that the two groups bear identical molecular lesions, perhaps arising through marriage of an Ashkenazi Jew into the French Canadian group (*5*). Our results showing a generally intact α-chain gene in Ashkenazi patients but a deletion in the α-chain gene of the French Canadian patients of 5 to 8 kb at the 5′ end, demonstrate that in the cell lines studied this is not the case. However, generalizations about this finding cannot be made until larger numbers of patients and carriers from each group are analyzed at the DNA level to assess the homogeneity of the mutations in these two populations.

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An Apical-Membrane Chloride Channel in Human Tracheal Epithelium

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The mechanism of chloride transport by airway epithelia has been of substantial interest because airway and sweat gland–duct epithelia are chloride-impermeable in cystic fibrosis. The decreased chloride permeability prevents normal secretion by the airway epithelium, thereby interfering with mucociliary clearance and contributing to the morbidity and mortality of the disease. Because chloride secretion depends on and is regulated by chloride conductance in the apical cell membrane, the patch-clamp technique was used to directly examine single-channel currents in primary cultures of human tracheal epithelium. The cells contained an anion-selective channel that was not strongly voltage-gated or regulated by calcium in cell-free patches. The channel was also blocked by analogs of carboxylic acid that decrease apical chloride conductance in intact epithelia. When attached to the cell, the channel was activated by isoproterenol, although the channel was also observed to open spontaneously. However, in some cases, the channel was only observed after the patch was excised from the cell. These results suggest that this channel is responsible for the apical chloride conductance in airway epithelia.

CHLORIDE SECRETION BY AIRWAY epithelia and other epithelia is a two-step process. Chloride enters the cell across the basolateral membrane by means of an electrically neutral, Na^+-coupled cotransport step; the coupling to Na^+ energizes intracellular accumulation of Cl^- above electrochemical equilibrium. Chloride then leaves the cell passively, moving down a favorable electrochemical gradient by means of an electrically conductive apical exit step (*1*). Apical Cl^- conductance is a primary regulatory step that controls the rate of transepithelial Cl^- secretion and the response to neurohumoral mediators. Although most agents that stimulate secretion increase intracellular levels of cyclic AMP and cytosolic Ca^{2+} concentrations, the identity of the second messenger that directly regulates the apical Cl^- permeability is uncertain.

In cell-attached and excised patches of membrane, I observed unitary current steps with the patch-clamp technique (*2, 3*). Figure 1 shows the current-voltage (*I–V*) relation of a Cl^- channel in an excised, inside-out cell-free patch bathed with symmetrical 145 m*M* NaCl solutions. The *I–V* relation rectified strongly; conductance increased with outward currents. The slope conductance measured at 0 mV with symmetrical 145 m*M* Cl solutions was 25 ± 2 picosiemens (pS) (mean ± SEM, n = 37 patches). No consistent differences were observed for channels obtained from different tracheas. Figure 1 also shows that unilateral changes in the Cl^- concentration shifted the reversal potential in the direction expected for a Cl^- channel. The selectivity of the channel for Cl^- over cations and the sulfate and gluconate anions is a property similar to that of the apical Cl^- conductance (*1*).

Isoproterenol stimulates Cl^- secretion by increasing the apical Cl^- conductance in intact and cultured epithelia (*1, 4*). Figure 2 shows that isoproterenol also activated previously quiescent channels in a cell-attached patch. In six patches that appeared to contain only a single channel (*5*), the probability (P) of finding the channel open before addition of isoproterenol was zero. However, at a mean time of 59 ± 13 seconds after the addition of isoproterenol, the value of P was at a peak of 0.46 ± 0.16. In five other multichannel patches in which isoproterenol increased activity, the value of P in three patches was zero during the 56 ± 14 seconds of observation before addition of isoproterenol (Fig. 2). In the other two patches, there was a brief burst of activity of one channel before the addition of isoproterenol, followed by the opening of several channels after isoproterenol addition. Although Fig. 2 shows a dramatic activation of the Cl^- channel by isoproterenol, this was not always observed. From 40 patches in which the Cl^- channel was observed, the channel was activated by isoproterenol in 28% and opened spontaneously in the cell-attached mode of untreated cells in 30%. However, in 42% of the patches, the channel was observed to open only after excision of the patch from the cell.

Agents that stimulate secretion increase the intracellular Ca^{2+} concentration (*6, 7*) and produce small changes (5 to 15 mV) in apical membrane voltage (*1*). However, I found no consistent effect of internal Ca^{2+} on channel activity in excised, inside-out, cell-free patches (Fig. 3). In five excised patches that contained either one or two channels, the value of P was 0.73 ± 0.06 with 1 m*M* Ca^{2+} bathing the internal surface of the patch and 0.78 ± 0.07 when the

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