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Tay-Sachs Disease in Moroccan Jews: Deletion of a Phenylalanine in the α-Subunit of β-Hexosaminidase

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Summary

Tay-Sachs disease is an inherited lysosomal storage disorder caused by defects in the β-hexosaminidase α-subunit gene. The carrier frequency for Tay-Sachs disease is significantly elevated in both the Ashkenazi Jewish and Moroccan Jewish populations but not in other Jewish groups. We have found that the mutations underlying Tay-Sachs disease in Ashkenazi and Moroccan Jews are different. Analysis of a Moroccan Jewish Tay-Sachs patient has revealed an in-frame deletion (ΔF) of one of the two adjacent phenylalanine codons that are present at positions 304 and 305 in the α-subunit sequence. The mutation impairs the subunit assembly of β-hexosaminidase A, resulting in an absence of enzyme activity. The Moroccan patient was found also to carry, in the other α-subunit allele, a different, and as yet unidentified, mutation which causes a deficit of mRNA. Analysis of obligate carriers from six unrelated Moroccan Jewish families showed that three harbor the ΔF mutation, raising the possibility that this defect may be a prevalent mutation in this ethnic group.

Introduction

Tay-Sachs disease is an autosomal recessive disorder caused by an absence of the lysosomal enzyme, β-hexosaminidase A (reviewed by Neufeld [1989] and Sandhoff et al. [1989]). The enzyme, a heterodimer composed of noncovalently associated α- and β-subunits, acts on a variety of substrates containing terminal N-acetylglucosaminides and N-acetylgalatosaminides, including G_{M2} ganglioside. In Tay-Sachs disease, mutations in the α-subunit gene cause the enzyme deficiency, and, as a consequence, undegraded G_{M2} ganglioside accumulates in lysosomes. This process is particularly injurious to neurons and leads to nervous system deterioration. In affected individuals, mental and motor impairment begins in the first year of life and progresses rapidly, with death ensuing in early childhood.

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Among the general population the Tay-Sachs disease carrier frequency, calculated from the incidence of the disease, is estimated to be 1/300 (Sandhoff et al. 1989). This frequency is elevated among certain ethnic groups. The most well-known example is the Ashkenazi Jewish population, in which ~3% (1/31) of the population carry an α-subunit mutation. The major mutation, a 4-bp insertion in exon 11 of the α-subunit gene, accounts for ~70% of the mutant alleles. Most of the remaining Tay-Sachs alleles in the population contain a splice-junction mutation at the 5′ end of intron 12 (Arpaia et al. 1988; Myerowitz 1988; Myerowitz and Costigan 1988; Ohno and Suzuki 1988; Paw et al. 1990; Triggs-Raine et al. 1990). A second well-characterized group with an increased incidence of Tay-Sachs disease is within the French-Canadian population in eastern Canada, where the carrier frequency is similar to that in the Ashkenazi population (Andermann et al. 1977). In the French-Canadian isolate, as with Ashkenazi Jews, one particular mutation predominates, in this case a 7.6-kb deletion at the 5′ end of the α-subunit gene (Myerowitz and Hogikyan 1987; Hectman et al. 1989).

It has been established that Moroccan Jews, a sub-

group of Sephardic Jews, also have an elevated carrier frequency, estimated to be ~1/60, for Tay-Sachs disease (Vecht et al. 1983; R. Navon and E. Ackstein, unpublished data). The high carrier frequency in Ashkenazi and Moroccan Jews does not extend to other Jewish populations. Evidence has been presented suggesting that the Ashkenazi and Moroccan mutations may be distinct (Bach et al. 1976).

In the present study we demonstrate that the molecular defects that cause Tay-Sachs disease in Ashkenazi and Moroccan Jews are different. Analysis of a Moroccan Jewish Tay-Sachs patient has demonstrated an in-frame phenylalanine codon deletion (ΔF) in the α-subunit gene. The mutation impairs the folding and assembly of the α-subunit, resulting in an absence of enzyme activity. The Moroccan patient was also found to carry, in the other allele, a different, and as yet unidentified, mutation, which causes a deficit of mRNA. Analysis of Tay-Sachs disease obligate carriers from six unrelated Moroccan Jewish families revealed that three carry the ΔF mutation.

Experimental Procedures

Reagents

Tissue culture medium and reagents were from Biofluids (Rockville, MD). Immuno-Precipitin was from Bethesda Research Laboratories. Enlightening was from Amersham [^{35}S]Methionine (1,000 Ci/mmol), [^{32}P]ATP (3,000 Ci/mmol), and GeneScreenPlus membranes were from New England Nuclear. The reagents for the PCR technique were from Perkin Elmer–Cetus. Reagents for DNA sequencing were from U.S. Biochemicals. Low-melting-temperature agarose (SeaPlaque) was from FMC BioProducts (Rockland, ME). DEAE-dextran was from Pharmacia. AMV reverse transcriptase and oligo(dT)$_{15}$ were from Promega. Restriction enzymes, DNA ligase, and T4 DNA kinase were from New England Biolabs. T4 DNA polymerase was from Bio-Rad. 4-Methylumbelliferyl-6-sulfo-2-acetamido-2-deoxy-D-glucopyranoside was from Toronto Research Chemicals.

Moroccan Patient and Carriers

The Moroccan Jewish Tay-Sachs patient was born in Israel. Both parents were born in Morocco—the father (MD) in Rabat, the mother (AD) in Casablanca. The patient had classical signs of infantile Tay-Sachs disease, and the diagnosis was confirmed by a deficiency of β-hexosaminidase A in her serum, leukocytes, and skin fibroblasts.

All of the Moroccan Jewish carriers were obligate heterozygotes for Tay-Sachs disease. The carriers were found to have heterozygote levels of β-hexosaminidase A, both in serum and in leukocyte samples. All were born in Morocco—SiM in Rabat, SY and SM in Marrakech, AR and HA in Casablanca, and AM in Fez. MD and SiM are first cousins, as are SY and SM.

Cell Culture

Normal human fetal lung fibroblasts, IMR-90, were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). A skin fibroblast culture was derived from the Moroccan Jewish patient with Tay-Sachs disease. COS 1 cells were obtained from the American Type Tissue Culture Collection. Cells were cultured at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 25 mM HEPES, 10% FBS, and antibiotics (growth medium).

Fibroblast Labeling and Immunoprecipitation

Human fibroblast cultures in 100-mm dishes were labeled with 400 μCi of [^{35}S]methionine in 5 ml DMEM (formulated without methionine) containing 5% dialyzed FBS. After 16 h the medium was collected, and the cells were rinsed twice with PBS. The cell monolayers were incubated with PBS containing 1% Nonidet P-40 and 10 mg BSA/ml for 15 min at room temperature. The resulting cell extract and medium samples were preabsorbed with 100 μl of Immuno-Precipitin. To determine the association state of the α-subunit, the samples were divided in half and were first immunoprecipitated with 3 μl of either anti-β-hexosaminidase A, which recognizes all forms of the α-subunit, or anti-hexosaminidase B, which will precipitate only α-subunits that are associated with β-subunits (Proia et al. 1984). After incubation for 2 h, 40 μl of Immuno-Precipitin was added, and the immune complexes were collected by centrifugation. The precipitate was washed twice with PBS containing 0.1% Nonidet P-40. The labeled proteins were solubilized by heating at 95°C for 5 min in 100 μl of PBS containing 1% SDS and 10 mM DTT. The Immuno-Precipitin was removed by centrifugation, and 0.9 ml of PBS containing 2% Nonidet P-40, 10 mg BSA/ml, and 10 mM iodoacetamide was added to the supernatant. The samples were preabsorbed with Immuno-Precipitin as before and then incubated overnight at 4°C with 3 ml of anti-denatured α-subunit serum. After incubation with 40 μl of Immuno-Precipitin, the immune complexes were washed three times with a buffer containing 20 mM Tris-HCl, 0.6 M NaCl,

0.05% Nonidet P-40, and 0.1% SDS, pH 8.6. The labeled proteins were solubilized in sample buffer (1% SDS, 0.125 M Tris-HCl, pH 6.8, and 10% glycerol), were reduced with 10 mM DTT, and were electrophoresed on 8%–16% gradient gels in the presence of SDS. The gels were saturated with Enlightening, were dried, and were exposed to Kodak X-Omat AR film at 70°C.

Site-directed Mutagenesis

Introduction of the ΔF mutation into the α-subunit was accomplished by site-directed mutagenesis according to a method described elsewhere (Kunkel et al. 1987) by using single-stranded M13 containing the α-subunit (Navon and Proia 1989) and the mutagenic oligonucleotide (antisense), 5′ GCT GAC TTC TAA GAA TGT GCT CAT GAA CTC 3′.

Cell Transfections

The normal α-subunit cDNA and the α-subunit with the ΔF mutation were cloned into the expression vector, pSVL, and were transfected into COS 1 cells according to a method described elsewhere (Navon and Proia 1989). Cell extracts were assayed for β-hexosaminidase activity by 4-methylumbelliferyl-6-sulfo-2-acetamido-2-deoxy-D-glucopyranoside according to a method described elsewhere (Navon and Proia 1989).

Mutation Detection by DNA Sequence Analysis

Total RNA was prepared from human fibroblast cultures by the guanidine isothiocyanate/CsCl method (Chirgwin et al. 1979), and the polyA$^+$ fraction was isolated using an oligo(dT)-cellulose column (Aviv and Leder 1972). The polyA$^+$ RNA (2 μg) was reverse transcribed using AMV reverse transcriptase and oligo$(dT)_{15}$. Segments of the α-subunit were amplified by the PCR technique (Saiki et al. 1988) using *Taq* DNA polymerase and α-subunit-specific oligonucleotide primers which contained an additional *Eco*RI sequence at their 5′ end. The DNA was subjected to amplification on a Perkin Elmer—Cetus thermocycler for 39 cycles. Each cycle was 2 min at 94°C for denaturation, 3 min at 60°C for annealing, and 6 min at 72°C for primer extension. The amplified segments were cleaved with *Eco*RI and isolated from a low-melting-temperature agarose gel and were ligated into the *Eco*RI site of the M13mp18 vector. From the recombinant clones, single-stranded DNA was isolated and sequenced by the dideoxy-chain termination method (Sanger et al. 1977) using Sequenase.

Direct sequencing of DNA derived from asymmetric PCR amplification was accomplished according to a method described elsewhere (Kadowaki et al. 1990). The first amplification was accomplished using 5′ GCA GGT GAA ATC AAC CTC ATC TCC TCC (primer 1) and 5′ACT CCT GGA TTA CTG ACT CCT TGC TAC (primer 2). A portion of the first reaction was subjected to asymmetric amplification using only primer 1. Sequencing was performed using a [^{32}P] end-labeled internal primer, 5′TGC TAC TCT GGG TCT GAG CCC.

Mutation Detection by Hybridization with Allele-specific Oligonucleotide Probes

For detection of the ΔF mutation, cDNA prepared as described above, or genomic DNA was amplified using primer 1 and primer 2. The DNA was subjected to amplification on a Perkin-Elmer Cetus thermocycler for 39 cycles. Each cycle was 2 min at 94°C for denaturation, 2.5 min at 60°C for annealing and 3 min at 72°C for synthesis. The Ashkenazi infantile Tay-Sachs mutations were amplified according to a method described elsewhere (Myerowitz 1988; Myerowitz and Costigan 1988). The conditions for mutation detection by dot blotting and hybridization were as described elsewhere (Navon and Proia 1989). The sequence of the oligonucleotide probe specific for the ΔF mutation was 5′ATG AGC ACA TTC TTA GAA GTC. The sequence of the corresponding normal probe was 5′AGC ACA TTC TTC TTA GAA GTC. The sequences of the probes for detection of the Ashkenazi infantile mutations were as described elsewhere (Myerowitz 1988; Myerowitz and Costigan 1988).

Results

Control IMR-90 fibroblasts and a fibroblast culture derived from a Moroccan patient with Tay-Sachs disease were labeled with [^{35}S]methionine for 16 h, and the cell extracts and medium were immunoprecipitated with antiserum prepared against either hexosaminidase A, which precipitates all forms of the α-subunit (total), or anti-hexosaminidase B, which precipitates only α-subunits associated with β-subunits (associated) (Proia et al. 1984). In contrast to the IMR-90 fibroblast extracts, which contained both precursor and mature α-subunits, the extracts from the Moroccan Tay-Sachs fibroblasts contained only precursor α-subunits (fig. 1). The α-subunits synthesized by the Tay-Sachs fibroblast culture were not immunoprecipitated by antibodies against the β-subunits (associated), demonstrating that the α-subunits pro-

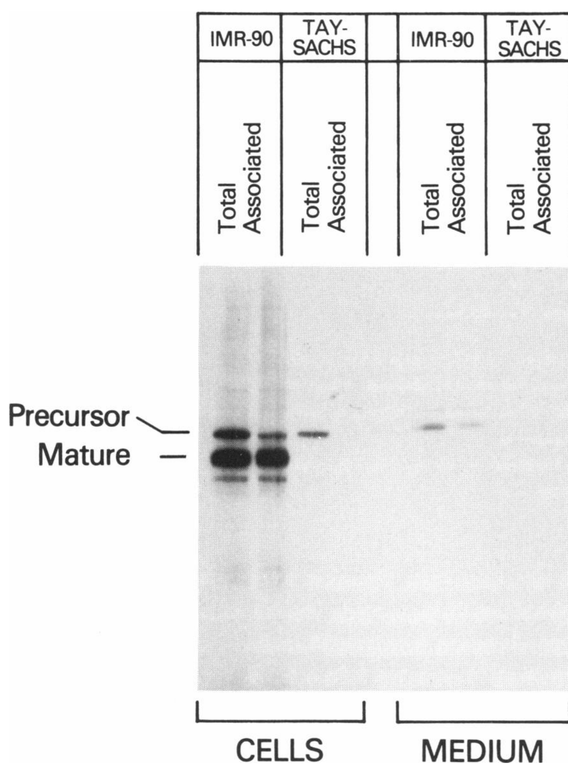

Figure 1 Synthesis and assembly of α-subunit in normal and Moroccan Tay-Sachs fibroblasts. Fibroblast cultures in 100-mm dishes were labeled with 400 μCi [^{35}S]methionine for 16 h. Cell extracts and medium samples were divided in half and immunoprecipitated with either anti-hexosaminidase A (total) or anti-hexosaminidase B (associated). The immune precipitates were solubilized and then reimmunoprecipitated with anti-denatured α-subunit serum.

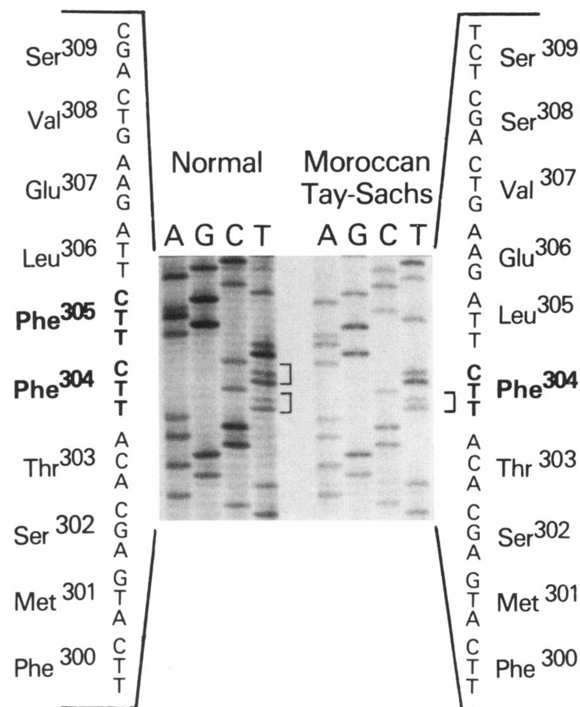

Figure 2 Nucleotide sequences of α-chain cDNA from Moroccan patient with Tay-Sachs disease. A portion of an autoradiograph is shown for the sequencing gel of the normal cDNA and of the Moroccan Tay-Sachs patient's cDNA. The two adjacent TTC codons in the normal sequence and the single TTC codon in the mutant sequence are indicated.

duced by these cells were not competent for assembly with β-subunits.

The cell-labeling experiment demonstrated that α-subunit protein was produced by these cells. As expected, northern analysis revealed easily detectable α-subunit mRNA in the Moroccan Tay-Sachs fibroblasts (not shown). To identify the mutation carried by the Moroccan patient, mRNA was isolated from the fibroblast culture and was reverse transcribed into cDNA, and portions of the α-subunit cDNA were amplified by the PCR technique and were cloned and sequenced. This analysis revealed a deletion of one of the two adjacent phenylalanine codons (TTC) that are present at positions 304 and 305 in the normal α-subunit sequence (Myerowitz et al. 1985) (fig. 2). To verify the result, genomic DNA was amplified in the region of the mutation, and the amplified material was sequenced directly (fig. 3). The resulting ladder demonstrated the sequence containing phenylalanine deletion (ΔF) superimposed on a normal sequence (N). Thus, the patient carries two different α-subunit alleles—one with the ΔF mutation and the other with a normal sequence in this region.

The ΔF mutation was introduced into the normal α-subunit cDNA by oligonucleotide-mediated site-directed mutagenesis. The normal and the mutated cDNAs were expressed in COS 1 cells under the control of the SV40 early promoter. As shown in table 1, the ΔF mutation rendered the α-subunit enzymatically inactive. In the transfected COS 1 cells the α-subunit carrying the ΔF mutation was found only as precursor in the cells, with none secreted into the medium (fig. 4), a finding similar to results obtained with the Moroccan Tay-Sachs fibroblast culture.

Genomic DNA from Moroccan Jewish individuals who were obligate carriers for Tay-Sachs disease were tested for the presence of the ΔF mutation. The region of the mutation was amplified by the PCR technique,

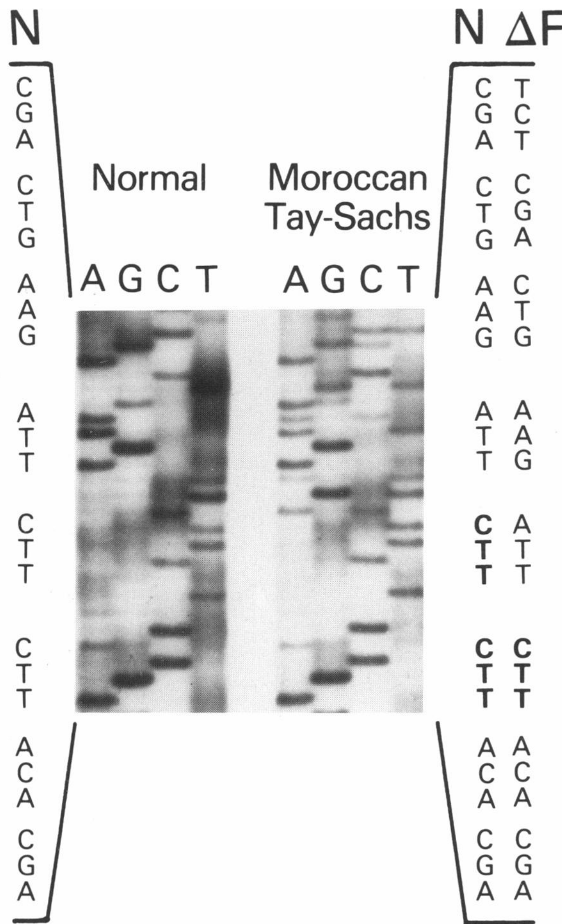

Figure 3 Direct sequencing of PCR products derived from genomic DNA. Genomic DNA from a normal individual and from a Moroccan Tay-Sachs patient was subjected to asymmetric PCR amplification in the region of the ΔF mutation, and the products were sequenced. A portion of the autoradiograph is shown for the sequencing gel of the normal cDNA and of the Moroccan patient's DNA. The normal (N) and the mutant (ΔF) sequences are displayed.

and the product was blotted onto duplicate hybridization membranes. The samples were hybridized with allele-specific oligonucleotide probes to detect either the sequence containing the ΔF mutation or the corresponding normal sequence (fig. 5). Amplified genomic DNA of the Moroccan patient and the father (MD) were positive for the mutation in this assay. The mother (AD) tested negative for the ΔF mutation and, therefore, must be carrying the other, as yet unknown, mutation. The Moroccan patient's DNA hybridized with the normal probe, which was expected because of compound heterozygosity. In total, five of eight Moroccan Jewish obligate carriers were positive for

Table 1

Expression of α-Chain-associated β-Hexosaminidase Activity in Transfected COS 1 Cells

DNA	β-Hexosaminidase Activity (units[a]/mg protein)	
	Experiment 1	Experiment 2
pSVLα	47.3	44.5
pSVLαΔF	3.1	2.7
Mock transfection	4.1	4.3

Note.—COS 1 cells were transfected with pSVLα or pSVLαΔF or were mock transfected. After 48 h the cell extracts were assayed with 4-methylumbelliferyl-6-sulfo-2-acetamido-2-deoxy-D-glucopyranoside.

[a] Defined as the activity that releases 1 nmol of 4-methylumbelliferone/min.

the ΔF mutation. Because MD and SiM as well as SY and SM are first cousins, the ΔF mutation was found in three of the six unrelated families carrying a Tay-Sachs mutation. Genomic DNA from 10 normal Moroccan Jews was negative for the mutation. The patient and

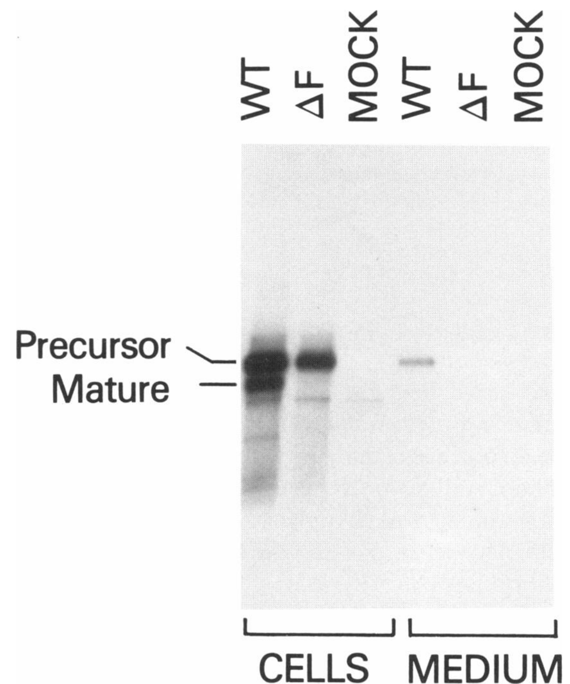

Figure 4 Expression of α-subunit carrying ΔF mutation in COS 1 cells. COS 1 cells were transfected with pSVL carrying the wild-type α-subunit or the α-subunit containing the ΔF mutation or were mock transfected. After 48 h the cells were labeled with [^{35}S]methionine for 16 h, and cell extracts and medium samples were immunoprecipitated with anti-hexosaminidase A.

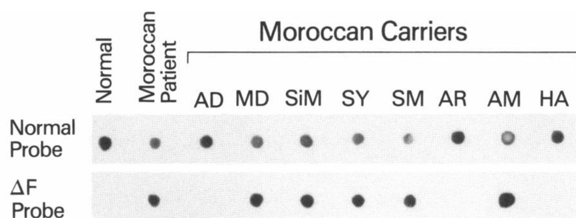

Figure 5 Identification of ΔF mutation in Moroccan carriers. PCR-amplified genomic DNA from a clinically normal individual, the Moroccan Jewish Tay-Sachs patient, the patient's mother (AD) and father (MD), and six other obligate Moroccan Jewish carriers was assayed for the presence of the ΔF mutation. The DNA samples were hybridized with an allele-specific oligonucleotide probe specific for the ΔF mutation (ΔF Probe) and with an oligonucleotide specific for the corresponding normal region (Normal Probe).

the carriers did not carry either of the two Ashkenazi mutations—the splice-junction mutation or the 4-bp insertion (not shown).

We next determined whether RNA expressed from the other mutant allele carried by the patient could be detected. PolyA$^+$ RNA from the Moroccan patient and from normal fibroblasts was reverse transcribed, and the region surrounding the ΔF mutation was amplified by PCR. Equal amounts of the amplified DNA were hybridized with allele-specific oligonucleotide probes (fig. 6). As expected, the probe specific for the ΔF mutation hybridized only to the PCR product

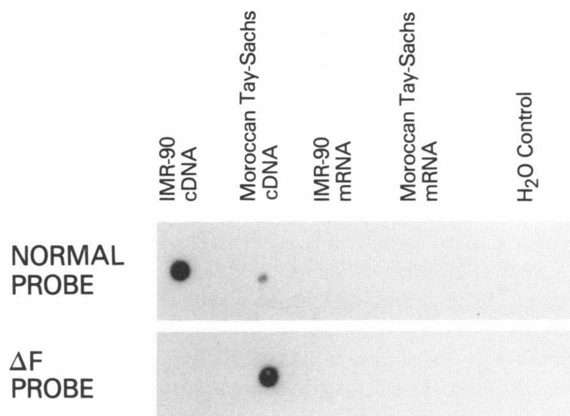

Figure 6 Allele-specific expression of mRNA in fibroblasts from Moroccan Tay-Sachs patient. The samples indicated on the top of the figure were subjected to amplification by PCR in the region of the ΔF mutation. Equal amounts of the indicated samples were dot blotted and hybridized with an allele-specific oligonucleotide probe specific for the ΔF mutation (ΔF Probe) and with an oligonucleotide specific for the corresponding normal region (Normal Probe).

derived from the Moroccan patient's cDNA. Hybridization with the probe carrying the normal sequence, which should hybridize to sequences expressed from the unknown mutant allele, demonstrated a low level of expression, compared with that in normal fibroblasts. No signal was detected by direct amplification of mRNA done without reverse transcription, ruling out contamination with genomic DNA.

Discussion

The Moroccan Jewish Tay-Sachs patient that we have described carries two different Tay-Sachs alleles. In one allele a 3-bp deletion results in the in-frame removal of one of the two successive phenylalanine codons that occur at positions 304 and 305 in the normal α-subunit sequence. The other allele contains an as yet unidentified mutation. We found that this unidentified mutation caused a significant decrease in the amount of α-subunit mRNA, suggesting a defect in transcription or mRNA stability. The unidentified mutation in both the Moroccan patient and obligate heterozygotes was not one of the two Ashkenazi infantile mutations. Different α-subunit mutations in the Ashkenazi and Moroccan Jewish populations are consistent with the hypothesis that Tay-Sachs disease mutations originated and expanded in the Ashkenazi Jewish population after the migration of their predecessors to Europe (Myrianthopoulos and Aronson 1966; Petersen et al. 1983).

The ΔF mutation occurred in the context of a stretch of pyrimidines, TTC TTC TT, containing direct trinucleotide repeats. The deletion of a short direct repeat is consistent with a "slipped mispairing" model in which strand slippage and mispairing during DNA replication causes a loop out of a repeat sequence which is subsequently excised (Farabaugh and Miller 1978; Efstatiadis et al. 1980).

The fibroblast culture derived from the Moroccan patient synthesized an α-subunit that is unable to assemble with the β-subunit to form β-hexosaminidase A. Although the patient is a compound heterozygote, all or most of the α-subunit protein detected in the fibroblasts is likely to be derived from the ΔF allele because expression of transcript from the other allele is severely depressed. Also, the ΔF protein, in precursor form, is relatively stable as determined from the transfection experiment, arguing against the possibility that the ΔF protein might be rapidly degraded—and thus undetectable—in the patient's fibroblasts. It cannot be determined at this time whether the mutation induces

a global alteration in the folding of the α-subunit or whether this change has a more direct effect on the assembly process. The two adjacent phenylalanines at positions 304 and 305 in the human α-subunit are conserved in the homologous human and mouse β-subunits (Myerowitz et al. 1985; Korneluk et al. 1986; Bapat et al. 1988; Proia 1988), suggesting that this region may be important for folding or attainment of the final enzyme structure. It is interesting that in the mouse α-subunit (S. Yamanaka and R. L. Proia, unpublished data) and in the single *Dictyostelium* hexosaminidase gene (Graham et al. 1988) the second phenylalanine is conserved, with the first being replaced by a leucine.

Among Ashkenazi Jews, three α-subunit mutations account for nearly all of the alleles that cause Tay-Sachs disease and its milder variant, adult G_{M2} gangliosidosis (Neufeld 1989). It has been suggested that a DNA-based assay, because of its high specificity and ability to distinguish between the adult and infantile disorders, may be very useful when used in conjunction with enzyme screening for Tay-Sachs disease carriers among Ashkenazi Jews (Paw et al. 1990; Triggs-Raine et al. 1990). The finding that the ΔF mutation was carried by three of six unrelated Moroccan Jewish families harboring a Tay-Sachs allele raises the possibility that this may be a prevalent mutation among this population. At the present time there are 500,000 Moroccan Jews and 1,500,000 Ashkenazi Jews living in Israel, with significant intermarriage between the populations (Vecht et al. 1983). DNA-based testing of enzymatically identified carriers among Israeli Jews should include the ΔF mutation, as well as the three Ashkenazi mutations. In addition, it is important that the other Moroccan mutation(s) be identified for incorporation into the DNA-based testing.

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