The Major Defect in Ashkenazi Jews with Tay-Sachs Disease Is an Insertion in the Gene for the α-Chain of β-Hexosaminidase*

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The Ashkenazi Jewish population is enriched for carriers of a fatal form of Tay-Sachs disease, an inherited disorder caused by mutations in the α-chain of the lysosomal enzyme, β-hexosaminidase A. Until recently it was presumed that Tay-Sachs patients from this ethnic isolate harbored the same α-chain mutation. This was disproved by identification of a splice junction defect in the α-chain of an Ashkenazi patient which could be found in only 20–30% of the Ashkenazi carriers tested. In this study we have isolated the α-chain gene from an Ashkenazi Jewish patient, GM515, with classic Tay-Sachs disease who was negative for the splice junction defect. Sequence analysis of the promoter region, exon and splice junctions regions, and polyadenylation signal area revealed a 4-base pair insertion in exon 11. This mutation introduces a premature termination signal in exon 11 which results in a deficiency of mRNA in Ashkenazi patients. A dot blot assay was developed to screen patients and heterozygote carriers for the insertion mutation. The lesion was found in approximately 70% of the carriers tested, thereby distinguishing it as the major defect underlying Tay-Sachs disease in the Ashkenazi Jewish population.

Mutations in the α-chain of the A form of β-hexosaminidase (β-N-acetyl-β-hexosaminidase, EC 3.2.15.2), a lysosomal enzyme composed of two polypeptides (α, β), result in Tay-Sachs disease, an inherited disorder displaying clinical and biochemical variation (1, 2). The disease is inherited in an autosomal recessive manner. Heterozygote carriers harbor one α-chain mutant allele and are asymptomatic, while individuals in whom both α-chain alleles are defective display neurodegeneration due to accumulation of undegraded gangliosides (1). Patients with a severe type of Tay-Sachs disease called the “classic” form show clinical symptoms as early as 6 months of age, and death follows in early childhood. Ashkenazi Jews with Tay-Sachs Disease Is an Insertion in the Gene for the α-Chain of β-Hexosaminidase*der, underlies classic Tay-Sachs disease in this ethnic group (1). This was disproved by discovery of a splice junction defect (3–5) in the α-chain gene of approximately 20–30% of the Ashkenazi carriers tested (3, 4). This report describes a 4-base pair insertion in exon 11 of the α-chain gene of an Ashkenazi patient. Since 70% of the carriers tested are positive for this mutation, it appears to be the major defect underlying classic Tay-Sachs disease in the Ashkenazi Jewish population.

EXPERIMENTAL PROCEDURES

DNA Sources

Fibroblast cultures GM2968, GM3051, GM3052, GM515, and IMR90 were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Camden, NJ). Conditions for culture were as described (6). Blood samples of Ashkenazi patients with classic Tay-Sachs disease and of their parents were obtained from Lyndon Badal of Kingsbrook Jewish Medical Center, Brooklyn, NY. Leukocyte pellets from Ashkenazi Jewish obligate heterozygote carriers and normal Ashkenazi Jews were kindly provided by Eugene Grebner (Thomas Jefferson University, Philadelphia, PA).

Construction of Genomic Library and Isolation and Characterization of Clones

Genomic DNA was isolated from cultured fibroblasts of Ashkenazi Jewish patients with classic Tay-Sachs disease, GM515, and used to construct a library in vector YEMBL3 (Promega Biotec) as described (7). Recombinant clones were screened with a cDNA clone that encodes the entire α-chain for β-hexosaminidase, pβHex-5 (8). The probe was labeled with [α-32P]dCTP (6000 Ci/mmol, Du Pont-New England Nuclear) by the random primer method (9). Positive clones were purified and assigned to their appropriate location on the α-chain gene map (10) by analyses as previously reported (7). α-Chain inserts or portions thereof were transferred into pTZ-18 vectors by standard methods (11), and plasmids so generated were purified as described (12, 13).

Amplification of Specific Genomic DNA Sequences

Genomic DNA isolated from either cultured cells (11), whole blood, or leukocyte pellets (14) was used (1 μg) as the initial template in the polymerase chain reaction (15) to amplify a segment of exon 11 encompassing the insertion defect. The reaction was carried out for 27 cycles utilizing two 23-base primers, 5'-GTTGCTGGAGAGAGTATTCAGT-3' and 5'-TTCAAATGCCCAGGGTTTACACTA-3' (Midland Certified Reagent Co.) and TaqI polymerase along with a DNA amplification kit (Gene Amp, Perkin-Elmer Cetus) as described by the manufacturer with the exception that the annealing step was performed at 55 °C. Following amplification, one-tenth of each sample was electrophoresed in a low-melting agarose (4% NuSieve, FMC Corp.) and stained with ethidium bromide to assay for reaction specificity and yield.

DNA Sequencing

Plasmids—Plasmid DNA was used as template in the sequencing reaction (16) with dATP (500 Ci/mmol) and either avian myeloblastosis virus reverse transcriptase (Promega Biotec) or sequenase (United States Biochemicals) as described by the manufacturer. α-Chain exon-specific oligomers (18 or 19 bases, prepared by the Midland Certified Reagent Co.) were utilized as primers without purification.

Amplified Genomic DNA—Amplified DNA was freed of deoxyribonucleotides and salts by spin dialysis of the sample in a Centricon 30 (Amicon Corp.) microconcentrator as described (17). Direct sequencing of the sample was performed utilizing a sequencing primer, 5'-GGAAAAGTTGACTATATGAAAGGC-3' end-labeled with [γ-32P]dATP (6000 Ci/mmol) and sequencing reaction (16) with dATP (500 Ci/mmol) and either avian myeloblastosis virus reverse transcriptase (Promega Biotec) or sequenase (United States Biochemicals) as described by the manufacturer. α-Chain exon-specific oligomers (18 or 19 bases, prepared by the Midland Certified Reagent Co.) were utilized as primers without purification.

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mmol), and avian myeloblastosis virus reverse transcriptase by the method of Saiki et al. (18).

Dot Blot Assay for a-Chain Insertion Defect

One-twentieth of each amplified DNA sample was applied in duplicate to a Biotrans nylon membrane (0.2 μm, ICN), desatured, and fixed to the membrane according to the dot blot protocol supplied by the manufacturer. One set of samples was hybridized to mutant probe (5'-GAGCCTATATTCTCCTG-3') with a sequence complementary to that found in mutant exon 11. The other set of samples was hybridized to normal probe (5'-GAGCCTATATTCTCCTGGC-3') with a sequence complementary to that found in normal exon 11. Both probes were end-labeled to a specific activity of 5 × 10^6 cpm/μg with [γ-32P]ATP (6000 Ci/mmol). The blots were washed as described (3) and exposed to x-ray film at −70 °C in the presence of a Cronex intensifying screen.

RESULTS AND DISCUSSION

Although previous studies (3, 4) did not exclude the possibility that a multiplicity of defects underlies classic Tay-Sachs disease in the Ashkenazi Jewish population, we began our search by assuming that in addition to the splice junction defect only one other lesion was present in this ethnic group. The mutant a-chain gene of an Ashkenazi Jewish patient, GM515, was selected for isolation and study because it was negative for the splice junction mutation (3) and therefore presumably homozygous for the second unknown defect. A genomic library was constructed in λEMBL3 with DNA isolated from fibroblast cultures of GM515 and screened for the mutant a-chain gene by using a-chain cDNA, ρβHa-a-5, as probe (8). Clones spanning the entire 40-kilobase a-chain mutant gene were isolated. GM515 as well as fibroblasts from other Ashkenazi patients was previously shown to have an intact a-chain gene by Southern analysis (19, 20) and a deficiency of a-chain mRNA by Northern analysis (20–22). Therefore we hypothesized that a subtle defect affecting transcription or RNA processing would be likely and sequenced the promoter region, all of the 14 exons including the splice junction regions, and the polyadenylation signal area. Comparison of these sequences to those of the corresponding region in the normal a-chain gene revealed only one difference, a 4-base pair insertion (5'-TATC-3') in exon 11 (Fig. 1) identical in sequence to the four bases preceding it. The insertion causes a shift in the reading frame which results in a nonsense mutation 9 base pairs preceding the insertion (Fig. 1).

We developed an assay to screen for the 4-base pair insertion in other Ashkenazi Jewish patients and carriers. A 113-base pair region of exon 11 encompassing the insertion is amplified from genomic DNA samples by the polymerase chain reaction (15). A fraction of the amplified product is applied in duplicate to a nylon membrane; one set of samples is hybridized to an oligonucleotide probe with a sequence specific for normal exon 11, the other set to a probe with a sequence specific for mutant exon 11. DNA samples from 20 normal Ashkenazi controls were negative for the insertion mutation. Fourteen of the twenty obligate heterozygote carriers screened were positive for this defect. These insertion-positive carriers were negative for the splice junction mutation. This result was anticipated because a carrier can have only one mutant allele. Similarly, those carriers that were negative for the insertion were positive for the splice junction mutation. A family which had tested negative for the splice junction lesion (3) was evaluated for the insertion defect. Both alleles of the affected child, R.B., harbor the insertion defect while the father, B.B., and the mother, T.B., both have one normal allele and one bearing the insertion (Fig. 2). In a family where the affected child, GM2968, demonstrated the splice junction mutation in only one allele inherited from the father, GM3051, we predicted the presence of an unknown mutation in the other allele which must have been inherited from the mother, GM3052 (3). Indeed, the child and the mother bear the insertion defect in one allele. Both alleles from the father and one each from mother and child exhibit normal exon 11 (Fig. 2). This dot blot assay is simple and rapid and may be useful clinically in conjunction with the splice junction assay for heterozygote screening when the enzymatic test is indeterminant.

Examination of GM515, the cell strain of the Ashkenazi Tay-Sachs patient originally used to clone the mutant gene, gave a surprising result. Because it was negative for the splice junction defect, we presumed that it would be homozygous for the insertion. But our assay shows that only one allele in GM515 bears the insertion (Fig. 2). Since GM515 is an affected patient and must have two mutant a-chain alleles, we predict the presence of a third a-chain defect in this patient, different from the splice junction and insertion defects. The third mutation must be present in very low frequency in the Ashkenazi Jewish population or is a private
family mutation. The parents of GM515 were unavailable for testing.

The region of exon 11 that had been amplified in the genomic DNA samples was sequenced directly. As expected, the sequence found in the amplified sample of an Ashkenazi Jew with noncarrier status is normal (Fig. 3), whereas that observed in an Ashkenazi patient with classic Tay-Sachs, R.B. (homozygous for the α-chain exon 11 defect by the dot blot assay (Fig. 2)), does indeed contain the 4-base pair insertion (Fig. 3). In an obligate heterozygote carrier, B.B. (the father of R.B.), both normal and mutant alleles are equally amplified by the polymerase chain reaction so that sequencing ladders derived from the normal and mutant alleles (Fig. 3).

Because the insertion introduces a premature termination signal, one would have expected to find a truncated α-chain polypeptide (10 kDa shorter than normal) in cultured cells of Ashkenazi patients. In fact, no α-chain polypeptide can be observed by immunological techniques in GM515 or other Ashkenazi patient fibroblasts (23). Rather, the insertion results in a deficiency of α-chain mRNA (19). The thalassemias provide similar examples of nonsense mutations associated with mRNA deficiency (24). The mechanism by which a premature termination codon within an exon affects mRNA levels is unknown but may involve mRNA stability, intranuclear mRNA metabolism (25), or mRNA processing.

The etiology of the high gene frequency of classic Tay-Sachs disease in the Ashkenazi population has been the subject of controversy, i.e., the founder effect versus selective advantage (26, 27). Identification of the major mutant α-chain allele (~70%) and suggestion of a very low-frequency allele in this report, along with the description of the splice junction allele, appear to support the selective advantage theory.

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