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A SPLICING DEFECT DUE TO AN EXON-INTRON JUNCTIONAL MUTATION RESULTS IN ABNORMAL β-HEXOSAMINIDASE α CHAIN mRNAs IN ASHKENAZI JEWISH PATIENTS WITH TAY-SACHS DISEASE*

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SUMMARY: Abnormal β-hexosaminidase α chain mRNAs from an Ashkenazi Jewish patient with the classical infantile Tay-Sachs disease contained intact or truncated intron 12 sequences. Sequence analysis showed a single nucleotide transversion at the 5' donor site of intron 12 from the normal G to C. This provides the first evidence that this junctional mutation, also found independently in two other laboratories by analysis of genomic clones, results in functional abnormality. Analysis with normal and mutant oligonucleotides as probes indicated that our patient was a compound heterozygote with only one allele having the transversion. The patient studied in the other two laboratories was also a compound heterozygote. Another Ashkenazi Jewish patient was normal in this region in both alleles. Thus, the splicing defect is the underlying genetic cause in some but not all Ashkenazi Jewish patients with Tay-Sachs disease.

The classical infantile Tay-Sachs disease prevalent among Ashkenazi Jews is the prototype of human sphingolipidoses [1]. The disease is caused by a genetic deficiency of β-hexosaminidase α subunit [2,3]. The cDNA and the gene encoding the normal human β-hexosaminidase α chain have been isolated and characterized [4-6]. A major deletion in the 5' end of the gene was described for Tay-Sachs disease occurring in the French-Canadian population [7,8]. We identified specific point mutations responsible for the disease within the protein-coding sequence of the gene in two mRNA-positive variants of the disease [9,10]. However, the gene abnormality underlying the classical Ashkenazi Jewish form of the disease remains elusive. Even though the β-hexosaminidase α mRNA is nearly undetectable in this form [11], a diffuse smear is usually visible on Northern analysis, consistent with a recent report of normal transcription of the gene [12]. We isolated a series of abnormal β-hexosaminidase α cDNAs from fibroblasts of a patient and examined their structure.

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MATERIALS AND METHODS

Materials. The fibroblast cell lines obtained from the Human Genetic Mutant Cell Depository, Coriell Institute for Medical Research, Camden, NJ, included three cell lines from infantile Jewish patients (GM0502B, GM2968, GM0515A). Control cell lines were from our own collection. All cultures were maintained in our laboratory under the standardized conditions before use. Bethesda Research Laboratories (Gaithersburg, MD), Boerhinger Mannheim (Indianapolis, IN), International Biotechnologies Inc. (New Haven, CT) and New England Biolab (Beverley, MA) were the main sources for enzymes, reagents and other molecular biological supplies. Radioisotopes were obtained from ICN Radiochemicals (Irvine, CA). Sources for non-standard materials will be indicated below as needed.

Northern Analysis. Poly A+ RNA was prepared from fibroblasts from 100 150-ml flasks [13]. For routine Northern analysis, approximately 1 μg of poly A+ RNA/sample was electrophoresed in 1% agarose/formaldehyde denaturing gel [14]. The amount of RNA was increased to 10 μg/sample to visualize the faint signals in Jewish Tay-Sachs disease samples. Transfer of RNA to BIOTRANS^{TM} Nylon membrane (1.2 μm)(ICN, Irvine, CA), hybridization with a full-length normal β-hexosaminidase α cDNA (pβHα-5)[4] labelled with [α-^{32}P]dATP by nick-translation [15], and subsequent washing were carried out as described by the manufacturer. Blots were exposed to Kodak X-ray film at -70° using a Cronex Hi-Plus intensifying screen. An actin cDNA was used as the control probe.

Isolation of cDNA Clones. The poly A+ RNA fraction was isolated from fibroblasts from an Ashkenazi Jewish patient (GM0502B). Because of the expected low frequency of the β-hexosaminidase α message, a cDNA library was constructed from 10 μg of the mRNA fraction essentially according to the procedure of Gubler and Hoffman [16] but ligated into the lambda gt11 phage through the Eco RI linker. The library was screened, without amplification, with the ^{32}P-labelled normal full-length β-hexosaminidase α cDNA (pβHα-5)[4]. Contrary to our expectation of a low yield, more than 40 positive clones were identified and 31 of them were purified. Several clones were selected for further studies on the basis that they were positive for the probes for the 5' terminus, the middle portion, and the 3' terminus of the normal cDNA . After purification, the inserts were transferred to the pUC-13 plasmid vector.

DNA Sequence Analysis. The DNA sequence analysis was carried out by the Sanger dideoxy chain-termination method [17] with appropriate M13 vectors, the 17-mer sequencing primer, and ^{35}S-labelled dATP [18]. The DNA polymerases used were either the Klenow enzyme or the commercial Sequenase (US Biochem. Corp., Cleveland, Ohio).

Genomic DNA Analysis with Oligonucleotide Probes. In order to examine possible genetic heterogeneity of Tay-Sachs disease in the Ashkenazi Jewish population, two 21-mer oligonucleotide probes with the normal and mutant sequences, centered around the mutation, were synthesized. Genomic DNA was isolated according to the standard procedure [19] and digested with Hpa I and Xba I. Agarose gel electrophoresis, direct drying of the gel, labelling of the probes with multiple ^{32}P-nucleotides, hybridization to the separated DNA, and autoradiography were done essentially as described by Thein and Wallace without modification [20].

RESULTS

Northern Analysis. While β-hexosaminidase α mRNA is nearly undetectable in the Ashkenazi Jewish form of Tay-Sachs disease on the standard Northern analysis, positive signals could be visualized when 10 μg of the mRNA fraction

was applied, or when a probe of very high specific activity or a long exposure time was used (Fig. 1). The signal was usually a diffuse smear ranging from the area substantially larger than the normal β-hexosaminidase α mRNA to the area of the normal α chain message. Positive signals of the abnormally large sizes in the area of 3 kb were not obvious in controls even when 10 μg of mRNA was applied, suggesting that they might represent abnormally large and unstable mRNA produced by the Tay-Sachs fibroblasts.

Structure of cDNAs. Restriction mapping of three cDNA clones obtained from the unamplified cDNA library indicated that they contained an abnormal segment of several hundred bases not present in the normal full-length cDNA. Its location was between the downstream Kpn I and the unique Hinc II sites and contained several restriction sites (Stu I, Pvu II, Hinc II, Sau3A I, Apa I)(Fig. 2). The normal full-length cDNA did not hybridize to the Pvu II-Apa I

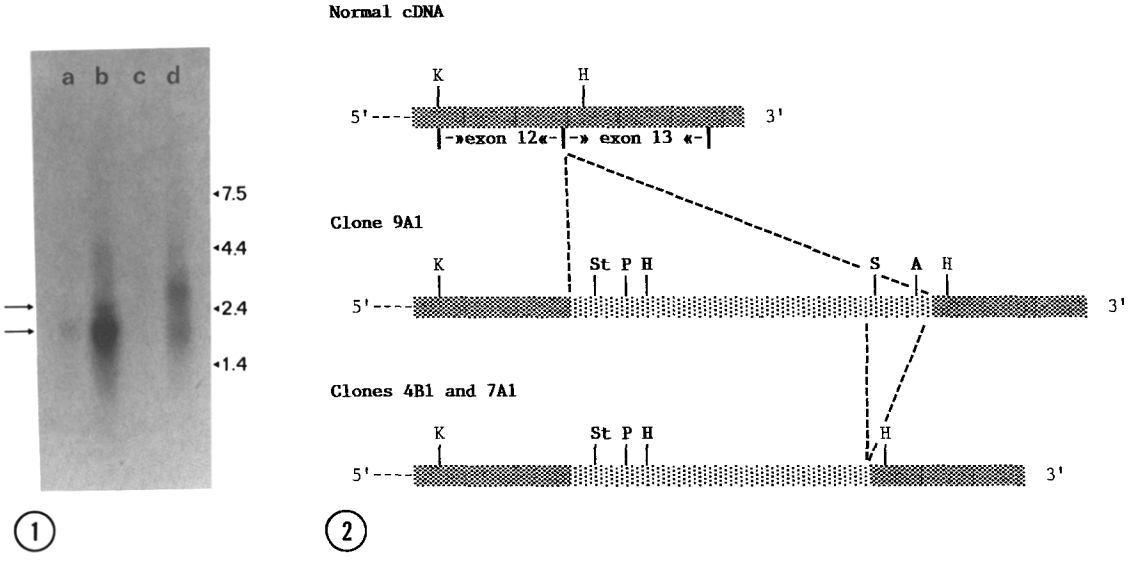

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Fig. 1. Northern blotting analysis of β-hexosaminidase α mRNA from normal and Tay-Sachs fibroblasts. Technical details are described in the text. Lane a: normal fibroblast poly A+ RNA fraction, 1 μg, Lane b: normal, 10 μg, Lane c: Tay-Sachs fibroblasts (GM0502B) poly A+ RNA fraction, 1 μg, Lane d: Tay-Sachs, 10 μg. Signal is essentially undetectable in the Tay-Sachs disease sample at the 1 μg level but a diffuse signal is clearly visible at the 10 μg level. The numbers indicate the positions of the standard poly(A)-tailed RNA molecular size ladders. The two arrows indicate positions of normal β-hexosaminidase α chain mRNA. The larger of the normal mRNA species is due to an alternate poly A site and constitutes approximately 5% of total in human fibroblasts. The size distribution is abnormal in the Tay-Sachs samples.

Fig. 2. Restriction maps of abnormal β-hexosaminidase α cDNAs isolated from a Ashkenazi Jewish Tay-Sachs disease. Compared to the normal sequence, three clones contained an extra segment (light shaded bar) located between the Kpn I in exon 12 and Hinc II in exon 13 in the normal cDNA. The abnormal segment appeared shorter in clones 7A1 and 4B1 at the 3' end, missing the Sau3A I and Apa I sites. Names of restriction sites are abbreviated: K, Kpn I; H, Hinc II; St, Stu I; P, Pvu II; S, Sau3A I; A, Apa I. Sequence analysis indicated that the extra segment is intron 12, intact in Clone 9A1 and truncated in Clones 4B1 and 7A1.

restriction fragment within this segment. Since the downstream Kpn I site is at the 5' end of exon 12 and the unique Hinc II site is within exon 13, it was plausible that this abnormal segment might be intron 12. Sequence analysis of the segment between the Kpn I and the unique Hinc II sites showed that the extra segment is indeed intron 12, either intact or truncated at the 3' end (Fig. 3)[6]. No sequence abnormalities were detected around the junction of intron 12 and exon 13 in clone 9A1. However, the first nucleotide at the 5' donor site of intron 12 was consistently C, instead of the normal G. In a recent survey of nearly 1500 exon-intron junctional sequences, the first two nucleotides at the 5' donor site of introns were always GT [21]. Thus, the single-base transversion from G to C at the exon 12-intron 12 junction can explain the retention of intron 12 in these mRNAs.

Genomic DNA Analysis for the Junctional Transversion. Hybridization of the normal and mutant oligonucleotide probes to genomic DNA digests indicated that the cell line we studied (GM0502B) was heterozygous with respect to the transversion at the exon 12-intron 12 junction (Fig. 4). The other allele was normal in this region. The same result was obtained for GM2968. In another cell line from an infantile Ashkenazi Jewish Tay-Sachs patient (GM0515A), both

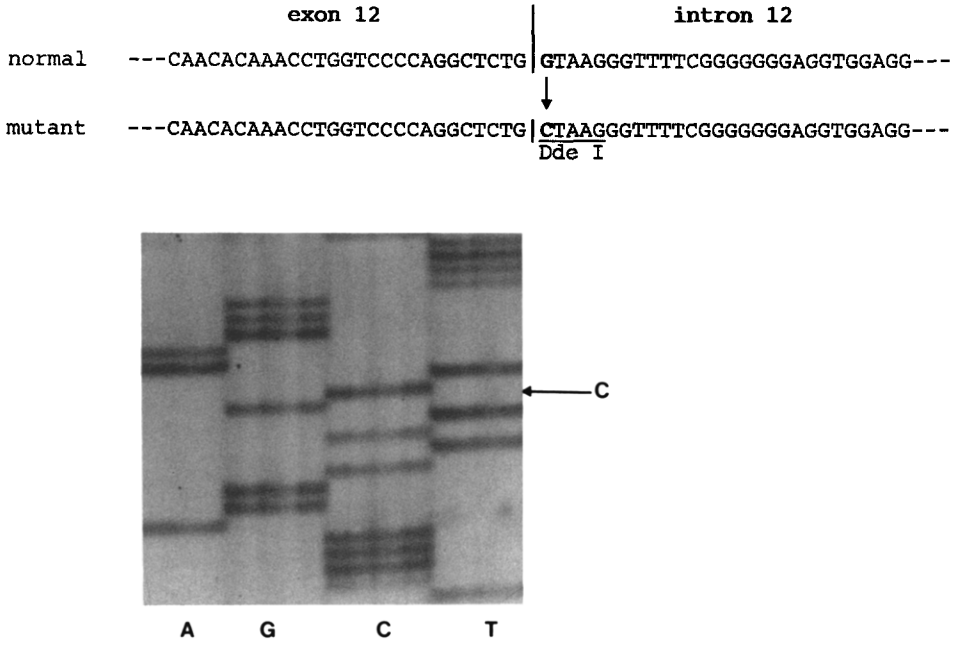

Fig. 3. The exon 12-intron 12 junctional sequence in Tay-Sachs disease. The junction of exon 12 and intron 12 was sequenced in the three abnormal β-hexosaminidase α cDNAs from the Tay-Sachs patient that retained intron 12. In all three clones, there was a single nucleotide transversion at the 5' donor site of intron 12. The top row shows the normal sequence, and the bottom row the mutant sequence. The transversion from the normal G to C is indicated by an arrow. The mutation generates a new Dde I site. The photograph shows the portion of a sequencing gel with the abnormal C indicated by an arrow.

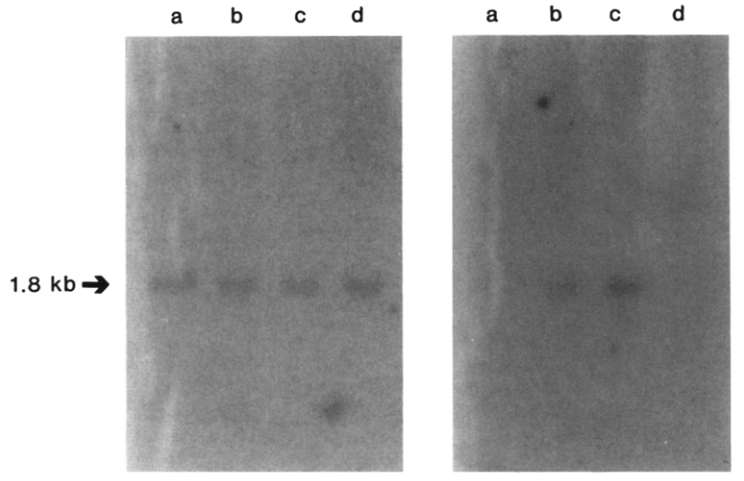

Fig. 4. Analysis of genomic DNA with oligonucleotide probes. Genomic DNA, double-digested with Hpa I and Xba I, was electrophoresed in 1% agarose gel, dried and directly hybridized to labelled probes in 5 x SSC, 0.1% SDS and 100 µg/ml salmon sperm DNA at 59°C [22]. The conditions of differential washing for the matched and single-base mismatched probes were in 6 x SSC at 59°C for 3 min. The double digestion results in a 1.8 kb fragment containing the exon 12-intron 12 junction of the β-hexosaminidase α gene [8]. Left panel, normal probe; right panel, mutant probe. a, normal control; b, GM0502B; c, GM2968; d, GM0515A. Only the normal junctional sequence is present in the control and GM0515A, while both GM0502B and GM2968 are compound heterozygotes giving positive signals for both the normal and the mutant sequences.

alleles were normal in this region. These results indicated that the Ashkenazi Jewish form of infantile Tay-Sachs disease is genetically heterogeneous.

DISCUSSION

In a recent meeting, two groups of investigators independently reported a single-base transversion of G to C at the 5' donor site of intron 12 (R. Gravel, Toronto and R. Myerowitz, NIH)[22]. Coincidentally, these groups studied the same cell line (GM2968). They sequenced isolated genomic clones. In the present study, we have independently found the same junctional mutation by taking an entirely different approach. We had observed that the nearly undetectable smear of the β-hexosaminidase α mRNA signal on Northern analysis of Tay-Sachs fibroblast lines had an abnormal size distribution, including some clearly larger than normal mRNA. We reasoned that these abnormal mRNA should provide insight into the nature of the mutation responsible for the unstable mRNA and thus for the disease. In three cDNA clones obtained from an unamplified library, we found unexcised intron 12 with the single-nucleotide transversion at the 5' donor site. Since the first GT at the 5' donor site of introns appears to be obligatory [21], the transversion is expected to result in splicing failure at the junction. Nevertheless, our findings provide the

first experimental evidence that the junctional mutation in fact results in a series of abnormal and unstable β-hexosaminidase α chain mRNAs. Preliminary results on these and additional cDNAs indicate that the consequences of the splicing defect are complex and not limited to retention of intron 12. Detailed characterization of a series of abnormal β-hexosaminidase α chain mRNA is underway.

The high incidence and the uniform clinical/pathological features of infantile Tay-Sachs disease among Ashkenazi Jews were suggestive of a single genetically homogeneous disorder. The founder effect and other factors have been proposed to explain the unusually high concentration of the disease in a single ethnic group. However, the analysis of genomic DNA with the normal and mutant oligonucleotide probes showed clearly that two patients were compound heterozygotes with only one allele carrying the exon 12-intron 12 junctional mutation and that another patient was normal in this region. Our results are in agreement with those of Myerowitz who, taking advantage of the new Dde I site generated by the mutation (Fig. 3), found only six out of the twenty heterozygous carriers with the junctional mutation [22]. The infantile Tay-Sachs disease among the Ashkenazi Jewish population is thus genetically heterogeneous. The junctional mutation is only one of the two or possibly more different mutations responsible for Ashkenazi Jewish Tay-Sachs disease.

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