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Multiple Abnormal β-Hexosaminidase α Chain mRNAs in a Compound-Heterozygous Ashkenazi Jewish Patient with Tay-Sachs Disease*

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Abnormal β-hexosaminidase α chain cDNA clones were isolated from fibroblasts of an Ashkenazi Jewish patient with Tay-Sachs disease. Four abnormal cDNA clones were sequenced in their entirety. We showed previously that three of these mRNAs retained intron 12 with a mutation from G to C at the 5′ donor site and that the patient was heterozygous with respect to this splicing defect (Ohno, K., and Suzuki, K., (1988) *Biochem. Biophys. Res. Commun.* 153, 463–469). One clone retained, in addition to intron 12, intron 13, which was truncated and polyadenylated due to a polyadenylation signal within intron 13. The fourth clone did not contain intron 12 and was missing exon 12. Some of these abnormal mRNAs were also missing one or more of upstream exons. The regions of exon 12-intron 12 and of upstream exons were evaluated in a total of 30 clones, including those completely sequenced, by restriction mapping and Southern analysis with appropriate probes. Of the 25 cDNA clones that included the exon 12-intron 12 region, 11 contained the exon 12-intron 12 sequence with the junctional transversion, and 11 were missing both exon 12 and intron 12. Among the 12 clones that included the region of exon 3-exon 9, 7 were missing one or more of upstream exons. Three clones gave results expected of normal cDNA in the region of exons 12 and 13. One of the three, furthermore, was 3.6-kilobases long and contained the completely normal β-hexosaminidase α chain mRNA sequence on the 3′ side and an abnormal 1.7-kilobase segment at the 5′ end. These findings suggest that the splicing defect results in either retention of intron 12 or skipping of exon 12 in approximately equal proportions and that remote upstream exons are also frequently excised out. The three clones that were normal in the exon 12-intron 12 region could have derived from the other yet-to-be-characterized mutant allele. However, we were unable to obtain firm evidence that the abnormal upstream sequence is directly related to Tay-Sachs disease.

β-Hexosaminidase (β-*N*-acetylhexosaminidase, EC 3.2.1.52) is a lysosomal hydrolase consisting of two subunits, α and β. Of the two catalytically active isozymes, β-hexosaminidase A is a heterodimer (αβ), while β-hexosaminidase B is a homodimer (ββ). Genetic defects in the α subunit result in loss of β-hexosaminidase A activity, and that of the β subunit abolishes activities of both the A and B isozymes. The classical form of the genetic β-hexosaminidase α chain defect is Tay-Sachs disease that occurs at an unusually high frequency among Ashkenazi Jews (1). Described more than a century ago (2, 3), it is the prototype of the human genetic sphingolipidoses. Affected patients accumulate GM_2-ganglioside in neurons as the consequence of the catalytic deficiency of β-hexosaminidase A (4, 5).

Both cDNA and genomic clones of normal β-hexosaminidase α subunit have been characterized (6–8). A clinically typical but genetically distinct infantile form of α subunit abnormality, occurring among the French-Canadian population, was found to be due to a major deletion at the 5′ end of the β-hexosaminidase α chain gene (9, 10). More recently, point mutations within the coding sequence for the mature α subunit protein have been reported in a patient with an enzymatically unique form of GM_2-gangliosidosis (B1 variant) and in another patient with the "insoluble α chain" variant (11, 12). A splicing defect at the 5′ donor site of intron 12 has been described in some Ashkenazi Jewish patients either by cloning and sequencing of genomic sequences (13, 14) or by isolation and characterization of abnormal cDNAs (15). However, all three reports arrived at the same conclusion that the splicing defect accounts for some but not all infantile Ashkenazi Jewish patients with Tay-Sachs disease. The patient we studied was a compound heterozygote with only one allele carrying the splicing defect (15). In this report we describe in detail the structures of the abnormal cDNAs isolated from fibroblasts of this patient. They indicate highly complex consequences of the splicing defect.

EXPERIMENTAL PROCEDURES

Materials—The fibroblast cell line from the infantile Jewish patient was obtained from the Human Genetic Mutant Cell Depository, Coriell Institute for Medical Research, Camden, NJ (GM0502B). The culture was maintained in our laboratory under the standardized conditions before use. Bethesda Research Laboratories, Boehringer Mannheim, International Biotechnologies Inc. (New Haven, CT), Pharmacia LKB Biotechnology Inc. 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 nonstandard materials will be noted below as needed.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J04178.

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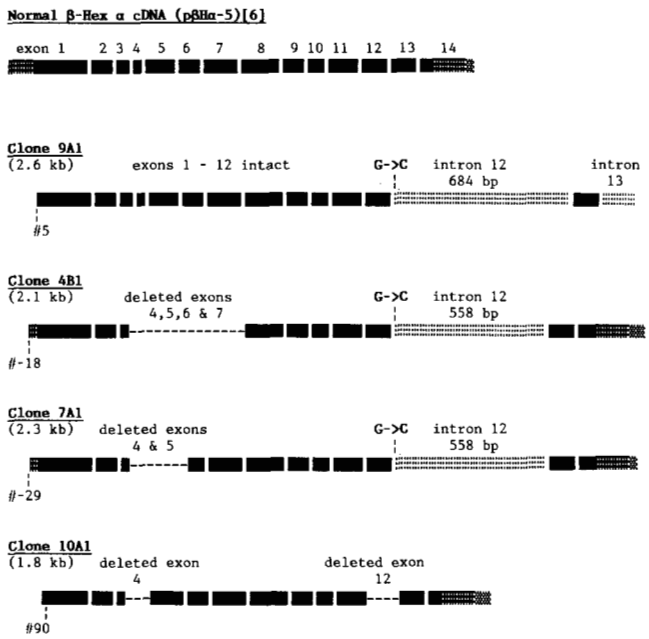

FIG. 1. **Schematic representations of β-hexosaminidase α cDNAs isolated from a Jewish Tay-Sachs patient.** The four clones shown in this figure were sequenced in their entirety. *Dark bars* represent protein-coding sequences, *intermediate bars* the 5′- and 3′-untranslated exon sequences, and *light bars* introns. Details are described in the text. In all of the three clones which retained intron 12, there was a single nucleotide transversion of G to C at the 5′ donor site of intron 12. The 5′ terminus of each clone is indicated by a nucleotide *number under the bar* near the terminus. The A of the initiation codon, ATG, in the normal cDNA is nucleotide 1. Thus, negative numbers indicate that the 5′ terminus of those clones extended beyond the initiation codon. Clone 9A1 was polyadenylated after 216 bp of intron 13 sequence.

Isolation of cDNA Clones—The poly(A)$^+$ RNA fraction was isolated essentially according to Aviv and Leder (16). Because of the very small quantity of β-hexosaminidase α mRNA in these cells (15), a cDNA library was constructed from 10 μg of the poly(A)$^+$ RNA fraction according to a procedure based on that of Gubler and Hoffman (17) and ligated into the λgt11 phage through the *Eco*RI linker. The library was screened, without amplification, with the ^{32}P-labeled normal full-length β-hexosaminidase α cDNA (pβHα-5) (6). More than 40 positive clones were initially identified and 30 of them were eventually purified. Four clones were selected for complete sequencing on the basis that they hybridized to 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. The remaining 26 clones were purified from the λgt11 vector and studied directly without recloning into the plasmid.

DNA Sequence Analysis—The DNA sequence analysis was carried out by the Sanger dideoxy chain-termination method (18) with appropriate M13 vectors, the 17-mer sequencing primer, and ^{35}S-labeled dATP (19). The DNA polymerase used was either the Klenow enzyme or the commercial Sequenase (US Biochemical Corp., Cleveland, OH).

Southern Analysis—Either intact or suitably digested cDNA clones were electrophoresed in 1% agarose or in 3% Nu-Sieve agarose (FMC Bioproducts, Rockland, ME). Transfer of DNA to BIOTRANS® Nylon membrane (1.2 μm) (ICN, Irvine, CA), hybridization with appropriate probes labeled with [α-^{32}P]dATP by nick translation (20), and subsequent washing were carried out as described by the manufacturer. Blots were exposed to Kodak x-ray film at −70 °C using a Cronex Hi-Plus intensifying screen.

RESULTS

Characterization of cDNAs—Considering the very low β-hexosaminidase α mRNA level in these cells (15), the number of positive cDNA clones isolated was unexpectedly large. With the full-length β-hexosaminidase α cDNA as a probe, we usually detect 3–4 positive clones/100,000 from normal fibroblast λgt11 libraries. The number of positive clones from the patient's library was similar to that expected from normal libraries (3/100,000). Four clones, ranging in size from approximately 2.6 to 1.8 kb,[1] were selected for complete sequencing studies. Three of them contained the intron 12 sequence, either intact or truncated and all with the single nucleotide transversion at its 5′ donor site from the normal G to C, as we reported earlier (Fig. 1) (15). The size of the intact intron 12 in clone 9A1 was 684 bp, consistent with the size reported for the normal intron 12 (8). In addition, the downstream sequence of 9A1 showed presence of intron 13 after exon 13 without sequence abnormality at the junction. The clone was terminated prematurely by polyadenylation because of a typical polyadenylation signal (AATAAA) present within intron 13. The intron 12 sequence in the other two cDNA clones (4B1 and 7A1) was truncated at the 3′ end followed by exon 13. The truncation in both clones was at the same site between two Gs of a sequence, AAG | GTA, which satisfies the consensus 5′ splice site sequence for an intron (21). This site must have been used for splicing because the normal 5′ donor site is obliterated by the mutation. These sequence abnormalities of clones, 9A1, 4B1, and 7A1, are shown in detail in Fig. 2. On the other hand, the structure of the fourth clone (10A1) was different from the other three. Not only did it not retain intron 12 but it also lacked exon 12 (Fig. 1). Minor and probably insignificant variations were noted in the polyadenylation sites of these clones from the site reported for the normal β-hexosaminidase α mRNA. In normal human fibroblasts, most of the β-hexosaminidase α mRNA is polyadenylated immediately after nucleotide 1776, while a small proportion is polyadenylated at 2229 (6). The corresponding nucleotide numbers were 1779 for clone 7A1 and 1800 for both clones 4B1 and 10A1.

In addition to these abnormalities, three of the clones were missing substantial segments of upstream sequences away from the exon 12-intron 12 region. These were not random deletions but one to four exons were specifically and contig-

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Clone 9A1  Exon 12 - Inrton 12 (intact) - Exon 13 - Intron 13 - poly A
Clone 4B1  Exon 12 - Intron 12 (truncated) - Exon 13 - Exon 14 - poly A
Clone 7A1  Exon 12 - Intron 12 (truncated) - Exon 13 - Exon 14 - poly A

CCCTGAGCAGAAGGCTCTGGTGATTGGTGGAGAGGCTTGTATGTGGGGAGAATATGTGGACAACACAAACC
TGGTCCCCAGGCTCTG|CTAAGGGTTTTCGGGGGGGAGGTGGAGGGTTGGGCCTGAGAGCAGGAGTTCTCC
CTTAGAGACCCAATCCCATCTAGCCACCTCTGGACTAGTACCCTTCTTTCAGGCCTGAGAGAGAGCAGGCC
GTGCAACGAGCCTTCTGAAGATTTGTGTGCAATATTCACTCTCCACATGACTTCCGTCTATATCCCTCCAG
CTGCCCTTTGGTATGTGGGATAGGGATTGTTAACCTTACTTCCCAGAGAGATAATGAGGCCTGGAGAACAT
AGGTGAGTTGCTCAAGACCCAGCACAAGCATCCATGATTCCTGTAATAAGGCTTCCCTCTGCTGTTTTCAC
TGCAGCCTTACCAAGTATGGTTGGGTGTGCAAAGTTTACATTTTAAGGACCTCTGCTGCTGCCACTGTCAT
TGTGGTTAATGATGGCTTTAAGGTTTATTCTTATTCCCGTATCTTTGAGAGAGGAAGAGAGTGGGATTGCT
ACCCACATTTTAATGAAGGTGGAGCTGAGCCGTAGAACTCTCTGGGAGCCATCCAACCTGGCTGTGGCTCA
TAACAAGGTATTGATCACTTCCTTTGGCCTGAGTGAGTCCAGGGTGCCTAGACAAGAGGTAGCAGCCTGTG
GATGTCCAGCACCTTTGCAGGGAATACAGGGCCCAATCTGGCACATGCCCCTTTTCCTCCAG|GCCCAGAG
CAGGGGCTGTTGCCGAAAGGCTGTGGAGCAACAAGTTGACATCTGACCTGACATTTGCCTATGAACGTTTG
TCACACTTCCGCTGTGAGTTGCTGAG|GTAAGCAAGCTGTGGGGCCTTCGCAAGGCGGAGCAGGCCAGATC
CAGGGCTGGGGAACCCCTTAGAGAGAGGAAGACAATAATTAACAATAGCTAACACTTACAGAGGCTTATAG
TCAGCCCTCATCCACGGATTCAACCAACTGCGATAAAAAGTATTCAGGAAAAAAAAAATACAACAAAAAAT
GGTACAAATAAAAAATACTGTATAACAACTAAAAAAAAAA-
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FIG. 2. **Structures of Clones 9A1, 4B1, and 7A1.** The sequence depicts the complete sequence of Clone 9A1 from exon 12 to the 3′ terminus. The exons 12 and 13 sequences are in *bold typeface* and the intron 12 and 13 sequences are in *normal typeface*. Clone 9A1 not only included intact intron 12 but also intron 13, which was truncated by polyadenylation (poly(A) signal *underlined*). Intron 12 in Clones 4B1 and 7A1 was truncated at the same place. The *underlined* sequence of intron 12 was missing in these clones, apparently spliced out at the 5′ donor site consensus sequence. Clones 4B1 and 7A1 did not contain intron 13, and exon 13 was followed by exon 14 as in the normal cDNA. There was a single-base transversion at the 5′ donor site of intron 12 from the normal G to C in all three clones (*double-underlined*).

[1] The abbreviations used are: kb, kilobase pair(s); bp, base pair(s).

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uously missing (Fig. 1). One clone with the junctional transversion and the retained intron 12 (9A1), however, was completely normal in the 5′ sequence from exon 1 through 12.

Southern Analysis of Other Clones—Five probes were prepared from the normal full-length β-hexosaminidase α cDNA (Fig. 3). They were probe 1 (5′-*Kpn*I), probe 2 (*Hinc*II-3′), probe 3 (5′-*Sac*I), probe 4 (*Sac*I-*Sac*I), and probe 5 (*Sac*I-*Pvu*II). Probe 6 was prepared from Clone 9A1 (*Kpn*I-*Stu*I in intron 12) (Fig. 3). These probes were designed to assess 1) whether or not the exon 12-intron 12 junctional region is present (intact clones and probes 1 and 2), 2) if so, whether the region is normal, contains intron 12 with the junctional transversion, or is missing exon 12 (*Dde*I digestion and probe 6), and 3) whether the upstream exons 3–7 area is normal or abnormal (*Sac*I/*Pvu*II digestion and probes 3, 4, and 5). Of the total of 30 clones, five were too short and did not include the exon 12-intron 12 region. The sizes of *Dde*I fragments from remaining clones that hybridized to exon 12-intron 12 sequences (probe 6) indicated that 11 clones contained unexcised intron 12 with the junctional transversion (similar to Clone 9A1, 4B1, and 7A1), while 11 were missing both exon 12 and intron 12 (similar to Clone 10A1) (Fig. 4, Table I). There were 12 clones in which integrity of the upstream exons could be assessed. Seven of them were missing one or more of upstream exons and five were normal in the region of exons 3–7 (Fig. 5, Table I). Upstream abnormalities occur equally in clones with retained intron 12 or skipped exon 12.

Three clones were normal in the region of exon 12–13. Two of these clones were relatively short but one (15A1) was 3.6-kb long. Furthermore, the probes designed to assess integrity of upstream exons indicated that 15A1 is normal also in the upstream regions. Because of the potential significance of Clone 15A1, it was then sequenced in its entirety. The sequence of the 3′ half was identical with that of the full-length normal cDNA precisely from nucleotide −168 to the 3′ terminus (pβHα-5) (6). The sequence immediately upstream of the normal cDNA sequence, however, was not that of the normal gene (8). This abnormal 1.7-kb upstream sequence could not be identified with any sequence registered in the Genbank. A preliminary series of Southern analysis of genomic DNA from several patients failed to give firm evidence for its association with Tay-Sachs disease. The possibility of this sequence being an unrelated artifact cannot yet be excluded.

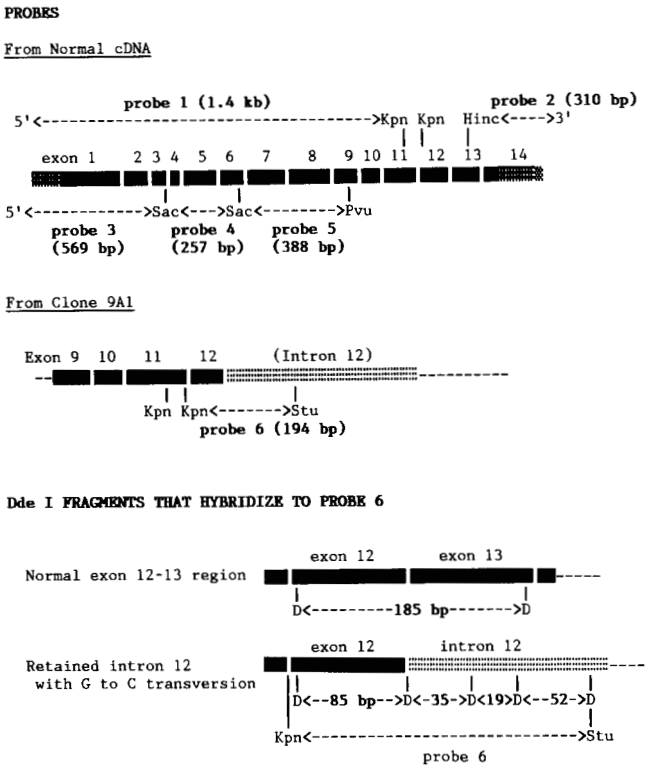

FIG. 3. **Strategies for characterization of cDNA clones.** Either probe 1 or 2 provides size estimate of clones. Positive hybridization of both probes 1 and 2 indicates that the clone includes the region of exons 12 and 13. Then the Southern analysis of *Dde*I-digested clones with probe 6 gives a single 185-bp fragment, if the clone has normal exons 12 and 13 structure. It gives four smaller fragments if intron 12 is retained and if there is the junctional transversion, which generates a new *Dde*I site. In practice, the two smaller fragments (35 and 19 bp) are difficult to visualize. If clones that are long enough to include the exon 12–13 region do not give positive *Dde*I fragments, it indicates absence of exon 12 and intron 12. Hybridization of probes 3–5 to clones double-digested with *Sac*I and *Pvu*II provides information on the presence or absence of exons 4–7. A positive band for probe 3 assures that the clone extends to exon 1–3 region. The normal exon structure gives 257-bp (*probe 4*) and 388-bp (*probe 5*) bands. Then, other sizes of fragments that hybridize to probes 4 and 5 indicate the presence or absence of either or both of the *Sac*I sites, and from the known sizes of the exons, as to which of the upstream exons are missing. The sizes of normal exons are 66 bp (*exon 3*), 47 bp (*exon 4*), 111 bp (*exon 5*), 102 bp (*exon 6*), 133 bp (*exon 7*), 181 bp (*exon 8*), and 87 bp (*exon 9*). The positions of the two *Sac*I sites are 54 bp from the 5′ end of exon 3 and 90 bp from the 5′ end of exon 6, respectively.

DISCUSSION

Three laboratories recently described a single-nucleotide transversion at the 5′ donor site of intron 12 in the β-hexosaminidase α chain in some Ashkenazi Jewish patients with classical infantile Tay-Sachs disease (13–15). Our data indicated that the junctional mutation indeed results in defective splicing of the transcripts (15). The more detailed characterization of the series of abnormal mRNAs as described in this report show that the consequences of the junctional transversion are complex and include abnormalities in apparently unrelated remote upstream sequences.

The abnormalities in the β-hexosaminidase α chain mRNA in this patient can be categorized into three major groups, retention of intron 12, skipping of exon 12, and skipping of one or more of remote exons. The failure to excise intron 12 is the most predictable outcome of the junctional abnormality. In a recent survey of 1446 exon-intron junctions ranging from primates to viruses and plants, the first two nucleotides at the 5′ donor site of introns were invariably GT (21). Even though a few exceptions in which the first two bases were GC have been reported (22–24), we are not aware of normal exon-intron junctions where the first base on the donor side is not G. Once the 5′ donor site is obliterated, the entire intron may remain unexcised. Alternatively, a portion of the intron may be spliced out from a 5′ donor site consensus sequence present within the intron to the normal 3′ end of the intron. At least in one instance, intron 13 was also unexcised but was truncated by polyadenylation due to a polyadenylation signal within intron 13.

The skipping of exon 12 cannot be interpreted definitively because these cDNAs do not give direct evidence for the junctional mutation and because we know that our patient was a compound heterozygote with only one of the two alleles carrying the junctional mutation (15). Thus, we cannot logically exclude the possibility that these cDNAs with skipped exon 12 came from the other allele. We tentatively interpret, however, that they did derive from the allele with the junctional mutation, because skipping of the exon immediately

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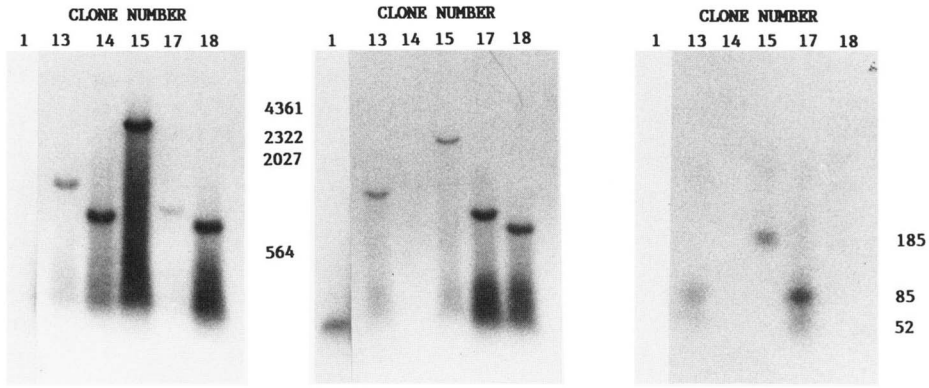

FIG. 4. **Examples of the Southern analysis of the exon 12-intron 12 region.** Clones that gave different results are selected for this figure. Inserts were excised from the λgt11 vector, electrophoresed without digestion in 1% agarose, blotted, and hybridized to probe 1 (*left panel*) or probe 2 (*middle panel*). A clone that includes both the 5′ and 3′ regions of the normal cDNA should give a positive band of the same size for both probes. Then, the inserts were digested by *Dde*I, electrophoresed in 3% Nu-Sieve agarose gel, blotted, and hybridized to probe 6, which spanned the sequence of exon 12 and 5′ end of intron 12 (*right panel*). The *numbers* between the left and middle panels indicate the positions of the size standards from the *Hin*dIII-digested λ phage. The *numbers* of the right panel are estimated sizes of the three visible bands. Absence of positive bands indicates missing exon 12 and intron 12 (skipped exon 12). A single 185-bp band derives from the normal exon 12-exon 13 structure, while two bands of 85 and 52 bp indicate presence of intron 12 with the junctional transversion. Two other smaller fragments (35 and 19 bp) are not visualized in this system. Refer to Fig. 3 and Table I for interpretations.

TABLE I

Summary of abnormalities in the region of exon 12-intron 12 and upstream exons of GM0502B β-Hexosaminidase (β-Hex) α cDNA clones

Refer to Fig. 4 and its legend for descriptions of probes and the criteria for characterization of the regions of exon 12-intron 12 and of upstream exons.

Clone no.	Size	Hybridization with normal Hex α cDNA Fragment		*Dde*I fragments hybridized with 9A1 *Kpn*I-*Stu*I	Exon 12	Intron 12	*Sac*I/*Pvu*II fragments hybridized with			Skipped exons between 3 and 9
		5′-*Kpn*I	*Hin*cII-3′				5′-*Sac*I	*Sac*I-*Sac*I	*Sac*I-*Pvu*II	
	bp									
1	300	−	+	−	(too short)					
2	1500	+	+	85/52	+	+		(too short)		
3	1100	+	+	−	−	−		(too short)		
4 (4B1)	2100	+	+	85/52	+	+	+	−	245	4–7
5	280	−	+	−	(too short)					
6	1700	+	+	85/52	+	+		(too short)		
7 (7A1)	2300	+	+	85/52	+	+	+	92	388	4 and 5
9 (9A1)	2700	+	+	85/52	+	+	+	257	388	None
10 (10A1)	1800	+	+	−	−	−	+	213	388	4
11	550	+	+	185	+	−		(too short)		
13	1600	+	+	85/52	+	+		(too short)		
14	1200	+	−	−	(too short)					
15 (15A1)	3600	+	+	185	+	−	+	257	388	None
17	1300	+	+	85/52	+	+		(too short)		
18	1100	+	+	−	−	−	+	−	630	3–6+
19	400	+	−	−	(too short)					
20	400	−	+	−	(too short)					
21	1300	+	+	−	−	−	+	−	−	4–9+
23	1500	+	+	85/52	+	+		(too short)		
25	1250	+	+	−	−	−	+	−	720	3–6+
27	950	+	+	−	−	−		(too short)		
28	900	+	+	−	−	−		(too short)		
31	1800	+	+	85/52	+	+	+	460	388	3+
32	1350	+	+	85/52	+	+		(too short)		
34	800	+	+	−	−	−		(too short)		
35	1700	+	+	−	−	−	+	257	388	None
37	1000	+	+	185	+	−		(too short)		
41	1200	+	+	−	−	−	+	257	388	None
44	1250	+	+	85/52	+	+		(too short)		
45	1700	+	+	−	−	−	+	257	388	None

preceding a mutation at the intron 5′ donor site has been reported in human phenylketonuria (25), the skipping of exons 3–7 occurred equally in both the intron 12-retaining and the exon 12-missing cDNAs, and because the three other cDNAs that were normal in the exon 12-intron 12 region might well have come from the other allele. Furthermore, both retention of an intron and exclusion of the preceding exon were reported in human adenosine deaminase mRNA even in

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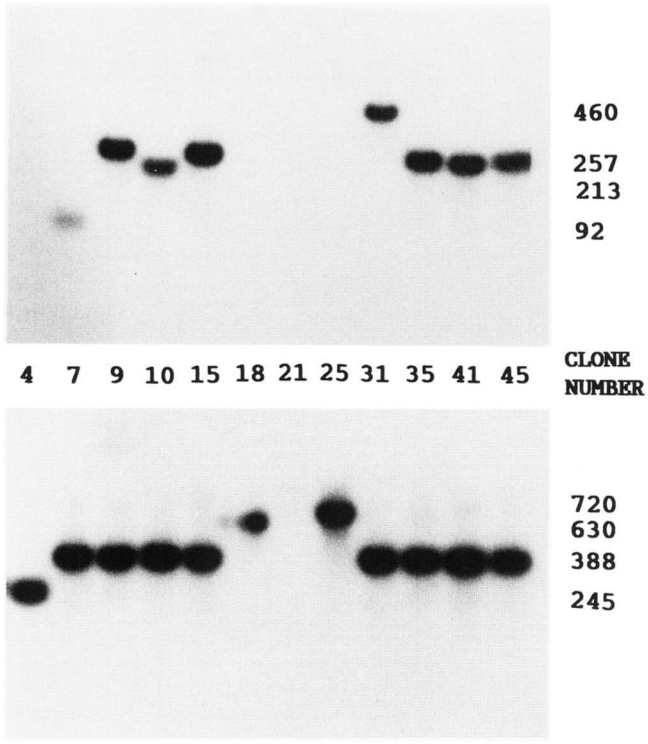

FIG. 5. **Integrity of upstream exons.** The clones that were judged long enough to include the regions of exons 3–9 by their length and positive hybridization to probe 3 were double-digested by *Sac*I and *Pvu*II, electrophoresed in 3% Nu-Sieve agarose gel, blotted, and hybridized to probe 4 (*top panel*) or probe 5 (*bottom panel*). The *numbers* on the right side of the panels are estimated sizes of the fragments. A 257-bp band for probe 4 and a 388-bp band for probe 5 indicate the normal upstream exon structure. Refer to Fig. 3 for criteria for interpretations and Table I for the findings.

the absence of a mutation, when the size of the intron was close to the minimum size required for normal splicing (26). If our interpretation is correct, retention of the intron and skipping of the preceding exon occurs with approximately an equal frequency as the result of the junctional mutation.

Skipping of apparently unrelated upstream exons was found in the majority of the clones both with retained intron 12 and skipped exon 12. This appears to occur without intrinsic abnormalities in the upstream region, because at least one clone (9A1), when sequenced in its entirety, had the junctional mutation and a completely normal upstream exon structure. The number of missing exons ranged from one to four, and possibly more. Although the mechanism with which the upstream exons are excised is not clear, random degradation of unstable mRNA is unlikely, because the missing segments precisely corresponded to individual exons. If these exons are skipped as the result of the junctional mutation, the finding is consistent with splicing taking place after transcription is completed. A remote possibility exists that such mis-splicing occurs normally at a significant frequency but is not detected because the resultant mRNA is unstable, while in the mutant there is an apparent enrichment of such abnormal mRNAs because other mRNA molecules are also unstable due to the junctional mutation. We consider the possibility highly unlikely because, judged from the number of clones isolated from the library, the mutant mRNA may not be as unstable as it appears on the Northern blotting and because a majority (7:11) of the mRNA with the exon 12-intron 12 abnormalities were also missing upstream exons. Since mRNAs with double abnormalities (retained intron 12 and missing upstream exons) would be at least as unstable as those with retained intron 12 only, these findings are inconsistent with missing upstream exons being an apparent enrichment of normally occurring mis-splicing, unless more than half of mRNAs are normally mis-spliced and degraded rapidly to almost undetectable levels.

The three cDNA clones that were normal in the region of exon 12-intron 12 aroused our interest. Although two were too short for further meaningful characterization, one was 3.6-kb long, and its 3′ half was identical with the normal full-length β-hexosaminidase α cDNA. The sequence of the upstream 1.7-kb segment did not match the sequence of the normal gene above exon 1 as published by Proia and Soravia (8). It is tempting to speculate that these clones derived from the other abnormal allele and that the "foreign" upstream sequence might give a clue as to its abnormality. However, a preliminary series of experiments so far have failed to show positive association of this sequence with Tay-Sachs disease. It is equally possible now that it represents an artifact totally unrelated to Tay-Sachs disease or even to β-hexosaminidase α chain. If these mRNAs also came from the allele with the junctional mutation, they suggest that a very small proportion of precursor mRNA is spliced correctly even in the presence of the junctional mutation. Patients homozygous with respect to the splicing defect could answer some of these uncertainties but we have not encountered such patients.

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