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A Novel Mutation in the Invariant AG of the Acceptor Splice Site of Intron 4 of the β-Hexosaminidase α-Subunit Gene in Two Unrelated American Black G_{M2}-Gangliosidosis (Tay-Sachs Disease) Patients

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Summary

Samples of genomic DNA from three unrelated American black infants having both biochemical and clinical features of classical infantile Tay-Sachs disease were sequenced following PCR amplification. A G → T transversion was observed in the AG acceptor splice site preceding exon 5 of the β-hexosaminidase α-subunit gene in the first black family. This transversion changed the acceptor splice site from the consensus sequence, AG, to AT, thereby interfering with splicing at this intron 4/exon 5 junction. The proband was homozygous for this mutation; his mother and a brother are heterozygous. The same mutation was found in a second, apparently unrelated, black G_{M2}-gangliosidosis patient. The second patient was a compound heterozygote, as only one allele carried this mutation. The mother and a brother in this second family are carriers for this mutation, while the father and a noncarrier sister are normal for this region of the gene. The third proband did not have this mutation; nor did the mother of a fourth black proband. Eight other independently ascertained non-black, non-Jewish, G_{M2}-gangliosidosis families did not have this mutation. The observation of the same novel mutation in two unrelated black G_{M2}-gangliosidosis patients indicates that the American black population has segregating within it at least one G_{M2}-gangliosidosis mutation which may be specific to this population and not a result of migration.

Introduction

We are aware of at least seven cases of Tay-Sachs disease in American black families. While each of these patients appears to have both the biochemical and clinical findings of classical infantile Tay-Sachs disease, the exact nature of the mutation(s) has not, until now, been determined. It has, therefore, remained unknown whether the presence of Tay-Sachs disease in the American black population results from mutations arising from racial admixture or is due to the presence of one or more mutations unique to the black population.

We now describe a novel mutation which appears to be specific to the American black population. This mutation is present in two, apparently unrelated, black infantile G_{M2}-gangliosidosis patients and is not present in eight other independently ascertained non-black, non-Jewish families.

Material and Methods

Clinical Summary

Patient 77-620 (family A, III-1; fig. 1) was noted to have developmental delay at age 5 mo, including failure to smile, respond to sounds, or roll over. By 12 mo, he was profoundly mentally retarded with cerebral palsy of the rigid type and was noted to have problems with choking and gagging. A cherry red spot

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Table 1

HEX A Levels in Family A

Member[a] or Category	% Heat-labile HEX A in[b]			Interpretation
	Serum	WBC	Fibroblasts	
I-1	45	52	ND	Inconclusive
I-2	32	38	ND	Carrier TSD
II-1	31	ND	27	Carrier TSD
II-2	33	38	ND	Carrier TSD
II-3	47	ND	ND	Inconclusive
II-4	50	58	ND	Inconclusive
II-5	45	57	ND	Inconclusive
II-6	28	40	ND	Carrier TSD
II-7	51	58	ND	Noncarrier
II-8	34	33	ND	Carrier TSD
II-9	45	58	ND	Inconclusive
II-10	32	38	27	Carrier TSD
III-1	4	ND	6	Affected TSD
III-2	26	ND	39	Carrier TSD
Control noncarriers	51–65	58–70	50–65	Noncarrier
Control inconclusive	44–50	50–57	46–49	Inconclusive
Tay-Sachs carriers	29–43	38–49	25–45	Carrier TSD
Tay-Sachs affected	0–5	0–12	0–12	Affected TSD

[a] Pedigree numbers are as in fig. 1.
[b] ND = not determined.

was observed at 15 mo, at which time his hexosaminidase A (HEX A) enzyme levels were determined to be consistent with the diagnosis of G_{M2}-gangliosidosis (table 1). At the age of 25 mo the patient was admitted to a pediatric hospital because his degenerative condition made feeding difficult, resulting in malnutrition and dehydration. He died at 4 years of age.

Patient 90-269 (family B, II-3) was evaluated at 15 mo for developmental delay, congenital nystagmus, and cherry red spots on the macula, bilaterally. Enzyme analysis at this time was consistent with the diagnosis of G_{M2}-gangliosidosis (M. R. Natowicz, personal communication). At 18 mo, hospital admission was required for evaluation of choking episodes and seizures. General examination was notable for acoustically induced myoclonus and deficient visual fixation and tracking responses. Loss of milestones was noted at 20 mo, with social skills assessed at being at the 10–11-mo level, fine motor skills and language skills at the 10-mo level, and gross motor skills at the 8-mo level. He is now 3½ years old.

Enzyme Analysis

HEX A (4-methylumbelliferyl-N-acetyl-β-D-glucosaminidase) activity was determined according to a method described elsewhere (Thomas et al. 1982).

PCR and Direct Sequencing of PCR Products

Genomic DNA from a panel of various G_{M2}-gangliosidosis cell lines (i.e., cell lines with unknown mutations in the β hexosaminidase α-subunit gene) was examined by amplifying individual α-subunit exons and flanking intron sequences by using the PCR and then directly sequencing the PCR products. Cell lines studied in the two families reported here were fibroblasts from the proband of family A (III-1), his mother (II-1), and putative father; amniocytes from his brother (III-2); and lymphoblasts from the proband of family B (II-3), his mother (I-2), father (I-1), sister (II-1), and brother (II-2) (fig. 1). In addition, both DNA from a third black G_{M2}-gangliosidosis proband (DH) and a lymphoblastic cell line (L90-371) from the mother of a fourth black proband were examined.

The mutation described in the present report was detected by using both a sense PCR primer (primer 1) specific for the 3′ end of intron 3 (5′-GCTACATTG-AGAACCTTCCA-3′) and an antisense PCR primer (primer 2) which spanned both the 3′ end of exon 5 and the adjacent intron 5 sequence (5′-GGTTAC-CAGAGTGTCCAGGA-3′). The PCR was performed according to GeneAmp specifications, and sequencing used an internal primer (primer 3) specific to the 3′ end of intron 4 (5′-TAAGAATCCTGGGAGAGTTG-3′)

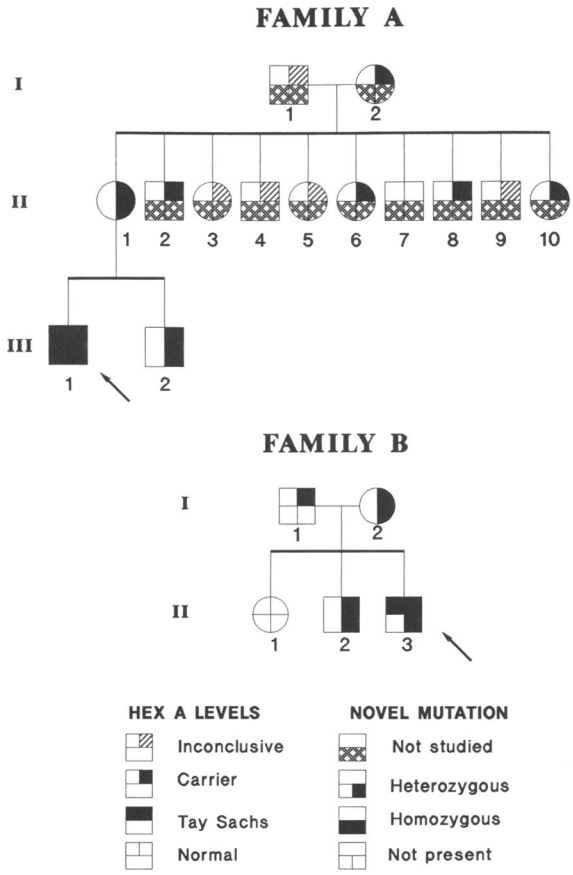

Figure 1 Pedigrees of families A and B. Probands are indicated by arrows.

(as in Wong et al. 1987). To rule out heterozygosity for the Ashkenazi Jewish 4-bp insert in family B and in the mother of the fourth proband (L90-371), exon 11 was amplified (5′-TATAACAGATTCAGCCAG-AC-3′ and 5′-TTTCACCTTCAAATGCCAGG-3′) and subjected to electrophoresis on a 2% Nusieve, 1% agarose minigel. Presence of the insert results in formation of heteroduplexes which can be visualized as a doublet when the gel is stained with ethidium bromide and evaluated under UV light (as in Shore and Myerowitz 1990). The splice-site junction of exon/intron 12 was amplified (5′-CAGGTACCCCTGAG-CAGAA-3′ and 5′-TCCTGCTCTCAGGCCCAAC-3′); digested with *Dde*I, electrophoresed on a 2% Nusieve, 1% agarose minigel; stained with ethidium bromide; and evaluated under UV light to check for the Ashkenazi splice-site mutation which is at the 5′ end of intron 12 and which creates a new *Dde*I site at that location.

Single-Strand Conformation Polymorphism (SSCP) *Analysis for Detection of Mutations*

A 207-bp DNA fragment was amplified by using ^{32}P-labeled primers flanking exon 5 (primers 3 and 2) and was diluted, denatured, and electrophoresed on a 6% acrylamide, 10% glycerol gel (as in Orita et al. 1989).

DNA Polymorphism Analysis for Paternity Testing

Two different DNA polymorphisms due to $(GT)_n$ dinucleotide repeats (Litt and Luty 1989; Tautz 1989; Weber and May 1989) were studied by using PCR. These polymorphisms were a $(GT)_n$ repeat in IVS-5 of the HMG14 gene (HMG14-GT2) on human chromosome 21 (Landsman et al. 1989; Petersen et al. 1991) and a $(GT)_n$ repeat of locus D21S156 (Lewis et al. 1990). These VNDR (variable number of dinucleotide repeat) polymorphisms are highly informative, with 10 and 12 different alleles and with observed heterozygosity (in the 40 CEPH families) of 74% and 76%, respectively. PCR programs, primers, end-labeling of primer, PAGE of reaction products, and autoradiography were done according to methods described elsewhere (Lewis et al. 1990; Petersen et al. 1991).

Results

Enzyme Analysis

Serum and fibroblast HEX A analysis of family A is presented in the table and illustrated in figure 1. No paternal relatives were available for testing. Plasma, leukocyte, and fibroblast analysis of the proband of family B (II-3) revealed absence of HEX A activity, consistent with G_{M2}-gangliosidosis (M. R. Natowicz, personal communication). Analysis of his, his parents', and his siblings' HEX A activity was also performed on transformed lymphoblasts (fig. 1).

Sequence of Normal and Mutant Alleles at Intron 4 Acceptor Splice Site

The intron 4/exon 5 DNA sequence of the proband of family A (III-1) revealed homozygosity for a G → T transversion in the acceptor splice site of intron 4 (fig. 2*A*), changing the invariant acceptor splice site, AG, to AT (fig. 2*B*). The proband's mother (II-1) and sib (III-2) were both shown to be heterozygous at this location. The DNA sequence of the proband's putative father was normal in this region. However, DNA polymorphism analysis with HMG14-GT2 and D21S156 failed to show inheritance of DNA from

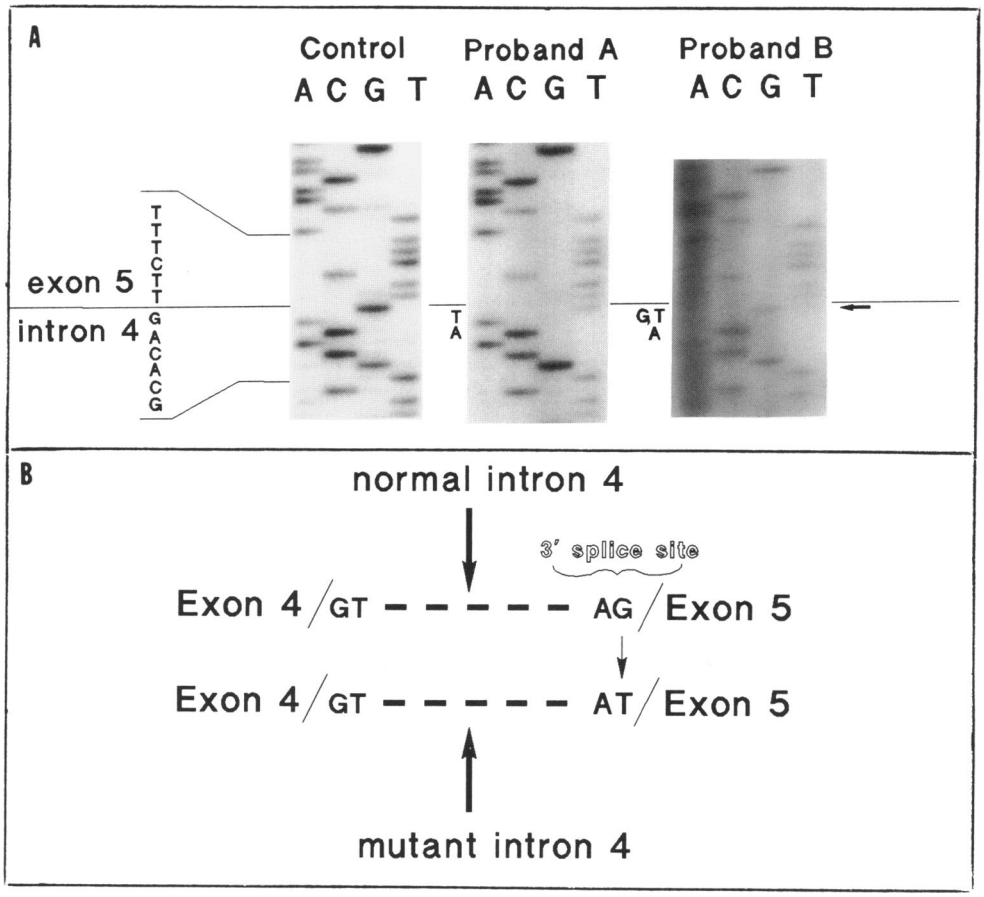

Figure 2 Sequence analysis of black G_{M2}-gangliosidosis mutation. *A*, Sequences from amplified PCR products comprise 3′ end of intron 4 and 5′ end of exon 5. The letters represent the sense strand and show the region with the base transversion. Proband A is homozygous for this mutation, showing a band only in the T lane at the 3′ boundary of the intron. Proband B is a compound heterozygote for this mutation, showing a band in the G lane and the T lane at this location. *B*, Diagram indicating G → T transversion in acceptor splice site of intron 4.

the putative father by the proband (data not shown), indicating nonpaternity.

The same G → T transversion at nucleotide position −1 of the intron 4/exon 5 splice site was observed in the proband of family B (II-3). However, in this patient a G band was also noted at the same position, indicating heterozygosity for this mutation. The proband's mother (I-2) and carrier brother (II-2) were also noted to be heterozygous for this base change; his noncarrier sister's (II-1) and his father's (I-1) DNA were normal in this region. Analysis of a third black proband (DH) and of the mother of a fourth black proband (L90-371) revealed normal DNA sequence for this region of intron 4 and exon 5.

To rule out the possibility that the unknown mutation(s) in family B and in L90-371 was one of the two major Ashkenazi Jewish mutations, exons 11 and 12 were examined as described above. No evidence for either mutation was found. Presence of either mutation in DH was ruled out in the laboratory of Eugene Grebner (personal communication).

SSCP Analysis

SSCP analysis of exon 5 and of the preceding intron 4 sequence revealed a band shift in patients with family members having the G → T transversion, indicating that this method can be utilized as a means of rapid screening for this mutation.

Discussion

A new mutation, a G → T transversion in the 3′ acceptor splice site of intron 4 of the β-hexosaminidase α-subunit gene, has been found in two independently

ascertained, apparently unrelated black G_{M2}-gangliosidosis patients. One proband was homozygous for this mutation, while the other was a compound heterozygote for this mutation and another, as yet undefined, mutation. Both probands were ascertained on the basis of clinical signs indistinguishable from those of classical, infantile G_{M2}-gangliosidosis. Segregation of this mutation with carrier status in these two families is compatible with the interpretation that this splice-site mutation is responsible for deficient enzyme activity.

The AG-to-AT transversion found in these individuals is yet another mutation that can result in classical Tay-Sachs disease. The fact that it was found in two apparently unrelated black patients (representing 3/7 black Tay-Sachs alleles tested)—but neither in eight unrelated non-Jewish non-black G_{M2}-gangliosidosis families nor in the literature—suggests that this mutation may be more common in, if not unique to, the American black population. Compound heterozygosity in the proband of family B, along with absence of this mutation in both the other black proband (DH) and the mother of a fourth proband (L90-371), indicates that this is not the only mutation present in this population. The frequencies of both this newly defined mutation and the other(s) must be quite low, however, as the occurrence of G_{M2}-gangliosidosis in American blacks is rare; we are aware of only seven black probands. The incidence of this disease in African blacks is unknown.

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