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Six Novel Deleterious and Three Neutral Mutations in the Gene Encoding the α-Subunit of Hexosaminidase A in Non-Jewish Individuals

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Summary

Initial investigations demonstrated that only 3/34 "Tay-Sachs chromosomes" in 22 unrelated, non-Jewish patients or carriers of some form of G_{M2}-gangliosidosis (7 black and 15 non-Jewish Caucasian) had either of the two mutations commonly found in the Jewish population. To determine the nature and incidence of the alterations in this non-Jewish population we have utilized PCR, single-strand conformation polymorphism analysis and sequencing to detect new mutations in genomic DNA. Fourteen primer sets have been utilized to analyze 80% of the coding region and 23/26 splice sites of the gene coding for the alpha chain of hexosaminidase A. Presumed deleterious mutations were discovered in 17/34 chromosomes believed to be carrying a β-hexosaminidase A α-subunit gene mutation. Ten had abnormalities which have been described previously. In the remaining 24 Tay-Sachs disease alleles, six novel mutations predicted to be deleterious were discovered. These include two small deletions (a single-base frameshift and a three-base deletion removing an amino acid), two different nonsense mutations, an initiation codon mutation (ATG→GTG), and a missense mutation (Arg499Cys) in a highly conserved residue. In addition, three presumed nondeleterious mutations were found.

Introduction

Defects in the α-subunit of β-hexosaminidase A (Hex-A) are responsible for classical Tay-Sachs disease (TSD), as well as for later-onset and biochemically variant (i.e., juvenile/adult-onset and B1 variant) G_{M2}-gangliosidoses. The heterozygote frequency of these disorders in the Ashkenazi Jewish population (1/30–1/40) is approximately 10 times that in the general non-Jewish population. Previous investigations have shown that two mutations—a 4-bp insert in exon 11 and a splice-site mutation (G→C) at the 5′ end of intron 12—in the Hex-A α-subunit gene (HEXA) are responsible for most cases of TSD in the Ashkenazi Jewish population (Arpaia et al. 1988; Myerowitz 1988; Myerowitz and Costigan 1988; Ohno and Suzuki 1988*b*; Paw et al. 1990*b*; Triggs-Raine et al. 1990; Grebner and Tomczak 1991). This study was undertaken to determine what proportion of TSD (or related variants) in the non-Jewish population is accounted for by these two mutations and, if present, the nature and distribution of additional mutations.

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Materials and Methods

Thirty-four alleles believed to harbor mutations in HEXA from a group of non-Jewish individuals were evaluated (table 1). Cell lines from 12 non-Jewish probands with various forms of G_{M2}-gangliosidosis accounted for 24/34 non-Jewish alleles, while 6 alleles were from non-Jewish obligate heterozygotes. The remaining 4 were from "carriers" ascertained by enzyme analysis only.

Primer sets were designed to amplify by PCR the 14 exons and most flanking intron sequences of HEXA

Table 1

Phenotypes of Non-Jewish Individuals Studied

Clinical/Biochemical Phenotype	No. of Individuals	No. of Mutant Alleles
Classical TSD probands	10	20
Obligate carriers of classic TSD	4	4
B1 variant TSD proband	1	2
Obligate carriers of B1 variant TSD	2	2
Adult-onset TSD proband	1	2
Carriers by enzyme assay	4	4
Total	22	34

(table 2). The PCR was performed according to GeneAmp specifications. Initial denaturation was carried out at 98°C for 6 min, followed by 30 cycles, each of which comprised 98°C denaturation (30 s), 55°C annealing (30 s), and 72°C extension (30 s, for PCR products <500 bp, or 1 min, for PCR products >500 bp).

Single-strand conformation polymorphism (SSCP) analysis was performed by using ^{32}P-labeled primers or incorporation of ^{35}S during PCR amplification, followed by dilution, denaturation, and electrophoresis on a 6% acrylamide, 10% glycerol gel (Orita et al. 1989). Exons exhibiting band shifts on SSCP analysis were amplified and sequenced using the PCR primers (as in Wong et al. 1987). A few regions that remained ambiguous were resolved by cycle sequencing with *Taq* polymerase (Perkin Elmer Cetus) and by the incorporation of dye-labeled dideoxynucleotide terminators on an ABI model 373 fluorescent DNA sequencer (Applied Biosystems).

Screening for the two common Ashkenazi Jewish TSD alleles was performed using PCR primers and methods described by Mules et al. (1991). PCR of exon 11 4-bp insert heterozygotes results in heteroduplex formation which is visualized when the PCR product is subjected to electrophoresis on a 2% Nusieve, 1% agarose minigel. Homozygotes for this mutation were detected by SSCP analysis. The Ashkenazi Jewish intron 12 splice-site mutation, which creates a new *Dde*I site, was evaluated by *Dde*I digestion of the exon 12 PCR product.

To rule out the presence of the French-Canadian 7.6-kb deletion at the 5′ end of the α-subunit gene (Myerowitz and Hogikyan 1987), Southern blots were prepared. Genomic DNA was digested with *Eco*RI, was electrophoresed on a 0.8% agarose gel, was transferred to a nylon hybridization transfer membrane (GeneScreen Plus; Dupont), and was hybridized to a ^{32}P-labeled, 300-bp probe, the sequence of which is outside the deletion in intron 1 (courtesy of R. Myerowitz).

The genomic DNA of 36 unrelated American black sickle-cell anemia carriers or patients (courtesy of C.

Table 2

PCR Primers for Hex-A Alpha-Subunit

Exon	5′ Primer[a]	3′ Primer[a]	Size of PCR Product (bp)
1	CGTGATTCGCCGATAAGTCA	TCCGACTCACCTGTGAGGTA[b]	352
2	TGTGAGCTGAGGGCTAGAGC	CCAGGCCATCCAGAGTTACA	253
3	TCTGGTCTATAATCTGAGAA	TTGCAGTGAGCAGGGACTGG	227
4	GCTACATTGAGAACCTTCCA	ACAGTGATTCCAAACAGACC	314
5	TAAGAATCCTGGGAGAGTTG	GGTTACCAGAGTGTCCAGGA[b]	207
6	TGAGAGCTGAGGCAGGTGAA	AACTGGCTGGTTAGGATGAG	329
7	GCATCTTCTACTCTGCTAGC	AAGCTTCACTCTGAGCATAA	252
8	GACACTCATATGGGGTTTTC	GAGTAAGCAACTGATCAGGC	260
9	CAGGCATTAGGCTTTCAGGA	GGCCTGACTCGGTATGGAAA	223
10	CAGTCTAGAACCCATCAGAG	ACTGCTGGTGGCTTCTTCTC	170
11	ACTGCCATTTGACCTTTTTA	CCATCCTGTGGCCCAACCCA	268
12	GAAACAACTTAGCTGGGGTG	TCCTGCTCTCAGGCCCAAC	239
13	TGTGGATGTCCAGCACCTTT	CTCAGCAACTCACAGCGGAA[b]	171
14	TGACTGGTGTGAAAAGTGTTGCTG[c]	AGGGAGGTGGATGAGTATGC	690

[a] Sequences flank exons unless otherwise noted and are given 5′ to 3′.

[b] Spans exon and intron; therefore splice site and portion of exon are not examined.

[c] Courtesy of B. Triggs-Raine.

Boehm) was analyzed by SSCP for frequency of the presumed nondeleterious mutations in exon 11. The PCR primers used were the same used to screen for the Ashkenazi Jewish 4-bp insert (Mules et al. 1991).

Results

We have evaluated approximately 80% of the coding sequence and 23/26 splice sites in 34 presumed mutant Hex-A α-subunit alleles from non-Jewish individuals. Mutations associated with deficient Hex-A enzyme activity were detected in 17/34 alleles.

Previously Described Mutations

Ten of these 17 alleles carried mutations described elsewhere (table 3). The 4-bp insert in exon 11, associated with approximately 70% of Ashkenazi Jewish TSD, accounted for three alleles (table 3, number 1). The G→A(805) transition in exon 7, associated with adult-onset TSD in Ashkenazi Jews and non-Jews, accounted for one allele (Navon et al. 1990; table 3, number 2). An acceptor splice-site mutation in intron 4 was present in three alleles of two American blacks (table 3, number 4), described in detail by Mules et al. (1991). Two alleles carried the B1 variant mutation, G→A(533) in exon 5 (Tanaka et al. 1990; table 3, number 3). The three-base in-frame deletion (amino acid 304 or 305) in exon 8, reported in the Moroccan Jewish population (Navon and Proia 1991) and recently in several French TSD families (Akli et al. 1991), was detected in one allele of an individual with Irish and French Catholic origins (table 3, number 5). Neither the Ashkenazi Jewish intron 12 splice-site mutation nor the French-Canadian 7.6-kb deletion was detected in these subjects.

Novel Presumed Deleterious Mutations

Seven of the remaining 24 alleles carried mutations which are believed to be deleterious and which have not been described elsewhere (table 4). Two of these mutations were found in American black individuals. One of 12 alleles from American blacks (five black classic TSD probands, one black obligate carrier, and one black carrier established by enzyme analysis) was an A→G transition, in exon 1, that changed the initiating methionine to valine (table 4, number 1; fig. 1*A*). A second new mutation found in the black population, a G deletion at bp 436 of exon 4, creates a frameshift

Table 3

Previously Published Mutations Found in Present Study

Number	Mutation	Phenotype of Individual	Ethnic Origin(s)	Alleles with Mutation	Cell Line	Reference(s)
1	4-bp insert, exon 11	Classic TSD	American black	2	TC90-2156	Myerowitz and Costigan 1988
		Classic TSD	French-Canadian and German	1	SR	
2	G→A(805), exon 7	Adult-onset TSD	?Slavic, Irish, English, and Polish	1	L89-23[a]	Navon and Proia 1989; Paw et al. 1989; Navon et al. 1990
3	G→A(533), exon 5	B1 variant TSD	Hungarian	1	TC86-1522[b]	Ohno and Suzuki 1988*a*; Tanaka et al. 1988
		Obligate carrier (B1 variant proband)	Irish	1	L90-497[c]	Tanaka et al. 1990
4	AG→AT (acceptor splice, intron 4)	Classic TSD	American black	2	TC77-620[d]	Mules et al. 1991
		Classic TSD	American black	1	L90-269[d]	
5	del TTC (amino acid 304 or 305)	Classic TSD	Irish and French	1	TC86-1577	Akli et al. 1991; Navon and Proia 1991
Total				10		

[a] Mutation in this patient originally reported by Navon et al. (1990).
[b] Mutation in this patient originally reported by Tanaka et al. (1990, case 6).
[c] Clinical and biochemical features of proband reported by Charrow et al. (1985).
[d] Mutation in this patient originally reported by Mules et al. (1991).

Table 4

New Presumed Deleterious Mutations

Number	Mutation	Consequence	Phenotype of Individual	Ethnic Origin(s)	Alleles with Mutation	Cell Line
1	ATG→GTG (exon 1)	Initiating Met1Val	Classic TSD	American black	1	L90-269
2	del(G)(436) (exon 4)	Frameshift→Term in exon 6	Classic TSD	American black	2	TC73-267[a]
3	C→T(409) (exon 3)	Arg137Term	Classic TSD	?Irish	1	TC83-1240
4	C→T(1495) (exon 13)	Arg499Cys	Adult-onset TSD	?Slavic, Irish, English, and Polish	1	L89-23
5	G→A(987) (exon 9)	Trp329Term	B1 variant (late onset)	?English and German	1	TC86-1522
6	del(GGA)(amino acid 320 or 321) (exon 8)	del Gly	Obligate carrier (B1 variant proband, late onset)	?Irish	1	L90-496
Total					7	

[a] Same as GM00077 from NIGMS Human Genetic Mutant Cell Repository.

resulting in a termination codon in exon 6. Proband TC73–267 was homozygous for this mutation (table 4, number 2; fig. 1*B*). These two new mutations, plus the previously described intron 4 splice-site mutation found in two unrelated black families (table 3, number 4), as well as the common Ashkenazi 4-bp insert found in one homozygous black proband (table 3, number 1) account for 8/12 alleles from American black individuals investigated.

The four remaining novel presumed deleterious mutations were found in non-Jewish Caucasian individuals. One classical TSD proband (TC83-1240) carried a C→T(409) transition in exon 3 on one allele (table 4, number 3). This nonsense mutation was inherited from her father, who has an Irish surname (fig. 1*C*). (This mutation has been described just recently in one allele of a French TSD patient [Akli et al. 1991].) A previously undescribed C→T(1495) transition in exon 13, changing arginine to cysteine at codon 499, was detected in a non-Jewish adult-onset TSD proband (L89-23) whose mixed ethnic heritage included Slavic, Irish, English, and Polish contributions (table 4, number 4; fig. 1*D*).

A nonsense mutation (G→A(987), exon 9) that would result in premature termination (Trp329Term) was observed in a B1 variant proband (table 4, number 5; fig. 1*E*). This transition was inherited from his mother, whose origins were German and English. In a second B1 variant family, only the parents (obligate carriers) were available for study (fig. 1*F*). The mutation detected in the mother was a 3-bp in-frame deletion of codon 320 or 321 (del(GGA), exon 8), resulting in the deletion of a glycine (table 4, number 6). Both parents were of Irish extraction.

New Variant (?nondeleterious) Alleles

A silent mutation, a C→T transition at bp 9, was identified in exon 1 of an American black TSD obligate heterozygote (L90-371), leaving serine at codon 3 unchanged (table 5, number 1; fig. 1*G*).

Two base changes were detected in exon 11 in several American black families. An A→G(1195) transition was seen in two black families (table 5, number 2). This mutation resulted in an Asn399Asp change and was inherited in the same allele as was the Met1-Val mutation (table 4, number 1) by L90-269 from his father (fig. 1*A*). It was also detected in the obligate carrier, L90-371, who did not have the Met1Val mutation (fig. 1*G*). A G→A(1306) transition, resulting in the replacement of valine (436) with isoleucine, was observed in three black families being analyzed for defective α-subunit genes (table 5, number 3). In the families of TC77-620 and L90-269, this Val436Ile change was inherited in the same allele as was the deleterious intron 4 splice-site mutation (table 3, number 4; fig. 1*A* and *H*). Individual L90-2031, a "carrier" by enzyme assay, was homozygous for this mutation (fig. 1*I*). No other mutation has yet been detected in this individual. To determine the frequency of these amino acid substitutions in the American black popu-

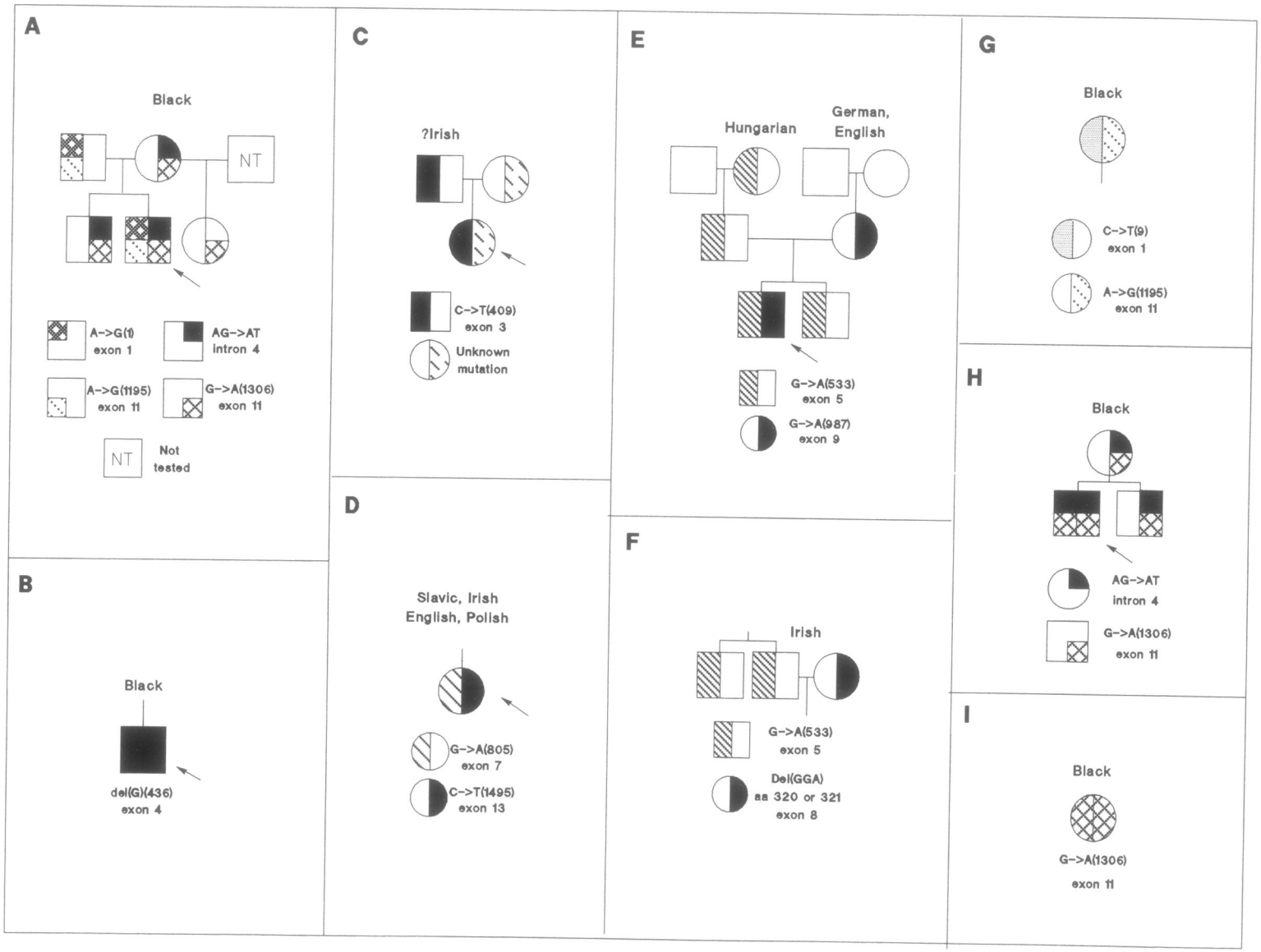

Figure 1 Pedigrees of families with novel mutations. *A*, Family of L90-269. *B*, TC73-267. *C*, Family of TC83-1240. *D*, L89-23. *E*, Family of TC86-1522. *F*, Family of L90-496. *G*, L90-371. *H*, Family of TC77-620. *I*, TC90-2031. Probands are indicated by arrows.

Table 5

New Variant (?nondeleterious) Mutations

Number	Mutation	Consequence	Phenotype of Individual	Ethnic Origin	Alleles with Mutation	Cell Line
1	C→T(9) (exon 1)	Ser3Ser	Obligate carrier (classic TSD proband)	American black	1	L90-371
2	A→G(1195) (exon 11)	Asn399Asp	Classic TSD	American black	1	L90-269
			Obligate carrier (classic TSD proband)	American black	1	L90-371
3	G→A(1306) (exon 11)	Val436Ile	Classic TSD	American black	2	TC77-620
			Classic TSD	American black	1	L90-269
			Carrier, enzyme assay	American black	2	TC90-2031

lation, 72 alleles were evaluated in 36 unrelated black sickle-cell anemia patients or carriers: 40% carried the G→A(Val436Ile) transition, and 10% carried the A→G(Asn399Asp) transition in exon 11, leaving 50% of alleles from black individuals with the exon 11 amino acid sequence previously described in the non-black population.

Discussion

These data indicate that the 4-bp insert in exon 11, which accounts for the majority of Jewish TSD alleles, is responsible for only 3/34 (<9%) TSD alleles in the non-Jewish population. The second most common Ashkenazi Jewish abnormality, the intron 12 splice-site mutation, was not detected in any of these non-Jewish alleles.

While functional assays have not been performed, four of the six presumed "deleterious" mutations described here (table 4) are of types associated with defective translation. Two are termination mutations (C→T(409) [table 4, number 3] and G→A(987) [table 4, number 5]), one a frameshift leading to a termination codon 3′ of the mutation (del(G) (436) [table 4, number 2]) and one a mutation changing the initiating methionine to valine (ATG→GTG [table 4, number 1]).

The two remaining mutations are also believed to be deleterious. The Arg499Cys (table 4, number 4) occurs in a residue which is conserved in the Hex-A β-chain and in β-N-acetylhexosaminidase A, a presumed common ancestor from *Dictyostelium discoideum* (Graham et al. 1988). Moreover, the resulting substitution of cysteine for arginine is likely to result in inappropriate disulfide bonds. The deletion of an amino acid (del(Gly), codon 320 or 321 [table 4, number 6]) also occurs in a conserved residue of the α-subunit. This in-frame codon deletion is similar to (and in the same exon as) the del(Phe), codon 304 or 305, described in the Moroccan Jews and in several French individuals, which has been reported to cause impaired subunit assembly (Akli et al. 1991; Navon and Proia 1991).

Although two of the mutations believed to be "variants" result in amino acid changes, the Asn399Asp and the Val436Ile mutations occur in residues which are not conserved. Furthermore, both are present in the same allele as is a deleterious mutation. These "variants" were also present in individuals lacking the associated deleterious mutations, thus indicating that the deleterious mutations occurred on chromosomes already carrying the "variant" DNA changes. The Val436Ile substitution is common in the American black population (40% of alleles tested), while the Asn399Asp substitution is present in 10% of these alleles. To date, neither has been detected in 22 unrelated nonblack, non-Jewish alleles studied.

Of the 14 mutations in this non-Jewish population, 3 are in CpG dinucleotides (C→T(409), C→T(1495), and G→A(1306)) conforming to the CG hot-spot rule. Twelve additional mutations in CpG dinucleotides of HEXA have been reported: C→T(508), G→A(509), C→T(532), G→A(533), G→A(598), G→A(intron-9+1), C→T(1177), G→A(1444), G→A(1496), del C(1510), C→T(1510), and G→A(1511), confirming that, as in other genes, these sequences are responsible for a large number of mutations (Barker et al. 1984; Cooper and Youssoufian 1988; Nakano et al. 1988, 1990; Koeberl et al. 1989; Lau and Neufeld 1989; Ainsworth and Coulter-Mackie 1990; Paw et al. 1990*a*, 1991; Tanaka et al. 1990; Akli et al. 1991; Fernandes et al. 1991). Two mutations are codon de-

letions within a region of direct trinucleotide repeats (del TTC [amino acid 304 or 305] and del GGA [amino acid 320 or 321]). Slipped mispairing, previously described as a mutational mechanism, may account for these mutations (Farabaugh et al. 1978; Efstratiadis et al. 1980).

In the present study, 11 different deleterious mutations responsible for half (17/34) of the non-Jewish alleles have been identified. These results are consistent with others' recent findings that TSD in the non-Jewish population is a highly heterogeneous genetic disorder.

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