HUMAN MUTATION 9:195–208 (1997)

*MUTATION UPDATE*

# Tay-Sachs Disease-Causing Mutations and Neutral Polymorphisms in the Hex A Gene

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*Communicated by R.G.H. Cotton*

**Tay-Sachs disease is an autosomal recessive disorder affecting the central nervous system. The disorder results from mutations in the gene encoding the α-subunit of β-hexosaminidase A, a lysosomal enzyme composed of α and β polypeptides. Seventy-eight mutations in the Hex A gene have been described and include 65 single base substitutions, one large and 10 small deletions, and two small insertions. Because these mutations cripple the catalytic activity of β-hexosaminidase to varying degrees, Tay-Sachs disease displays clinical heterogeneity. Forty-five of the single base substitutions cause missense mutations; 39 of these are disease causing, three are benign but cause a change in phenotype, and three are neutral polymorphisms. Six nonsense mutations and 14 splice site lesions result from single base substitutions, and all but one of the splice site lesions cause a severe form of Tay-Sachs disease. Eight frameshift mutations arise from six deletion- and two insertion-type lesions. One of these insertions, consisting of four bases within exon 11, is found in 80% of the carriers of Tay-Sachs disease from the Ashkenazi Jewish population, an ethnic group that has a 10-fold higher gene frequency for a severe form of the disorder than the general population. A very large deletion, 7.5 kilobases, including all of exon 1 and portions of DNA upstream and downstream from that exon, is the major mutation found in Tay-Sachs disease carriers from the French Canadian population, a geographic isolate displaying an elevated carrier frequency. Most of the other mutations are confined to single pedigrees. Identification of these mutations has permitted more accurate carrier information, prenatal diagnosis, and disease prognosis. In conjunction with a precise tertiary structure of the enzyme, these mutations could be used to pin insight into the structure–function relationships of the lysosomal enzyme. Hum Mutat 9:195–208, 1997.** © 1997 Wiley-Liss, Inc.

KEY WORDS: **inherited, lysosomal disorder, Hex A, polymorphism, mutations, Tay-Sachs**

## INTRODUCTION

Tay-Sachs disease is a genetic disorder inherited in an autosomal recessive fashion caused by a deficiency of the lysosomal enzyme β-hexosaminidase A (Hex A). The enzyme is composed of two polypeptide chains designated the α- and β-subunits, encoded on chromosomes 15 and 5, respectively. These subunits associate to form the active enzyme that hydrolyzes terminal β-N-acetylglucosamine, β-N-acetylgalactosamine and the corresponding 6-sulfated β-N-acetylglucosaminides from a variety of the water-soluble and water-insoluble substrates, including GM2 ganglioside. In the latter reaction, Hex A requires the assistance of an activator protein to make the lipophilic GM2 ganglioside accessible for hydrolysis in the hydrophilic environment of the lysosome. The β-subunits can dimerize to form another active enzyme species called β-hexosaminidase B (Hex B), which is catalytically active predominantly on water-soluble neutral substrates containing terminal β-N-acetylglucosamine or β-N-acetylgalactosamine. Mutations in the β-subunit result in the genetic disorder known as Sandhoff's disease, characterized by a deficiency of both Hex A and Hex B. A very minor form of the enzyme, β-hexosaminidase S (Hex S), is formed by dimerization of α-subunits whose physiologic function is unclear (for review, see Gravel et al., 1995).

Mutations in the α-chain gene (Hex A) impair the catalytic function only of Hex A, while Hex B remains fully active and capable of degrading the water-soluble substrates. The major physiological effect of a lack of Hex A is therefore the accumulation of GM2 ganglioside. Since the cells of neuronal tis-

Received 29 January 1996; accepted 19 June 1996.

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sue contain the highest concentration of GM2 ganglioside, they are most adversely affected by the absence of Hex A. The pathological effect of this situation is neuronal deterioration resulting in mental and motor retardation. The clinical course of the disorder is variable. The severity of the disease is inversely proportional to the amount of residual Hex A activity, which in turn is determined by the degree of catalytic crippling a particular mutation inflicts upon the enzyme. Mutations that obliterate Hex A activity cause a severe form of the disorder generally known as the classic or infantile form of Tay-Sachs disease, characterized by an early age of onset, 6 months, and a rapidly progressing clinical course leading to death in early childhood. The juvenile and adult forms commence later and generally manifest a less severe course. The number of Tay-Sachs carriers in the general population has been predicted to be at 1 in 300. However, certain ethnic and geographic isolates display a significantly higher number. The best known of these are the Ashkenazi Jewish and French Canadian populations, which display an estimated carrier frequency of 1 in 30.

In 1985, a cDNA clone coding for the entire α-chain of β-hexosaminidase was isolated (Myerowitz et al., 1985). The cDNA has an open reading frame of 1587 base pairs (bp) corresponding to 529 amino acids; the first 17–22 amino acids satisfy the requirements of a signal sequence. Three potential glycosylation sites have been identified in the sequence, and all are used (Weitz and Proia, 1992). A 65% sequence homology between the α- and β-chains at the DNA level suggest that both polypeptides evolved from a common ancestor (Myerowitz et al., 1985). The coding sequence is divided into 14 exons within a gene approximately 35,000 bp in size (Proia and Soravia, 1987). Exons 2–14 are clustered together at the 3′ end of the gene, while exon 1 is separated from the group by a very large intervening sequence of 20,000 bp. The arrangement of the exons within the α-chain gene is echoed in the β-chain gene reiterating their similar genetic ancestry (Proia, 1988). Isolation of a cDNA clone coding for the α-chain of human β-hexosaminidase followed by characterization of the α-chain gene provided the tools necessary to identify mutations causing Tay-Sachs disease. Their characterization has clarified the basis of the clinical heterogeneity of the disease, added to our knowledge of structure–function relationships of the enzyme and molecular epidemiology of the disease, and enabled more accurate carrier identification and prognoses for affected individuals.

## MUTATIONS IN HEX A GENE OF B-HEXOSAMINIDASE

To date, 78 different mutations have been reported in the Hex A gene and are listed in Table 1. Sixty-seven are classified as disease causing, three as benign but producing a phenotypic change, and eight as neutral polymorphisms. Most of the mutations are private in nature, present in one or at most a few familes of diverse backgrounds. Some are common in Tay-Sachs disease carriers of a particular ethnic origin or geographic location. Approximately 50% of the abberations occur at CpG dinucleotide sequences corroborating their reputation as "hot spots" for mutation via spontaneous deamination of methylcytosine to thymidine. The mutations present in the Hex A gene include 63 single base substitutions, two small insertions and ten small deletions, 1–18 bp in size, and one large deletion of 7.6 kilobases (kb). The effects of these changes include substitution of amino acids (missense mutations), introduction of stop codons (nonsense mutations), inclusion of incorrect sequences of amino acids (frameshifts mutations), abnormal splicing, and no change in codon sense (silent mutations) and are discussed below.

### Missense Mutations

Of the 77 documented mutations, 39 are missense mutations caused by a single base substitution, making this type of mutation the one most commonly found in the Hex A gene. These missense mutations are sprinkled throughout the 14 exons with greater density in exons 5, 6, and 7 (Fig. 1). Thirty-three are disease causing, three are clinically benign but produce a change in phenotype (the pseudodeficient lesions discussed in detail below), and three are neutral polymorphisms. Two of the disease-causing missense mutations occur in the methione initiation codon, Met1Val (Mules et al., 1992) and Met1Thr (Harmon et al., 1993) and presumably obliterate translation. All the B1 variant phenotypes discussed below are produced by missense mutations. Many of the Hex A genes harboring missense mutations yield α-chain peptides that can be detected immunologically but are present in less than normal quantities due to their instability. Many such α-chain peptides lack some post-translational modification such as phosphorylation Gly250Asp (Trop et al., 1992) and Glu482Lys (Nakano et al., 1988), or maturation Leu39Arg (Akli et al., 1993a), Pro25ser (Harmon et al., 1993). Others cannot associate properly Gly269Ser (Navon and Proia, 1989), Arg504Cys (Paw et al., 1991). An interesting effect of a missense mutation occurring in 509C→A, Arg170Gln

TABLE 1. Mutations in the Human α-Chain Gene of β-Hexosaminidase

| Intron/ exon | Mutation | Expected effect | Comments | Clinical phenotype | Origin | References |
|---|---|---|---|---|---|---|
| 5′ to IVS-1 | 7.6 kb | Lack of mRNA | Undetectable mRNA, absence of DNA fragment in Southern blot | Infantile | French Canadian | Myerowitz and Hogikyan (1986, 1987) |
| E 1 | 1A→G | Met1Val | | Infantile | Black American | Mules et al. (1992) |
| E 1 | 2T→C | Met1Thr obliteration of initiation, no translation | Determined in patient who is a a compound heterozygote with 73C→T in E 1 | Infantile (predicted in homozygous patients) | English | Harmon et al. (1993) |
| E 1 | 9C→T | Ser3Ser | Neutral polymorphism | | Black American Ashkenazi, and Sephardiic Jews | Mules et al. (1992); Grinshpun et al. (1995) |
| E 1 | 73C→T | Pro25Ser | 2.5% Hex A activity; SDS gel shows normal size α-precursor but no mature form; compound heterozygote with 2T→C in E 1 | Late infantile | English | Harmon et al. (1993) |
| E 1 | 78G→A | Trp26 stop | | Infantile | United Kingdom Arab | Triggs-Raine et al. (1991); Drucker and Navon (1993) |
| E 1 | 116T→G | Leu39Arg | Decreased amount of α-chain precursor and lack of mature polypeptide (short half-life) | Infantile | Polish | Akli et al. (1993a) |
| IVS-2 | +1G→A | Abnormal splicing | Low levels of mRNA; observed transcripts lack E 2 | Infantile | French | Akli et al. (1991) |
| IVS-2 | +1G→C | Abnormal splicing | | Infantile | Germany/Irish/ English | Triggs-Raine et al. (1991) |
| E 3 | 380T→G | Leu127Arg | Mutation in 2nd allele not detemined | Infantile | Italian | Akli et al. (1993a) |
| E 3 | 409C→T | Arg137stop | mRNA deficient | Infantile | Irish, French | Akli et al. (1991); Mules et al. (1992) |
| IVS-3 | +1G→T | Abnormal splicing | 66 bp shortened mRNA with absence of E 3 | Infantile | Japanese | Tanaka et al. (1994) |
| E 4 | ΔTT(424–425) | Frameshift; immediate premature termination | <1% mRNA via Northern blot | Infantile | French | Akli et al. (1993a) |
| E 4 | ΔG436 | Frameshift premature termination in E 6 | | Infantile | Black American | Mules et al. (1992) |
| IVS-4 | +5G→A | Abnormal splicing | 3% mRNA, transcripts lack E 4 | Infantile | French | Akli et al. (1991) |
| IVS-4 | –1G→T | Abnormal splicing | | Infantile | Black American | Mules et al. (1991) |
| E 5 | ΔTG(477–478) | Frameshift premature termination 2 codons downstream | | Infantile | United Kingdom | Triggs-Raine et al. (1991) |
| E 5 | 508C→T | Arg170Trp | | Infantile | Italian, French Canadian | Akli et al. (1993a) Fernandes et al. (1992) |

*(continued)*

TABLE 1. Mutations in the Human α-Chain Gene of β-Hexosaminidase (*Continued*)

| Intron/ exon | Mutation | Expected effect | Comments | Clinical phenotype | Origin | References |
|---|---|---|---|---|---|---|
| E 5 | 509C→A | Arg170Gln | Expression studies show inactive, unstable, and shortened mature protein; aberrant transcripts with 51-bp deletion at 5´ end of E 5 produced by activation of cryptic acceptor splice site within E 5 | Infantile | Japanese, Moroccan Jewish, Scottish | Nakano et al. (1990); Drucker et al. (1992); Akli et al. (1993a) |
| E 5 | 532→T | Arg178Cys | B1 variant[a] phenotype; expression studies show lack of α-subunit catalytic activity | Infantile | Czechoslovakian | Tanaka et al. (1990) |
| E 5 | 533G→A | Arg178His | B1 variant[a] phenotype; expression studies show absence of α-subunit catalytic activity | Juvenile; late infantile when compound heterozygote with null allele | Common in Portugal and wide geographic distribution | Ohno and Suzuki (1988);Tanaka et al. (1988, 1990a) dos Santos et al. (1991) |
| E 5 | 533G→T | Arg178Leu | B1 variant[a] phenotype | Infantile | United Kingdom | Triggs-Raine et al. (1991) |
| E 5 | 540C→G | Tyr180 stop | Decreased quantity of mRNA | Infantile | Moroccan Jewish | Drucker et al. (1992) |
| E 5 | +A (inserted after 547) | Premature termination 6 bp downstream of insertion | No immunoprecipitable α-subunit in patient fibroblasts | Infantile | Chinese | Akalin et al. (1992) |
| E 5 | 570G→A | Leu190Leu | 3% mRNA of normal size containing silent mutation, 7% mRNA lacks E 5; 2.5% Hex A activity due to normal transcripts | Late infantile | Tunisian | Akli et al. (1990) |
| IVS-5 | –1G→T (3´ end) | Abnormal splicing | mRNA lacking E 5; expression studies show inactive α-subunit | Infantile | Japanese | Tanaka et al. (1993) |
| IVS-5 | +1G→A | Abnormal splicing | | Infantile | Turkish | Ozkara et al. (1995) |
| E 6 | 574G→C | Val192Leu | B1 variant[a] phenotype | Infantile | German/Romanian, English/Irish | Ainsworth and Coulter-Mackie (1992); Coulter-Mackie (1994) |
| E 6 | 587A→G | Asn196Ser | | Unknown[b] | U.S. French Canadians | Triggs-Raine et al. (1995) |
| E 6 | 590A→C | Lys197Thr | | Adult | Dutch | Akli te al. (1993a) |
| E 6 | 598G→A | Val200Met | Neutral polymorphism; found in same allele containing 574G→C in E 6; expression studies show 80% Hex A activity with MUG and 100% activity with MUGS | | German/Romanian English/Irish | Ainsworth and Coulter-Mackie (1992) |

(*continued*)

TABLE 1. Mutations in the Human α-Chain Gene of β-Hexosaminidase (*Continued*)

| Intron/ exon | Mutation | Expected effect | Comments | Clinical phenotype | Origin | References |
|---|---|---|---|---|---|---|
| E 6 | 611A→G | His204Arg | | Infantile | German | Akli et al. (1993a) |
| E 6 | 629C→T | Ser201Phe | | Infantile | North African | Akli et al. (1991) |
| E 6 | 632T→C | Phe211Ser | | Infantile | Italian | Akli et al. (1993a) |
| IVS-6 | +1G→A | Abnormal splicing | mRNA lacking E 6 | Juvenile[d]/ adult | American | Akli et al. (1993a) |
| IVS-6 | +30T→G | ? | Detected in obligate Tay-Sachs carrier harboring another disease causing allele, therefore probably benign | Unknown[b] | U.S. French Canadians | Triggs-Raine et al. (1995) |
| E 7 | 739C→T | Arg247Trp | Pseudodeficient[c] | Normal (in compound heterozygote with null allele) | Wide distribution | Triggs-Raine et al. (1992) |
| E 7 | 745C→T | Arg249Trp | Pseudodeficient[c] | Normal (in compound heterozygote with null allele) | French Canadian | Cao et al. (1993) |
| E 7 | 748G→A | Gly250Ser | Tentative pseudodeficient[c] status | Unknown[b] | U.S. French Canadians | Triggs-Raine et al. (1995) |
| E 7 | 749G→A | Gly250Asp | Expression studies show no Hex S activity; if cotransfected with β-subunits, show 12% Hex A activity; unstable, unphosphorylated α-subunit | Juvenile | Lebanese Maronite | Trop et al. (1992) |
| E 7 | 772G→C | Asp258His | Expression studies show B1 phenotype[a] | Infantile | Scottish/Irish | Fernandes et al. (1992) |
| E 7 | 805G→A | Gly269Ser | Reduced level of α-subunit; defective processing and association with β-chain | Adult | Ashkenazi Jewish, wide distribution | Navon and Proia (1989); Navon et al. (1990); Paw et al. (1989) |
| IVS-7 | +1G→A | Abnormal splicing | No detectable mRNA | Infantile | French Canadian | Hechtman et al. (1992) |
| IVS-7 | +1G→C | Abnormal splicing | mRNA almost undetectable; observed transcripts lack E 7 | Infantile | Portuguese | Ribeiro et al. (1995) |
| E 8 | 902T→G | Met301Arg | | Infantile | Yugoslav | Akli et al. (1993a) |
| E 8 | ΔTTC (910–912 or 913–915) | ΔPhe (304 or 305) | Expression studies show no Hex S activity; defective processing and association with β-chain | Infantile | Moroccan Jewish, French/Irish, Portuguese/Italian | Navon and Proia (1991); Akli et al. (1991) |
| E 8 | ΔCT (927–928) | Frameshift premature termination 35 bases downstream | | Infantile | French Canadian | Fernandes et al. (1992) |
| E 8 | ΔGGA (958–960 or 961–963) | ΔGly320 or 321 | | Late infantile | Irish | Mules et al. (1992) |

(*continued*)

TABLE 1. Mutations in the Human α-Chain Gene of β-Hexosaminidase (*Continued*)

| Intron/ exon | Mutation | Expected effect | Comments | Clinical phenotype | Origin | References |
|---|---|---|---|---|---|---|
| E 9 | 987G→A | Trip329stop | | Late infantile | German, English | Mules et al. (1992) |
| E 9 | 1003A→T | Ile335Phe | Evidence that nucleotide change is disease causing: a carrier produced four fetuses determined prenatally to be affected | Unknown[b] | American | Tomczak and Grebner (1994) |
| E 9 | ΔGACTTC AAGCAGC TGGAG (1039–1056) | AspPheLys GlnLeuGlu (347–352) | Evidence that nucleotide change is disease causing: carriers produced a fetus that was determined prenatally to be affected | Unknown[b] | Ashkenazi Jewish | Tomczak and Grebner (1994) |
| IVS-9 | ΔTCTCC (–8 to –12) | Abnormal splicing | Low amount of normal mRNA, and most transcripts lack E 10 | Infantile | Polish | Triggs-Raine et al. (1991) |
| IVS-9 | +1G→A | Abnormal splicing | mRNA undetectable via Northern blot; observed transcripts aberrant due to activation of two cryptic donor splice sites in E 9 and IVS-9 | Infantile | French, Italian Irish, Scottish, Welsh, Cajun, Dutch | Akli et al. (1993b); Akerman et al. (1992); McDowell et al. (1992) |
| IVS-9 | –1G→T | Abnormal splicing | | Infantile | Irish, French | Brown et al. (1995) |
| IVS-10 | + 18A→G | ? | | Unknown[b] | U.S. French Canadians | Triggs-Raine et al. (1995) |
| E 11 | 1164C→G | Ile388Met | | Unknown[b] | U.S. French Canadians | Triggs-Raine et al. (1995) |
| E 11 | 1176G→A | Trp392stop | Almost undetectable mRNA via Northern blot | Infantile | Ashkenazi Jewish | Shore et al. (1992) |
| E 11 | 1177C→T | Arg393stop | mRNA deficient | Infantile | French | Akli et al. (1991) |
| E 11 | ΔG(1182 or 1183) | Frameshift premature termination 10 bp downstream from deletion | Nucleotide change found in obligate carrier | Infantile | American | Tomczak and Grebner (1994) |
| E 11 | 1195A→G | Asn399Asp | Neutral polymorphism | | Black American | Mules et al. (1992) |
| E 11 | 1260→G | Trp420Cys | Expression studies show no catalytically active enzyme; normal size mRNA; allele 2 unknown | Infantile | Irish/German | Tanaka et al. (1990b) |
| E 11 | + TATC (after 1273) | Frameshift premature termination 4 codons downstream | mRNA deficient; expression studies show mRNA deficiency caused by premature termination codon | Infantile | Ashkenazi Jewish (80% of alleles) wide distribution | Myerowitz and Costigan (1988); Boles and Proia (1995) |

(*continued*)

TABLE 1. Mutations in the Human α-Chain Gene of β-Hexosaminidase (*Continued*)

| Intron/ exon | Mutation | Expected effect | Comments | Clinical phenotype | Origin | References |
|---|---|---|---|---|---|---|
| E 11 | 1306G→A | Val436Ile | Neutral polymorphism | | Black American | Mules et al. (1992) |
| E 12 | 1338T→C | Pro446Pro | Probably benign, but may affect splicing | Unknown[b] | U.S. French Canadians | Triggs-Raine et al. (1995) |
| E 12 | 1360G→A | Gly454Ser | | Infantile | Italian | Akli et al. (1993a) |
| E 12 | 1373G→A | Cys458Tyr | Normal-sized mRNA; expression studies show no enzyme activity | Infantile | Japanese | Tanaka et al. (1994) |
| IVS-12 | +1G→C | Abnormal splicing | Almost undetectable mRNA, abnormal transcripts delecting E 12 or including intron 12 | Infantile | Ashkenazi Jewish (15% of alleles) | Myerowitz, 1988 (Arpaia et al. 1988); Ohno and Suzuki (1988b) |
| E 13 | 1444G→A | Glu482Lys | α-Polypeptide retained in ER not processed, not phosphorylated, mRNA normal size and quantity, expression studies show lack of Hex S activity | Infantile | Italian, Chinese | Nakano et al. (1988); Akalin et al. (1992) |
| E 13 | 1451T→C | Leu484Gln | Expression studies show no enzyme activity | Infantile | Japanese | Tanaka et al. (1994) |
| E 13 | 1453T→C | Trp485Arg | Expression studies show Hex S reduced to 2% | Infantile | Chinese | Akalin et al. (1992) |
| E 13 | 1495C→T | Arg499Cys | | Infantile | Slavic, Irish, English, Polish | Mules et al. (1992); Akli et al. (1993) |
| E 13 | 1496G→A | Arg499His | α-Chain not processed unstable, unable to exit ER | Juvenile | Jewish/Scottish, Irish | Paw et al. (1990) |
| E 13 | 1510C→T | Arg504Cys | Expression studies shows no α-catalytic activity; defective α-subunit dimerization | Infantile | German, French/Algerian | Paw et al. (1991); Akli et al. (1991) |
| E 13 | ΔC1510 | Frameshift causing premature termination 4 codons downstream from deletion | Truncated α-polypeptide, not phosphorylated and not processed to mataure form, unstable; similar findings in expression studies | Infantile | Italian | Lau and Neufeld (1989) |
| E 13 | 1511G→A | Arg504His | Defective association phosphorylated and secreted; expression studies show that α-subunit fails to dimerize | Juvenile | Assyrian, Lebanese, American | Paw et al. (1990); Boustany et al. (1991) |
| E 13 | 1520G→A | Glu506Glu | Neutral polymorphism | | German | Paw et al. (1991) |
| IVS-13 | –6T→C | Neutral polymorphism | | | Askhenazi Jews, Scots-Irish | Kaplan et al. (1993) |
| E 14 | 1684T→C | 3´UTR | | Unknown[b] | U.S. French Canadians | Triggs-Raine et al. (1995) |

[a]Normal Hex A activity with synthetic substrate 4MUG but inactive against 4MUGS or natural substrate GM2 ganglioside.
[b]Mutation determined in a carrier.
[c]Inability to hydrolyze synthetic substrate 4MUG but normal hydrolysis of 4MUGS and natural substrate, GM2 ganglioside.
[d]Second allele unidentified but most likely responsible for patient phenotype.

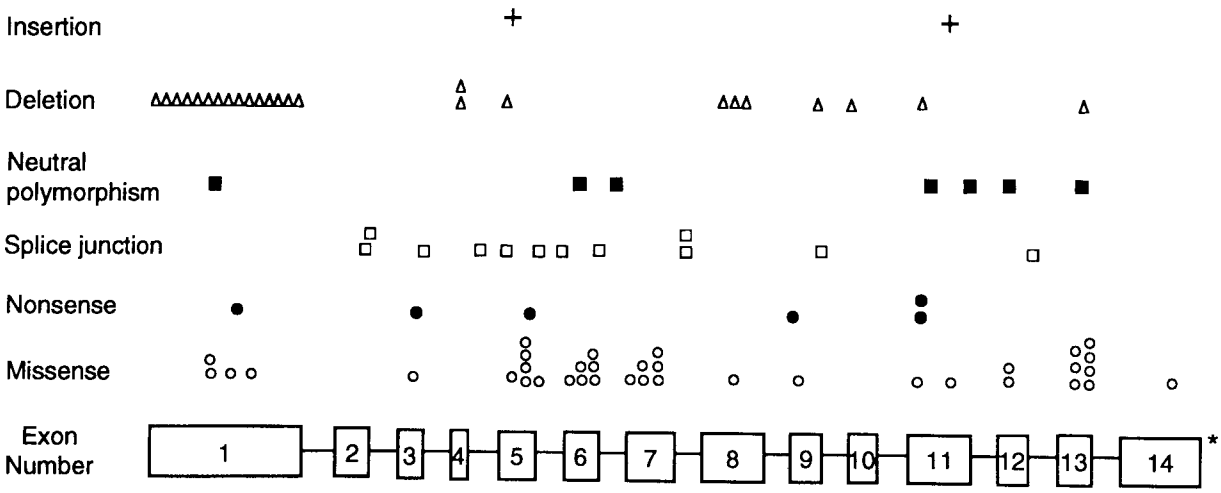


FIGURE 1. **Structure of the Hex A gene and mutations identified therein. Blocks indicate the exons and their relative size. Introns are designated by lines without consideration to their relative size.**

(Nakano et al., 1990), is activation of a cryptic acceptor splice site in exon 5 to produce aberrant transcripts with 51 bp deleted at the 5′ end of exon 5. With the exception of one late infantile form of the disease caused by an inframe deletion of 3 bp, ΔGly320 (Mules et al., 1992), all the milder forms of Tay-Sachs disease are caused by missense mutations.

### Nonsense Mutations

Six single base substitutions, one each in exons 1, 3, 5, 9, and two in exon 11 (Fig. 1), that result in nonsense mutations in the Hex A gene have been defined. In contrast to the missense mutations, which in many instances lead to the production of immunologically detectable albeit unstable α-chain polypeptides, the nonsense mutations exert their detrimental effect at the mRNA level. Deficiency of mRNA (<1%) has been found in cells harboring the following α-chain gene nonsense mutations: Arg137-stop, Try180stop, Trp392stop, and Arg393stop. mRNA levels in Trp26stop α-chain mutants have not been studied. Premature termination codons causing several other human diseases (Baserga and Benz, 1992; Belgrader et al., 1993) have been correlated with reduced levels of mRNA due to increased mRNA instability.

### Abnormal Splicing

Thirteen splice junction mutations have been documented. Most occur in the intervening sequences at the 5′ half of the gene (Fig. 1). Only three, IVS-4, –1G→T (Mules et al., 1991), IVS-5, –1G→T (Tanaka et al., 1993), and IVS-9, –1G→T (Brown et al., 1995) occur at 3′ splice sites. Transcripts resulting from alleles harboring the splice junction mutations display aberrant splicing and often lack exons germane to the splice site defect. Moreover, the level of mRNA itself is generally very reduced (<1%) due to instability of the defective transcripts. Many species of aberrant transcripts, some lacking exon 12, others lacking upstream exons, are produced from IVS-12, + 1G→C (Ohno and Suzuki, 1988b), the lesion found in 15% of the Ashkenazi Jewish carriers for Tay-Sachs disease. The IVS-9, + 1G→A splice site mutation, the predominant Tay-Sachs disease causing aberration in individuals from the British Isles, results in the activation of two cryptic donor splice sites, one in exon 9 and the other in IVS-9 (Akerman et al., 1992).

### Deletions and Insertions

Eleven deletions and two insertions have been found in the Hex A gene (Fig. 1). One of the deletions, a 7.6-kb DNA fragment encompassing exon 1 and surrounding upstream and downstream DNA, is the major mutation found in French Canadian carriers of Tay-Sachs disease. Four small deletions do not produce frameshifts but nonetheless cause a severe form of the disorder. The remaining six deletions (1 or 2 bp), as well as two small insertions (1 and 4 bp), cause frameshifts. A stop signal is inevitably found either immediately or up to 10 codons downstream from the site of the frameshift resulting in premature termination of the α-chain polypeptide. All of these described frameshifts have yielded the infantile form of Tay-Sachs disease. The most well-known frame-

shift-causing mutation is a 4-bp insertion in exon 11 (+ TATC, Myerowitz and Costigan, 1988) found in 80% of the Ashkenazi Jewish carriers of Tay-Sachs disease.

### Neutral Polymorphisms

Of the seven neutral polymorphisms in the Hex A gene (Fig. 1), three arise from base substitutions that do not change the sense of the codon, Ser3Ser (Mules et al., 1992), Glu506Glu (Paw et al., 1991) and Pro446Pro (Triggs-Raine et al., 1995). A caveat to the inclusion of the 1338T→C, Pro446Pro mutation (detected in the New England French Canadian population) in the category of neutral polymorphism is that proximity of the aberration to the 3´ splice site of IVS-12 may affect splicing. The rationale for inclusion of the three missense mutations, Val200Met (Ainsworth and Coulter-Mackie, 1992), Asn399Asp, and Val436Ile (Mules et al., 1992), in the category of neutral polymorphism is that all were found in alleles already containing a known disease causing mutation. Moreover, Asn399Asp and Val436Ile were also present in normal individuals, most frequently those belonging to the American black population. Tentatively included in the category of benign mutations is IVS-6, + 30T→G, by virtue of the fact that it was detected in an obligate Tay-Sachs carrier harboring a known disease-causing mutation (Triggs-Raine et al., 1995).

## MUTATIONS COMMONLY FOUND IN ETHNIC OR GEOGRAPHIC POPULATION ISOLATES

The first mutant alleles to be characterized were those present in two populations that exhibited a carrier frequency for Tay-Sachs disease 10-fold over that found in the general population. A 4-bp insertion in exon 11 was found in 80% of the Ashkenazi Jewish carriers (Myerowitz and Costigan, 1988), while a splice junction at the 5´ end of exon 12 was found in 15% of the Ashkenazi Jewish carriers (Myerowitz, 1988). A 7.6 Kb deletion including exon 1, part of intron 1 and a portion 5´ upstream of exon 1 was discovered to be the major mutation causing Tay-Sachs disease in the French Canadian population (Myerowitz and Hogikyan, 1986). A later study identified a 3-bp deletion coding for a phenylalanine residue in exon 8 in 50% of the Moroccan Jewish carriers of Tay-Sachs disease (Navon and Proia, 1991). It has been estimated that this population has a fivefold higher gene frequency for the disorder. More recent studies have shown that carriers for Tay-Sachs disease from some geographic isolates harbor one type of mutation more often than others, although the disease gene frequency in these groups is not elevated. For example, IVS-9, + 1G→A mutations has been reported to represent 42% of the alleles of non-Jewish Tay-Sachs carriers in the British Isles (Landels et al., 1993). Seventy-nine percent of mutant alleles found in the Japanese population contain Cys458Tyr (Tanaka et al., 1994), a mutation that has not been found in any other group. The Arg178His allele responsible for a B1 variant phenotype discussed below is in general a mutation rarely found but is often present in Tay-Sachs disease carriers from the Portuguese population (dos Santos et al., 1991).

## MUTATIONS ASSOCIATED WITH LATER-ONSET FORMS OF TAY-SACHS DISEASE

While the vast majority of disease-causing mutations in the Hex A gene result in the severe infantile form of Tay-Sachs disease, nine described mutations are associated with later onset, often less severe forms of the disorder. These milder phenotypes arise from the presence of a mutant form of the enzyme that still retains a measure of catalytic activity.

Four mutations have been reported associated with the late infantile form of Tay-Sachs disease. The age of onset and time of demise of patients with this form of Tay-Sachs disease are generally delayed at 1–2 years relative to that of the strict infantile form. A missense mutation in exon 1, Pro25Ser (Harmon et al., 1993), a 3-bp deletion (GGA) coding for a glycine residue in exon 8 (Mules et al., 1992) and a nonsense mutation in exon 9 (Mules et al., 1992) all cause a late infantile form. The missense mutation in exon 1 was detected in a patient who had 2.5% of normal Hex A activity. This residual activity is presumed to arise from this missense mutation as the second allele of the patient contained a Met1Thr (Harmon et al., 1993) missense that would presumably obliterate translation initiation. An interesting mutation that results in the late infantile form of Tay-Sachs disease was found in exon 5, Leu190Leu (Akli et al., 1990). In this instance, the adenine residue that replaces the guanine residue at position 510 is the last nucleotide of exon 5 before the invariant 5´ splice site dinucleotide of IVS-5. (A G residue adjacent to the 5´ splice site is found in 80% of human genes.) The G-to-A change causes inefficient as well as aberrant splicing. mRNA lacking exon 5 has been demonstrated in a Tunisian patient homozygous for this mutation. The late infantile phenotype presumably results from the presence of a small amount of correctly spliced mRNA that can be translated to provide 2.5% of normal Hex A activity.

The juvenile form of Tay-Sachs disease has a later age of onset and displays a pervasive but more pro-

tracted clinical course that is usually fatal in childhood or early adulthood. Three missense mutations, one in exon 7, Gly250Asp (Trop et al., 1992), and two in exon 13, Arg499His, and Arg504His (Paw et al., 1990), have been associated with this phenotype. Cotransfection of α-chain cDNA containing the Gly250Asp mutation and normal β-chain cDNA in Cos-1 cells allowed for detection of some residual activity. The substitution is detrimental to the enzyme activity because a large, charged residue has replaced a small neutral residue in a hydrophobic section of the α-chain. Both mutations in exon 13, Arg488His, and Arg504His do not impair the biosynthesis of α-chain polypeptide. However, the former mutation yields a polypeptide defective in its ability to associate with the β-polypeptide, while the latter mutation interfered with exit of the α-chain from the endoplasmic reticulum. Both mutations are located at CpG dinucleotide mutagenic hot spots. In contrast to the juvenile phenotype of the Arg499His missense, when Arg499 is replaced by a Cys (Paw et al., 1991), the resulting phenotype is the severe form. Appearance of a cysteine residue may create an illegitimate disulfide bridge and destroy the proper tertiary structure of the enzyme wiping out all enzyme activity.

The hallmark of the adult form of Tay-Sachs disease is its clinical variability. The disorder becomes clinically apparent between the second and third decade of life. The course is highly variable, generally with less motor and neurological deterioration than the other forms. Psychosis is frequently the first manifestation of the disease. Missense mutations cause this form of Tay-Sachs disease. A Gly269Ser, 805G→A, mutation in exon 7 is the most frequently found change associated with adult type Tay-Sachs. Originally characterized in an Ashkenazi Jewish patient and thought to be more prevalent in this ethnic group (Navon and Proia, 1989), the mutation has been shown to have a wide geographic distribution (Navon et al., 1990). Moreover, the Gly269Ser is found more often among non-Jewish (5%) than Jewish carriers (2%) (Kaback et al., 1993). Similar to the Leu190Leu, 570G→A, the Gly269Ser, 805G→A, mutation affects the last nucleotide at the 3′ end of an exon that provides the potential for synthesis of improperly spliced mRNA. However, the Gly-Ser mutation gives rise to an α-subunit which is defective in association when expressed in Cos-1 cells. The importance of this amino acid is underscored by its conservation in the human β-chain subunit and in β-Hex from slime mold (*Dictyostelium discoideum*). The second mutation associated with adult Tay-Sachs is a Lys197Thr in exon 6. Since this mutation was found in a Dutch patient in compound heterozygosity with the Arg499His, the juvenile allele described above, it must provide the residual activity that results in adult Tay Sachs phenotype.

## MOLECULAR LESIONS CAUSING THE B1 VARIANT PHENOTYPE

Patients classified as B1 variant phenotypes produce Hex A, which has normal catalytic activity when assayed with the commonly used synthetic substrate 4-methylumbelliferyl N-acetylglucosaminide (4MUG) but is catalytically inactive against 4-methylumbelliferyl N-acetylglucosaminide sulfate (4MUGS) or $GM_2$ ganglioside. The explanation for this phenomenon postulates that mutations causing this phenotype affect the active site in the α-subunit without affecting its ability to undergo normal processing and association with the β-subunit. Since $GM_2$ ganglioside and 4MUGS are normally hydrolyzed at the catalytic site present on the α-subunit, these substrates remain intact. On the other hand, 4MUG is normally hydrolyzed by Hex A at the catalytic site present on the β-subunit, which is fully operational. Compound heterozygotes of the B1 variant allele with a functionally null allele appear as carriers when assayed with 4MUG.

Three of the five mutations reported to produce the B1 variant phenotype occur in exon 5 and are due to base substitutions that change the sense of codon 178 from an Arg to an His, Cys, or Leu. Ohno and Suzuki (1988a) suggested that this region of the enzyme is important in defining a portion of the α-subunit catalytic site and an amino acid substitution at codon 178 drastically alters the tertiary structure of the polypeptide. Replacement of codon 178 with the Cys (Tanaka et al., 1990a) or Leu (Triggs-Raine et al., 1991) results in the infantile form of Tay-Sachs disease, while substitution with His (Ohno and Suzuki, 1988a) produces a milder juvenile phenotype, perhaps because it is more chemically similar to Arg than the Cys or Leu. Yet the amount of residual Hex A activity that the Arg178His allows for must be very marginal because a compound heterozygote with a null allele produces a late infantile phenotype (dos Santos et al., 199 1). The Arg178His alteration has a wide geographic distribution (Tanaka et al., 1988) but is a lesion which is commonly found among Portuguese individuals who have been distinguished as carriers (dos Santos et al., 1991). More recently, two additional base substitutions causing missense mutations, one in exon 6, Val192Leu (Ainsworth and Coulter-Mackie, 1992) and the other in exon 7, Asp258His (Fernandes et al., 1992), have been associated with the B1 variant phenotype of the severe

infantile form. These results enlarge our conception of the amino acids, which may be important for glycosidic cleavage at the α-chain catalytic site.

## MUTATIONS CAUSING HEX A PSEUDODEFICIENCY

Some mutations in the α-chain subunit prevent Hex A from hydrolyzing the synthetic substrate 4MUG but do not hinder it from hydrolyzing the sulfated synthetic 4MUGS or the natural substrate GM2 ganglioside. Genes coding for the α-chain that contain this type of lesion are called pseudodeficient alleles. Individuals bearing this type of allele along with a second disease-causing allele are healthy despite the fact that they lack Hex A activity when assayed with 4MUG. Such individuals are described as Hex A pseudodeficient. In addition, carriers of pseudodeficient alleles cannot be distinguished from those harboring disease causing mutations by conventional screening tests.

Two missense mutations, Arg247Trp, (Triggs-Raine et al., 1992) and Arg249Trp (Cao et al., 1993), causing the pseudodeficient phenotype have been described in exon 7. Both are present in a highly conserved region of the α subunit. The mechanistic basis for their effects is unknown but presumably involves interference with substrate recognition and/or binding by the α-subunit. The Arg247Trp missense accounts for 32% of the non-Jewish enzyme defined carriers but only 2% of the Jewish enzyme-defined carriers (Kaback et al. , 1993). The Arg249Trp has never been found in Jewish enzyme-defined carriers but is present in 4% of the non-Jewish carriers. A third mutation, also in exon 7, Gly250Ser (Triggs-Raine et al., 1995), has recently been documented in Tay-Sachs disease carriers living in New England of French Canadian background. This mutation holds tentative status as a pseudodeficient allele because it has been detected almost as often as the most common French Canadian disease-causing mutation, the 7.6-kb deletion but has never been found in a French Canadian patient with Tay-Sachs disease.

Elucidation of the pseudodeficient alleles has clarified a puzzling inconsistency between the predicted carrier frequency of Tay-Sachs disease in the non-Jewish population based on disease incidence (1/300) and the observed carrier frequency based on carrier definition by enzyme assay (1/167). When corrections are made for the observed frequency of the pseudodeficiency alleles in the non-Jewish population, the observed carrier frequency becomes 1/277 (Kaback et al., 1993), a number that agrees more closely with the predicted frequency. No such discrepancy between predicted and enzyme-defined carrier frequency has been observed in the Ashkenazi Jewish population since pseudodeficient alleles account for very few enzyme-defined carriers.

## CLINICAL AND DIAGNOSTIC RELEVANCE

Owing to the great molecular heterogeneity of Tay-Sachs disease (Table 1), enzymatic assay for Hex A rather than mutational analysis is still the best method as an initial screen for distinguishing carriers of the disorder. Several years after the enzymatic defect causing the disease was discovered (Okada and O'Brien, 1969), a screening program and availability of prenatal diagnosis targeted at the Ashkenazi Jewish population because of their elevated carrier frequency was established. Screening programs for French Canadians have since been established. Individuals from other than these two groups are candidates for screening if they have reason to suspect from their family history that they might indeed be carriers. Screening for such carrier status is achieved by assaying an individual's serum for Hex A activity with the synthetic substrate 4MUG. The increased heat stability of Hex B relative to Hex A allows for distinction of carriers, noncarriers, and affected individuals. However, the enzymatic screen does have limitations. First, enzymatic screening cannot discriminate between carriers of the infantile or less severe forms of the disorder. For example, results of Hex A assay for carriers of the Gly269Ser mutation causing adult Tay-Sachs disease and for carriers of the severe infantile form of Tay-Sachs disease would be similar. A simple DNA test can distinguish between the two and would be helpful in predicting the clinical course of the progeny issuing from such carriers. In addition, individuals harboring the pseudodeficiency allele will appear as carriers, when in fact any progeny issuing from such individuals will be healthy. Moreover, carriers of the B1 variant phenotype will be presumed normal in the enzymatic carrier screen with 4MUG. The use of molecular analysis in conjuction with the enzymatic screen for these categories of mutations can resolve these ambiguities and provide more accurate information upon which individuals make their reproductive choices. Epidemiological studies that have determined which alleles, if any, are commonly found in particular populations allow for better selection of the battery of mutations that should be analyzed for at the DNA level.

Prenatal diagnosis of Tay-Sachs disease prior to embryo implantation has only become possible because of our knowledge of the molecular lesions causing the disorder. Screening embryos for homozygosity of the 4-bp insertion by gene amplification via PCR

has been performed on fertilized oocytes biopsied at the four to eight cell stage (Gibbons et al., 1995). Enzymatic assay for Hex A is impossible at this stage due to an insufficient amount of material. A successful pregnancy and birth resulted after analysis. Although an expensive technique requiring in vitro fertilization, it is nonetheless an option for individuals desiring to avoid abortion but who have children biologically rather than by adoption.

## BIOLOGICAL RELEVANCE AND FUTURE DIRECTIONS

In contrast to the clinical and diagnostic benefits reaped from delineation of the large number of naturally occurring α-chain gene mutations, their elucidation has not led to a precise picture of the amino acid residues involved in important biological functions of Hex A such as location of substrate binding, catalysis, and subunit association, or mechanism of catalysis. At best, what has been achieved in the way of mapping structure to function using these mutations is a hypothetical notion of the enzyme domains that might be involved in these processes. For example, correlation between the mutations producing the B1 variant and pseudodeficient phenotypes have led to the suggestion that exons 5–7 are involved in substrate binding and perhaps catalysis. Lack of progress in this area can in part be blamed on both the absence of a model of the tertiary structure of the polypeptide and the paucity of studies that have thoroughly analyzed the functional deficits of mutant proteins. Together, such information would allow for full exploitation of the already defined and yet to be discovered mutations for structure–function correlations and should be a focus for future research.

The recent creation of a Tay-Sachs mouse model via targeted disruption of the α-chain gene has set another direction for future research (Yamanaka et al., 1994). Such models will allow for closer study of the pathogenesis of Tay-Sachs disease and development of new treatments. Moreover, the Tay-Sachs mice will be useful for the development of both ex vivo and in vivo somatic cell gene therapy (Friedmann, 1994).

## ACKNOWLEDGMENT

I express my deep appreciation to Jack Y. Vanderhoek for his endless encouragement and support in everything I choose to undertake.

## NOTE ADDED IN PROOF

A recent report by Tews et al. (1996) describing the crystallization and structure of *Serratia marcescens* chitobase, a bacterial enzyme homologous to the hexosaminidases, has allowed for modeling of the catalytic domain of human Hex A.

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