Juvenile GM2 Gangliosidosis Caused by Substitution of Histidine for Arginine at Position 499 or 504 of the α-Subunit of β-Hexosaminidase* (Received for publication, January 10, 1990)

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Juvenile GM2 gangliosidosis is a rare neurodegenerative disorder closely related to Tay-Sachs disease but of later onset and more protracted course. The biochemical defect lies in the α-subunit of the lysosomal enzyme β-hexosaminidase. Cultured fibroblasts derived from patient A synthesized an α-subunit which could acquire mannose 6-phosphate and be secreted, but which failed to associate with the β-subunit to form the enzymatically active heterodimer. By contrast, fibroblasts from patient B synthesized an α-subunit that was retained in the endoplasmic reticulum. To identify the molecular basis of the disorder, RNA from fibroblasts of these two patients was reverse-transcribed, and the cDNA encoding the α-subunit of β-hexosaminidase was amplified by the polymerase chain reaction (PCR) in four overlapping fragments. The PCR fragments were subcloned and shown by sequence analysis to contain a G to A transition corresponding to substitution of histidine for arginine at position 504 in the case of patient A and at position 499 in the case of patient B. The mutations were confirmed by hybridization of allele-specific oligonucleotides to PCR-amplified fragments of DNA corresponding to exon 13 of the α-subunit gene. The Arg504 → His mutation was found on both alleles of patient A as well as of another unrelated patient; the homozygosity of this mutant allele is attributable to consanguinity in the two families. The Arg500 → His mutation was found in patient B in compound heterozygosity with a common infantile Tay-Sachs allele. There is additional heterogeneity in juvenile GM2 gangliosidosis, as neither mutation was found in the DNA of a fourth patient. The Arg → His mutations at positions 499 and 504 are located at CpG dinucleotides, which are known to be mutagenic "hot spots."

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§ Listed as P-N-acetylhexosaminidase (EC 3.2.1.52) and also as N-acetyl-β-galactosaminidase (EC 3.2.1.30).

1 The abbreviations used are: GM2 ganglioside, N-acetylgalactosaminyl β1-4(N-acetylneuraminyl-α2-3)galactosyl-β1-4-glucosyl-β1-1-ceramide; PCR, polymerase chain reaction.

2 The GM2 gangliosidoses are autosomal recessive disorders caused by mutations in any of the three genes required for lysosomal degradation of the GM2 ganglioside: the α- and β-subunits of β-hexosaminidase and the activator protein. The subunits must be associated into the heterodimeric (αβ) isoenzyme, β-hexosaminidase A, to catalyze the removal of the β-GalNAc residue from the glycolipid; this function cannot be performed by the homodimeric isoenzymes B (ββ) or S (αα). Deficiency of the β-hexosaminidase A isoenzyme results in lysosomal accumulation of the undegraded GM2 ganglioside, particularly in the nervous system. Tay-Sachs disease, the infantile form of β-hexosaminidase A deficiency, is the best known and most common form of GM2 gangliosidosis; its neurologic manifestations become evident in infancy and lead to death in early childhood. Other forms of β-hexosaminidase deficiency are classified as "juvenile," "chronic," or "adult-onset" depending on age of onset and clinical course. The clinical, pathological, biochemical, and genetic aspects of the GM2 gangliosidoses have been summarized in a recent review (1).

Recent studies have shown molecular heterogeneity within the β-hexosaminidase A deficiency diseases, the consequence of mutations in the α-subunit gene (reviewed in Ref. 2). An insertion of 4 nucleotides in exon 11 (3) is the most common mutation among Ashkenazi Jews, accounting for over two-thirds of the carriers (3, 4) or about 2% of that population as a whole. The same population also harbors a splice site mutation in intron 12, but at a lower frequency (5-7). Deletion of exons 1 and its flanking sequences has been found among some French-Canadians (8, 9). These are null mutations, resulting in absence of mature α-subunit mRNA and lack of synthesis of α-subunit polypeptide and cause the infantile form of Tay-Sachs disease. Other less common allelic mutations that cause infantile Tay-Sachs disease encode defective polypeptides; they are not associated with any particular demographic group. These mutations include a substitution of lysine for glutamic acid at position 482 (10) and a deletion of cytosine in codon 504 resulting in premature termination (11); in both cases, the α-subunit is defective in its intracellular transport from the endoplasmic reticulum to the Golgi (11-13). In contrast, the "B1" mutation allows normal processing and transport of the α-subunit and association into the heterodimeric A isoenzyme; however, a substitution of histi...
dine for arginine at position 178 (14) abolishes its catalytic activity toward the G\textsubscript{M2} ganglioside (15). Finally, a substitution of serine for glycine at position 269 (16, 17) causes the synthesis of a defective \(\alpha\)-subunit that can be transported through the biosynthetic organelles and be secreted, but which fails to associate with the \(\beta\)-subunit (18); this mutation has been found in patients with adult-onset/chronic G\textsubscript{M2} gangliosidosis, usually in compound heterozygosity with one of the common Ashkenazi infantile mutations (16, 17), but occasionally in homozygous form (19).

In the present study, we have characterized at the level of polyepitide, mRNA, and DNA the mutations of two patients, Juvenile A and Juvenile B, with enzymatically diagnosed \(\beta\)-hexosaminidase A deficiency and clinically diagnosed G\textsubscript{M2} gangliosidosis of the juvenile type; for two additional patients, the mutations were examined at the level of DNA only. Data on the biosynthesis of the Juvenile B \(\alpha\)-subunit were included in a previous study (18) where her fibroblasts were identified as "California Juvenile." Identification of the molecular defects was achieved by amplification of the entire \(\alpha\)-subunit cDNA (after reverse transcription) by the polymerase chain reaction (20) and sequence analysis of the subcloned PCR fragments. Hybridization with allele-specific oligonucleotide probes confirmed the respective mutations in the genomic DNA.

**MATERIALS AND METHODS**

**Clinical Summary**—The patient identified here as Juvenile A is of Assyrian origin, and her parents are related as first cousins. She had already developed progressive ataxia, spastic paraplegia, dysarthria, and cherry-red macula at the time of referral at age 10. Juvenile B, who is of mixed Jewish and Scottish-Irish origin, manifested a progressively severe neurologic deterioration similar to that of her affected brother (21), that began at age 3-5 and progressed until her death at age 26. A third patient, a 12-year-old male, for whom only genomic DNA was examined, is of Armenian extraction. His parents are first cousins; symptoms began at age 4 with clumsiness of hands, followed by progressive ataxia, spasticity, and dementia. A fourth patient is a 4-year-old non-Jewish Caucasian female, with onset of tremors and psychomotor retardation beginning at age 2.

**Reagents**—Restriction and modifying enzymes were purchased from Promega Biotec or Stratagene, Thermus aquaticus (Taq) and modified T7 (Sequenase) DNA polymerases were from United States Biochemical, and avian myeloblastosis virus reverse transcriptase was from Biochemical and other cell culture reagents were from GIBCO. Cells and Cell Culture—The strain of normal human fetal lung fibroblasts (IMR 90) was obtained from the Cornell Institute for Medical Research, Camden, NJ. The strain of skin fibroblasts from a patient with juvenile G\textsubscript{M2} gangliosidosis designated Juvenile A was kindly provided by Dr. R. Gatti (UCLA). The IMR 90 strain of fibroblasts from the patient designated Juvenile B was initiated in the laboratory of one of the authors (M. K.). Fibroblasts were cultured at 35 °C in 5% CO\textsubscript{2} in Eagle's minimal essential medium supplemented with 10% fetal bovine serum, pyruvate, nonessential amino acids, and antibiotics.

Peripheral blood leukocytes from two additional juvenile G\textsubscript{M2} gangliosidosis patients and their parents, as well as from enzymatically proven noncarriers, were prepared by standard methods (24).

**Biosynthesis Studies**—Radioiodelabeling of fibroblasts, immunoprecipitation of \(\beta\)-hexosaminidase and its subunits, electrophoresis, and fluorographic visualization of the polypeptides were carried out as described (23) except for a change in the labeling medium. The medium used was Eagle's minimal essential medium with Earle salts, prepared from a GIBCO Selectamine kit to be free of either leucine or phosphate for labeling with \(^{3}H\)leucine or \(^{32}P\)phosphate, respectively; it was supplemented with antibiotics, pyruvate, nonessential amino acids, and 5% fetal bovine serum.

**RNA Preparation and Northern Blot Analysis**—Total RNA was isolated from cultured fibroblasts by a published procedure (25). About 20 \(\mu\)g of total RNA was electrophoresed in a 1% agarose, 0.6 M formaldehyde gel and transferred onto a nitrocellulose filter. Radiolabeling (26), blot hybridization (27, 28) with a full-length \(\alpha\)-subunit cDNA probe and washing (29) were performed according to standard procedures.

**Reverse Transcription and PCR Amplification of cDNA**—The enzymic reaction was reverse-transcribed and amplified in four overlapping pieces, using the four sets of oligonucleotide primers identified in group I, Table I. About 20 \(\mu\)g of total RNA was incubated for 1 h at 42 °C with 40 units of reverse transcriptase and one set of primers (250 pmol each of sense and antisense primers) in 50 \(\mu\)l of 50 mM Tris, pH 8.3, 50 mM KCl, 1 mM MgCl\textsubscript{2}, 1 mM dithiothreitol, 10 ng/ml bovine serum albumin, 0.2 \(\mu\)mol each of dNTP. The RNA template was digested with 1.5 \(\mu\)g of RNase A at 37 °C for 30 min. Taq polymerase (4 units) was added in 50 \(\mu\)l of PCR amplification buffer (42 mM Tris, pH 8.7, 3.7 mM MgCl\textsubscript{2}, 17 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 10 mM 2-mercaptoethanol, 0.17 mg/ml bovine serum albumin, 10% dimethyl sulfoxide). PCR amplification (20) of the cDNA was performed in a Perkin Elmer-Cetus DNA Thermal Cycler using the following profile: 1-min denaturation at 94 °C, 2-min annealing at 48 °C, 4-min extension at 72 °C for 40 cycles.

**Subcloning and Plasmid Sequencing**—The products of PCR amplification were purified by electrophoresis in a 5% nondenaturing polyacrylamide gel. Eluted DNA fragments were sequentially 5'-phosphorylated with T4 polynucleotide kinase, treated with T4 DNA polymerase, and blunted-end-ligated into the dephosphorylated SalI restriction site of pGEM3Z. The plasmid DNA was sequenced by the dideoxy method (31) with Sequenase as recommended by the manufacturer.

**Isolation and PCR Amplification of Genomic DNA**—High molecular weight genomic DNA was isolated from fibroblasts and leukocytes as described (32). The DNA from relatives of Juvenile A was provided by Dr. R. Gatti. Genomic DNA (1 \(\mu\)g) was annealed to 500 pmol each of sense and antisense primers (Table I, group 2) and amplified with 2.5 units of Taq polymerase in 100 \(\mu\)l of 67 mM Tris, pH 8.7, 6.7 mM MgCl\textsubscript{2}, 17 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 10 mM 2-mercaptoethanol, 0.17 mg/ml bovine serum albumin, 10% dimethyl sulfoxide, 0.2 \(\mu\)M each dNTP. Amplification was carried out for 35 cycles as follows: 2-min denaturation at 94 °C, 2-min annealing at 48 °C, and 3-min extension at 72 °C. The PCR products were purified by phenol/chloroform extraction and ethanol precipitation.

**Allele-specific Oligonucleotide Hybridization**—A minor modification of a published procedure (33) was used. About 10 ng of genomic PCR products were dotted onto Hybond nylon membrane in a Schleicher and Schuell dot blot manifold and hybridized with oligonucleotide probes listed in Table I, group 3. Filters were washed as described (33) at 54 °C and subjected to autoradiography.

**RESULTS**

**Defective \(\alpha\)-Subunits Identified by Biosynthetic Studies**—As shown in Fig. 1, fibroblasts of Juvenile A synthesized as much \(\alpha\)-subunit in a 3-h labeling period as did IMR 90 control fibroblasts (panel A, "pulse" lanes). But in contrast to the normal \(\alpha\)-subunit, the patient's \(\alpha\)-subunit was not processed proteolytically to the mature form and disappeared almost entirely from the cell during overnight incubation (panel A, "chase" lanes); it was not found extracellularly (not shown) and was presumed to have been degraded. Normal synthesis and maturation of the \(\beta\)-subunit served as internal control in these experiments. The Juvenile A \(\alpha\)-subunit could undergo a late posttranslational event, acquisition of the mannose 6-phosphate marker, in normal fashion (panel B, "P" lanes). Secretion of the Juvenile A \(\alpha\)-subunit could be induced by the presence of 10 mM NH\textsubscript{4}Cl in the medium; as shown by use of
TABLE I

Synthetic oligonucleotides

<table>
<thead>
<tr>
<th>Segments covered</th>
<th>Oligonucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exons 1-5</td>
<td>Sense: 5'ACGTGATTCGCCGATAAGTCACGG3'</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'GAGTGTCCAGGATGCTAGAGAG3'</td>
</tr>
<tr>
<td>Exons 5-9</td>
<td>Sense: 5'GACTGAGATTGAGGACTTTCCCGC3'</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'GCTGCTTGAAGTCCTCACCG3'</td>
</tr>
<tr>
<td>Exons 8-11</td>
<td>Sense: 5'GTCAGGGCCATAGGATACGGTTCAG3'</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'GAAGCCTGGCTCCACTACCAT3'</td>
</tr>
<tr>
<td>Exons 11-14</td>
<td>Sense: 5'GTGAACTATATGAAGGAGCTGGAACTG3'</td>
</tr>
<tr>
<td></td>
<td>Antisense: 5'GAAGCCTGGCTCCACTACCAT3'</td>
</tr>
</tbody>
</table>

Group 2: primers for PCR amplification of genomic DNA

| Introns 12-13    | Sense: 5'GAGGTAGCAGCCTGTGGATG3' |
|                  | Antisense: 5'TCCGCCTTGCGAAGGCCCCACAGCT3' |
| Exon 11–Intron 12| Sense: 5'GTGAACTATATGAAGGAGCTGGAACTG3' |
|                  | Antisense: 5'GGACAACTCCTGCTCTCAGG3' |

Group 3: allele-specific oligonucleotides

| Arg<sup>506</sup> → His (Juvenile A) | Normal: 5'CACACTTCCGCTGTGAGTT3' |
|                                    | Mutant: 5'CACACTTCCGCTGTGAGTT3' |
| Arg<sup>199</sup> → His (Juvenile B) | Normal: 5'CCTATGAACGTTTGTCACA3' |
|                                    | Mutant: 5'CCTATGAACGTTTGTCACA3' |
| Exon 11 Insertion (Tay-Sachs)       | Normal: 5'GAACCGTATxTCCTATGGC3' |
|                                    | Mutant: 5'GAACCGTATxTCCTATGGC3' |

Fig. 1. Synthesis of the α-subunit by fibroblasts derived from juvenile G<sub>M2</sub> gangliosidosis patient A. Normal (IMR 90) and Juvenile A confluent fibroblast cultures in 100-mm Petri dishes were labeled with 0.25 mCi of [<sup>3</sup>H]leucine/dish for a 3-h pulse, followed by an 18-h chase (panel A) and for the same period of pulse and chase in the presence of 10 mM NH<sub>4</sub>Cl (panel C). In panel B, the cells were labeled for 3 h with 0.25 mCi of [<sup>3</sup>H]leucine or 1.0 mCi of [<sup>32</sup>P]phosphate, as indicated. Antiserum raised against β-hexosaminidase A was used for immunoprecipitation from cell extracts, shown in panels A and B. Panel C shows immunoprecipitation from NH<sub>4</sub>-induced secretions using three different antisera, anti-A, anti-B, and anti-α, as indicated. The antisera precipitate: A, all forms of the α- and β-subunits; B, α-subunit that is associated with β, as well as all forms of the β-subunit; α, only monomeric α-subunit. Abbreviations: α<sub>n</sub>, β<sub>n</sub> = precursor α-, β-subunit; α<sub>m</sub>, β<sub>m</sub> = mature α-, β-subunit.

Fig. 2. Synthesis of the α-subunit by fibroblasts derived from juvenile G<sub>M2</sub> gangliosidosis patient B. Normal (IMR 90) and Juvenile B confluent fibroblast cultures in 100-mm Petri dishes were labeled with 0.25 mCi of [<sup>3</sup>H]leucine or 1.0 mCi of [<sup>32</sup>P]phosphate for a 3-h pulse followed by an 18-h chase, as indicated (panel A); antisera against α-hexosaminidase A was used for immunoprecipitation from cell extracts. Labeling with [<sup>3</sup>H]leucine and chase were carried out in the presence of 10 mM NH<sub>4</sub>Cl in panel B; free or associated α-subunit was precipitated from the secretions using specific antisera, as described in Fig. 1.

Identification of the Mutations in α-Subunit cDNA—The α-subunit mRNA from fibroblasts of the two index patients was found by Northern analysis to be of normal size (Fig. 3, left panel). The amount of α-subunit mRNA was found to be normal in fibroblasts of Juvenile A but reduced in fibroblasts of Juvenile B. The β-subunit mRNA served as internal control (Fig. 3, right panel).

The entire α-subunit mRNA was reverse-transcribed and amplified by PCR in four overlapping fragments. Sequence analysis of the subcloned PCR fragments from the mRNA of Juvenile A revealed a single change from the normal: a G to A transition at nucleotide position 1511, resulting in substitution of histidine (CAC) for arginine (CGC) at codon 504.
amplified DNA was analyzed by allele-specific oligonucleotide hybridization. As shown in Fig. 5, Juvenile A, whose parents are related to each other, had inherited the $\text{Arg}^{504} \rightarrow \text{His}$ mutation in homozygous fashion. The identical mutation was found in an unrelated juvenile $\text{GM}_2$ gangliosidosis patient, whose parents are also consanguineous. The amplified DNA from the parents of both patients hybridized to both normal and mutant probes as expected for obligate heterozygotes (Fig. 5). Amplified DNA from 63 enzymatically proven noncarriers of $\beta$-hexosaminidase deficiency hybridized solely to the normal oligonucleotide probe, confirming that this mutation was not a neutral polymorphism (data not shown).

The PCR fragment from the DNA of Juvenile B hybridized to both the normal and mutant oligonucleotides for codon 499, showing her to be a compound heterozygote for the $\text{Arg}^{499} \rightarrow \text{His}$ mutation (Fig. 6). This mutation was inherited from her mother; the other allele, inherited from her Jewish father, carried the more common 4-base pair insertion in exon 11 that underlies infantile Tay-Sachs disease. The reduced amount of $\alpha$-subunit mRNA (Fig. 3) is consistent with compound heterozygosity with a null allele. Amplified DNA from 63 noncarriers did not hybridize to the $\text{Arg}^{499} + \text{His}$ oligonucleotide probe, indicating that this change likewise is not a polymorphism (not shown). The genomic DNA of Juvenile B was also tested for base changes observed in some of the subcloned PCR fragments (see above), using oligonucleotides designed specifically for that purpose. In all instances, the genomic DNA was found to hybridize only to the normal oligonucleotide, confirming that the changes which had been observed in occasional clones were the result of Taq polymerase errors (not shown). Amplified DNA from the fourth juvenile $\text{GM}_2$ gangliosidosis patient failed to hybridize to mutant probes at codon 499 or 504 (data not shown).
We have identified mutations of the α-subunit gene of β-hexosaminidase, guanine to adenine transitions in exon 13, that are present on one or both alleles of patients with juvenile GM2 gangliosidosis. Two unrelated patients were shown to be homozygous for the Arg499 + His mutation. In both families, the parents are consanguinous. Another patient was shown to be a compound heterozygote for the Arg504 + His allele with the c.111insG allele. The absence of either substitution in a fourth patient shows that yet other mutations may give rise to juvenile GM2 gangliosidosis.

The defective α-subunit of Juvenile A (Arg499 + His) acquires the mannose 6-phosphate recognition marker but fails to associate with the β-subunit and is not processed to the lysosomal to the secretory pathway (22, 34), the monomeric α-subunit of Juvenile A is secreted. This shows that it can be transported out of the endoplasmic reticulum and Golgi complex. Overall, the abnormal α-subunit of Juvenile A resembles the association-defective α-subunit (18) caused by the Glu142→Ser substitution found in patients with chronic/adult-onset GM2 gangliosidosis (16, 17).

Because the α-subunit synthesized by fibroblasts of Juvenile B (identified in the earlier publication as “California Juvenile”) was previously found not to be associated with the β-subunit, it had been grouped together with the association-defective α-subunit of chronic and adult-onset GM2 gangliosidosis (18). However, the Juvenile B α-subunit (Arg504 + His) is not secreted in the presence of NH4Cl. It is retained in an early biosynthetic compartment, probably the endoplasmic reticulum. Therefore it resembles more closely the defective α-subunits resulting from some infantile Tay-Sachs disease mutations, deletion of cysteine in codon 504 (11, 13), and substitution of lysine for glutamic acid in position 482 (10, 19). Retention in the endoplasmic reticulum is thought to be due to misfolding of the mutant α-subunits, as has been observed for other defective proteins (reviewed in Ref. 35). It is clear that the effect of amino acid substitutions on the fate of the α-subunit is not correlated with their position in the linear sequence and will have to be interpreted in terms of the three-dimensional configuration of the polypeptide. Unfortunately, the requisite x-ray crystallographic information is not available at the present time. Despite the lack of knowledge regarding secondary and tertiary structure of the protein, the importance of arginine residues at positions 499 and 504 can be inferred from their evolutionary conservation. The arginine at position 504 in the α-subunit is conserved in the homologous β-subunit of human (30, 36) and murine (37) origin; the arginine at position 499 is even more highly conserved, as it is found in the human (30, 36) and murine (37) β-subunit, and also in the β-hexosaminidase of the slime mold, Dictyostelium discoideum (38).

The mutations described here give rise to a juvenile form of GM2 gangliosidosis, which is intermediate in clinical course between the catastrophic infantile form (Tay-Sachs disease) and the relatively mild adult-onset form. The clinical phenotype cannot be correlated with the linear position of the mutation nor with the properties of the polypeptide observed in radiolabeling studies. It is generally believed that clinical severity is inversely related to the ability of the mutant β-hexosaminidase to degrade GM2 ganglioside in vivo (1). We have been unable to demonstrate residual activity in fibroblast homogenates of the juvenile GM2 gangliosidosis patients, using a fluorogenic substrate, 4-methylumbelliferyl 6-sulfo-β-N-acetylglucosaminide (not shown). However, a mutant enzyme might not survive the standard extraction or assay conditions developed for the normal enzyme, or it might not function with a synthetic substrate. The fibroblasts of patient B had been shown previously to have 3% the normal activity with the physiological substrate, namely, GM2 ganglioside complexed to the activator protein (1).

The two G to A transitions identified in this study have occurred at CpG sites. Other G to A transitions at CpG sites have been found in exon 5 (the “B1” mutation, Arg504 + His (14)) and in exon 13 (Glu142→Lys (10)) of the β-hexosaminidase α-subunit gene and in intron 12 of the β-subunit gene (39). CpG sites are known to be mutagenic “hot spots,” accounting for a disproportionate number of human polymorphisms (40) as well as of disease-producing mutations (e.g. of hemophilia due to deficiency of Factor VIII (41–43) or Factor IX (44)). Such mutations reflect the tendency of 5-methylcytosine, frequently found at CpG sites, to deaminate spontaneously to thymidine (45). Depending on the DNA strand on which the deamination occurs, the result is either a C to T or a G to A transition on the sense strand. Such mutations occur sporadically, and the same base change in different individuals can be the result of separate mutational events, as has been observed in Factor VIII (43); thus the Arg499 + His mutation might have occurred independently in the two kindreds in which it was observed. A two-step repair system that excises the mismatched TMP and fills the gap with CMP has recently been described in mammalian cells (46); the deletion of cysteine at the CpG site in codon 504 (11) could have resulted from an error in the second phase of this repair process. Since the coding sequences of the two subunits of β-hexosaminidase each contain about three dozen CpG dinucleotide sites, additional mutations can be anticipated, even though not all the sites would be methylated and many of the mutations would be silent.

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Note Added in Proof—The Arg504 + His mutation was independently discovered by R. M. Boustany and K. Suzuki (personal communication) in a juvenile GM2 gangliosidosis patient from a consanguinous Lebanese Christian family.

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Molecular Basis of Juvenile G_{M2} Gangliosidosis


Juvenile GM2 gangliosidosis caused by substitution of histidine for arginine at position 499 or 504 of the alpha-subunit of beta-hexosaminidase.

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