



Tay-Sachs and Sandhoff diseases: Examples of genetically-determined diseases

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We received a thank you card from a client who expressed joy at the knowledge the baby she was carrying was unlikely to be affected and later went on to have a second normal baby. Years earlier, she had been devastated on learning that her first born had a rare, fatal disorder totally unknown to her and her husband. In addition, each of her subsequent pregnancies had predicted an affected fetus. Her first born died at the age of three despite excellent nursing and home care. She and her husband were found to be carriers of infantile Tay-Sachs disease (TSD).

Infantile Tay-Sachs disease and infantile Sandhoff disease are the major forms of lysosomal storage disorders collectively known as the GM2 gangliosidoses, so-named due to the massive storage of GM2 ganglioside, a sugar-containing fatty material that is a normal, albeit minor (<5% of the total) constituent of the brain gangliosides (at least 30 different species of gangliosides are known), the function of which is not completely understood (1).

Neurons of the central nervous system have the highest concentrations and the most diverse array of these substances in the body. When degradation of GM2 ganglioside is impaired, it accumulates in the lysosomes of neurons. Over time, the affected neurons become engorged with ganglioside-laden lysosomes, the neuronal structure becomes disturbed, and the neurons become dysfunctional (2). This slow unrelenting process results in the clinical conditions known as Tay-Sachs and Sandhoff diseases. In both, infants appear normal for the first few months of life after which time they display loss of head control, decrease in muscle tone, decreasing attentiveness, and often an exaggerated startle response. As the disease progresses, patients lose their ability to see, neurological deficits

and seizures become more prominent leading ultimately to a vegetative state and death before 4 years of age. While the infantile forms of GM2 gangliosidosis are the most severe, both of these disorders are clinically heterogeneous and include later onset juvenile and adult variants.

All forms of Tay-Sachs and Sandhoff disease are inherited as autosomal recessive traits, meaning one mutant allele is inherited from each parent, who, while carriers, are completely normal and do not manifest any features of the diseases (1).

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Tay-Sachs disease, named after the co-discoverers Warren Tay, in 1881 and Bernard Sachs, in 1896, is due to mutations in the HEX A gene that resides on chromosome 15 (15q23-24) and encodes the α -subunit of the heterodimeric ($\alpha\beta$) enzyme, Hex A (see review by Mahuran 3). Sandhoff disease, named after Konrad Sandhoff (4,5), is due to mutations in the HEX B gene that resides on chromosome 5 (5q13) and encodes the β -subunit of Hex A. A second form of Hexosaminidase, called Hex B, is also composed of 2 subunits but both are β -subunits. Normal individuals have both Hex A and Hex B in all tissues in the approximate ratio of 60 parts Hex A: 40 parts Hex B. In Tay-Sachs disease and its variants, only Hex A is defective (as mutations occur in the α -subunit alone) while Hex B is normal; whereas in Sandhoff disease, both Hex A and Hex B are defective (as both contain the defective β -subunit). Tay-Sachs disease has a carrier frequency of about 1:30 in the Ashkenazi Jewish (AJ) population and about 1:256 in the general population. Sandhoff disease does not show any ethnic predilection and has a carrier frequency of about 1:300. Hex A alone has the ability to degrade GM2 ganglioside, and since Hex A is defective in both Tay-Sachs and Sandhoff disease, GM2 ganglioside is stored in both conditions.

Over 100 different mutations have been identified in the HEX A gene, but within the AJ population, three mutations account for ~98% of all disease mutations (the remaining ~2% are rare and occur in single families as "private" mutations) (3). These are (i) a 4 bp insertion in exon 11 (+TATC1278), representing 70-80% of infantile TSD alleles; (ii) a splice junction mutation (G to C transversion) at the 5' end of intron 12 (+1 IVS-12), comprising 13-17% of infantile TSD alleles; and (iii) a G805A (Gly269Ser) mutation in exon 7, totaling 5-6% of AJ mutations. The

latter is associated with adult-onset TSD (6). When any two of these mutations are found in the same individual the disease ensues. Neither the 4bp insertion nor the splice junction mutation generate a viable Hex A α -subunit whereas the adult onset Gly269Ser mutation is potentially a target for therapeutic interventions. Similarly, at least 29 mutations, mostly considered "private" in the HEX B gene have been described, the majority of which do not generate a viable β -subunit.

Biochemical diagnosis of GM2 gangliosidosis can be achieved by a simple enzyme assay that uses a non-sulfated synthetic substrate and takes advantage of the difference in the heat stability of Hex A and Hex B, where Hex A is heat labile and Hex B is heat stable under the same conditions (7,8). Alternatively it has been found that Hex A hydrolyzes a sulfated form of the synthetic substrate whereas Hex B does not (9). These analyses can be performed using serum or peripheral blood leukocytes as enzyme source. In Tay-Sachs disease, the total level of Hexosaminidase activity (Hex A + Hex B) is within normal limits but all of it is heat stable (i.e. only Hex B is present) whereas Sandhoff disease

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is suspected if the total serum Hex is very low using either or both non-sulfated and sulfated substrates and is completely heat labile (an unstable non-physiological form of the enzyme containing only heat unstable α -subunits contributes to the detectable activity). Based on the differences in heat lability of serum Hex A and B, carrier detection programs were launched in the early 1970's in Los Angeles (Dr. Mike Kaback), Toronto (Dr. J.A. Lowden), Montreal (Dr. C.R. Scriver), and many centres around the world resulting in a marked decline in the incidence of Tay-Sachs disease worldwide (7,8). In current carrier screening programs, maximal ascertainment is achieved when both serum and leukocyte based enzyme analysis and molecular diagnostics are used to identify the carrier state. Molecular diagnostics is the preferred ap-

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proach for carrier screening in the AJ population as it takes advantage of the high incidence of a few specific mutations in this population. However, where no mutations are found, it is recommended that the enzyme analysis be performed to exclude the occurrence of the more rare private mutations. When both of the mutations are known in families, molecular diagnostics is the preferred method for carrier detection and for monitoring pregnancies at risk for the disease.

Through research, significant progress has been made in the understanding of how the various mutations in the HEX A and HEX B genes affect the structure, function, and lysosomal localization of the respective enzymes. Arising from this, great strides have been made in developing treatments for some storage diseases over the last 15 years; particularly noteworthy is the successful development of enzyme replacement therapy for Gaucher disease, the most common lysosomal disease known (10). This has led to additional enzyme replacement therapies (for lysosomal

diseases such as Hurler disease, Fabry disease, among others) and the development of new approaches to therapy, such as enzyme enhancement/chemical chaperone treatments. Of particular note, with respect to Tay-Sachs and Sandhoff disease, is the recent work of Don Mahuran and his group at the Hospital for Sick Children, Toronto, where they have demonstrated enhanced levels of Hex A in cultured cells from late-onset patients with various genotypes, treated with two different competitive inhibitors of the enzyme, one being a drug previously approved by the US FDA for another disease which acts as pharmacological chaperones for the mutant enzyme (11,12). Cultured fibroblasts from late onset Tay-Sachs patients with the most common missense mutation (G805A (Gly269Ser) in exon 7) respond well. Additionally, unlike enzyme replacement therapy, these small molecules often can cross the blood brain

of mutations causing the GM2 gangliosidosis. *Biochim Biophys Acta.* 1455:105-138, 1999.

barrier to act on the storage products. While this therapeutic approach only applies to this missense mutation and not to those that cause the infantile forms of Tay-Sachs and Sandhoff disease, further studies using similar approaches are warranted and offer the potential for future positive intervention in some forms of these devastating disorders. ❖

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