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Tay-Sachs Disease: From Molecular Characterization to Ethical Quandaries and the Possibility of Genetic Medicine

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Abstract

Tay-Sachs disease (TSD) is a rare neurodegenerative disorder caused by mutations in the HEXA gene, which encodes the α subunit of the enzyme β-hexosaminidase A. Lacking this key enzyme in GM2 ganglioside catabolism, individuals who are homozygous for HEXA mutations suffer from abnormal accumulation of GM2 ganglioside in brain and nerve cells, ultimately resulting in the progressive deterioration of the central nervous system. TSD is one of three disorders characterized by β-hexosaminidase deficiency; Sandhoff disease (SD) and the AB variant arise by mutations in the HEXB and GM2A genes respectively, which disrupt other points of GM2 ganglioside degradation. Characterized by developmental delay and stagnation, muscular weakness, coordination deficits, seizures, and eventual hearing and vision loss, these three disorders are clinically indistinguishable and occur in three forms defined by age of onset. While there is a much higher incidence of TSD in the Ashkenazi Jewish population, community carrier screening and counseling initiatives have reduced disease prevalence to about the equivalent of non-Jewish populations; however, such efforts have raised ethical concerns in the Jewish community that are increasingly relevant in light of scientific and medical advancements. Currently, treatments for TSD and its related disorders focus on symptom management, with gene therapies and the application of modified CRISPR-Cas-9 technology being explored.

Introduction

Named after ophthalmologist Warren Tay and neurologist Bernard Sachs, Tay-Sachs disease (TSD) is a neurodegenerative disorder caused by the excessive accumulation of gangliosides in the nerve cells of the central nervous system[1]. Following Tay's 1881 description of "a conspicuous, tolerably defined, large white patch, more or less circular in outline, and showing at its centre a brownish-red, fairly circular spot, contrasting strongly with the white patch surrounding it," the cherry-red spot which is now the

hallmark of the disease, in the eyes of a one-year-old infant who displayed increasing weakness of the neck and limbs[2], Sachs published his paper on "arrested cerebral development" in 1887[3]. Presenting the case of "patient S," Sachs' work provides a detailed description of the clinical progression of this newly discovered condition. Concluding his paper with a report of the postmortem examination of the child, Sachs noted the inability to find any neurons with a normal histologic appearance[3]. Instead, his search of the brain revealed ballooned nerve cells with the nuclei of these cells obliterated by masses of particulate material: the first observation of the abnormal lipid accumulation that characterizes TSD[3]. A Jewish physician working among New York's large Jewish population, Sachs encountered nineteen cases of the disease over the next nine years in individuals of this ethnic community, with notable consanguinity among the parents of affected children, leading him to the discernment of its familial and ethnic nature[4].

Nearly ninety years after Tay and Sach's initial discoveries, neuroscientist John O'Brien and colleague Shintaro Okada uncovered the genetic basis of the disorder in 1969[5]. As a result of mutations in the HEXA gene, individuals with Tay-Sachs disease are deficient in the lysosomal beta-hexosaminidase A (HexA) enzyme which is a requisite for breaking gangliosides in the nerve cells of the brain and spinal cord[6]. Lacking this catabolic enzyme, the brains of affected individuals suffer from excessive accumulation of GM2 gangliosides, a group of neuronal cell membrane glycosphingolipids having key roles in normal central nervous system functioning[7]. Ultimately, this buildup results in significant neuronal degeneration and death. Despite significant advances in understanding the disease, the progression from ganglioside accumulation to progressive neurodegeneration and death is not yet fully characterized[8]. Generally, the accumulation of gangliosides is viewed as the primary insult to neurons, while other neuropathologies like demyelination and inflammation are considered consequences of neuronal deterioration. However, evidence suggests that both demyelination, arising by the arrest and inhibition of myelin formation, and inflammation are also active participants in disease progression and the destruction of neurologic functioning, opening up another avenue for targeted therapeutic intervention[8].

Primarily involving the progressive deterioration of motor and cognitive functions, the clinical manifestations of Tay-Sachs reflect this degradation of neurons. Inversely correlated with the level of HexA enzyme activity, the severity of TSD takes on three forms: infantile, juvenile, and adult[9]. Each form is distinguished by the general age of onset and manifests symptoms that are similar but vary both between type and within type[1]. While Tay-Sachs disease is the most common, two additional disorders are characterized by beta-hexosaminidase deficiency, GM2 ganglioside accumulation, and resulting neurodegeneration: Sandhoff disease (SD) and GM2-activator protein deficiency (AB variant)[7]. Sandhoff disease arises from mutations in the hexosaminidase (HEXB) gene and GM2-activator protein deficiency from mutations in the GM2A gene[7]. This group of disorders known as the GM2 gangliosidoses represent different points of molecular failure; however, they are clinically indistinguishable.

When the molecular basis of Tay-Sachs disease was discovered, prenatal detection and the development of carrier screening programs became possible[1]. An autosomal recessive disorder, Tay-Sachs disease occurs at significantly higher proportions in individuals of eastern European (Ashkenazi) Jewish descent[10]. Reflecting the inverse relationship between the level of residual HexA activity and the severity of the disease, different allele combinations contribute uniquely to the form of Tay -Sachs affecting an individual[1]. Generally, individuals who are homozygous or compound heterozygous for null alleles will present the infantile form of the disease, whereas juvenile-onset results in individuals with one null allele and one missense allele[1]. In the least severe form, adult-onset, a combination of either two missense alleles or one null allele and one missense allele associated with residual HexA activity is present[1]. These varying levels of

hexosaminidase A in the body tissues and fluids of individuals with Tay-Sachs disease have enabled a highly sensitive, straightforward, and inexpensive method for prenatal diagnosis and identification of heterozygous individuals through enzymatic testing[11]. Within the general population, about one in 300 individuals is a carrier, and one in 360,000 individuals is born with Tay-Sachs[1]. Among the Ashkenazi Jew population, the carrier prevalence is ten times more frequent at one individual out of every thirty, resulting in an incidence of one affected individual for every 3,600[1]. As a result of carrier screening programs, genetic counseling, and prenatal diagnosis, the incidence of affected births in this population has declined by more than 90% over the last three decades as the Ashkenazi community has engaged in preventative measures[12]. Currently, most cases of Tay-Sachs occur in populations that are not considered high risk as individuals are not aware of their carrier status due to these disorders being caused by rare, recessive alleles[13].

Genetic & Molecular Basis

Mutations occurring in the 35-kb long HEXA gene, located on chromosome 15q23-24, are the root of Tay Sachs disease[14]. The HEXA gene encodes the alpha subunit of the beta-hexosaminidase A enzyme[9]. Including partial deletion, splicing, nonsense, and missense mutations, 181 HEXA mutations resulting in a dysfunctional HexA protein have been reported[7]. In Ashkenazi Jews, the ethnic group demonstrating a high prevalence of the disease, three specific mutations are pervasive, accounting for 94% to 98% of cases: a four base-pair insertion in exon 11, a G→C transversion at the 5' splice site of intron 12, and a G→A transition at the 3' end of exon 7[14]. Present in roughly 80% of Tay-Sachs alleles among Ashkenazi Jews, the 4-bp TATC insertion induces a frameshift producing a downstream premature stop codon and ultimately the degradation of the mRNA via the nonsense-mediated decay pathway. This insertion is also the most prevalent mutation in non-Jewish individuals with the disease[14]. As a result of G→C transversions, abnormal splicing and subsequent mRNA instability occur[14]. Causing an amino acid substitution of serine for glycine at codon 269, G→A transitions result in reduced enzyme activity as α-chain mRNA levels are decreased and the ability of the mutant α-subunits to form stable αβ heterodimers is impaired[14]. The mutations in exon 11 and intron 12 typically manifest in the infantile and juvenile forms of Tay-Sachs, while the mutation in exon 7 is associated with the onset of the disease in adulthood[10]. In another population showing high frequencies of Tay-Sachs, the French Canadians of southeastern Quebec, the major mutation is a 7.6 kb deletion of the 5' end of the HEXA gene that removes the promoter region, exon 1, and part of intron 1[10]. Occurring in 70% of infantile-onset cases, this mutation is incompatible with mRNA synthesis; therefore, HEXA protein translation is not possible[15].

The GM2 gangliosidoses, TSD, SD, and the AB variant, arise when GM2 gangliosides are not catabolized due to deficient β-hexosaminidase enzyme[9]. The major glycolipids of the neuronal cell membrane, the GM2 gangliosides fulfill structural roles, such as membrane organization and cell adhesion, and are involved in numerous neurodevelopmental processes, including the formation of the neural tube, neuritogenesis, axonogenesis, synaptogenesis, and myelination[7]. Normally, these lipids are catabolized by the heterodimeric (αβ) β-hexosaminidase A enzyme (HexA), which must be in dimeric form to function[9]. When HexA is not present in adequate quantities, abnormal storage occurs in the lysosomes, and GM2 ganglioside aggregates in cells[6]. Occurring particularly in neurons of the brain and spinal cord due to those cells having the highest GM2 ganglioside concentration, this excessive build-up is toxic, leading to cell degeneration and death through a number of pathways, including lysosomal degradation and adaptive and innate immune response[7]. In cases of GM2 gangliosidoses, the prevalence of GM2 is significantly increased, representing 90% of nervous system gangliosides instead of the normal 5%[9]. In individuals with the more severe infantile form of the disease, the GM2 ganglioside buildup in the brain is ubiquitous, while storage in the brains of individuals affected with the

later-onset forms is less excessive[1]. In these cases, GM2 ganglioside buildup may be limited to specific regions of the brain such as the brain stem, hippocampus, or spinal cord while others, like the neocortex, remain unaffected[1].

Requiring the concerted action of three gene products – the α and β subunits of HexA and the GM2 activator protein, hydrolysis of GM2 ganglioside can also be interrupted by mutations in the gene encoding the β subunit of HexA, HEXB, and the gene encoding the activator protein, GM2A. In cases of Sandhoff disease, mutations in the HEXB gene, located at chromosome 5q13, disrupt the production of the β subunit of the HexA enzyme as well as the homodimeric (ββ) hexosaminidase (HexB) enzyme[9]. Thus, individuals with Sandhoff disease are deficient in the enzyme that degrades gangliosides (HexA) and the enzyme that degrades globosides (HexB), which are another type of glycosphingolipid[7]. Currently, 103 mutations have been identified for the HEXB gene, including missense, nonsense, splicing, small indels, and gross deletion[7]. The least common of the three GM2 gangliosidoses, the AB variant, arises from mutations in the GM2A gene that encodes the lipid transporter protein GM2-AP[7]. For complete GM2 ganglioside degradation, GM2-AP must also be present to extract the lipids from the cell membrane and solubilize them to their substrate form[9]. Mutations in the GM2A gene are extremely rare and have only been observed in nine different allele combinations across eleven clinical cases[7].

Symptoms

Because the accumulation of GM2 ganglioside occurs in the central nervous system, TSD, SD, and the AB variant are particularly severe disorders[16]. Both the severity of clinical phenotype as well as the age of disease onset are dictated by the extent of HexA activity, 10-15% of which is necessary for the prevention of GM2 ganglioside build-up[16]. While the disorders are categorized into three forms, infantile, juvenile, and adult-onset, the clinical manifestations occur on a continuous spectrum[9]. Most commonly, this group of disorders presents in the infantile form and are clinically indistinguishable from one another[9].

Characterized by extremely low levels of HexA, less than 0.5%, the classic or infantile form of Tay-Sachs produces initial symptoms between three and six months of age and results in early-childhood death[1]. As GM2 ganglioside builds up in neuronal cells, deteriorating them, the infant's nervous system development and function stagnate, resulting in mental and motor regression[16]. Commonly, infants will first lose various motor skills such as sitting, crawling, turning over, and raising the chest and head[1]. An increased or exaggerated startle response is often observed, and disease progression is accompanied by slow growth, muscle weakness and spasms, and gradual loss of vision[6]. With age, come more serious symptoms, including seizures, deterioration of cognitive capacities, paralysis, blindness, unresponsiveness to the environment, and respiratory failure, all of which occur on an ever-quickening timeline[6]. Generally, individuals with infantile Tay-Sachs are relegated to a vegetative state by age two and do not survive past the age of four, typically succumbing to aspiration bronchopneumonia in year two or three of life[16]. In addition to the accumulation of GM2 gangliosides in the brain structures, the macular cells of the retina are also subject to build-up[16]. The subsequent deterioration of these cells exposes the choroid, which contains the blood vessels responsible for supplying blood to the retina, producing the cherry-red spots in the eyes that are displayed in about 90% of infants with Tay-Sachs[2].

In individuals with juvenile or adult-onset Tay-Sachs disease, minimal residual activity of the HexA enzyme, typically 5-15%, hinders the rapid progression of symptoms[1]. According to the extent of HexA deficiency, the symptoms present in these two less common forms of Tay-Sachs vary. Despite having a mean age of onset of 21 years, subtle motor symptoms may arise during childhood in cases of the adult form thus resulting in overlap in the age of onset for juvenile and adult TSD[9]. Clumsiness and coordination deficits present as some of the first signs of juvenile Tay-Sachs as ataxia emerges in the individual, typically between the ages of two and five years following a period of normal development[1]. From initial symptoms,

gains in motor and speech abilities plateau before regressing. Additional symptoms manifested as the disease runs its course include dysarthria, dysphagia, muscle spasms, and brain atrophy[12]. In a small natural history study of all three of the GM2 gangliosidoses, 100% of individuals developed disturbances in gait and speech, 87% developed intellectual impairment, and about 50% presented with behavioral and psychiatric problems[9]. Death typically occurs around age fifteen[12].

In contrast to infantile Tay-Sachs disease which displays generally consistent symptoms on a regular timeline among affected individuals, late-onset/adult Tay-Sachs is associated with a highly variable age of onset, clinical course, and prognosis[14]. While one affected individual may present symptoms in early childhood or adolescence, another may not show any signs until their 20s or 30s, and another may reach their 60s or 70s experiencing only minor muscular complications[6]. Regardless of the age of symptom onset, the adult-onset form is considerably less clinically aggressive than the infantile or juvenile forms, as a result of high residual activity of the HexA enzyme, resulting in a gradual rather than rapid decline in central nervous system function; early signs and symptoms often go unnoticed, receiving no medical attention[1]. Like the other forms of the disease, ataxia, muscle spasticity, dysarthria, and dysphagia are typical symptoms, usually progressing in severity as the individual ages[1]. Mental deterioration is also commonly observed, as an individual experiences memory deficits and changes in behavior and personality[6]. About 50% of affected individuals are subject to psychiatric symptoms, including anxiety, depression, bipolar episodes, and various forms of psychosis such as hallucinations, paranoia, and withdrawal from reality[14]. As with all other characteristics of this form of Tay-Sachs, lifespan varies ranging from shortened to unchanged[6]. Ultimately, the life expectancy for an individual with late-onset TSD is unknown due to the high variance of the disease symptoms and presentation.

Symptom Management

Currently, treatment options for Tay-Sachs disease have no curative effect and are primarily palliative. Given the neurodegenerative course of the disease, individuals with TSD and the other GM2 gangliosidoses generally do not survive past the age of four. Because disease progression cannot be slowed, treatments are focused on managing the symptoms specific to the individual and improving their quality of life. In cases of infantile Tay-Sachs, the four general priorities of physicians are maintaining adequate nutrition and hydration, protecting the airway, preventing infectious diseases, and controlling seizures[1]. Feeding tubes are often necessary not only for nutritional support but also to prevent aspiration[6]. While anticonvulsants may be used to treat seizures, complete seizure control is rarely achieved as seizure frequency increases over time[1]. Individuals may also be attended to by ophthalmologists, audiologists, and speech pathologists, who address the feeding and swallowing problems associated with the disease, and physical therapists who work to improve mobility and flexibility[6]. With the goal of maximizing function and maintaining the ability to engage in the activities of daily living, treatment for Tay-Sachs in the juvenile and adult forms is provided by psychiatrists, physical, occupational, and speech therapists according to the symptoms that manifest in the individual[1]. Though treatments for late-onset Tay-Sachs are unable to cure the disease, it is important that diagnosis is early and accurate as certain drugs, particularly neuroleptics, can exacerbate neurological symptoms in patients with the late-onset form, who would otherwise experience a prolonged disease progression[12].

Diagnosis, Screening, & Ethical Implications

For both Tay-Sachs and Sandhoff disease, enzymatic assay to detect the level of β-hexosaminidase activity in an individual's blood is considered the best method for diagnosis and carrier identification[9]. Using the synthetic substrates for the HexA and HexB enzymes, 4-methylumbelliferyl-N-acetyl-β-glucosamine (4-MUG) and 4-methylumbelliferyl-N-acetylglucosaminide-6-sulfate (4MUGS), the enzyme assay can be performed with isolated serum or leukocytes[9]. Individuals with

infantile-onset TSD or SD present absent or extremely low HexA activity, 0%–5%, as well as normal to elevated levels of HexB, while those with later-onset forms show minimal residual HexA activity, typically between 5% and 15%[9]. Sandhoff disease is characterized by low activity of both the HexA and HexB enzymes. Diagnosis of TSD and SD carriers also considers the percent of HexA activity in combination with the total hexosaminidase activity. In carriers of TSD, HexA constitutes 20%-50% of total hexosaminidase activity as opposed to 60%-65% in noncarriers, while carriers of SD typically display a decrease in total hexosaminidase activity in conjunction with a high proportion of HexA activity (75%–80%). If these conditions are suspected before birth, amniocentesis and chorionic villus sampling (CVS) may be used to determine levels of enzyme activity for diagnosis. Confirmation of a diagnosis or resolution of inconclusive results may be obtained by familial variant testing, panel tests for common HEXA and/or HEXB pathogenic variants, and sequencing analysis of the HEXA, HEXB, and/or GM2A genes.

With the capabilities for carrier identification by serum quantification of HexA and fetal diagnosis through amniocentesis and the recognition of the high incidence rate in the Ashkenazi Jewish population, a community-based TSD heterozygote screening program was conceived and implemented[11]. First introduced in Baltimore, Maryland in 1970, this program was made possible by the efforts of many individuals and organizations, from the scientists who developed a fully automated assay for serum hexosaminidase to the volunteer educators who recruited participants and promoted the initial screening event. To elicit high compliance, organizers deemed education to be a critical component and thus relied heavily on local rabbis and Jewish women's organizations to inform and direct members of their communities. Additionally, carriers themselves played a role in furthering the screening, finding themselves responsible for communicating their positive status and the need to be tested to their relatives[17]. In the first thirty years of carrier screening for Tay-Sachs disease after the relevant enzymatic defect was discovered in 1969, more than 1.4 million individuals across the world were screened, the majority being Jewish and of child-bearing ages greater than eighteen[13]. Ultimately, the program, consisting of the test itself, genetic counseling, and strategic integration of its members into its educational aims, resulted in a 90% reduction of Tay-Sachs incidence in the U.S./Canadian Jewish population and prompted program development in Jewish communities in other countries.

The first effort of its type in the United States, this prototype community testing and education program has served as a model for other areas of public health education, heterozygote screening, and reproductive counseling for avoiding fatal childhood diseases[11]. Despite its success, carrier screening raised numerous ethical issues in the Jewish community, not least among them the association of the Jewish community with another stigmatized disease. For the Hasidic and ultra-Orthodox communities, distrust of information originating outside their community and worry over the marriageability of their children and the issue of abortion prevented them from experiencing the general decline of TSD births brought about by carrier testing[17]. To mitigate these concerns and thereby encourage increased participation, screening was eventually aimed at high school youth through the premarital, anonymous Dor Yeshorim screening program that seeks to avoid the risk of TSD births without exposing individuals to community, insurance, or employer social stigmatization and discrimination[18]. While Dor Yeshorim has been successful in Orthodox communities, the program has been criticized on numerous levels[17]. For example, by withholding users' own test results due to the potential emotional burden, stigmatization of families, and jeopardizing of future marriages[19] – even when finally utilized to judge the genetic compatibility of a marriage match – the program reinforces the very stigma it professes to circumvent[17].

In the absence of a cure, the soundest public health approach and most ethically appealing alternative were determined to be avoiding Tay-Sachs births via

screening, education, and genetic counseling. Indeed, population-based genetic screening has virtually eliminated TSD from the Jewish community but not without raising a broad range of social, medical, and ethical concerns that have become increasingly relevant in light of genetic advances. As individuals consider the implications of the inheritance patterns and clinical progression of TSD for themselves, their family – both real and potential – and even their community, they must navigate the tension between personal agency and a sense of responsibility to others. While genetic counseling enables the individualized and informed autonomy to make responsible genetic decisions, the social implications of TSD constrain so-called freedom of choice as genetic self-knowledge and management are reframed within the context of moral and social obligation according to current trends in values. In a society where healthy children are expected and suffering is to be avoided, questions over human value as related to suffering, normality, and functionality, optimization of reproduction, and the place of genetic compatibility in marriage arise. The acts of genetic counseling and prenatal screening involve inherent judgments about the value of lives that are genetically impaired, the kinds of individuals who should be allowed to exist, and who should be allowed to make such judgments. For a disease tied to ethnic and religious influences as TSD is, the ethics of genetic decision-making are even more complex. On one hand, the benefits of genetic testing have been profound. Besides dramatically decreasing the incidence of TSD, genetic counseling options lead to the births of 2500 unaffected children between 1969 and 1998 that may have otherwise not been conceived or brought to term[11] . On the other hand, to resist intervention, participation in advocacy and education efforts, or the testing of one's children, to make marital and reproductive decisions that oppose wider perceptions of right and wrong, characterizes one as irrational and even immoral when the potential genetic disposition of an individual seems to indicate that the highest quality of life is to not be born at all.

Investigative Therapies

For many families affected by TSD or SD, the goal is not a cure but rather stability and symptom improvement. To that end, treatments restoring a mere 2% of normal enzyme activity would dramatically improve the outlook for those affected with these diseases, considering the small increments of hexosaminidase activity that separate the severe infantile forms from the variable juvenile and adult forms.

Enzyme Replacement Therapy

Currently, enzyme replacement therapy is being investigated as a potential treatment for Tay-Sachs disease, with the goal being to introduce a replacement for the deficient HexA enzyme responsible for ganglioside build-up[16]. While this route has been used to treat other lysosomal storage diseases, such as Gaucher disease, Hurler syndrome, and Fabry syndrome, enzyme replacement therapy has not been a success in Tay-Sachs-affected individuals[6]. In Gaucher disease, the fat-laden cells accumulate in the spleen, liver, and bone marrow as opposed to the brain where the replacement enzyme is unable to traverse the blood-brain barrier due to its size[16]. Though enzyme replacement therapy in Tay-Sachs patients has been ineffective, researchers consider the success of the therapy in other lysosomal storage disorders as a cause to continue examining the mechanism and investigating possibilities for a working model of enzyme administration in brain cells[16].

Substrate Reduction Therapy

Another route of research is substrate reduction therapy, which involves slowing the synthesis of glycolipids by the introduction of small molecules that are able to bypass the blood-brain barrier[16]. Whereas enzyme replacement therapy attempts to digest the cell waste via a replacement enzyme, substrate reduction therapy seeks to inhibit the production of the harmful waste in the first place, reducing it to a level where the residual enzyme activity is enough to prevent pathologic accumulation[14]. By decreasing the amount of substrate synthesized, the

therapy works to enable adequate metabolization of GM2 gangliosides by residual HexA activity, thus preventing the build-up that results in neuron death[16]. While Tay-Sachs mouse models have demonstrated success in reducing ganglioside storage, decreasing neurological symptoms, and extending life through substrate reduction therapy, the translation of success to humans has been unreliable thus far[16]. Currently, however, the oral inhibitor of the glucosylceramide synthase enzyme that catalyzes an early step in the synthesis of GM2 gangliosides, Venglustat, is undergoing a phase 3 clinical trial[20]. Including participants with adult and juvenile-onset GM2 gangliosidosis, the trial seeks to assess the pharmacodynamics, pharmacokinetics, and safety of Venglustat as well as its efficacy in decreasing or improving neurologic and motor symptoms in cases of late-onset TSD and SD.

Gene Therapy

Gene therapy, which replaces the defective gene with a functional gene, offers a potentially permanent solution for correcting Tay-Sachs disease and its GM2 counterparts. Using a viral vector, the therapeutic HEXA gene is introduced into the organism, stimulating regular production of the lysosomal enzyme that is otherwise deficient[16]. Whereas lysosomal enzymes are impermeable to the blood-brain barrier, gene therapy can circumvent this issue. For the GM2 gangliosidoses and other lysosomal storage disorders, Adeno-associated virus (AAV) intracranial gene therapy, delivered by injections to specific central nervous system areas, such as the thalamus, cerebral spinal fluid (CSF), and deep cerebellar nuclei, has emerged as the most promising approach[21]. Utilizing the mechanism by which secreted lysosomal enzymes, especially when overexpressed, may be taken up by neighboring cells through the mannose-6-phosphate receptor and targeted to the lysosome, gene therapy enables the delivery of the functional gene to a small subset of cells to supply the entire body with the replenished enzyme[22]. By targeting structures that are highly interconnected with other regions of the brain, such as the striatum, deep cerebellar nuclei, thalamus, and cerebral spinal fluid, infusion approaches capitalize on the natural pathways of the brain – diffusion, CSF flow, and axonal transport – for distribution of AAV vectors and the therapeutic enzyme and minimize the number of injections necessary to achieve widespread enzyme distribution[22]. AAV gene therapy for GM2 Gangliosidosis has proven effective in various animal models, including SD mice and cats and TSD sheep, demonstrating that axonal transport is an evolutionarily conserved mechanism for the widespread distribution of lysosomal enzymes in large brains[23].

A study by McCurdy and colleagues (2015) examined the extent of biochemical disease correction following intracranial injections of AAVrh8 vectors in feline models of Sandhoff disease[21]. After bilateral injections in the thalamus and deep cerebellar nuclei with monocistronic AAVrh8 vectors encoding feline Hex alpha and beta subunits, widespread distribution of the vector and enzyme was observed by histochemical staining. Quantitative analyses showed that residual Hex activity against a synthetic fluorogenic substrate was significantly higher in treated SD cats than in untreated SD cats at 2.7-35-fold normal in the brain and 4.2-14-fold normal in the spinal cord. In correlation with the increased HexA levels, AAV-treated SD cats exhibited a significant reduction of GM2 ganglioside in the brain at 89-99%. Furthermore, AAV-treated cats demonstrated clinical benefit as they came out of the study with preserved neurological function and gait, having had the disease progression stabilized by the therapy. Providing the first report of unequivocal therapeutic effect in a non-rodent model of gangliosidosis, this work was a critical step toward human clinical trials.

In 2019, researchers from UMass Chan Medical School and Auburn University administered the first-in-human test of AAV gene therapy for Tay-Sachs disease, providing the basis for future trials of AAVhr8-HEXA/HEXB vectors in patients with TSD and Sandhoff disease[23]. Two children with infantile TSD, each at a different point of disease progression, underwent treatment. Whereas Patient TSD-001 (2½ years old) demonstrated the developmental delays, macrocephaly, seizures, and

abnormal myelination characteristic of Tay-Sachs patients, Patient TSD-002 (7-months old) was clinically well at the time of trial enrollment, having been diagnosed because of two preceding siblings with the disease. Both individuals were administered an equimolar mix of the AAVrh9-HEXA and AAVrh8-HEXB vectors, with Patient TSD-001 injected intrathecally only due to thalamic degeneration and Patient TSD-002 treated with both thalamic and intrathecal injections at half the dosage of her trial counterpart. Prior to treatment, baseline HexA activity in the CSF was about 0.3 nmol ml^{-1} per hour. After treatment, levels of HexA activity were observed to be 0.5-0.6 nmol ml^{-1} per hour, and Western blots of CSF showed increases in HEXA protein levels from 0 to 6 months after treatment. Between the two patients, there were differential rises in various GM2 ganglioside species in the CSF, with greater increases in the 2½-year-old than in the 7-month-old, indicating a relationship between GM2 levels and disease state. However, as GM2 ganglioside levels are known to increase exponentially, potentially reaching about 200-fold higher levels than normal at the time of death, even the modest increases or stable levels observed in the patients are indicative of a therapeutic effect. This therapeutic effect was evident in plateaued disease progression, in the form of increased myelination for TSD-002 and resistance to seizures for TSD-001 at a point where TSD patients are normally refractory to antiepileptic medications. No serious adverse events or antitransgene immune responses arose in relation to the vector, and the mild adverse events that did emerge were determined to be related to procedural aspects of the treatment. Preclinical safety studies in nonhuman primates indicate that a thalamic injection 10 times higher than was given is safe and most efficacious; however, as the integration and relay center of the cerebral cortex, the thalamus is a high-risk target. As patient safety dictated a reduced injection volume, dosage increases of both the thalamic and intrathecal AAV injections are one of the next steps for future clinical trials.

Based on this proof-of-concept data from Flotte and colleagues[23], the U.S. Food and Drug Administration initiated a registrational study of gene therapy for individuals with Tay Sachs and Sandhoff disease in November of 2020 by lifting a clinical hold and clearing an Investigational New Drug (IND) application for the Axovant Gene Therapies AXO-AAV-GM2 therapy[24]. The first investigational gene therapy to achieve IND clearance for Tay-Sachs and Sandhoff disease and the first potentially curative treatment for these diseases to enter the clinic, AXO-AAV-GM2 delivers two AAVrh8 vectors encoding the HEXA and HEXB genes directly to the central nervous system to restore HexA function, as demonstrated by the 2019 expanded access study[23]. In this new registrational trial, a dose-ranging cohort to evaluate safe dose-escalation will be followed by an efficacy cohort. The study will enroll both infants (6 months to 20 months old) and juveniles (2 years to 12 years old) with TSD or SD.

Gene Editing

Though still in its initial stages, the first preclinical studies of genome editing tools have demonstrated potential for the treatment of GM2 gangliosidoses, addressing some of the limitations of AAV gene therapy. CRISPR/Cas9 uses an RNA-guide nuclease (sgRNA-Cas9) to induce double-strand breaks (DSB) into a specific locus of the genome which can be repaired either through non-homologous end joining or homologous direct repair mechanisms[7]. Used to knock out target genes, the CRISPR/Cas9 system has been used to create *in vitro* and *in vivo* models of GM2 gangliosidoses[7]. While double-stranded cuts are useful for disrupting genes or moving large segments of DNA, they can lead to unfavorable off-target effects, including complex mixtures of indel products, translocations, and activation of p53[26]. Limited by its inability to make precise genome edits, CRISPR/Cas9 is not a suitable approach for pathologies caused by hundreds of mutations, such as the GM2 gangliosidoses[7]. However, two refinements in the CRISPR system present new routes of research for treating Tay-Sachs and Sandhoff disease[26,28]. The knock-in strategy using genomic safe harbors, loci with high transcriptional activity and the capacity to accommodate new therapeutic genes without causing unintended alterations to the host genome,

addresses the limitations of off-target effects and editing of alleles that is insufficient for beneficial levels of protein expression[27]. Prime editing, which can facilitate indels and conversions without cutting both strands of DNA or using DNA templates, circumvents shortcomings in targeting flexibility and editing precision[26].

In 2019, Ou and colleagues described the novel PS813 gene-editing system to treat Tay-Sachs and Sandhoff disease using the albumin locus as an integration site for a promoterless HEXM cDNA to produce constitutive expression of HEXM[28]. Consisting of a modified α subunit incorporating a partial sequence of the β subunit that can form a stable dimeric enzyme capable of degrading GM2 gangliosides, HEXM is placed under the control of the albumin promoter for secretion by hepatocytes. By integrating the corrective donor cassette into a locus with high transcriptional activity (i.e. safe harbor), this approach addresses the limitations of episomal AAV vectors: namely, diluted vector expression in dividing cells and restrictions in packaging capacity. After the intravenous administration of two AAV8 vectors (AAV8-SaCas9 and AAV8-HEXM-sgRNA) in neonatal Sandhoff mice, increased enzyme activity in the liver, heart, spleen, (up to 144- and 17-fold of wildtype levels) and brain and a reduction of GM2 gangliosides in the liver, heart, and spleen to normal levels were observed. Though this same level of brain GM2 ganglioside reduction was not observed, it was hypothesized that due to the substantial amount of gangliosides in neuronal membranes, even very small reductions in the total amount of GM2 gangliosides may result in therapeutic benefit in light of significant coordination, motor function, and motor memory improvements in the treated mice. Demonstrating the potential of developing *in vivo* genome editing as a treatment for Tay-Sachs and Sandhoff patients, this research presents the alternatives to delivering therapeutic genes across the blood-brain barrier, packaging both HEXA and HEXB cDNA into one AAV vector, and dealing with the risks and costs associated with the use of two vectors.

Mediating targeted insertions, deletions, and all twelve base-to-base conversions without requiring double-strand breaks (DSB) or donor DNA templates, prime editing uses a catalytically impaired Cas9 endonuclease, which only nicks a single strand of the double helix rather than cutting both, coupled to a reverse transcriptase[26].

The reverse transcriptase is programmed with a prime editing guide RNA (pegRNA) that specifies the target site and encodes the desired edit. Through these modifications to the original CRISPR/Cas9 system, more flexible and precise editing is enabled. As proof of principle, Anzalone and colleagues performed over 175 edits in human cells to correct the primary genetic causes of numerous diseases, including Tay-Sachs. In TSD cells, efforts were directed toward the most common mutation that causes TSD, the 4-bp insertion in HEXA ($HEXA^{1278+TATC}$). This mutation was created in HEK293T cells with 31% efficiency and only 0.8% indels and subsequently corrected by 19 of the 43 pegRNAs tested with an efficiency of 20% or more. In comparison to CRISPR/Cas9 DSB-dependent repair systems, off-target effects at predicted regions of the genome were nearly undetectable.

The potential for avoiding undesirable changes in the genome while employing such specific editing is a significant advancement in the possibility of therapeutic gene editing. Since its development, prime editing has been examined for its capacity to repair disease-causing variants in cells, organoids, and mice embryos, and numerous online tools have been developed to design pegRNAs[29]. Much additional research is required for prime editing to prove its utility and amend difficulties in application: optimization of the pegRNAs for their targets, determination of the cellular factors that facilitate prime editing efficiency, prediction and evaluation of genome-wide off-target effects, and delivery methods for the large construct of RNA and enzymes into living cells.

Conclusions

Linked to a number of important scientific discoveries, technical innovations, and sociohistorical events, the characterization of Tay-Sachs disease has lead to successful preventative measures and targets for

therapeutic intervention. However, effective treatments have yet to be realized. While there is excitement over the developments in gene therapy, the long-term effects of these treatments are still unknown, as is whether they will merely be another means of symptom management or actually cure the diseases. Generating clinical trial data is made difficult by the small number of patients and the rapid progression of the disease. Moreover, there are also questions about safe and effective dosages, whether reversing the clinical course of these diseases will ever be possible, if prevention will be the only option, and how narrow the treatment window is. If a gene therapy drug is eventually approved for Tay-Sachs and/or Sandhoff disease, the cost will no doubt be an issue; when Zolgensma, the gene therapy for spinal muscular atrophy affecting about 60,000 children every year, was approved by the FDA in 2019, became the most expensive drug in U.S. history at $2.1 million per patient.

As the science of gene therapy continues advancing, the successful development and application of this treatment for TSD may lie in universal newborn screening for the condition[25]. Because most cases of TSD occur in non-high-risk populations due to heightened awareness and carrier screening in the Ashkenazi community, TSD may be an unsuspected condition for affected infants in many cases. Consequently, accurate diagnosis, particularly for those affected with the juvenile and adult-onset forms, may be delayed, allowing the disease state to progress beyond treatment capabilties and clinical trial qualifications. Due to the lack of treatment for TSD and the rarity of the disease, many national health authorities are thus far reluctant to fund such efforts; however, in order for gene therapy to become a viable option for patients, the industry needs to identify affected individuals to test the treatment in while their disease state remains within study parameters. Though universal newborn screening involves various concerns of cost and ethics, it would facilitate greater participant numbers for clinical trials by identifying individuals affected with TSD and doing so before the aggressive progression of the disorder excludes them from the parameters of the study. With evidence from other diseases and clinical trials suggesting that gene therapy will generally only be able to suspend neurological damage rather than reverse it, early detection and diagnosis will be critical for the application of the treatment as well[25].

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Competing Interests

The authors declare no competing interests.

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