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REVIEW ARTICLE

CRISPR-Cas9 Mediated Gene Therapy: Current Advancements and Applications Towards Tay-Sachs and Sandhoff Disease

Richelle Bertly Josefano [1], Abigail Yoel [1], Beatrice Miranda Japri [1], Felicia Lael Belva [1], Clara Ayu Widjojo[1], Rio Hermantara[2*]

[1]Department of Biotechnology, Institut Bio Scientia Internasional Indonesia, Jakarta, Indonesia

[2]Department of Biomedicine, Institut Bio Scientia Internasional Indonesia, Jakarta, Indonesia

**Corresponding author: rio.hermantara@i3l.ac.id*

A B S T R A C T

Tay-Sachs disease and Sandhoff disease are neurodegenerative diseases that are classified as autosomal recessive lysosomal storage disorders. They are commonly caused by a mutation in the HEXA and HEXB genes, which are responsible for encoding beta-hexosaminidase-A (Hex A) and beta-hexosaminidase-B (Hex B). Furthermore, Sandhoff's disease symptoms include spinocerebellar ataxia, motor degeneration, sensorimotor neuropathy, tremor, dystonia, and psychosis, which are comparable to Tay-Sachs disease symptoms. The current treatment of Tay-Sachs includes enzyme replacement therapy, bone marrow transplantation, and administration of genetically modified stem cells with HexA, which do not impede neurological dysfunction and are ineffective in the long run. On the other hand, there is no standard treatment for Sandhoff, but it utilizes bone marrow transplantation, which is ineffective. So far, there is only one available gene editing treatment. Therefore, it might be necessary to consider gene editing as a prospective treatment for both diseases, with CRISPR being a primary method. By utilizing Adeno-associated viruses (AAV) as the delivery method for the CRISPR-Cas9 system, it can replace the defective HEXA or HEXB gene with a modified gene termed HEXM, which was found to be the gene codes for the Hex subunit of the same enzyme that is missing in Tay-Sachs and Sandhoff disease. Several challenges of implementing CRISPR-Cas9 technology to treat Tay-Sachs and Sandhoff disease include off-target mutations, unintentional cleavage of the non-targeted sites, and bioethical challenges. Further studies can be explored using various CRISPR-Cas9 systems to improve its efficiency.

K E Y W O R D S

CRISPR-Cas9, Gene therapy, Tay-Sachs disease, Sandhoff disease

H I G H L I G H T S

- Tay-Sachs and Sandhoff diseases are autosomal recessive neurodegenerative lysosomal storage disorders caused by mutations in the HEXA and HEXB genes with similar neurological symptoms, making it hard to distinguish without genetic testing.
- Current treatments, such as enzyme replacement therapy and bone marrow transplantation, have not proven effective in addressing the neurological dysfunctions of Tay-Sachs and Sandhoff diseases.
- CRISPR-Cas9 gene editing presents a potential solution, replacing defective genes (HEXA or HEXB) with a modified HEXM gene through Adeno-associated virus (AAV) delivery.

INTRODUCTION

In the general population, Tay Sachs disease is uncommon, with an incidence of around 1 in 100,000 live births and a carrier frequency of about 1 in 300. People of Ashkenazi Jewish ancestry, such as those of central or eastern European background, are more likely to be affected by Tay-Sachs disease, with them being 1 in 30 carrier genes (Resnik et al., 2019). However, Kumar and Rodriguez (2018) mentioned that currently, many cases occur in people of other ethnic backgrounds, such as French-Canadian and Cajun descent. On the other hand, Sandhoff disease is considered to be the “severe form” of Tay-Sachs disease and is estimated only to affect 1 in 1,000,000 people; it occurs in a multitude of populations, including the Creole population, the Canadian population, and individuals with Lebanon ancestry (National Organization for Rare Disorders, 2020).

The rising occurrence of Tay-Sachs and Sandhoff diseases among various groups, such as those of French Canadian and Cajun heritage, is influenced by multiple genetic and demographic factors. The founder effect, which occurs when a small subset of individuals establishes a new population carrying specific genetic traits, has increased the prevalence of certain mutations within these communities (Matute, 2013). For instance, the insertion mutation in exon 11 of the beta-hexosaminidase A alpha-subunit is not only prevalent among Ashkenazi Jews but also found in populations like the Cajun and French-Canadian communities (Hoffman et al., 2013). Genetic drift, a random change in allele frequencies over time, can further intensify the impact of genetic drift, contributing to a higher rate of recessive diseases in smaller founder populations (Robinson et al., 2023). Moreover, some mutations are unique to particular populations, such as the intron 9 mutation in the Hex A alpha-subunit seen in the Cajun group, which emerged within the last century (McDowell et al., 1992). Intermarriage and consanguinity (marriage between close relatives) also promote the chances of inheriting two copies of a recessive gene, thus increasing disease prevalence (Anwar et al., 2014).

Over time, genetic mutation accumulation has further increased the frequency of these conditions in specific groups. For instance, the exon 11 insertion mutation is common in the Cajun population, having been present since the community's formation more than 200 years ago (McDowell et al., 1992). Likewise, there is a high carrier rate for Tay-Sachs disease in the French-Canadian population, suggesting a significant genetic burden of the disease-causing alleles (Hoffman et al., 2013).

Both diseases are quite concerning as they affect mostly infants. Children who usually suffer from this disease only have a short life span of up to 4 years of age (Lyn et al., 2020). Up until now, there has yet to be an effective treatment for both diseases but more towards easing the symptoms; hence, an efficacious treatment is needed, which can be done through gene therapy (Picache et al., 2022; National Institutes of Health (NIH), 2023).

Since its development as a genetic editing tool in 2012, Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) has been the current choice for developing novel gene editing and gene therapy treatments. It has provided promising results for other genetic diseases, including sickle cell disease, HIV, cystic fibrosis, and many more (Wu et al., 2020).

In December 2023, CASGEVY, a therapeutic that utilizes CRISPR by Vertex Pharmaceuticals, was approved by the FDA as a treatment for sickle cell disease. Vaso-occlusive crisis (VOC) is an inflammatory response and a painful complication as a result of suffering from sickle cell disease. However, according to the clinical study conducted with 31 patients, when patients were administered with CASGEVY, it resulted in 93.5% of patients not experiencing severe VOC, and 100% were not hospitalized (Parums, 2024; Vertex Pharmaceuticals, n.d.). Compared to Zinc Finger Nucleases (ZFN) and Transcription Activator-Like Effector Nucleases (TALEN), CRISPR-Cas9 is less time-consuming as it does not need to be paired with a cleaving enzyme, has high specificity, and has a low cytotoxic activity (Lino et al., 2018). Hence, observing the

success of CRISPR and its benefits, it is believed to have the same promising efficiency and effect on Tay-Sachs and Sandhoff disease (Wong et al., 2021).

The review will provide an in-depth evaluation of the current advancement and application of CRISPR-Cas9- mediated gene therapy toward Tay-Sachs disease and Sandhoff disease. This review will discuss the utilization of CRISPR-Cas9-mediated gene therapy towards Tay-Sachs disease and Sandhoff disease, its efficiency, opportunities, affordability, and challenges. Lastly, future considerations, including ongoing clinical trials, will be explored, as well as possible methods to overcome the challenges.

TAY-SACHS AND SANDHOFF DISEASE

Tay-Sachs and Sandhoff diseases are autosomal recessive lysosomal storage disorders that are clinically and diagnostically almost indistinguishable. According to Ramani & Sankaran (2021), Tay-Sachs and Sandhoff diseases are neurodegenerative disorders caused by a mutation occurring in the HEXA and HEXB genes, respectively, which encode the enzymes beta-hexosaminidase-A (Hex A) and beta-hexosaminidase-B (Hex B). Inside the endoplasmic reticulum, processing and assembly of these subunits result in three isoforms: b-hexosaminidase A (ab, HexA), b-hexosaminidase B (bb, HexB), and b-hexosaminidase S (aa, HexS). For the enzyme to become catalytically active, it must first form a dimer consisting of two chains. Properly folded and assembled enzymes are rapidly transported to the Golgi apparatus, where they acquire the mannose-6-phosphate recognition marker and then directed to the lysosomes for final processing, ultimately producing the mature enzymes (Maier et al., 2003). The hexosaminidase enzyme, which comprises two subunits, Hex A and Hex B, is considered a hydrolase found in the lysosomes that helps the catabolism of sphingolipids, which in this case are GM2 gangliosides. When the HEXA gene is mutated, the activity of Hex A and Hex B is inhibited; the mechanism by which it inhibits other Hex proteins is not fully known; however, it is thought to have been linked to the loss of Hex A activity, which affects the stability and function of Hex B (Dersh et al., 2016; Kumari, 2017; Lemieux et al., 2006). It is also hypothesized that the inhibition results from the disruption of protein folding, assembly of the enzyme, and/or intracellular trafficking in the alpha chain of Hex A (Dersh, 2016). As a result, the ability of the cells to degrade GM2 gangliosides is reduced, resulting in an excess of GM2 gangliosides (Lyn et al., 2020). Accumulation of GM2 gangliosides within the lysosome of neurons induces toxicity and leads to cell death and neurological damage in the brain and spinal cord. (Demir et al., 2020).

The HEXA and HEXB genes encode precursor proteins that are produced during the development of hexosaminidase (Maier et al., 2003). The process starts with the precursor proteins that are generated in the cell's ER, which are the hexosaminidase subunits that are encoded by the HEXA and HEXB genes. In the ER, the precursor proteins go through processing and subunit assembly. Hexosaminidase B's beta chains undergo dimerization to produce the catalytically active enzyme (Sonderfeld-Fresko & Prioa, 1988). The alpha and beta subunits of hexosaminidase A are encoded by the genes HEXA and HEXB, respectively. The hexosaminidase A enzyme is made up of the alpha and beta subunits (Lemieux et al., 2006).

Tay-Sachs disease

There are three proteins required for the hydrolysis of GM2 ganglioside: the two subunits of Hexosaminidase A (HexA) and the GM2 activator protein (GM2A). GM2A is a small glycolipid transport protein that acts as a substrate-specific cofactor for the enzyme. **Figure 1** shows the idea of lysosomal metabolism of GM2. HexA is an enzyme composed of two distinct subunits, an α chain and β chain, forming a heterodimeric structure. Unlike some enzymes that directly interact with their substrates, HexA does not directly engage with the membrane-bound GM2 ganglioside. Instead, the GM2 activator protein extracts the GM2 ganglioside from the membrane, forming an activator-lipid complex (Chavany & Jendoubi., 1998). This complex structure consists of a ceramide backbone linked to an oligosaccharide unit made of four sugar

molecules: glucose, galactose, N-acetylneuraminic acid, and N-acetylgalactosamine (Tettamanti & Anastasia, 2009). This complex then serves as the actual substrate for HexA, enabling the enzyme to catalyze the GM2 degradation by removing N-acetylgalactosamine from GM2.

In Tay-Sachs disease, the lack of HexA results in the accumulation of GM2 gangliosides. Initially, HexA is transported to the Golgi body after the process of glycosylation, the formation of intramolecular disulfide bonds, and dimerization inside the ER. The enzyme must undergo post-translational alteration along with mannose-6-phosphate, which is crucial for helping the lysosome detect the HexA enzyme. The activator protein GM2A facilitates the binding of GM2 ganglioside to the active site of HexA, resulting in lipophilic GM2 ganglioside that may be hydrolyzed in the hydrophilic media of the lysosome (Dersh et al., 2016; Sandhoff, 2016; **Figure 2**). Accumulation of GM2 gangliosides inside lysosomes forms characteristic inclusions within the cells known as membranous cytoplasmic bodies, which are enlarged lysosomes packed with gangliosides. Progressive accumulation of gangliosides in neurons will cause neurodegeneration and, ultimately, neuronal death (Demir et al., 2020).

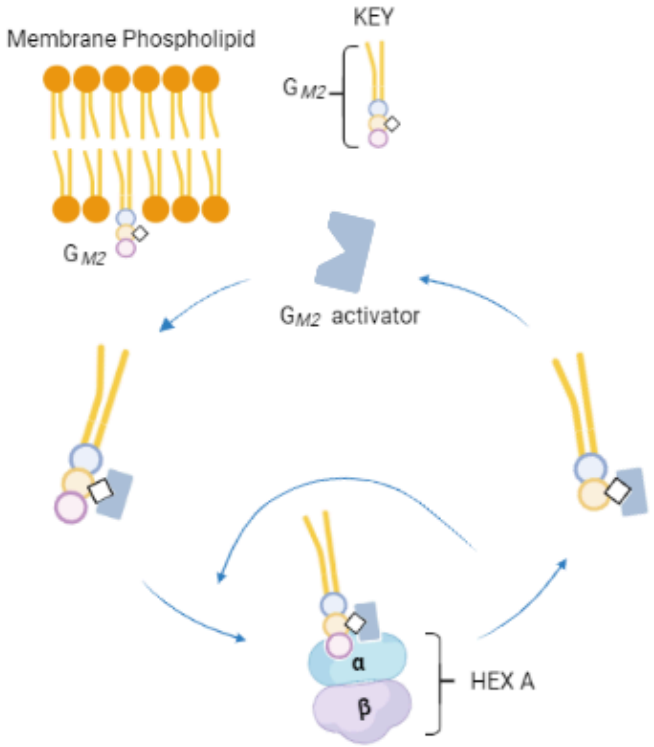

Figure 1. **GM2 ganglioside metabolism.** GM2 activator binds to the GM2 gangliosides, forming an activator-lipid complex that can be a substrate for HexA, which is the enzyme used for degrading GM2 (Created using Biorender and adapted from Chavany & Jendoubi., 1998)

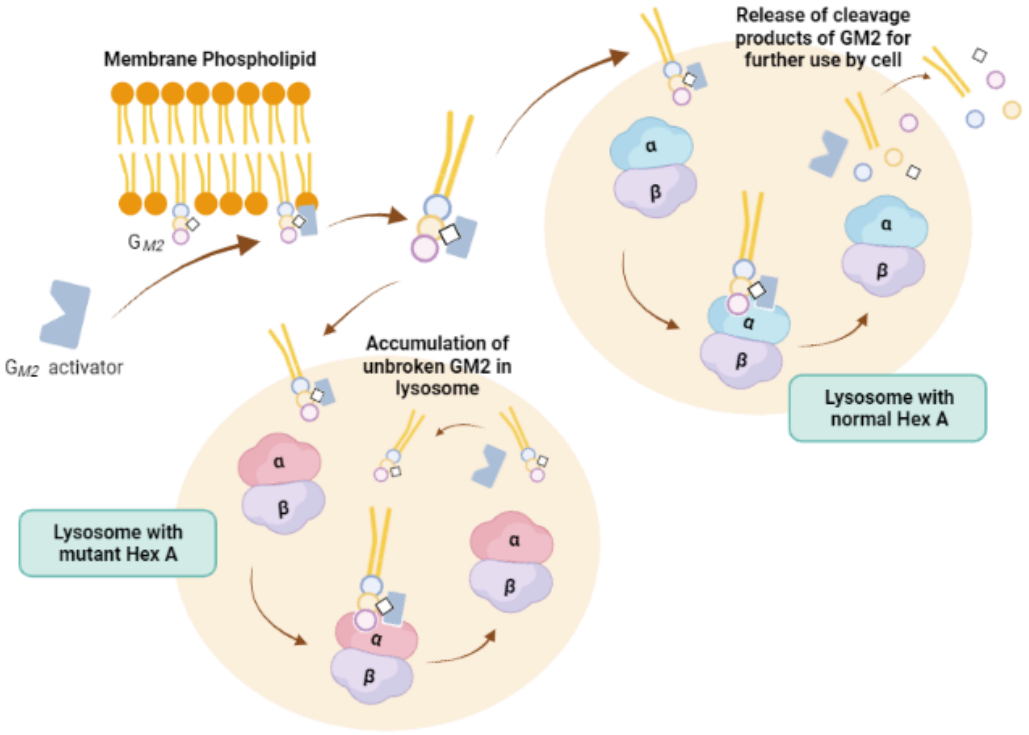

Figure 2. Tay-Sachs disease pathogenesis. Initiated by the mutation in HEXA, which results in disruption of HexA activity. Lysosomes with normal HexA release GM2 cleavage products for further cellular use, while in lysosomes with mutant HexA, GM2 gangliosides accumulate in neurons, causing neurodegeneration. (Created using Biorender and adapted from Solovyeva et al., 2018)

Tay-Sachs disease can be categorized into three types: infantile, subacute juvenile, and late-onset. In infantile Tay-Sachs, symptoms emerge within the first few months of life, and the disease advances rapidly, leading to significant and severe neurological decline in a short period. Most children with infantile Tay-Sachs do not survive past early childhood, often passing away before the age of 4. Conversely, subacute juvenile Tay-Sachs shows symptoms typically in late childhood or adolescence, usually between 2 and 10 years old. Progression is slower than in the infantile form, with symptoms gradually worsening over time. Though less severe than the infantile type, affected individuals may still survive into late teens or early adulthood (Picache et al., 2022). Late-onset Tay-Sachs presents symptoms during early adulthood, usually between ages 20 and 30, and progresses slowly over several years. The symptoms are milder compared to the early-onset forms (Vasquex, 2020). This disease can be distinguished by acute neurodegeneration that is accompanied by the activation of macrophages and astrocytes, expansion of microglia, and the production of inflammatory mediators (Solovyeva et al., 2018). The main characteristic of Tay-Sachs disease is the "cherry red" spot in the retina because the macula and choroid usual color has faded, contrasting with the pallor produced by the enlarged ganglion cells in the rest of the retina. Additionally, other symptoms happen in late-onset form, including ataxia, dysarthria, muscle weakness, tremors, atrophy, and psychosis (Lyn et al., 2020).

Sandhoff disease

Sandhoff disease, like Tay-Sachs disease, is caused by an accumulation of GM2 ganglioside in neurons in the brain and spinal cord, but the mutations happened in two different genes. Tay-Sachs disease is caused by HEXA gene mutations, whereas Sandhoff is caused by HEXB gene mutations (Yasui et al., 2013). Sandhoff disease, like Tay-Sachs, is divided into three kinds with varied symptoms: infantile, juvenile, and adult. Progression of the disease starts early, at 3-6 months of age. After that, they will show symptoms like the loss of motor skills such as turning over, crawling, and sitting (Tim-Aroon et al., 2021). They also have a

stronger startle response to loud noises. Children with Sandhoff illness have seizures, vision and hearing loss, and intellectual disability as the disease develops. Sandhoff illness in both juvenile and adult stages is extremely rare, and the signs and symptoms are usually milder than in the infantile form. The juvenile stage can start between the ages of 2 and 10. Speech difficulties, loss of cognitive function (dementia), seizures, and lack of motor coordination are all common symptoms. Adult Sandhoff's illness is characterized by mobility impairments as well as psychological concerns (Kang et al., 2013).

CURRENT TREATMENT OF SANDHOFF AND TAY-SACHS DISEASE

Tay-Sachs disease

There have been several therapeutic approaches to Tay-Sachs disease; however, very few of them have undergone clinical trials. One of the treatment options that has undergone clinical trials is substrate reduction therapy (SRT), which prevents a mutant enzyme from forming certain substrates, which lessens the demand for the enzyme to hydrolyze its substrate and lowers the buildup of substrates associated with lysosomal storage disorder (Picache et al., 2022). For instance, miglustat (N-butyldeoxynojirimycin, NB-DNJ), a glucosylceramide synthase immunosugar inhibitor, lowers the buildup of the substrate caused by glucocerebrosidase mutations in Gaucher disease. SRT for TSD entails lowering GM2, which is formed in TSD, in order to prevent the creation of the accumulating substrate, but it could not stop the progression of the neurological dysfunction (Maegawa et al., 2009, as cited in Solovyeva et al., 2018). Furthermore, there have been other therapeutic approaches, including enzyme replacement therapy (not yet undergone clinical trials for Tay-Sachs) and administration of genetically modified stem cells with HexA (succeeded in increasing HexA in the leukocytes in a 3-year-old patient but did not impede neurological symptoms) (Pichache et al., 2022). Similarly, like the stem cell therapy, it did not have registered clinical trials available, and most of the results only showed an increase in HexA activity and survival rate of the model mice; this did not impede neurological dysfunction (Matsuoka et al., 2011; Norflus et al., 1998; Wada et al., 2000; Stepien et al., 2018). Moreover, gene therapy has been tested on both small and large animal models, which yielded a positive result and can be used to treat two GM2 ganglioside diseases (and Sandhoff disease), unlike other treatment options (Gray-EdTay-Sachswards et al., 2018; Lahey et al., 2020).

Sandhoff disease

Most current treatments for Sandhoff disease are palliative, focusing on symptom relief and improving quality of life rather than curing the disease. These include anticonvulsants to manage seizures, muscle relaxants to help manage spasticity, and proper nutrition and hydration (Karimzadeh et al., 2014). Although no treatments have been proven to extend life expectancy, clinical trials are ongoing (Parker & Parker, 2007; Prabu et al., 2022). One of them is SRT; as mentioned above, it is a clinically approved treatment for Gaucher disease (Cox et al., 2003). The therapy aims to lower GL1 levels in Gaucher cells by blocking the enzyme responsible for its production (GCS). GL1 is the anabolic precursor to the glycosphingolipids that develop in Sandhoff disease. Thus, inhibiting the glycosphingolipid pathway in the CNS may result in a lower lipid accumulation. Marshall et al. (2019) treated Sandhoff mice with the GCS inhibitor Genz-682452, which has demonstrated CNS access. The GCS inhibitor Genz-682452 successfully reduced GL1 and GM2 levels, delayed motor function loss, and increased lifespan by 17.5%. Although there were no current clinical trials in human patients, this result successfully showed the development of SRT to treat Sandhoff disease. Furthermore, Ou et al. (2020) used a novel PS813 gene editing system with the HEXM subunit to treat Sandhoff mice. By delivering the CRISPR system via AAV, HEXM cDNA was integrated into the albumin locus, enabling lysosomal enzyme production. The result showed an increase in brain activity, motor coordination improved, and tissue analysis showed reduced vacuolation in the brain and

liver, highlighting the potential of in vivo gene editing for treating Sandhoff disease. Most Sandhoff disease treatments are still in clinical trials with mouse models. However, Villamizar-Schiller et al. (2015) tested miglustat and a ketogenic diet in a 6-year-old Sandhoff patient, resulting in significant improvements in seizure control, neurological and cardiac function. The mechanism behind these effects remains unclear, and further research is needed.

INTRODUCTION TO CRISPR

CRISPR-Cas9 is a gene-editing tool that consists of two primary components, which are a guide RNA and Cas9 (CRISPR-associated protein 9 nuclease). A guide RNA aids in identifying the intended target gene, and Cas9 causes double-strand breaks that allow the genome to be changed (Redman et al., 2016). As genetic diseases arise from changes in the genetic sequences, CRISPR gene editing offers a promising potential solution/treatment for these types of diseases (Cox et al., 2015). For instance, sickle cell disease is a genetic disorder that has been successfully treated with CRISPR-Cas9 gene therapy (Frangoul et al., 2021). The therapy aims to restore fetal haemoglobin expression to relieve the disease's symptoms. CRISPR-Cas9 was applied to knock out the BCL11A transcription factor, a gene that suppresses fetal haemoglobin production. Results showed that it reduces disease symptoms, improves life quality, and reduces the need for blood transfusions.

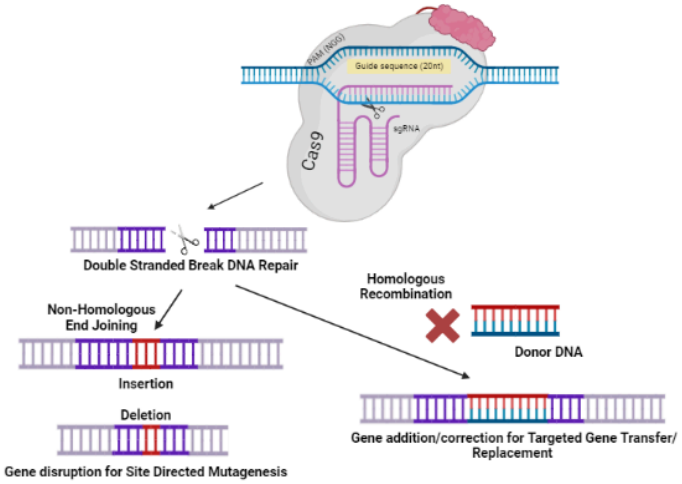

Figure 3. **The Cas9/sgRNA system's fundamental strategy.** There are two mechanisms to repair DSBs: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ directly ligates broken DNA ends without the need for a homologous template; it is efficient but error-prone as it can cause small changes in sequence, such as insertion or deletion. On the other hand, HDR uses a homologous DNA template to accurately repair DSBs by replacing the targeted gene sequence (created using Biorender and adapted from Khatodia et al., 2016)

Together with the Protospacer Adjacent Motif (PAM), a 5'-NGG sequence, crRNA, and tracrRNA can form gRNA. gRNA will then decide further the precision of the target sequence cleavage in the nucleic acid (Barrangou, 2013). The double-stranded target DNA's cleavage takes place within the protospacer region. Cas9, an endonuclease, will then cleave the double-stranded DNA, causing double-stranded breaks (DSBs) at the site targeted by gRNA (Cong et al., 2013). **Figure 3** illustrates the two pathways that could help repair DNA DSBs. The first pathway is through non-homologous end joining; it repairs the break by directly ligating the two broken ends of the DNA together without requiring a template. This process often results in the loss or addition of a few nucleotides at the repair site, leading to small insertions or deletions (indels) in the DNA sequence (Song et al., 2021). NHEJ is a relatively quick repair mechanism but can introduce random mutations, making it useful for disrupting genes or creating small alterations in the DNA (Tschaharganeh et

al., 2016). On the other hand, HDR (homologous-directed repair) is a more precise DNA repair pathway that utilizes a homologous DNA template to repair DSBs. This repair mechanism predominantly occurs during the S and G2 phases of the cell cycle when a sister chromatid or a homologous chromosome is available as a template. HDR can be harnessed for precise genome editing purposes, such as introducing specific genetic modifications or inserting new DNA sequences (Gray et al., 2022). An exogenous DNA template with the desired modifications is provided along with a nuclease (e.g., CRISPR-Cas9) to induce a DSB. The cell then utilizes HDR to repair the break using the exogenous template as a guide, resulting in precise modifications in the target DNA sequence (Maeder & Gersbach, 2016).

EFFICIENCY AND APPLICATION OF CRISPR-MEDIATED GENE THERAPY

The established treatments that are currently available for the diseases are limited to symptom management, which urges scientists to formulate cure options (Tay-Sachs disease, n.d.; Ou et al., 2020). Among them is CRISPR-mediated gene therapy. For the treatment of GM2-gangliosidoses, the genome repair system has been designed and tested to be delivered in vivo directly targeting specific organs, including the liver, spinal cord, or brain of the recipient (Ou et al., 2020; *Tay-Sachs and Sandhoff Disease*, n.d). The system would aim to eliminate the faulty HEXA or HEXB genes located in the Tay Sachs or Sandhoff patient's genome and insert copies of modified HEX genes that were engineered to be translated into functional hexosaminidase homodimers that comprise the inadequate enzyme production and their respective enzymatic activity.

Research done by Ou et al. (2020) utilized Adeno-associated viruses (AAV) as a CRISPR delivery method into the hepatocytes of Sandhoff mouse models. AAV is a gene-free viral vector that is modifiable to deliver genetic material; hence, despite its limited packing capacity, AAV has the least likelihood of causing an immune response or toxicity in the recipient's body compared to other types of viruses that have been studied as gene therapy delivery systems (Naso et al., 2017; Xu et al., 2019). Their liver-tropic AAV/CRISPR system called PS813, which was made from the combination of the AAV8 and Cas9 from Staphylococcus aureus (AAV-SaCas9) and AAV8-HEXM-sgRNA systems, was applied towards HEXB knockout (Hexb-/-) Sandhoff mouse models purchased from the Jackson Laboratory. This integrated gene editing system eliminates the faulty HEXA or HEXB gene and replaces them with a modified gene called HEXM, which they had found to code for the Hexμ, the homodimer of Hexα and Hexβ subunits of the same enzyme lacking in both Tay-Sachs and Sandhoff disease (Ou et al., 2020). As it codes for the homodimeric enzyme, the HEXM gene appeared to be capable of facilitating the synthesis of functional β-hexosaminidase and, subsequently, the appropriate processing of the GM2 gangliosides.

Their research model appeared to be effective in reducing the accumulation of GM2 gangliosides (Ou et al., 2020; Kumar et al., 2018). This was observed from the increase of Hex enzyme level in the targeted sites; the increase in 4-methylumbelliferyl-β-N-acetylglucosamine (MUG) and 4-methylumbelliferyl-β-N-acetylglucosamine-6-sulfate (MUGS) activities upon the administration of the research model, as well as GM2 ganglioside quantification—MUG and MUGS—helps to measure the Hex A and isoenzyme Hex S activity (Ou et al., 2020). Their study also found that only the complete treatment of both AAV systems resulted in increased Hex enzyme activity in the newborn diseased mice; the treatment of the HEXM gene without the company of the AAV8-SaCas9 system did not yield significant expression of the transplanted gene. However, the fenestrated structure of the liver blood vessels, as well as the major blood circulation and filtration centred in the liver, prompt the accumulation of the AAV8 and SaCas9 in the liver; the increased Hex activity was mostly observed in the liver, heart, and spleen of the mice and was less prominent in the brain (Kattenhorn et al., 2016; Ou et al., 2020). Thus, as a result, the impact was minimal when AAV-SaCAs9 or AAV8-HEXM-sgRNA were delivered alone.

Similarly, some studies used self-complementary adeno-associated viruses (scAAV), specifically scAAV9, to deliver the HEXM gene to Sandhoff mice models (Osmon et al., 2016; Kot et al., 2021). The expressed Hex μ subunits would interact with the GM2 activator proteins and enable the catalysis of the GM2 gangliosides (Osmon et al., 2016). Their trials on the diseased mice resulted in a rather minimal increase of the Hex enzyme level, yet there was a significant decrease in GM2 ganglioside accumulation. Osmon et al. (2016) also exhibited increased life expectancy as high as 250% of that of the untreated Sandhoff mice. On the other hand, scAAV9 packs a significantly smaller packing capacity, which became a consideration as to its utilization as the delivery mechanism of the gene therapy system. Moreover, a study by Kot et al. (2021) discovered that the recipient of this particular viral delivery/gene therapy system combination is susceptible to immune responses toward the viral particle of the vector itself.

Although hypothesized to be susceptible to the same treatment, both in vivo and in vitro studies and clinical trials were done separately on Tay-Sachs models to further investigate the efficacy of CRISPR-mediated gene therapy toward said disease (Flotte et al., 2022; Gray-Edwards et al., 2017; Solovyeva et al., 2018). As opposed to HEXA-deleted Tay-Sachs mice (Hexa-/-) that are asymptomatic due to minimal GM2 ganglioside accumulation, Tay-Sachs sheep models, also referred to as Jacob sheep, contain a single nucleotide mutation on the 3' splice site of exon 11, leading to the manifestation of clinical symptoms and observable TSD markers, which enables the analysis of the disease and treatment progression (Gray-Edwards et al., 2017; Solovyeva et al., 2018). In the research conducted by Gray-Edwards et al. (2017), various doses of Hex α and combined Hex α and Hex β copies of normal sheep cDNA were delivered separately into Tay-Sach sheep via a type of the AAV viral vectors called the AAVrh8, creating the AAVrh8-Hex α as well as AAVrh8-Hex α+Hex β therapy systems. Later, the Hex A enzymes in the brain and spinal cord of each sheep model were quantified; the combination of both cDNA evidently yielded in production of Hex A enzyme subunit in quantities and activities higher than that of a non-diseased sheep up to the one-year post-treatment mark. Meanwhile, the AAVrh8 with only the Hex α cDNA triggered the production of the Hex S subunit, the homodimer of the Hex α subunits; those treated with such a treatment system acquired Hex enzyme activities that are closest to the normal state. The Hex enzyme activities produced by those treatment groups successfully postponed or eliminated the disease symptoms, including physical manifestations, disease-related behaviours, and neural alterations in parts of the treatment target sites.

Human trials

Further, the AAVrh8-Hex α+Hex β gene therapy was applied in the first clinical trial of CRISPR Tay-Sachs treatment on humans in a study by the University of Massachusetts, after the successful trials on various animal models as well as in other disease treatments (Flotte et al., 2022). An equal combination of both cDNAs in the viral vector was delivered in vivo through the brain and spinal cord of two infants with Tay-Sachs disease, patients TSD-001 and TSD-002, in a ratio of injection allocation of 3:1, with prior bilateral thalamic injections in the case of TSD-002. Both of the trial subjects had different HexA gene mutations and different symptoms of development at shared age time points.

The performed enzyme assay found significant increases of HexA activity in the brain and cerebrospinal fluid (CSF) of the patients, measured roughly at 167 to 200% of the pre-treatment hourly enzyme activity. Acquiring 0.5 to 2% of normal HexA activity could delay symptoms manifestation and prolong the life expectancy of Tay Sachs patients; however, the assay was not specific in identifying only the HexA enzyme but also the HexS isoenzyme. Furthermore, both isoenzymes facilitate hydrolysis of different compounds, GM2 gangliosides by the Hex A isoenzyme and sulfated glycosphingolipids as well as anionic glycans for the Hex S. Seeing from a previous trial on Tay-Sachs sheep and noting that the brains of sheep and infants with Tay-Sachs have the same order of magnitude, the combination of Hex α + Hex β gene should produce the desired Hex A isoenzyme. However, Flotte's research was not able to confirm the result

of the enzyme assay due to the limitation of the subjects' biological material (Gray-Edwards et al., 2017; Flotte et al., 2022).

Additionally, little to no side effect was observed regarding the treatment procedure, genetic material, or viral vector, and immune responses were mild and quickly adapted. GM2 ganglioside accumulations, which were suggested to fluctuate proportionally along with the disease progression, were found to plateau in two GM2 ganglioside species and increased continually yet rather minimally in the others. Longitudinal data on GM2 gangliosides accumulation was not obtained due to the state of health of the patients, and their overall plateau or reduction was not absolute. Nevertheless, the minimum growth of GM2 gangliosides accumulation indicates a little yet observable efficacy of the treatment at the very least (Flotte et al., 2022).

TSD-001 had a more advanced progression of Tay-Sachs compared to TSD-002, therefore receiving roughly double the amount of vector received by TSD-002. As opposed to the supposedly increasing dysmyelination, TSD-001 acquired stabilized brain volume after the injection. Increased myelination was observed in TSD-002 through the 6-month observation period, in which the patient eventually acquired increased brain volume comparable to that of the healthy development of a 9-month-old child (Flotte et al., 2022).

In correlation with their disease progression, TSD-001 has about one-third of the motor function score of TSD-002, yet TSD-001's score was more stable throughout the observation period compared to TSD-002, whose score dropped upon the 6-month time point. In normal Tay-Sachs disease progression, seizures developed the latest by 22 months of age on average and would proceed to become insusceptible to treatments. TSD-001 has been on Keppra medication for seizure management prior to the treatment and still experienced seizures; however, the seizures stopped after the administration of gene therapy and have remained so. TSD-002, who had not developed seizures by the time of administration, experienced seizures 17 months post-treatment by the age of 24 months. The seizures stopped ever since Keppra started to be incorporated into the patient's regular treatment (Flotte et al., 2022).

It is important to note that this clinical trial aims to study four levels of doses: the starting dose, the low dose, the middle dose, and the high dose (Flotte, 2021). The dose incorporated in this research is the starting dose, which is estimated at 10% of the experimental dose with the highest efficacy due to the concern of adverse reactions against the thalamic injection (Flotte, 2021; Flotte et al., 2022). At the moment, the research is still in progress, with the clinical study of the starting dose at phase 1. The positive result of this first trial has prompted more clinical trials for treatments with higher doses.

Another study conducted by Queen University, Canada, began the clinical trials of their treatment model, TSHA-101. Similar to the model of the University of Massachusetts, they utilized AAV9 as the viral vector of their bicistronic gene therapy, consisting of healthy copies of both HEXA and HEXB genes. Patient 1, who was diagnosed with Sandhoff disease, and patient 2, who was diagnosed with Tay-Sachs disease, were both 15 months or younger and received the treatment through intrathecal injection. Post-administration observation captured increased Hex A enzymatic activity of nearly two folds at the first month and three folds at the third month of observation, as well as 1.25 fold of Hex A enzyme activity in patient 2 at the first month time mark. There were no significant drug or vector-related side effects evident in both patients; however, patient 1 had passed away due to a respiratory infection contracted at home and an MRSA infection acquired in the hospital during the treatment for said respiratory infection. The press publication released by Taysha Gene Therapies (2022), with whom this research is financially supported, also stated that the second patient was only able to participate in the analysis in the first month after treatment. This study, which has only one type of treatment scheduled for the test, is still ongoing and has reached the second phase of its clinical trial (Sehgal, 2021; Taysha Gene Therapies, 2022).

Although most of the papers said that both Tay-Sachs disease and Sandhoff disease can be treated by a CRISPR-Cas-mediated system and are considered efficient and effective, a study conducted by Qian et

al. (2021) has provided a more effective way of using the Prime Editing (PE) gene-editing tool in curing these diseases. However, it is to be noted that this study was done in an animal model, a rabbit, and no comparison between PE and CRISPR-Cas system results was made. As an improvement on the base editing therapy, comparing this technique to a regular CRISPR-Cas9-mediated system, it mediated 12 different base-to-base conversions without the use of DSBs (double-strand break) or donor DNA templates. This method employs the Cas9 nickase, revised RNA template, and reverse transcriptase to initiate a breakage on the specific bases on the non-complementary DNA strand, eliminating the need to cause a DSB (Scholefield & Harrison, 2021). CRISPR-Cas9 breaks both of the double strands of the DNA and then uses the cell's repair machinery to fix the broken parts and make the modifications (Ledford, 2019). As stated before, the treatment of Tay-Sachs disease uses the CRISPR-Cas9 modified gene, HEXM. However, the effectiveness in preventing neurodegeneration in the central nervous system was modest, and these techniques only partially restored the HexA activity and reduced the amount of the GM2 gangliosides inside the cells (Mani, 2021).

CHALLENGES AND FUTURE CONSIDERATIONS

General challenges

As previously mentioned, CRISPR is regarded as the simplest and one of the most efficient methods of genome editing by changing the nucleotide sequence of the sgRNAs (Wong et al., 2021). However, there are some challenges. Since CRISPR-Cas9 uses redesigned sgRNAs—a sgRNA that is redesigned due to the fact that their sequence did not properly target the specified gene—to genetically manipulate targeted genes, there could be an unintentional cleavage of the non-targeted sites. As a result, when working with CRISPR-Cas9, the efficacy of the sgRNAs can be uncertain (Nayarisseri & Limaye, 2019). From the perspective of bioethics, this efficacy concern has evidently raised persisting remarks in regard to the well-being of recipients and whether or not the application for treatments of such genetically inherited diseases would result in benefits greater than the potential harm (D'Souza et al., 2023; Lorenzo et al., 2022; Gonzalez-Avila et al., 2021). In such a sense, although recipients ought to be fully informed of the benefits and risks of the procedure, the unpredictability of the unintended edits as well as their repercussions, has led some researchers to argue that CRISPR genome editing is yet to be ready for human treatment administration. However, the somatic targeting of the treatments currently being tested is a notable aspect that could ease the risks at stake, as opposed to germline editing, which is more than likely to result in the inheritance of the modifications, be it intentional or unintentional.

The sgRNAs must be carefully modified with different strategies like the sgRNA length, GC contents, chemical modification, and truncated sgRNA to avoid off-target alterations (Naeem et al., 2020). Specifically in Tay-Sachs Disease and Sandhoff Disease, modifying the HEXA or HEXB gene could promote the rise of these hereditary diseases. The research conducted by Ou et al. (2020) shows that a genetically modified gene called HEXM that was created using the CRISPR-Cas9 system shows a positive result in treating both of these diseases. The challenge of this experiment is that this research was still conducted on neonatal lab mice that could have immune naivety and immunotolerance.

Another research conducted by Anzalone et al. (2019) results in a precise 'search-and-replace' genome editing strategy, also called prime editing, which is an improvement from the basic CRISPR-Cas9 system. They use this newfound strategy to correct or edit the primary mutations from the Tay-Sachs disease gene in the human embryonic kidney (HEK) cells. The CRISPR-Cas-mediated system also gives a possibility of huge deletions and other complicated rearrangements that can be caused by imprecise cutting and repair, which could manifest into pathogenic repercussions (Kosicki et al., 2018). In addition, there has been no indication of whether or not the research will be continued. Off-target mutation could also happen

when using the CRISPR-Cas9 system, where this mutation can inactivate the gene's functionality and interfere with normal gene expression (Cho et al., 2014). The off-target mutation is commonly caused by the likeliness of both the off-target sites with the target sites (Cong et al., 2013). Direct delivery of preassembled ribonucleoproteins (RNPs) using physical approaches such as microinjection has also been used in the CRISPR-Cas9 system for therapeutic gene editing (Kim & Kim, 2014; Zhang et al., 2021). Nevertheless, this technique can pose a challenge due to its high cost and time-consuming process (Kaneko & Nakagawa, 2020). Moreover, according to the study by Urnov (2021), CRISPR-Cas9 can cause chromothripsis, a mutational process that is known to play a role in congenital disorders that involve up to thousands of clustered chromosomal rearrangements occurring on one single event in localized and constrained genomic areas of one or more chromosomes. As mentioned, PE might be a better gene editing tool due to its ideal base insertions, deletions, and conversions in rabbit animal models for Tay-Sachs disease (Qian et al., 2021). However, no comparison study with the CRISPR-Cas system was performed. In order to create a more reliable statement that PE might be a better gene editing tool, it is necessary to perform this comparison study in the future.

Although CRISPR-Cas systems may successfully bestow double-strand breaks at a specific genomic sequence, homology-directed repair (HDR), especially in mammalian cells, is quite inefficient and unsuited due to the low inherent rate of HDR and difficulties in on-site delivery of dDNA, all the while a precise mutation delivered by a genome-editing tool is strongly dependent on the HDR occurring at the DSB locus site using the dDNA template encoding the desired product (Bollen et al., 2018; Savic et al., 2018; Cong et al., 2013). The rates of correction of standardized genome-editing techniques are 0.1-5%, and they often introduce a large number of arbitrary indels at the target genomic sequence as a result of the cellular reaction to DSBs (Hilton & Gersbach, 2015).

In terms of affordability, CRISPR-Cas9 provides a cheaper cost compared to other gene-editing tools with a high yield and precise result (Sander & Joung, 2014). CRISPR technology is currently only utilized by a few foundational patents, such as research and pharmaceutical corporations. These corporations will then provide therapeutic developers to govern the cost of CRISPR and how this technology will be turned into therapies. Usually, this technology comes at high prices due to personal economic desires (Contreras & Sherkow, 2017). Thus, the convenience, low cost, and long-term nature of CRISPR-mediated therapies may not necessarily imply that they will be less expensive than other therapies, especially when patents are involved (Sherkow, 2017). This concern has also raised the bioethical arguments on the social justice aspect of the treatment, as the non-affordability of this treatment would limit access to a smaller percentile of society, resulting in socioeconomic inequalities (Ayanoğlu et al., 2020; Lorenzo et al., 2022). However, with the increased demand for this technology, CRISPR-Cas9 is now 150 times cheaper than other genetic editing procedures, indicating a probability of improved affordability in the future (Wang, 2015).

Regarding its accessibility, the current CRISPR-Cas9 technology is still in its early phases. This implies that, even though the therapies have been proven safe and successful, there will be more years to come for the FDA (Food and Drug Administration) to approve this technology and for it to be widely spread to the public (Henderson, 2021). The FDA is pleased with the preliminary safety results; however, long-term reliability is still unknown. Since this medication is supplied by a viral vector, CRISPR-Cas components will be expressed in the eye indefinitely (Henderson, 2022). On the other hand, the public itself does not seem to be aware of the presence of this gene-editing technology. This raises two ethical concerns: the knowledge discrepancies could lead to the exclusivity and inequality of the treatment option or result in improper and unregulated application, leading to safety threats, inheritance of unwanted mutations, eugenics, and environmental damage should it be accessed by unqualified practitioners and/or recipients (D'Souza et al., 2023; Lorenzo et al., 2022; Gonzalez-Avila et al., 2021). To ease up the accessibility of CRISPR-Cas9, researchers should have the public's interest and agreement. For that, the US National Academies of Sciences, Engineering, and Medicine conducted a summit about the regulation of CRISPR-Cas9 gene-editing

technology. The result of this summit shows that processing with germline alteration without widespread community agreement on the suitability of this proposed technology would be irresponsible (Travis, 2015a; Travis, 2015b). These preliminary talks highlight the importance of knowing what the general public thinks about genetic alteration. Given the technical simplicity, low cost, and potentially widespread use of CRISPR-Cas9, understanding current public opinions is crucial for choosing where to target educational efforts and how to guide policy and laws so that CRISPR-Cas9 can be more accessible and affordable to the public (Salloch et al., 2014). However, in 2021, the WHO issued a new suggestion for editing the human genome for the development of public health. This suggestion has been consulted over the span of two years, and hundreds of participants from around the world with different perspectives have gathered to discuss this matter. Dr. Tedros Adhanom Ghebreyesus, WHO Director-General, stated that human genome editing has the potential to improve in treating and curing diseases; however, the true impacts will be realized only if it is used to benefit people rather than sparking more health disparities within and between nations (WHO, 2021). The suggestions center on system-level improvements that are required to build potential in nations, ensuring that human genome editing is used effectively, ethically, and safely. The suggestion also includes a new governance framework that highlights tools, organizations, and situations to demonstrate the challenges of implementing, controlling, and supervising human genome research.

Future considerations

Clinical trials of AAV-mediated gene therapy for Tay-Sachs disease on two recipients exhibited reduced signs of disease advancements (Shelton, 2019). Two years later, AXO-AAV-GM2 treatment on a Sandhoff disease infant was found to elicit a comparably stable condition among Sandhoff patients within the infant age group (UMass Medical School Communications, 2021). A trial conducted by Anzalone et al. (2019) utilized prime editing (a novel technique that is based on CRISPR-Cas9) to repair a mutation in the HEXA gene, one of the most prevalent common causative mutations in the Ashkenazi Jewish community. PE3 prime editing was utilized to replicate the mutation by inserting a 4bp insertion into the HEXA gene of lipo-infected HEK 293T cell lines, which resulted in 31% efficiency.

Aside from the limited successful clinical trials on GM2 gene therapy, CRISPR-Cas9 is yet to be legally approved, even further from being openly accessible to the public (Wong et al., 2021; American Society of Gene + Cell Therapy, n.d.). Therefore, it is highly suggested that further improvements are made toward the CRISPR-Cas9 system to minimize off-target mutations, for more clinical trials using both the CRISPR-Cas9 system and GM2-associated gene therapy to be done, and for the bioethical boundaries of such practices to be established, intended for authenticating CRISPR gene therapy as an approved cure option for Tay-Sachs and Sandhoff disease patients (Anzalone et al., 2019; Wong et al., 2021).

Before CRISPR may be utilized in people, ethical problems must be addressed. Somatic cell genome editing is presently employed in clinical settings because it is less likely to be abused than germline editing. With CRISPR, it is feasible that undetected off-target effects will be handed down to future generations, with serious repercussions (Wong et al., 2021; Manghwar et al., 2020). Before the moratorium can be removed, global debates involving scientists and ethicists are required to explore how germline editing should be accomplished and whether it is ethically acceptable.

Places that have a high prevalence of Tay-Sachs and Sandhoff disease could be potential markets for this research. According to Das et al. (2017) and Ostrer & Skorecki (2013), Ashkenazi Jews can be found in the Middle East and Eastern Europe. In terms of patent issues, there have yet to be any issues regarding it; however, there has been one patent made on the treatment of Tay-Sachs and Sandhoff disease that is utilized in animals that has conditions linked to a decrease in lysosomal hexosaminidase.

CONCLUSION

This literature review discusses the application and efficiency of CRISPR-Cas9 mediated gene therapy in treating Tay-Sachs and Sandhoff disease. CRISPR-Cas9 provides a cheaper cost compared to other gene-editing tools with a high yield and precise result. Despite its numerous benefits, CRISPR-Cas9 has certain challenges, including safety and efficacy, efficient delivery, and bioethical considerations. Future research can consider utilizing and providing a comparison of the various CRISPR-Cas systems (CRISPR-Cas9, CRISPR-Cas10, and CRISPR-Cas3) mediated gene therapy for Tay-Sachs and Sandhoff disease, which can further increase its efficacy and efficiency. Moreover, improvements to the CRISPR-Cas9 system are needed to reduce off-target mutations, obtain FDA approval, and perform more successful clinical trials.

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