Biomarkers And Gene Therapy With CRISPR-Cas9 To Treat Multiple Sclerosis

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Abstract:
Multiple Sclerosis (MS) is a partially heritable chronic autoimmune inflammatory disease characterized by the demyelination of neurons and axonal damage in the central nervous system. Treatment options such as antibody-mediated therapy, symptomatic therapy, and plasma exchange are partially effective in delaying or inhibiting the disease's progress; however, they have many drawbacks and are unsuitable for every MS patient. Many treatments and approaches are being developed to delay the disease progression. Biomarkers are one of those approaches. Biomarkers are regarded as an important indicator of MS and are used as drug targets. Finding highly heritable potential biomarker candidates is significant for the treatment and monitoring the progress of MS. Early diagnosis of MS is complicated and unreliable in most cases; biomarker studies are aiming to solve this problem by finding specific indicators that could distinguish types or other diseases from MS. Some of them are currently in clinical use and some promising candidates are still being studied. Another treatment option is gene therapy. The research focused on the heritability of MS shows that there are genetic factors in disease progression. Genome-wide association studies (GWAS) found more than 200 gene variants responsible for disease progression. Gene therapies using CRISPR-Cas9 gene-editing techniques are being developed to target the most causative genes to reduce inflammation to decrease neurological symptoms of MS. Briefly, biomarkers, and gene therapy with CRISPR-Cas9 provide new insights for advancements like patient-specific treatments, and could provide high success rates in treatments in MS and other neurodegenerative disorders.

Introduction

Multiple Sclerosis (MS) is a partially heritable demyelinating disease with progressive neurodegeneration caused by an autoimmune response to self-antigens (Malkani, 2022; Paul et al., 2019). Worldwide, there are 2.8 million people who have MS. While the cause of MS is unknown, many studies found that both environmental (latitude of childhood, EBV infection, salt, smoking) and genetic factors trigger MS (Parnell & Booth, 2017). There are four types of MS: Relapsing/Remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and progressive relapsing MS (PRMS). RRMS is the most common type (about 85% of cases). Patients with RRMS experience cycles of short-term attacks that worsen symptoms, followed by periods of remission where neurological functions do not worsen. In SPMS, patients experience initial relapses with gradual neurological impairment. In PPMS, patients show a steady functional decline from the beginning of the disease. In the last type, PRMS, functional decline and acute attacks are seen in patients (Loma & Heyman, n.d.). Treatment and symptoms vary depending on the type of MS.

The diagnosis of MS is made through a complex process of magnetic resonance imaging (MRI) and clinical characteristics such as cognitive degeneration, abnormal sensations, muscle spasticity, fatigue, loss of balance, and decreased mobility (Paul et al., 2019). Most of the time, the diagnosis process takes time and it is not precise because MS symptoms are similar to many autoimmune and neurodegenerative diseases. This situation delays treatment and increases risks. Treatment options for MS are antibody-mediated therapy, symptomatic therapy, and plasma exchange (Malkani, 2022). Although these therapies are effective, most of them decrease body resistance toward foreign antigens and cause many side effects like liver toxicity. They are also not suitable for every MS patient. For all of these reasons, it is significant to find new approaches for early diagnosis and treatment. Future advancements are focused on finding new biomarkers for early diagnosis and there are also ongoing gene therapies to propose new treatment options. In this review, we will be focusing on these options.

Heritability of Multiple Sclerosis

1. Genetics of MS

MS is a partially heritable autoimmune disease that mostly affects young adults and women (Parnell & Booth, 2017; Patsopoulos, 2018). It can be said that genetic variation may be responsible for about half of MS susceptibility. It has been concluded that MS is not a Mendelian disease because genetic sharing in the family does not show any linear relationship. However, the probability of MS could be higher if both parents have MS (Patsopoulos, 2018). The reason why MS is seen most commonly in women is still unknown. However, one study concluded that there could be an X-chromosome variant in MS. More studies regarding X-chromosome variants should be made to determine X-chromosome heritability in MS (Patsopoulos, 2018).

Studies have shown that the human leukocyte antigen (HLA) gene cluster on chromosome 6p21 has been most consistently identified as the strongest genetic locus for MS to study genome linkages (Parnell & Booth, 2017; Patsopoulos, 2018). The HLA genes are in the highly polymorphic major complex region (MHC) that has been associated with MS susceptibility for a long time (Ma et al., 2023; Patsopoulos, 2018; Umeton et al., 2022). Many MS genetic studies have demonstrated that MS is not a monogenic disease (dependent on a single gene); rather, it is a polygenic disease (caused by more than one gene) (Axisa & Hafler, 2016; Ma et al., 2023; Parnell & Booth, 2017; Patsopoulos, 2018). The theory of common disease-common variant (CDCV) was proposed to identify the genetic model of MS. This theory states that common diseases in a population are caused by several common gene variants (Patsopoulos, 2018). This hypothesis has been applied to many advancements in MS genome studies, such as genome-wide studies.

2. Genome-Wide Studies And Variants

Genome-wide association studies (GWAS) allow researchers to identify genes associated with a particular disease in a large population. The first GWAS-identified gene for MS was IL2RA, a gene that codes for a receptor that plays a role in several immune-related pathways. So far, GWAS studies identified 194 gene variants and 14 regions associated with MS (Axisa & Hafler, 2016). The largest genetic study in MS identified 233 genome-wide loci associated with MS susceptibility. Two hundred of these were in autosomal non-MHC regions of the genome. In one study, the GARFIELD algorithm was used to classify susceptibility genes according to their cell types (Patsopoulos, 2018). GWAS signals were significantly enriched in microglia, not in others (astrocytes, oligodendrocyte precursor cells, oligodendrocytes, or neurons) and the highest levels of enrichment were observed in B cells. When these are taken into account, it could be suggested that MS variants are mostly immune-related genes that alter the immune system pathways, and variants could change the expression of nearby genes (they could inhibit, and alter the splicing of exons) (Patsopoulos, 2018). Although these studies allowed researchers to study thousands of genetic variants, they can also lead to false positive results due to large samples. Identifying each variant within a region is difficult. For these reasons, many algorithms were developed to increase the accuracy of the studies. The Immunochip was designed to solve this problem (Axisa & Hafler, 2016). In addition,'' MS Chip'' was designed to replicate recent GWAS hits. This chip allowed for the identification of rare variants that have strong effects on some individuals. For instance, in one study, a variant in the CYP27B1 gene led to a complete gene function loss in one individual. Studies were unable to find this gene's negative effect on other individuals (Patsopoulos, 2018).

3. MS and Other Autoimmune Diseases

Several genes in autoimmune diseases are pleiotropic, meaning that they are associated with more than one disease (Ma et al., 2023; Patsopoulos, 2018). MS is one of the pleiotropic diseases. In parallel, many MS genome-wide variants were also found in other autoimmune diseases. For instance, TAGAP is a gene that has been associated with MS susceptibility; this gene's effect was also found in type I diabetes and rheumatoid arthritis (Patsopoulos, 2018). While it is not certainly known if autoimmune diseases have associations with neurodegenerative diseases, some studies indeed found significant correlations with other autoimmune and neuropsychiatric disorders.

4. Genetics And Environment

It is known that both environmental and genetic factors contribute to MS. Only nearly half of disease susceptibility is considered genetic (Axisa & Hafler, 2016; Ma et al., 2023; Parnell & Booth, 2017; Patsopoulos, 2018). The other factors and risks come from environmental factors. Some of these include latitude of childhood, EBV infection, salt, smoking, and vitamin D (Axisa & Hafler, 2016; Patsopoulos, 2018; Umeton et al., 2022). Researchers are trying to find correlations between genetic variants and environmental factors. They suggest that genetic

factors might function to mediate environmental risk. In one study, 110 MS risk genes were enough to conclude that vitamin D regulation contributes to MS susceptibility (Parnell & Booth, 2017). More studies are needed to prove this suggestion.

Biomarkers

1. Definition

Biomarkers are identified as indicators of biological processes and are often used to evaluate or monitor diseases (Paul et al., 2019). Therefore, biomarkers can be measured in body fluids like urine and blood, or they could be the measurement of a parameter such as blood pressure or brain activity. Biomarkers in MS are crucial as they can be helpful in early diagnosis. It is essential to have biomarkers that are qualified well enough to detect or monitor the disease progression. A good biomarker candidate should have at least two of these four qualities: easy to measure with multiple tests, highly sensitive and specific, correlate to the disease biology or pathogenesis (etc. inflammatory activity), and cost-effective (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019).

2. Biomarkers currently in clinical use

Magnetic resonance imaging (MRI) is the most important clinical tool to diagnose, monitor, and treat MS. The presence of white matter lesions indicates the progression of CIS to MS (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019). However, white matter lesions vary broadly from study to study. The presence of Gadolinium (Gd) lesions on MRI in MS is indicative of active inflammation. But this also has a drawback: it didn't show any positive correlation between Gd-enhancing lesions and cognitive decline in RRMS. Studies indicated that gray matter atrophy, rather than Gd and white matter lesions, could be a useful biomarker (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019). The presence of oligoclonal bands (OCB) is used as diagnosis criteria for MS. It has been known that OCBs occur in CFS (isoelectric focusing) analysis in patients (Axisa & Hafler, 2016; Ziemssen et al., 2019). It can be seen in nearly most patients with clinically definitive MS. For this reason, it is a useful biomarker. Natalizumab is a drug that is given to patients in the treatment process of MS. It is known as a successful drug, however, it can cause risks. Usage of Natalizumab causes MS patients to become vulnerable to many immune system diseases like progressive multifocal leukoencephalopathy (PML) (Axisa & Hafler, 2016). One indicator of PML progression is the John Cunningham virus (JCV). JCV antibodies are useful biomarkers for stratifying the risk of PML. However, it is important to conclude that while 50% of MS patients are JCV seropositive, less than 1% will develop PML (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019). This indicates that more specific biomarkers are needed for PML and MS.

3. Potential Biomarkers

There are several promising candidates besides clinically used ones. In this review, we will be focusing on YKL-40, CXCL13, Nfl, and miRNAs as potential biomarkers. YKL-40 is a glial marker expressed and secreted by astrocytes that is increased in response to inflammation. Levels of YKL-40 have been found to be higher in patients with MS and associates with active lesions (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019). In one study, CIS patients who converted to MS had significantly higher YKL-40, indicating a potential role for this protein in disease progression. Elevated YKL-40 levels could be found in serum. So far, it has been indicated as a promising candidate; however, it has a drawback. There hasn't been shown a clear association between MRI imaging and YKL-40 levels (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019). CXCL13 is a protein-ligand secreted by the CXCL13 gene (Pilz et al., 2020). It can be detected in blood and CSF (Axisa & Hafler, 2016). Also, CXCL13 levels were high in CIS, RRMS, SPMS, and PPMS patients compared to healthy controls (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019). For these reasons, it could be a potential biomarker. However, it is important to conclude that it is not specific. High levels of CXCL13 can also be an indicator of viral infections (Pilz et al., 2020). CNS neurofilaments (Nfl) are released after axonal damage (Axisa & Hafler, 2016; Ziemssen et al., 2019). Many studies have demonstrated that Nfl levels in CSF were increased in both RRMS and progressive MS. These studies suggest that Nfl could be a potential biomarker and correlate with treatment response to Fingolimod, natalizumab, and rituximab. However, Nfl studies didn't show consistency with active lesions seen in MRI (Axisa & Hafler, 2016; Paul et al., 2019; Ziemssen et al., 2019). Another candidate is miRNAs. miRNAs play many roles in important biological processes. Desultory miRNA expressions are associated with many diseases related to the immune system. Specific miRNA types are seen in MS patients. The possibility of being detected in human body fluids, such as saliva and serum also makes miRNAs potential biomarkers for MS (Paul et al., 2019; Ziemssen et al., 2019).

CRISPR Cas-9

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) is a mechanism that prokaryotes use to defend themselves and eliminate foreign genetic material from cellular invaders such as bacteriophages, a type of virus that infects bacteria (Abdelnour et al., 2021; Lee et al., 2022; Li et al., 2023; Malkani, 2022; Raikwar et al., 2019). Although CRISPR-Cas9 is not present in multicellular organisms, it was later discovered that this system could be used for gene editing at targeted parts of multicellular organisms. After this discovery, it was found that CRISPR-Cas9 has the potential to effectively alter clinical symptoms by changing the molecular factors that result in the progression of a particular disease. Since then, the CRISPR-Cas9 system has allowed for a breakthrough method of cost-effective and targeted genome editing.

By changing the nucleotide sequence of a small segment of guide RNA, CRISPR/Cas9 allows specific targeting to correct disease-causing mutations or silence genes associated with a disease (Li et al., 2023). This process is also called ‘’gene-editing.’’ CRISPR-Cas9 involves two main structures: a DNA-cutting protein called Cas9 and a guide RNA (Abdelnour et al., 2021; Li et al., 2023; Malkani, 2022) A single guide RNA (sgRNA) guides Cas9 (a DNA-cutting protein) to locate and bind to a specific sequence in the genome called PAM (protospacer-adjacent motif) (Li et al., 2023; Malkani, 2022). Once PAM is bound, the guide RNA unwinds the double helix. Cas9 cuts the DNA at a specific sequence, which causes the formation of breaks in both strands of the target DNA. Because of the damage in the base sequence of the gene, the gene becomes inactive. This triggers one of two repair mechanisms of DNA: homology-directed repair (HDR) or non-homologous end-joining (NHEJ) (shown in Figure 1) (Abdelnour et al., 2021; Fang et al., 2021; Li et al., 2023; Malkani, 2022). These repair mechanisms can lead to deletions and insertions in the targeted area. Another option to correct this damage is to introduce an artificial donor to the targeted area. This helps researchers to correct and change a specific sequence in the DNA. Furthermore, the delivery of this system is significant in terms of efficiency.

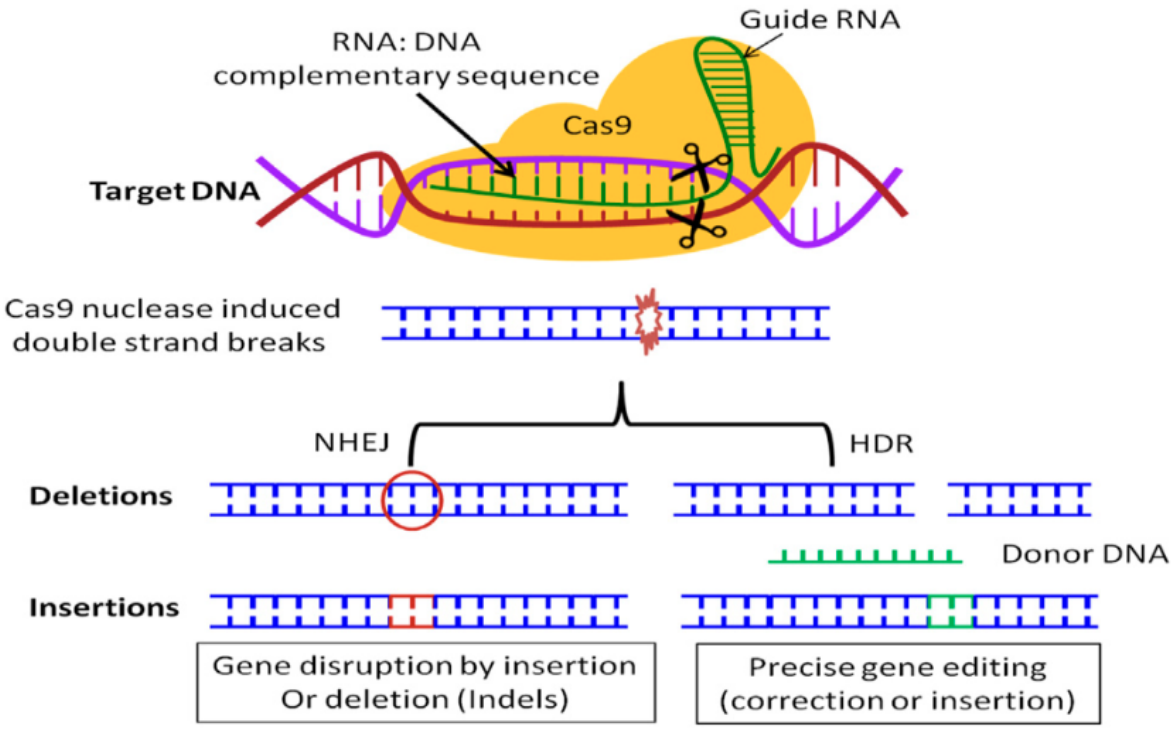

Figure 1: *Single guide RNA unwinds the DNA’s double helix. After that process, Cas9 locates a specific section with the guidance of a single guide RNA and cuts that part. This cut results in breaks in the DNA section. This triggers one of the two response mechanisms, NHEJ or HDR. NHEJ inserts or deletes the DNA section, whereas HDR inserts a donor DNA in the break. In terms of correction, HDR is more likely to correct the break than the NHEJ (Abdelnour et al., 2021).*

CRISPR-Cas9 requires the delivery of two macromolecules, gRNA, and Cas9, into the targeted cells. (Abdelnour et al., 2021; Li et al., 2023) The delivery process of CRISPR-cas9 includes three main stages to be effective: the carrier must remain stable without any degradation, the carrier must go to the target tissue or cell, and the CRISPR system should escape lysosome inside the cell to implement genome editing (Li et al., 2023). There are two different ways to deliver gene editing tools: non-viral and viral methods (Abdelnour et al., 2021; Li et al., 2023).

Non-Viral Methods

Electroporation is one of the non-viral methods. In this method, high-voltage electrical pulses are used to create temporary pores in the outer cell membrane. This helps molecules like gene editing tools to get inside almost any kind of cell. This method is highly effective, target-specific, and unlimited in size (Li et al., 2023). However, it can cause cell death; for this reason, it may cause some challenges. The other two non-viral methods are liposomes and liposome-based nanoparticles (LNPs) (Abdelnour et al., 2021; Li et al., 2023). Liposomes are frequently used to deliver drugs and LNPs are a promising candidate to deliver CRISPR-Cas9. LNPs are mainly composed of lipids, which help them to enter suitable cells. Even though it is useful, LNPs can only be used to deliver drugs for organ targets that have high lipid enrichment such as liver and brain. For this reason, it can be used to deliver gene-editing tools to the brain. In addition to these non-viral methods, polymeric nanoparticles, biomimetic nanomaterials, and exosomes have also been found effective for the delivery of CRISPR in some studies (Li et al., 2023)

Viral Methods

Viral methods are widely used to deliver gene-editing tools because they have evolved to be target-efficient. Viruses can enter cells and introduce their genetic material. The most commonly used viral vectors are adeno-associated vectors (AAV), lentiviruses, and baculovirus (Abdelnour et al., 2021; Li et al., 2023). AVV is one of the most common viral techniques used for delivering. It can easily infect cells and it has very low immunogenicity, which is less likely to trigger an inflammatory response. Although it is useful, it has a limited carrying capacity (around 4.4 to 4.8). Compared to other gene drugs, CRISPR-Cas9 is very large. Therefore, it exceeds AVV maximum carrying capacity (Li et al., 2023) On the contrary, lentiviruses have nearly 10kb loading capacity. This capacity is enough to load CRISPR; however, lentiviruses can trigger immune responses. Baculovirus is also one of the viral methods to deliver gene modification tools. It has been used to deliver CRISPR-Cas9 due to its nonpathogenic and extra load capacity (around 38kb) (Abdelnour et al., 2021; Li et al., 2023)

Other Therapy Approaches And Gene Therapies

The cause of MS is still unknown; however, research shows that the immune system responds to antigens in the myelin sheath, which damages neurons as a result. Active suppression of regulatory T cells plays a role in autoimmune responses (Axisa & Hafler, 2016; Ma et al., 2023;

Patsopoulos, 2018; Umeton et al., 2022). Lack of Treg function in MS patients is one of the characteristics of MS that may lead to impaired immune regulation. Genetic studies made with MS patients also supported that several genes for cytokine receptors are associated with Treg pathways (Axisa & Hafler, 2016; Ma et al., 2023; Patsopoulos, 2018; Umeton et al., 2022). It is demonstrated that early therapeutic interventions delay long-term disease progression. For this reason, many therapies for MS are focusing on regulatory Treg cell pathways to reduce neural damage by preventing these cells from triggering an autoimmune response to self-antigens. One such therapy is antibody-mediated. Antibody-mediated therapies use antibodies to specifically target T-cells. The most used ones are Fingolimod, Natalizumab, and Ocrelizumab (Axisa & Hafler, 2016). Fingolimod seizes lymphocytes in lymph nodes to prevent autoimmune responses. Usage of Fingolimod can lower relapses and reduce MRI-visible activity. The drawback of Fingolimod is that it can cause immune responses (Axisa & Hafler, 2016). Another antibody therapy is Natalizumab, which is a monoclonal antibody that targets the a4 integrin. This blockage affects T cells and reduces relapses seen in MS patients. However, it decreases body resistance and causes toxicity in the liver. Both Fingolimod and Natalizumab are not effective in PPMS treatments (Axisa & Hafler, 2016). On the contrary, Ocrelizumab can be used in PPMS treatments and it is the first approved drug for PPMS (Lamb, 2022). It delays MRI progression and reduces relapses by targeting CD20 surface antigen to reduce the function of CD20+ cells. It is also the first anti-CD20 mAb (monoclonal antibodies) to receive approval (Lamb, 2022). There are ongoing immunotherapy treatment combinations with Ocrelizumab. Antibody-mediated therapies can have various side effects. For this reason, it is essential to design the best therapeutic approaches to address the target cells.

Besides immunotherapies, gene therapies are becoming part of treatments as well. Studies show that a combination of these two therapies proposes new advances for MS and even for other autoimmune diseases (Axisa & Hafler, 2016; Hosseini et al., 2017; Keeler et al., 2018; Loma & Heyman, n.d.; Malkani, 2022; Marin-Bañasco et al., 2017; Parnell & Booth, 2017; Raikwar et al., 2019). In a few comprehensive genetic research studies, Th17, FOXP3 regulatory T cells, B cells, and macrophages were found to be involved in MS pathogenesis (Axisa & Hafler, 2016). Abnormalities in suppressive functions were seen in CD4+, CD25+, and FOXP3 Tregs (Axisa & Hafler, 2016; Berge et al., 2016; Hosseini et al., 2017; Keeler et al., 2018; Loma & Heyman, n.d.; Ma et al., 2023; Malkani, 2022; Parnell & Booth, 2017; Paul et al., 2019; Ziemssen et al., 2019). Studies have shown that CD4+ AND CD25+ Tregs could be used to prevent or reduce neurological symptoms of the disease. Moreover, studies with type 1 diabetes showed that transfer of CD4+ and CD25+ could be an effective treatment approach to treat autoimmune diseases (Keeler et al., 2018). In one study, this transfer technique was used as a hepatic gene transfer technique to treat mice with an EAE model (Keeler et al., 2018). In that study, liver cells were used as a target because of their antigen expression. They transferred a DNA coding sequence for a neuroprotein called myelin oligodendrocyte glycoprotein (MOG) in hepatocytes to see the effectiveness of the gene therapy. The vector

transfer resulted in an induction in FOXP3+ Tregs (Keeler et al., 2018). As a result, in mice with less neurological damage, the vector alone was enough to prevent and reverse the disease (Keeler et al., 2018). Combination with immunotherapy saved mice with an end-stage EAE disease and restored paralysis. Also, long-term effectiveness was seen in the models. This study showed how gene therapy could be effective in preventing and reversing MS development (Keeler et al., 2018). Further studies are needed to test the effectiveness of vector transfer therapies in more EAE models.

Proposed Targets for Gene Therapy

It has been demonstrated that antigen (Ag)-specific regulatory T cells (Tregs) have a significant role in modulating autoimmune CNS disease and, when used therapeutically, can be highly effective at treating MS (Hosseini et al., 2017; Keeler et al., 2018; Lévy et al., 2015; Mansilla et al., 2021; Marin-Bañasco et al., 2017). Like immunotherapies, gene therapies also aim to target these regulatory T-cells. The first proposal we state is using dead Cas9 accompanied by a specific guide RNA for GSTA4 to activate transcription and overexpression of GSTA4 (Carlström et al., 2020; Malkani, 2022). GSTA4 is a gene that secretes the GSTA4 enzyme (Carlström et al., 2020). It is a primary factor in oligodendrocyte (the cells responsible for myelination) apoptosis in differentiation. It promotes remyelination by increasing precursor cells that develop into mature, myelinating oligodendrocytes (Carlström et al., 2020). Previous studies showed that overexpression of GSTA4 prevents the apoptosis of oligodendrocytes (Carlström et al., 2020). This shows that increased oligodendrocyte differentiation could reverse the damage of MS. However, the delivery of gene-editing tools is the most challenging part because the brain is regarded as the most difficult organ for delivery due to its highly selective blood-brain barrier. This can be overcome by using AAV9 vectors (Malkani, 2022).

The second proposal, T regulatory cell gene-editing, aims to conduct a homology-directed repair on the receptors of Tregs to identify MOG and protect them from effector T cells, such as CD4+ and CD25+ (Marin-Bañasco et al., 2017), that recognize proteins as non-self antigens (Keeler et al., 2018; Malkani, 2022). This can be done by introducing a replacement sequence/HDR template that recognizes the receptor for MOG, using CRISPR-Cas9 gene-editing technology (Malkani, 2022).

Finally, we propose that anti-inflammatory cytokines could be used as CRISPR-Cas9 targets to treat MS. Inhibition of pro-inflammatory cytokines and overexpression of anti-inflammatory cytokines can be an effective approach (Hosseini et al., 2017). Many gene therapy studies demonstrated that IL-4 and LIF are involved in autoimmune responses (Hosseini et al., 2017). IL-4 is an anti-inflammatory cytokine that plays a role in CD4+ differentiation, which affects disease progression. Studies in animal models showed injection of recombinant AVV and plasmids coding IL-4 is effective in treating autoimmune diseases (Hosseini et al., 2017). LIF is a proinflammatory cytokine that inhibits TH17 differentiation (it was shown that TH17 is an

effective T cell subset to target) which enhances myelination by oligodendrocytes (Hosseini et al., 2017). These results showed that gene therapy using anti-inflammatory cytokines can be a promising approach against MS.

Additional CRISPR-Cas9 Targets

Many promising candidates could be used as a target in upcoming CRISPR-Cas9 treatments. Four studies concluded that the IR7R gene, the RNA helicase DEAD box polypeptide 39B, the IL2RA gene, and the TNFRSF1A gene could be appropriate gene therapy candidates to treat MS with CRISPR-Cas9 (Lee et al., 2022). These targets could be used in future gene therapy studies.

Conclusions

After its discovery, CRISPR Cas-9 became a great success and part of modern medicine techniques due to its ability to change a genome in a particular DNA section. This gave humans the ability to change and control their heritage material. Despite its success, CRISPR-Cas9 has also raised many ethical and safety issues, including whether treating individuals with it can have later impacts on future offspring. In addition, there are many aspects of the treatment to consider, such as its validity, applicability, biocompatibility, and off-target effects. However, future research is trying to design more efficient CRISPR-Cas9 gene-editing systems to propose new treatment options. Moreover, it is a promising candidate for treatment options for severe neurodegenerative diseases such as MS. Recent studies have shown that MS is a partially genetic disease, with more than 194 risk gene variants. These results allow researchers to focus on the genetics of the disease and specifically target the most causative genes with CRISPR-CAS9 to reduce the neurological damage caused by MS. In the future, we will be seeing CRISPR-Cas9 in therapeutic approaches to treat neurodegenerative and autoimmune diseases.

REFERENCES

[1] Abdelnour, S. A., Xie, L., Hassanin, A. A., Zuo, E., & Lu, Y. (2021). The Potential of CRISPR/Cas9 Gene Editing as a Treatment Strategy for Inherited Diseases. In *Frontiers in Cell and Developmental Biology* (Vol. 9). Frontiers Media S.A. https://doi.org/10.3389/fcell.2021.699597

[2] Axisa, P. P., & Hafler, D. A. (2016). Multiple sclerosis: Genetics, biomarkers, treatments. In *Current Opinion in Neurology* (Vol. 29, Issue 3, pp. 345–353). Lippincott Williams and Wilkins. https://doi.org/10.1097/WCO.0000000000000319

[3] Berge, T., Leikfoss, I. S., Brorson, I. S., Bos, S. D., Page, C. M., Gustavsen, M. W., Bjølgerud, A., Holmøy, T., Celius, E. G., Damoiseaux, J., Smolders, J., Harbo, H. F., & Spurkland, A. (2016). The multiple sclerosis susceptibility genes TAGAP and IL2RA are regulated by Vitamin D in CD4+ T cells. *Genes and Immunity*, *17*(2), 118–127. https://doi.org/10.1038/gene.2015.61

[4] Carlström, K. E., Zhu, K., Ewing, E., Krabbendam, I. E., Harris, R. A., Falcão, A. M., Jagodic, M., Castelo-Branco, G., & Piehl, F. (2020). Gsta4 controls apoptosis of differentiating adult oligodendrocytes during homeostasis and remyelination via the mitochondria-associated Fas-Casp8-Bid-axis. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-17871-5

[5] Fang, H., Bygrave, A. M., Roth, R. H., Johnson, R. C., & Huganir, R. L. (2021). An optimized crispr/cas9 approach for precise genome editing in neurons. *ELife*, *10*. https://doi.org/10.7554/eLife.65202

[6] Hosseini, A., Estiri, H., Niaki, H. A., Alizadeh, A., Zadeh, B. A., Ghaderian, S. M. H., Farjadfar, A., & Fallah, A. (2017). Multiple sclerosis gene therapy using recombinant viral vectors: Overexpression of IL-4, IL-10 and leukemia inhibitory factor in Wharton's jelly stem cells in the EAE mice model. *Cell Journal*, *19*(3), 361–374. https://doi.org/10.22074/cellj.2017.4497

[7] Keeler, G. D., Kumar, S., Palaschak, B., Silverberg, E. L., Markusic, D. M., Jones, N. T., & Hoffman, B. E. (2018). Gene Therapy-Induced Antigen-Specific Tregs Inhibit Neuro-inflammation and Reverse Disease in a Mouse Model of Multiple Sclerosis. *Molecular Therapy*, *26*(1), 173–183. https://doi.org/10.1016/j.ymthe.2017.09.001

[8] Lamb, Y. N. (2022). Ocrelizumab: A Review in Multiple Sclerosis. *Drugs*, *82*(3), 323–334. https://doi.org/10.1007/s40265-022-01672-9

[9] Lee, M. H., Shin, J. Il, Yang, J. W., Lee, K. H., Cha, D. H., Hong, J. B., Park, Y., Choi, E., Tizaoui, K., Koyanagi, A., Jacob, L., Park, S., Kim, J. H., & Smith, L. (2022). Genome Editing Using CRISPR-Cas9 and Autoimmune Diseases: A Comprehensive Review. In *International Journal of Molecular Sciences* (Vol. 23, Issue 3). MDPI. https://doi.org/10.3390/ijms23031337

[11] Lévy, C., Amirache, F., Girard-Gagnepain, A., Frecha, C., Costa, C., Bernadin, O., Negre, D., Gijsbers, R., Cosset, F.-L., & Verhoeyen, E. (2015). 1. Measles Virus Glycoprotein Pseudotyped Lentiviral Vectors Transduce Cytokine Stimulated and Resting Hematopoietic Stem Cells at an Efficiency Without Precedent. *Molecular Therapy*, *23*, S1. https://doi.org/10.1038/mt.2015.74

[12] Li, T., Yang, Y., Qi, H., Cui, W., Zhang, L., Fu, X., He, X., Liu, M., Li, P. feng, & Yu, T. (2023). CRISPR/Cas9 therapeutics: progress and prospects. In *Signal Transduction and Targeted Therapy* (Vol. 8, Issue 1). Springer Nature. https://doi.org/10.1038/s41392-023-01309-7

[13] Loma, I., & Heyman, R. (n.d.). *Multiple Sclerosis: Pathogenesis and Treatment*.

[14] Ma, Q., Shams, H., Didonna, A., Baranzini, S. E., Cree, B. A. C., Hauser, S. L., Henry, R. G., & Oksenberg, J. R. (2023). Integration of epigenetic and genetic profiles identifies multiple sclerosis disease-critical cell types and genes. *Communications Biology*, *6*(1). https://doi.org/10.1038/s42003-023-04713-5

[15] Malkani, S. (2022). *The Forefront of a Revolution: The Applications of CRISPR-Cas9 Technology to Treat Multiple Sclerosis*.

[16] Mansilla, M. J., Presas-Rodríguez, S., Teniente-Serra, A., González-Larreategui, I., Quirant-Sánchez, B., Fondelli, F., Djedovic, N., Iwaszkiewicz-Grześ, D., Chwojnicki, K., Miljković, Trzonkowski, P., Ramo-Tello, C., & Martínez-Cáceres, E. M. (2021). Paving the way towards an effective treatment for multiple sclerosis: advances in cell therapy. In *Cellular and Molecular Immunology* (Vol. 18, Issue 6, pp. 1353–1374). Springer Nature. https://doi.org/10.1038/s41423-020-00618-z

[17] Marin-Bañasco, C., Benabdellah, K., Melero-Jerez, C., Oliver, B., Pinto-Medel, M. J., Hurtado-Guerrero, I., de Castro, F., Clemente, D., Fernández, O., Martin, F., Leyva, L., & Suardíaz, M. (2017). Gene therapy with mesenchymal stem cells expressing IFN-ß ameliorates neuroinflammation in experimental models of multiple sclerosis. *British Journal of Pharmacology*, *174*(3), 238–253. https://doi.org/10.1111/bph.13674

[18] Parnell, G. P., & Booth, D. R. (2017). The Multiple Sclerosis (MS) genetic risk factors indicate both acquired and innate immune cell subsets contribute to MS pathogenesis and identify novel therapeutic opportunities. In *Frontiers in Immunology* (Vol. 8, Issue APR). Frontiers Research Foundation. https://doi.org/10.3389/fimmu.2017.00425

[19] Patsopoulos, N. A. (2018). Genetics of multiple sclerosis: An overview and new directions. *Cold Spring Harbor Perspectives in Medicine*, *8*(7). https://doi.org/10.1101/cshperspect.a028951

[20] Paul, A., Comabella, M., & Gandhi, R. (2019). Biomarkers in multiple sclerosis. *Cold Spring Harbor Perspectives in Medicine*, *9*(3). https://doi.org/10.1101/cshperspect.a029058

[21] Pilz, G., Sakic, I., Wipfler, P., Kraus, J., Haschke-Becher, E., Hitzl, W., Trinka, E., & Harrer, A. (2020). Chemokine CXCL13 in serum, CSF and blood-CSF barrier function: Evidence of compartment restriction. *Fluids and Barriers of the CNS*, *17*(1). https://doi.org/10.1186/s12987-020-0170-5

[22] Raikwar, S. P., Kikkeri, N. S., Sakuru, R., Saeed, D., Zahoor, H., Premkumar, K., Mentor, S., Thangavel, R., Dubova, I., Ahmed, M. E., Selvakumar, G. P., Kempuraj, D., Zaheer, S., Iyer, S. S., & Zaheer, A. (2019). Next Generation Precision Medicine: CRISPR-mediated Genome Editing for the Treatment of Neurodegenerative Disorders. In *Journal of Neuroimmune Pharmacology* (Vol. 14, Issue 4, pp. 608–641). Springer. https://doi.org/10.1007/s11481-019-09849-y

[23] Umeton, R., Bellucci, G., Bigi, R., Romano, S., Buscarinu, M. C., Reniè, R., Rinaldi, V., Pizzolato Umeton, R., Morena, E., Romano, C., Mechelli, R., Salvetti, M., & Ristori, G. (2022). Multiple sclerosis genetic and non-genetic factors interact through the transient transcriptome. *Scientific Reports*, *12*(1). https://doi.org/10.1038/s41598-022-11444-w

[24] Ziemssen, T., Akgün, K., & Brück, W. (2019). Molecular biomarkers in multiple sclerosis. In *Journal of Neuroinflammation* (Vol. 16, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12974-019-1674-2