

Review

## Evaluating CRISPR-Cas9's Ability for Identifying the Etiology of Multiple Sclerosis

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**Abstract:** Biomedical research has taken a new turn with the creation of the Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9 (CRISPR-Cas9) which is specifically important when trying to understand the origin of Multiple Sclerosis (MS). This review article summarizes recent studies that focus on the use of CRISPR-Cas9 technology to create accurate genetic models, study molecular pathways, and manipulate immune cells for therapeutic understanding. The search for relevant studies employed a review of database searches such as Scopus, Web of Science, and Google Scholar. Major primary papers Emerging discussions on the application of CRISPR-Cas9 in the treatment of autoimmune disorders concentrate on MS, RA, and systemic lupus erythematosus. Research is ongoing to solve the more technical issues such as efficiency, delivery mechanisms, and off target effects. Challenges remain, but their ability to serve as a therapeutic target in the case of MS represent an amazing opportunity for precision medicine. In conclusion, CRISPR-Cas9 is an invaluable asset for understanding MS because it provides unprecedented opportunities to unravel the enigma behind the condition and develop effective prescriptions.

**Keywords:** CRISPR-Cas9, multiple sclerosis, genetic models, autoimmune diseases, precision medicine, genome editing.

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### Introduction

Multiple sclerosis (MS), a chronic autoimmune disease, develops in the body due to damage to the central nervous system (CNS). Despite numerous studies, scientists still do not understand the cause of this disease. Advances in genome editing, including the use of the clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9), open up new ways to treat and understand the nature of this disease. Because CRISPR-Cas9 is the most precise, simplest and programmable method, it has become a fundamental step in the field of genome editing. Advances in this field have led to the emergence of the CRISPR toolkit, including new Cas proteins and new editing methods such as base editing and prime editing, as well as increased precision and a wider range of genetic changes [1–3]. Despite these advances, it has become easier to accurately model diseases and correct genetic mutations, which is of particular importance for studying such an enigmatic and complex disease as MS [4].

The effectiveness of CRISPR-Cas9 in the study of autoimmune diseases has been well documented. Thus, the technology successfully identifies and modifies genes involved in immune responses and provides information on the mechanisms of autoimmune disease development [4]. In the case of MS, CRISPR-Cas9 can be used to create precise genetic models that help researchers study the progression of the disease and identify the genetic factors involved in its occurrence. Given the potential of this technology to knock out and modify specific genes, it has become a valuable tool for elucidating the role of genetics in the

development of MS. The latter is characterized by an uncoordinated immune response directed against the CNS. CRISPR-Cas9 holds promise for engineering immune cells and modulating immune responses, which is critical for understanding and treating MS [5]. By editing genes in T cells and other immune cells, researchers can investigate how these changes affect immune function and contribute to the pathology of MS. This work helps to identify etiologic factors and facilitates the development of targeted immunotherapies [4].

To develop effective treatment, it is necessary to identify specific targets involved in MS. CRISPR-Cas9 allows for precise editing of candidate genes and facilitates the study of their role in MS [5]. Potential targets for CRISPR-Cas9 in MS include genes involved in immune regulation, myelin production, and neuroinflammation [6]. Thus, by editing these genes in animal models and human cells, researchers can gain a deep understanding of their role in MS to develop targeted treatment strategies.

Although CRISPR-Cas9 opens up new possibilities for disease treatment, despite its use in human disease research and treatment, it raises serious safety and ethical concerns. One of such concerns is the potential for off-target effects and unwanted genetic changes, which require careful consideration [7]. In addition, the ethical implications of gene editing, especially when modifications are made in the human germline, need to be carefully assessed [6]. For CRISPR-Cas9 to be successfully used in clinical practice, its safety and ethical integrity must be ensured [8].

Despite the limitations of CRISPR-Cas9 technology, it faces challenges that need to be addressed. These include improving the efficiency and specificity of gene editing, developing effective delivery methods, and overcoming immune responses to the Cas9 protein [8, 9]. Studies have been conducted to address these issues to improve the reliability and broaden the applicability of CRISPR-Cas9 in the study and treatment of MS [7].

Numerous studies have focused on the use of CRISPR-Cas9 in autoimmune diseases, but this study focuses on its application to understanding the genetic and molecular basis of multiple sclerosis (MS). Unlike previous reviews of gene editing in autoimmune diseases, this review takes a detailed look at emerging CRISPR-Cas9-based models of MS, targeted therapies for MS, and the current gaps and future developments in the field. This study discusses the evaluation of the use of CRISPR-Cas9 to study the genetic and molecular basis of MS, and the precise capabilities and gene editing of this process to identify disease causes and treatment targets.

## Methods

To assess the potential utility of CRISPR-Cas9 technology in determining the etiology of MS, we reviewed available publications. The methods used to conduct the review are described below: Scopus and Web of Science were searched for relevant articles based on a broad coverage of the scientific literature. Google Scholar was also used to collect resources from various sources, including both grey literature and articles not indexed in the above-mentioned databases. We used a combination of keywords with Boolean operators to formulate our search strategy. The main keywords were: "CRISPR-Cas9", "multiple sclerosis", "genome editing", "autoimmune disease", "etiology".

## Inclusion and exclusion criteria

- To ensure completeness of all relevant literature regarding CRISPR-Cas9 technology, only peer-reviewed papers published from 2016 to 2023 were included.
- Only articles in English were included.
- Studies focusing on the use of CRISPR-Cas9 in general genetics, autoimmune diseases, and MS specifically were included.
- Review articles, original studies, and meta-analyses were included.
- Articles not related to CRISPR-Cas9 technology and MS, editorials, commentaries, and other non-scientific publications were excluded.

## Data collection and analysis

Relevant data from the submitted articles were collected using a standardized form. Important information included the research goals, processes used, results, and their implications for CRISPR-Cas9 and MS. The data were analyzed to identify patterns, progress, applications, and barriers associated with the use of CRISPR-Cas9 in MS research.

## Article quality assessment

The quality of the selected studies was assessed using the Newcastle-Ottawa Scale (NOS), which is used for nonrandomized studies in meta-analyses, with modifications for this specific case.

Studies were assessed and ranked based on several factors, including the goal and objectives of the study, the methods used, and the results of the study.

## Article selection process

This review is based primarily on nine publications selected for review due to their direct relevance to CRISPR-Cas9 research in MS. These sources were examined to identify key research areas, such as technological innovations, features of autoimmune diseases, and features of MS. The literature was collected by topics and subtopics and analyzed for the development of CRISPR-Cas9 technology, its application in autoimmune diseases, modulation of the immune system, genetic components of MS, and safety and ethical issues. By synthesizing the results of these studies, the review aims to provide a comprehensive assessment of the potential of CRISPR-Cas9 to unravel the etiology of MS and develop therapeutic approaches. [Figure 1](#) illustrates the article selection process.

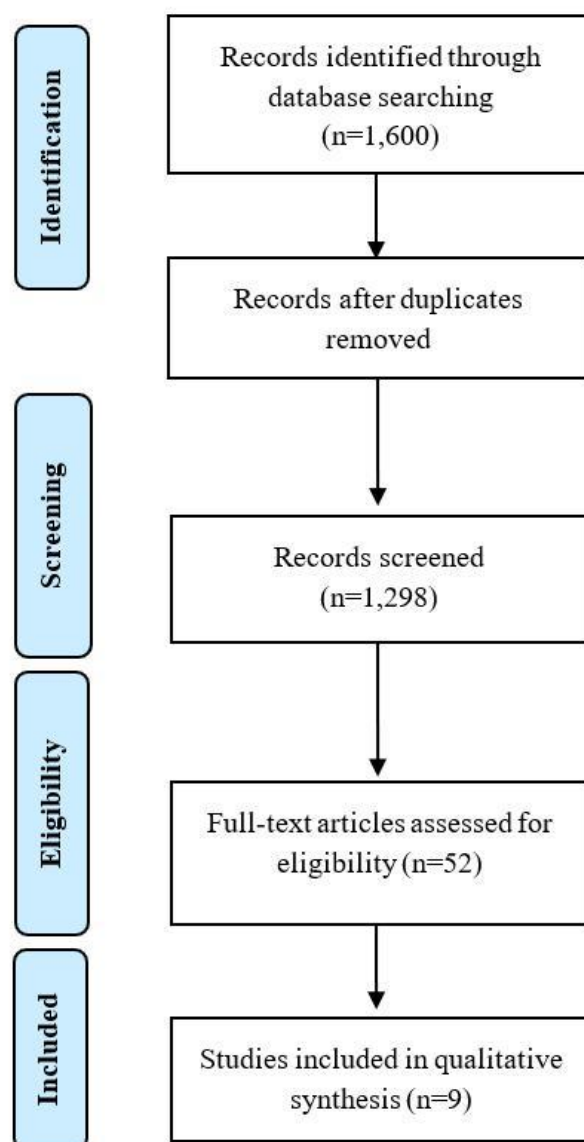


**Figure 1.** Flowchart of searching and selecting articles.

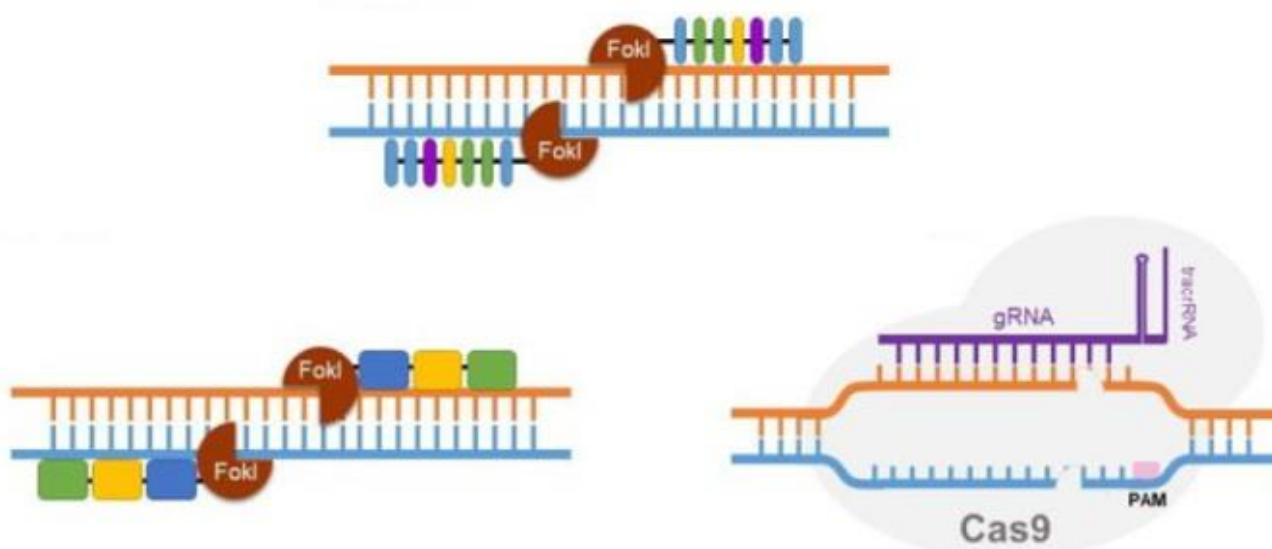


**Figure 2.** TALENs, ZFNs, CRISPR-Cas9.

### Precision and versatility of CRISPR-Cas9 in gene editing

#### High precision and versatility of CRISPR-Cas9

The outstanding adaptability and precision of CRISPR-Cas9 make it a new tool for genetic engineering. Unlike older gene editing technologies, CRISPR-Cas9 is much simpler because it uses RNA to identify target regions of the gene to be edited. The new technology allows for precise genome modification through double-strand breaks. This means that entire genes can be deactivated, inserted, or corrected. With the help of CRISPR-Cas9 guide RNA (gRNA), the Cas9 nuclease is able to localize and bind to a specific strand of DNA opposite the gRNA strand. As a result, specific changes can be made to target genes [3, 10].

The capabilities of CRISPR-Cas9 are greatly expanded by the inclusion of various modified Cas proteins, which broadens the range of possible modifications. For example, base editing and prime editing are complex modifications in the CRISPR system that perform targeted modification of specific DNA bases without double-strand cuts. This minimizes the likelihood of off-target effects and increases the likelihood of successfully introducing the desired genetic changes. [3, 11] These developments improve the precision of genomic edits, which is critical for understanding complex diseases such as MS.

The high precision of CRISPR-Cas9 is demonstrated by its use in a variety of cell types, especially human cells, making it a versatile tool in basic and translational research. It also facilitates the creation of accurate disease models due to its efficiency in creating targeted genetic changes and allows researchers to assess the genetic basis and molecular mechanisms of diseases [9].

#### Comparison with other gene editing technologies

Compared to previous gene editing technologies such as zinc finger nucleases (ZFNs) and Transcription activator-like effector nucleases (TALENs), the precision and versatility of CRISPR-Cas9 represent significant advantages.

ZFNs are engineered DNA-binding proteins consisting of a fingerprint domain that recognizes specific DNA sequences and a nuclease domain that introduces double-strand breaks. ZFNs can

indeed target specific DNA sequences, but their design and production are complex and time-consuming. Each zinc finger can recognize three nucleotides, and multiple zinc fingers are required to achieve specificity, making the process more complex and flexible than CRISPR-Cas9 [12, 13].

Another limitation to the use of ZFNs is the high cost and technical difficulties associated with their design and assembly. In contrast, CRISPR-Cas9's reliance on programmable guide RNAs simplifies design and allows for rapid and cost-effective customization of target sequences [9].

#### Transcription activator-like effector nucleases (TALENs)

Like ZFNs, TALENs cleave and modify nucleic acids. In the case of TALENs, it is the TALE that corresponds to a specific DNA sequence ([Figure 2](#)). Since each TALE domain is quite specific, binding to only one nucleotide, the level of specificity achieved by TALENs is significantly superior to ZFNs; however, the process of constructing TALENs is still very complex, since a set of TALE repeats is required to construct each target site [12, 14].

Although TALENs are more specific than ZFNs, they still cannot match CRISPR-Cas9 in efficiency and simplicity. CRISPR-Cas9 technology is versatile and easy to use, since a single Cas9 protein can be directed to different target sequences using different guides [9, 15].

**Ease of implementation.** Designing gRNA for CRISPR-Cas9 is much simpler than for ZFNs and TALENs, which require complex protein engineering. This ease of use speeds up research and reduces costs, making CRISPR-Cas9 more accessible to more scientists [9, 16].

**Higher efficiency and specificity.** Compared to ZFNs and TALENs, CRISPR-Cas9 exhibits a lower off-target profile and is easier to use to make edits. Increased efficiency due to greater flexibility in gRNA design and the inclusion of Cas9 variants increases specificity as genome editing becomes more precise [3, 17].

**Broad target organism coverage.** The wide range of organisms and cell types that CRISPR-Cas9 can be used with demonstrates its

effectiveness. From influenza and HIV models to gene therapy, it has proven effective in numerous studies, changing the landscape of the genetics field [3, 9].

### CRISPR-Cas9 in autoimmune diseases

#### *Using CRISPR-Cas9 in biology and medicine for autoimmune diseases: Assertion analysis*

The range and scope of biomedical research has been expanded by precise DNA manipulation using CRISPR-Cas9 technology, opening up new possibilities for studying and treating autoimmune diseases. Autoimmune diseases, which arise from the body's immune response to its own tissues, are often highly complex and involve multiple genes and environmental factors. Using CRISPR-Cas9, researchers can modify specific genes and then track changes in the development and progression of the disease.

Autoimmunity encompasses a wide range of diseases, and the cause is not fully understood. The efficiency of CRISPR-Cas9 increases the potential for unraveling this mystery. Diseases can be studied more thoroughly because animal models with modified genes related to the immune system can be developed to answer questions about the genes of interest and autoimmunity. Furthermore, the CRISPR-Cas9 system has the potential to serve as the basis for therapies that correct the genetic defects associated with these autoimmune diseases [4, 18].

#### *Examples of CRISPR-Cas9 use*

**Rheumatoid arthritis (RA).** RA is a chronic inflammatory disease of polyarticular joints and has a strong hereditary predisposition. CRISPR-Cas9 technology has been used to study genes involved in inflammatory processes in RA. For example, researchers have focused on genes encoding cytokines and cytokine receptors, which are essential for the immune system [4, 19].

Specific genes such as tumor necrosis factor alpha (TNF- $\alpha$ ) or interleukin 6 (IL-6) can be altered in animal models by deletion, and scientists have demonstrated the effect of these cytokines on the RA axiom. This research lays the foundation for targeting inflammation and preventing joint damage in RA patients [20].

**Type 1 diabetes mellitus (T1D).** A form of diabetes that results from autoimmune destructive processes affecting the insulin-producing  $\beta$ -cells of the pancreas is known as T1D. To study T1D, researchers use the CRISPR-Cas9 system to create biological models that have the genetic and immune hallmarks of T1D, which helps investigating the mechanisms of  $\beta$ -cell death [4].

One important application of this technology is the modification of genes associated with immune tolerance and autoimmunity using the CRISPR-Cas9 system. For example, altering the PTPN22 gene, which is thought to contribute to the risk of developing T1D, allows studying the interactions between immune cells and autoimmunity. This understanding is important in the development of gene therapies that enhance immune tolerance and protect  $\beta$ -cell activity [21].

**Systemic lupus erythematosus (SLE).** SLE is a complex autoimmune disease that affects the skin, joints, and various organs. SLE has many different genetic underpinnings due to the presence of multiple susceptibility genes. The functional role of these genes in the pathogenesis of SLE has been studied using CRISPR-Cas9 technology [4, 22].

For example, researchers have used CRISPR-Cas9 to knock out genes associated with SLE, such as interferon-induced helicase C domain-containing protein 1 (IFIH1) and signal transducer and activator of transcription 4 (STAT4). Animal models of gene knockout are under study to understand the pathobiology of SLE and to develop new ways to control the immune system to reduce symptoms and target therapies [23].

**Multiple sclerosis.** Similarly, MS is rather poorly understood compared with other autoimmune diseases, but offers great opportunities for studies using CRISPR-Cas9. MS is an immune-mediated disease that affects the CNS. Several researchers using CRISPR-Cas9 have focused on developing models with specific single nucleotide polymorphisms (SNPs) associated with MS to understand the genesis of the disease [7].

Editing several genes responsible for cartilage cell migration and inflammation, including interleukin 7 receptor (IL-7R) and CD40, has shed light on their role in the pathology of MS. These studies open up opportunities for the development of effective gene therapies aimed at correcting the immune response to prevent neurodegeneration in patients with MS [24].

### Genetic models and multiple sclerosis (MS)

#### *Construction of multiple sclerosis models with orthologous genes using CRISPR-Cas9 technology*

The development of MS defies the imagination, and CRISPR-Cas9 technologies are helping to overcome this problem. Like other complex diseases, MS is associated with inflammation and autoimmune attacks on the CNS, which cause demyelination and neurodegenerative changes. The pathogenesis of MS remains unknown. However, it is one of the diseases with a strong hereditary or genetic component. Using CRISPR-Cas9, precise genetic models can be created, allowing detailed investigation of the molecular mechanisms underlying MS.

**Developing animal models.** CRISPR-Cas9 has been used to create transgenic animal models with specific genetic mutations associated with MS. These models are critical to studying both the development of the disease and the gene elements that contribute to the development of MS. For example, mouse models with deletion or replacement of the IL-7R and CD40 genes, which are known to cause MS, provide valuable tools for understanding immune and neuroinflammatory processes [7]. These models allow investigating gene-environment interactions in MS and how the combination of specific genes and certain environmental conditions can cause its development.

**Knockout and knock-in models.** The CRISPR-Cas9 system can be used to create unique models by introducing precise genetic changes via developing knockout models in which certain genes are completely turned off, and knock-in models in which new genetic sequences are inserted [4]. In MS research, knockout models of immune regulatory genes such as T and B cells are informative for studying their brain autoimmune responses. Researchers can use these models, with mutations of interest specific to MS, to understand their role in vivo. This helps elucidating the molecular mechanisms by which specific genetic variations lead to the development of MS.

**Human cell models.** In addition to animal models, CRISPR-Cas9 technology can be applied to human cells to model MS in vitro. For example, human embryonic stem cells (ESCs) can be modified with mutations associated with MS. Subsequent differentiation of these

modified ESCs into neuronal or immunological cells allows studying the mechanisms of the disease in the context of human genetics [5, 25]. Such in vitro human cell models are useful for identifying the specific effects of genetic changes on cell types and for testing potential therapeutic measures aimed at eliminating the consequences of such changes.

#### ***Understanding the genetic components and mechanisms associated with multiple sclerosis***

The creation of precise genetic models of MS using CRISPR-Cas9 has greatly advanced our understanding of the genetic components and pathways of MS. These models allow for a high degree of control over the environment, which is essential for studying the relationships between the various elements that contribute to the complexity of the pathology of MS.

**Finding susceptibility genes.** Genetic models generated using CRISPR-Cas9 have facilitated the identification and validation of factors, target genes, and other susceptibility genes associated with MS. For example, many genes such as IL-7R, CD40, and tyrosine kinase 2 (TYK2) have been associated with MS using genome-wide association studies (GWAS). The CRISPR-Cas9 gene editing method allows functional validation of these disease determinants, further supporting their value in MS [7, 18]. By editing genes in animal models or human cells, researchers can assess the resulting phenotypic changes and immune response, making it easier to associate different gene variations with diseases.

**Pathway elucidation.** Genetic models allow for close examination of specific molecular pathways in MS. For example, the effect of the JAK-STAT signaling pathway of the TYK2 gene can be studied in CRISPR-Cas9-edited models. This may help to understand the immune dysregulation and neuroinflammatory components of MS, as well as to identify drug candidates [4, 26]. Moreover, CRISPR-Cas9 models allow studying the interactions between genetics and the immune system, as well as how certain gene alleles can alter the activity of T and B cells, which is critical for the pathological processes in MS [27].

**Mechanisms of the disease.** Genetic models at the ideal system level allow studying disease mechanisms at a miniature scale. For example, certain genes responsible for myelination and demyelination can be edited to observe how oligodendrocytes and myelin sheaths are preserved or damaged. Understanding this is fundamental to the neurodegenerative component of MS and other diseases [7]. They also facilitate the study of the functions of the blood-brain barrier in multiple sclerosis, especially how its genetic determinants at the biological level affect the immune functions of infiltrating cells in the CNS.

#### **Editing immune cells**

##### ***The role of CRISPR-Cas9 technology in modifying immune cells for functional and behavioral studies in autoimmune diseases***

Advances in CRISPR-Cas9 have revolutionized the study of autoimmune diseases at the level of immune cell function and behavior. With the ability to precisely modify specific genes using CRISPR-Cas9, researchers can now study the role of many genes and pathways involved in autoimmune responses of the body.

**Modifying T and B cells.** B and T cells play important roles in the adaptive immune response and are common effector cells in

many autoimmune diseases. CRISPR-Cas9 genes have been edited to study the processes occurring in these cells. For example, T cell receptor signaling, cytokine secretion, and even T cell differentiation can be modified or suppressed to observe the immune responses that result from this modification [5]. Scientists and clinicians can analyze changes in the function of Treg cells, which are vital for the control and action of suppressor T cells, by modifying genes such as FOXP3 and study the effects of autoimmune diseases, especially multiple sclerosis, in which immune regulation is already impaired [28].

**Studying immune cell functions.** With CRISPR-Cas9, it is now possible to alter immune cells by mutating or inserting relevant specific genes to track cell movement, interactions, and activity. Editing genes encoding chemokine receptors or adhesion molecules allows researchers to understand how immune cells travel to sites of inflammation, such as the CNS in patients with MS [4]. Additionally, CRISPR-Cas9 can be used to create immune cell models that carry other mutations common in patients with autoimmune diseases. These immune cell models allow researchers to study the effects of these mutations on immune system activity and how these changes lead to disease symptoms [29].

**Altering genes that affect immune cells activity.** CRISPR-Cas9 has been shown to work effectively in patients with autoimmune diseases on a large scale. High-throughput screening studies using CRISPR-Cas9 have revealed previously undocumented genes and signaling pathways in immune cells. For example, new genome-wide CRISPR screening methods in T cells have revealed new mechanisms that influence immune activation, tolerance, and even treatment [5]. With the development and implementation of these functional genomics methods, more and more genes are being studied and analyzed, confirming the influence of immune cells on the immune response and diseases requiring therapy.

##### ***Promising innovations in multiple sclerosis immunotherapy using CRISPR-Cas9***

Although multiple sclerosis is a chronic autoimmune disease characterized by damage to the CNS by the immune system, its treatment may be potentially effective due to the gene editing capabilities provided by CRISPR-Cas9.

**Changing the function of immune cells.** CRISPR-Cas9 can modify immune cells enhancing their regulatory functions or reducing their pathogenic potential. For example, the autoimmune process in MS can be improved by altering Treg cells, making them more suppressive and stable [5]. Similarly, CRISPR-Cas9 can be used to block genes that stimulate proinflammatory activity of T and B cells. Genes such as IFNG (interferon-gamma) and interleukin 17A (IL17A) can be used to limit inflammatory activity in patients with MS to a level at which it does not lead to neurodegeneration [30].

**Personalized immunotherapy.** The use of CRISPR-Cas 9 opens up the prospect of new immunotherapy strategies. Such approaches may include targeting immune cells obtained from patients and correcting their specific genetic defects or implanting beneficial cells. For example, transforming autologous T cells to express customized receptors or signaling molecules capable of inducing tolerance may be important for the treatment of MS [7]. Tailored CRISPR-Cas9-based immunotherapies aimed at self-targeting may also include the ablation of autoreactive myelin cells in MS by reprogramming immune cells [30].

**Safety and efficacy considerations.** Like many other aspects of CRISPR-Cas9-based immunotherapies, safety and efficacy require more careful analysis and broader scope. It is especially important to ensure that any modifications are within the intended scope. For example, creating highly accurate Cas9 variants and increasing the specificity of the guide RNA design are two methods to reduce off-target effects [6]. Further research and testing are needed to test the safety and efficacy of CRISPR-Cas9-based immune cell modifications in MS. In addition, these studies will help developing optimal strategies for delivering edited cells and ensuring the sustainability of their effects in the body [31].

### Target genes for immunomodulation

#### **Identification and modification of genes involved in immune regulation and response**

The autoimmune disease, MS, may benefit most from CRISPR-Cas9 technology due to its ability to accurately identify and modify genes associated with immune regulation. This will allow researchers to modify the immune response by specifically targeting genes responsible for the immunopathy needed to correct the autoimmune balance.

#### *Selecting important genes*

GWAS and functional genomics approaches have identified several genes associated with immune regulation and response in MS. For example, genes involved in T cell activation (such as CD4 and CD8), cytokine production (e.g., IFNG, IL17A) and immune cell migration (e.g., CCR5) are associated with them [4]. Functional validation of these candidate genes can be performed using CRISPR-Cas9 by mimicking MS-associated genetic variants in knockout or knock-in models. This strategy helps understanding the contribution of specific genes to disease susceptibility and progression [32].

**Approaches to modification.** Targeted knockout of proinflammatory genes: Genes encoding proinflammatory cytokines and receptors critical for autoimmune responses can be knocked out using CRISPR-Cas9. For example, IL-6 and TNF- $\alpha$  can be used to block inflammatory signaling to immune cells in MS [8]. Targeting Treg cell genes such as FOXP3 or CTLA4 can improve immune tolerance by enhancing regulatory T cell activity. This strategy aims to mitigate self-destructive responses and restore immune homeostasis to prevent the attack on myelin in MS [5, 33].

**Regulation of immune cell signaling pathways:** Altering immunomodulatory genes, including specific regions of the JAK-STAT signaling pathways such as TYK2, can influence immune cell activation and differentiation. Modification of these pathways may be useful in mitigating autoreactive responses in MS [7, 34].

**Potential CRISPR-Cas9 targets for treating different forms of multiple sclerosis.** IL-7R is important for T cell development and survival and is associated with MS. CRISPR-Cas9 allows assessing the effect of certain IL-7R variants on T cell responses and to explore the possibility of using IL-7R as a candidate for therapeutic modulation of the immune response in MS [7, 35].

CD40 is one of the molecules involved in B cell activation and also plays an important role in antigen presentation, which triggers autoimmune responses in MS. Using CRISPR-Cas9 to edit genes that regulate CD40 expression or function may help curb B cell-mediated inflammation and subsequent neurodegeneration in patients with MS [7, 36].

TYK2 is important for the function of the JAK-STAT signaling pathway, one of the major pathways regulating the immune response. Several genetic variants of the TYK2 gene are associated with MS. The CRISPR-Cas9 approach to TYK2 allows for modulation of cytokine signaling and immune cell activation, which may be useful in controlling the symptoms of MS [7].

These measures are supported by studies showing that underexpressed Treg cells can be induced to enhance the development of MS and other autoimmune diseases through CTLA4 knockout and transgenic modification [5].

### Potential genetic targets in multiple sclerosis for CRISPR-Cas9

#### **Specific genes associated with multiple sclerosis**

In MS, it is important to understand the genetic basis because it is a very complex and poorly understood autoimmune disease. In this disease, the immune system attacks the CNS, causing demyelination and eventually neurodegeneration. Here we review the genes involved in the development of MS that can be targeted using CRISPR-Cas9 technology and explain how editing these genes can help us understand the mechanisms of the disease.

IL-7R is essential for T-cell development, survival, and homeostasis. Genetic polymorphisms in IL-7R are associated with increased susceptibility to MS in GWAS. Editing IL-7R in immune cells using CRISPR-Cas9 may implicate it in regulating T cell activation and differentiation and autoimmunity in MS [7, 37].

CD40 is the tumor necrosis factor receptor, which is activated on antigen-sensitive B cells and dendritic cells. This activation is a factor in antibody production, differentiation and proliferation of B cells. Susceptibility to MS and disease progression are associated with a genetic variant of CD40. Editing CD40 in B cells and dendritic cells using CRISPR-Cas9 has led to theories about the role of CD40 in the autoimmune response and neuroinflammation in MS [7, 38].

FOXP3 is a transcription factor that determines the development and activity of regulatory T cells (Treg). T cells play an important role in maintaining immunity and preventing autoimmune diseases by suppressing inflammatory responses. Autoimmune diseases such as MS can be caused by T cell dysfunction due to mutations or genetic changes in FOXP3. The reason why changes in FOXP3 function or expression cause abnormal inflammation and neuronal responses in MS can be explained by editing FOXP3 in T cells using CRISPR-Cas9 [5].

**TYK2:** Cytokines such as type I interferons and interleukins (IL-12 and IL-23) that activate signaling pathways are influenced by TYK2, a member of the Janus kinase (JAK) family. Susceptibility to MS and altered immune response are associated with a genetic variant of TYK2. In immune cells, CRISPR-Cas9 editing of TYK2 may play a role in regulating T cell differentiation, cytokine signaling, and neuroinflammation in MS [7, 39].

**C-C chemokine receptor type 5 (CCR5):** Among the chemokine receptor types, CCR5 can be found on T cells and macrophages, which is involved in the trafficking of CCR5 to inflammatory cells. In addition, the progression of MS and the patient's susceptibility to this disease depend on the genetic type of CCR5. Addressing immune cell trafficking and CNS penetration allows us to correctly assess the impact of CRISPR-Cas9 editing of CCR5. Thus, it is effective in neuroinflammation in MS [4].

### ***How can editing these genes shed light on their role in the pathology of multiple sclerosis?***

Gene editing using CRISPR-Cas9 can shed light on the mechanisms of MS. Here are some of the ways:

**Functional validation.** Modifying genes in immune cells or in animal models in vivo helps researchers focus on specific genes that are suspected of playing a role in MS. The behavioral response of immune cells, including cytokine release and overall disease progression, classifies genetic variability in MS risk and its pathophysiological progression [7, 40].

**Elucidation of molecular pathways.** The pathology of MS can also be investigated using genetic engineering techniques. For example, editing TYK2 may provide a better understanding of JAK-STAT signaling and the cytokine response that contributes to the immune and neuroinflammatory disorders in MS [7].

**Identification of therapeutic targets.** The ability to study MS using CRISPR-Cas9 allows the identification of new genes that modify MS pathology as dominant therapeutic targets. Direct modification of these target genes may be useful for developing sophisticated MS treatment regimens that will balance the immune system, protect neurons, and repair inflamed tissues [7, 41].

### **Molecular mechanisms: Investigating molecular pathways and mechanisms affected by gene editing**

The precision and accuracy of investigating pathways and mechanisms altered by gene editing have been greatly enhanced by CRISPR technology. This precision facilitates the identification of new therapeutic targets for a variety of diseases with complex pathophysiological mechanisms, including autoimmune diseases such as MS.

### ***Applications of CRISPR-Cas 9 technology in molecular biology research***

**Immune cell signaling.** CRISPR-Cas 9 can be used to edit genes in important immune signaling pathways such as JAK-STAT, NF- $\kappa$ B, PI3K-Akt, and other immune cell signaling pathways. Interventions in key genes of specific molecular pathways help researchers understand the mechanisms underlying the immunosuppression and inflammatory responses present in MS [5].

**Production of cytokines.** Cytokine and receptor genes also play key roles in many immune processes, such as immune cell differentiation and activation. CRISPR-Cas9 gene knock-in and gene knockout strategies may help in engineering specific types of MS in which different cytokines, such as IFN- $\gamma$  and IL-17, are involved in neuroinflammation and demyelination [4, 18].

**Epigenetic modifications.** CRISPR-Cas9 technology, in combination with other epigenomic tools, can be used to study changes that modulate gene expression in immune cells. Knowing what changes occur at the epigenetic level in the immune system's response to MS helps to assess the likelihood of disease development and progression [12].

### ***Exploring new molecular targets vital for therapeutic strategies***

Use of CRISPR-Cas9-related genes in genomic functional studies: Novel molecular targets essential for the pathogenesis of multiple sclerosis can be identified by performing high-throughput

genome-wide gene function screening using CRISPR-Cas9 technologies in relevant cell types or animal models to identify genes modulating immune response, neuroprotection or myelin repair [5].

**Personalized medicine:** CRISPR-Cas9 can be used to generate patient-specific cell models containing disease-specific mutations or variants. This allows screening of therapeutic targets and development of personalized treatment strategies for MS patients using their characteristics [7].

**Drug target eradication:** CRISPR-Cas9 technologies are used to confirm the therapeutic efficacy of targeting a specific gene of interest or metabolic pathway predicted by genomic studies or bioinformatic approaches in preclinical models of MS, thereby facilitating the translation of high-throughput research approaches and basic research findings into clinical practice [42].

### **Risks and strategies in CRISPR-Cas9 gene editing**

Precision medicine involves many different components, including newer elements such as the CRISPR-Cas9 gene editing technology. Modifying DNA with CRISPR is much easier due to its high efficiency. However, therapeutic applications, on the other hand, are much more targeted. Unfortunately, off-target effects can be problematic. Off-target gene editing is far more precise, so there are a number of strategies that need to be implemented to protect against collateral damage when using the CRISPR-Cas9 system.

### ***Risks associated with off-target gene editing***

**Genomic instability:** Genomic regions similar to the target region are at risk of mutations, and due to these off-target effects, there is a potential for genomic instability and dysfunction at the cellular level [6, 43].

**Gene dysfunction:** Deletion or disruption of key genomic loci can lead to loss of gene activity in any biological system, leading to unintended phenotypic changes or consequences [3].

**Off-target immunogenicity.** CRISPR gene editing in clinical settings can be challenging as it may predispose patients to immune reactions or alter key genes in the body to prevent unwanted symptoms [4, 44].

### ***Strategies to minimize off-target effects***

**Improving CRISPR-Cas9 precision.** Improved gRNA design. Leveraging off-target gRNA tools for new gRNA sequences. gRNAs are designed to complement the genomic target without any attachments [5]. Cas9 variants, such as high-fidelity Cas9 nucleases, have been developed that have higher specificity and are less likely to misadapt [8, 45].

**Validation and screening procedures.** Next generation sequencing. Performing whole-genome sequencing or deep sequencing to detect unintended mutations following CRISPR-Cas9 application. This allows for a comprehensive assessment of potential off-target effects [12]. Testing of cellular or animal models to confirm the accuracy of the edits performed and the presence of other unwanted changes associated with living organisms before implementation [9, 46].

**Other gene editing methods.** Advanced and primed editing. Using alternative CRISPR-related technologies that have low levels of double-strand breaks, reducing the likelihood of off-target

effects compared to standard CRISPR-Cas9 systems [42]. Other genome editing tools such as ZFNs and TALENs can target specific genes and their specific actions under specific conditions with lower off-target effects [7, 47].

### Importance of precision and accuracy in CRISPR-Cas9 applications

**Clinical safety:** CRISPR-Cas9 application should be carried out with special emphasis on precision and accuracy as this will improve the safety and efficacy of gene therapy. Reducing side effects will make CRISPR-Cas9 more effective in treating genetic diseases, including autoimmune diseases such as MS [4, 31].

**Ethical considerations:** Interventions are more efficient, targeted, and ethical, meaning less harm is inflicted on the patients or experimental subjects, when accurate gene editing is performed [48].

### Technical challenges in CRISPR-Cas9 gene editing

The invention of CRISPR-Cas9 technology has opened a new era in genetics. It opens up many opportunities for the development of treatment protocols, but also has a number of drawbacks, including limitations in the implementation of therapy, insufficient efficacy and specificity, which is essential for the full realization of the risks and benefits of CRISPR-Cas9.

### Delivery methods

**Challenge:** One of the most difficult tasks is the efficient delivery of CRISPR-Cas9 components to target cells and tissues of the body. Various delivery methods have been tried, such as viral vectors (lentivirus, adenovirus) and non-viral methods (electroporation, lipid nanoparticles), but all of them have some drawbacks related to limitations in cell type specificity, immunogenicity or cargo capacity [6, 49].

**Strategies:** Current research is focused on the development of new delivery systems that can maximize efficacy while reducing side effects. For example, the development of new viral vectors with additional tissue-specific promoters will significantly increase the concentration of CRISPR-Cas9 in target tissues [3]. In addition, new nanotechnologies and biomaterials are developing non-viral delivery systems that are more biocompatible and provide improved targeting [4].

### Effectiveness of gene editing

**Challenge:** For practical applications in therapeutics and basic research, gene editing must be performed with high precision. The efficiency of gRNA editing, the activity of the Cas9 protein, and the accessibility of the target DNA sequence in chromatin play an important role in the overall efficiency [5].

**Strategies:** Researchers are actively using bioinformatics tools to optimize gRNA design to improve the efficiency of editing on-target and reduce off-target effects. In addition, the development of additional Cas9 variants with improved kinetics and processivity further improves the editing efficiency in different cell types and at more complex genomic loci [8, 50].

### Specificity and off-target effects

**Challenge:** One of the most challenging areas of CRISPR-Cas9 applications is achieving high specificity to reduce off-target effects. Off-target cleavage can not only lead to unintended mutations but also to changes in cellular function that may be detrimental for therapeutic purposes [12, 51].

**Strategies:** Work is underway, for example, to develop high-fidelity variants of Cas9 and other more complex nickases that can cleave only one strand of the DNA double helix. In addition, Digenome-seq and GUIDE-seq are more sophisticated methods that allow full validation and identification of genome-wide searches for potential off-target sites [42].

**Table 1. A comparative analysis of innovations, applications and ethical issues of CRISPR technology**

| Parameter                                       | Topics                                                                     | Common topics                                                                                                                                                                                | Differences                                                                                                       |
|-------------------------------------------------|----------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| Advances in CRISPR technology                   | The new associated CRISPR proteins, base editing and prime editing         | Expanding applications of CRISPR-Cas9 technology due to rapid advances in the field [3, 5, 8, 9]                                                                                             | Does not focus on recent advances [12]                                                                            |
| Applications in disease research                | Genetic disease models, disease treatments                                 | Broad applications in disease research and treatment [3, 4, 7-9]                                                                                                                             | Distinct focus on CRISPR melanoma (rather than other cancers) as opposed to other autoimmune disease targets [42] |
| Immune system and autoimmune diseases           | Autoimmunology, immunomodulation, and autoimmune diseases                  | CRISPR-based immune system research and potential applications in autoimmune diseases [4, 7]                                                                                                 | More focused on immunoengineering than other immunology researchers [5]                                           |
| Ethical and safety issues                       | Risks and concerns related to the ethical aspects of the technologies used | Ethical issues related to the use of CRISPR-Cas9 [7]                                                                                                                                         | Enrichment of p53-deficient cells and emphasis on its dangers [6]                                                 |
| Focus on technical vs. therapeutic applications | Development of technologies or treatments for diseases                     | Some references to technical aspects are distributed as [3, 5, 8, 9, 12], while others [4, 7] carry the burden of optimism, especially in the context of autoimmune diseases [3, 5, 7-9, 12] | In contrast to other reports on autoimmune diseases, studies on cancer (melanoma) have appeared [42]              |
| Disease-specific contexts                       | Multiple sclerosis, autoimmune diseases                                    | Covers multiple sclerosis in detail, while other sources take a broader approach [4, 5]                                                                                                      | Present some methodological nuances, but disagree on the disease context [4, 5, 7].                               |
| Challenges and limitations                      | Off-target effects, delivery methods, efficacy                             | The problems and limitations of CRISPR technology in current clinical practice are discussed [8, 9]                                                                                          | Provides contextual information rather than discussing topical issues [12]                                        |
| Immune cell engineering                         | Manipulation, immune mechanisms                                            | The application of CRISPR technology to manipulate the immune system and immune mechanisms is discussed [5]                                                                                  | More attention is paid to general applications of gene editing than to engineering immune cells [3, 4, 7-9]       |

Research is focused on addressing technology gaps to facilitate the use of CRISPR-Cas9 and ensure its safety. The next stage will focus on developing improved delivery systems with higher specificity and lower risk of immune response [52]. In addition, new editing tools will be developed for more complex DNA edits, such as new Cas9 variants and CRISPR-based systems [53]. Bioinformatics and computational tools will be used to optimize genome editing processes, using artificial intelligence and machine learning for automated targeting [54]. A more comprehensive analysis of recent developments, applications, and controversies surrounding CRISPR technology is presented in the following [Table 1](#).

## Conclusion

The revolutionary potential of CRISPR-Cas9 technology sheds light on the causes of MS and the development of targeted therapies. Precise genome editing with CRISPR-Cas9 allows researchers developing accurate models of MS, deeply analyze molecular pathways of the disease, and diagnose potential therapeutic approaches. Beyond modeling, the precision of CRISPR-Cas9 makes it possible to modify immune cells to study their dysfunction in autoimmune diseases such as MS, thereby opening up new avenues for immunotherapy. However, the effective application of CRISPR-Cas9 in MS research faces challenges such as off-target effects and other limitations related to the efficiency and accuracy of delivery. Fortunately, these challenges are now being addressed through the development of advanced tools, improved systems, and follow-up methods. These advances not only improve the safety and efficacy of CRISPR-Cas9, but also expand its practical application in the treatment of MS and other autoimmune diseases. Most importantly, the combination of CRISPR-Cas9 with new approaches such as single-cell genomics and high-throughput screening represents a revolution in the diagnosis and treatment of MS. It expands our understanding of the pathogenesis of this disease and changes the timing of the implementation of research results into practice.

## Conflict of interest

The authors declare no conflicts of interest.

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