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CRISPR: An Elixir for Autoimmune Diseases? A Systematic Review

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ABSTRACT: *Genetic studies have linked the gene polymorphisms and autoimmune disorders. In response, the Clustered Regularly Interspaced Short Palindromic Repeats and its associated protein 9 (CRISPR-Cas9) has become a promising tool for treating these diseases. The broad acceptance of CRISPR, due to its simplicity, precision, and adaptability, has significantly rushed scientific research, and fostered radical discoveries in both model species and human cells. CRISPR-Cas9 offers versatile applications for rare diseases like urea cycle disorders or hepatorenal tyrosinemia and in reducing cholesterol by targeting Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9). It can also immunomodulate the autoimmune diseases by specifically targeting genes associated with these conditions. This targeted approach holds the potential to modify the immune response, leading to the potential alleviation of disease progression. Our review underscores the ongoing exploration of CRISPR-Cas9 therapy for autoimmune disorders, emphasizing its transformative possibilities in this field. We specifically highlight the potential target genes for CRISPR-Cas9 immunomodulation in prevalent autoimmune disorders such as systemic lupus erythematosus, multiple sclerosis, insulin-dependent diabetes mellitus, psoriasis, type 1 coeliac disease, and rheumatoid arthritis. The future holds immense promise as the remarkable advances in CRISPR-Cas9 therapies pave the way for a revolutionary transformation in the treatment of various autoimmune disorders.*

Keywords: *Autoimmune disorders, CRISPR-Cas9 therapy, Immunomodulation, Gene therapy, RNA silencing*

INTRODUCTION

The understanding of the human genome has been greatly enhanced due to the continued advancement of genome editing techniques in recent decades, which has virtually enabled us to grasp a deeper comprehension of the role of genes and gene products in disease processes (Pavel-Dinu et al., 2023). The marvellous achievements of genetic engineering (the modification of nucleic acids) caused a breakthrough in the field of genome editing, back in the decade of 1970s. Over the past ten years, scientists are successfully able to perform various astonishing roles in the domains of biomedical research and applied biotechnology by using nucleases enzymes either synthetic or extracted from bacteria. All these achievements are achieved at a more rapid rate than ever imagined.

The essence of genome editing is the ability to permanently alter DNA at the molecular level. Scientists have successfully availed the two most powerful biological strategies including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 and Transcription Activator-like Effector Nuclease (TALEN) systems. Both technologies are being used widely nowadays (Doudna, 2020; Ustiugova et al., 2023).

The history of genome editing techniques takes us back to the early years of molecular biology, where the foundational principles were laid by scientists such as Crick. It was Crick who first articulated the central dogma of the field, which states that information flows from DNA/RNA (nucleic acid) to proteins in a sequential manner. This fundamental concept highlights the importance of permanent DNA modifications, as they have the potential to reshape protein sequences and thereby profoundly influence their functionality.

Gene Editing vis-à-vis Immunological Disorders: Research in immunology experienced a paradigm shift in the late 1990s with the completion of the human genome reference sequence. Although the 3.2 billion DNA bases in our bodies were discovered as part of the Human Genome Project, yet functions of most of the genes remained unknown (Feng et al., 2023). The focus of genomic research has been primarily on immune cells, which tackle invading foreign pathogens and are thus essential for maintaining human health. For mapping the DNA along with its associated genetic regulators responsible for the types, states, and functions of immune cells, various techniques, such as

transcriptional and chromatin state profiling, have been employed to study these cells (Vockley et al., 2023).

Conversely, genetic manipulation is the only way to provide answers to important concerns regarding immunity and DNA. What is the biological significance of the functional sequences in our DNA? What is the genetic framework—encoded in genes, noncoding sequences, and trans-regulators—that is responsible for wiring certain cellular pathways as well as performing specific roles in immune cells? What changes in cellular function and risks of immune-mediated illness can be attributed to variation in important coding and non-coding sequences? Can we reprogram natural immune cell genetic circuits using what we know about them to create the next wave of synthetic cellular therapies? The ability of emerging technology to edit immune cells' genomes will determine the solutions to all such queries. Immunologists are already starting to modify immune cell genomes to unveil the genetic basis of immunity thanks to one of these tools, CRISPR (Guo et al., 2022; Akram F et al., 2023).

Currently, several researchers are working to modify the underpinnings of genomes of immune cells so that immunological diseases can be treated using CRISPR. In this article, we review some autoimmune diseases that can be treated using CRISPR. However, before delving into those diseases and exploring how CRISPR technology can be employed to treat them, we first discuss the basics of the CRISPR technique.

CRISPR: The revolutionary technology known as CRISPR, in conjunction with its associated protein Cas9, originates from the bacterial cell's adaptive immune response system. To put it simply, the CRISPR sequence within the bacterium incorporates small segments of the viral genome, acting as memory sequences. These sequences enable the bacterium to recognize and mount a defence against future infections by the same virus. Furthermore, the Cas9 protein, serving as an endonuclease, plays a crucial role in this process by targeting and inducing double-strand breaks in the viral genome. This mechanism potentially renders the virus inactive, providing an effective defence mechanism for the bacterium (Katti et al., 2022).

The CRISPR sequence consists of multiple short repeating sequences interspersed with longer sequences known as spacers. When a host bacterium is exposed to a viral infection, small segments of the viral genome are integrated into the CRISPR region of the bacterium's genome, resulting in the production of

spacer sequences. These spacer sequences are transcribed into a long RNA molecule called pre-CRISPR RNA (pre-crRNA) (Khanzadi and Khan, 2020). The processing of pre-crRNA and the efficient functioning of the CRISPR/Cas9 system rely on the involvement of a small RNA molecule called trans-activating CRISPR RNA (tracrRNA). The tracrRNA is produced from the upstream region of the CRISPR sequence. Within the CRISPR sequence, the short repetitive sequences exhibit complementarity to specific regions of the tracrRNA. This complementary binding allows the pre-crRNA to form a duplex RNA structure with the tracrRNA, facilitating further processing and activation of the CRISPR/Cas9 system (Guo et al., 2022). The formation of the mature crRNA: tracrRNA complex involves sequential processing steps, including catalysis by RNASIII and an unidentified nuclease. Through these processing phases, the spacer region of the crRNA, derived from viral DNA fragments, serves as a crucial memory component for the bacterial cell. This memory function allows the bacterial cell to recognize and mount a specific immune response against future encounters with the corresponding virus (Hillary and Ceasar, 2023). Fig. 1 visually represents this process.

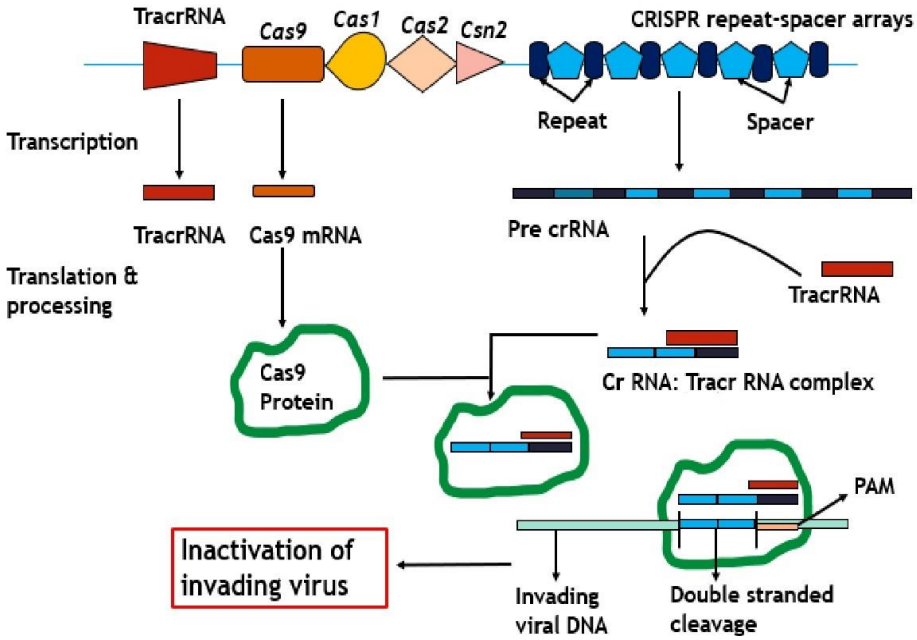

Fig. 1. CRISPR as a bacterial or archaeal adaptive immune system

The discovery of the CRISPR/Cas system was rooted in the intriguing observation of a distinct set of repetitive sequences downstream of the termination codon of the *Iap* gene's translation in *Escherichia coli* (*E. coli*) (Bharathkumar et al., 2022). These repetitive sequences, consisting of 14 base pairs and known as palindromic repeats, form the CRISPR locus. Throughout the genome of bacteria and archaea, these repeats are interspersed with 32-nucleotide sequences, creating a repetitive pattern. This CRISPR locus serves as a natural defence mechanism in these organisms, directed by RNA, to combat both RNA and DNA viruses (Pinilla-Redondo et al., 2022). Furthermore, the CRISPR locus not only consists of the palindromic repeats and spacer sequences but also contains foreign DNA segments known as CRISPR Array Regions. These CRISPR Array Regions are inserted between the palindromic repeats and play a crucial role in the system's functionality. By storing and translating information about previous infections, the CRISPR/Cas system serves as a paradigm for adaptive immune responses. This remarkable system enables the organism to retain a molecular memory of past encounters with specific pathogens, allowing for a more targeted and efficient immune response in future encounters (Sharma et al., 2021).

The technology has made significant progress since the initial report on CRISPR in 1987 (Fig. 2). The CRISPR-Cas9 technique has enabled genetic experiments to be conducted on a wide range of living species. This includes various organisms such as plants, *Drosophila*, zebrafish, mice, and even more complex organisms like humans. The versatility of CRISPR-Cas9 has expanded its applications across different species, facilitating precise genetic modifications and furthering our understanding of gene function and regulation.

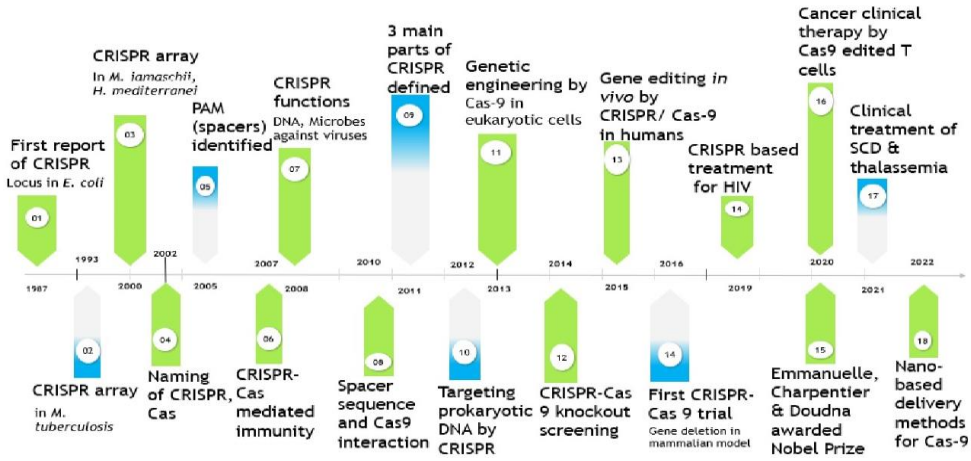

Fig. 2. Timeline of CRISPR-Cas9 System Development and Advancement

Phases of CRISPR mediated immunity: CRISPR-associated genes (Cas), which play a vital role in the CRISPR system, are typically found near the CRISPR region. These genes are known to be involved in various stages of adaptive immunity (Liu et al., 2019). The process of CRISPR-mediated adaptive immunity in bacteria or archaea can be broadly divided into three phases:

- Adaptation Phase: During this phase, the Cas genes, including Cas1 and Cas2, recognize and process the invading DNA. These Cas proteins facilitate the integration of short DNA fragments from the invading DNA into the spacer region of the CRISPR array, allowing the organism to acquire a genetic memory of past encounters with specific pathogens (Shmakova et al., 2022).
- Expression Phase: In this phase, the CRISPR array is transcribed into a precursor RNA called pre-crRNA. The pre-crRNA is then processed to generate mature CRISPR RNAs (crRNAs) with a specific 20-nucleotide sequence that targets the foreign DNA. The repeats in the crRNA interact with a trans-activating RNA molecule called tracrRNA. The Cas9 protein, along with the crRNA and tracrRNA complex, forms an active interference complex (Kanafi and Tavallaei, 2022).
- Interference Phase: In this final phase, the interference complex, consisting of the Cas9 endonuclease and the crRNA-tracrRNA complex, recognizes and binds to the

complementary sequence in the foreign DNA. The Cas9 endonuclease then cuts the double-stranded DNA, leading to the degradation or inactivation of the foreign genetic material (Dao et al., 2023).

Classification of CRISPR: The structure of CRISPR/Cas loci in bacteria and archaea can exhibit various variations, leading to the categorization of CRISPR systems based on their effector proteins. Presently, CRISPR is classified into two classes and six types, each characterized by a specific Cas protein (Makarova et al., 2020; Chaudhuri et al., 2022). The composition of the Cas protein involved in the interference phase serves as the primary distinguishing factor between Classes 1 and 2 (Burmistrz et al., 2020; Tian et al., 2022).

The Class 1 interference phase of the CRISPR system involves multiple Cas proteins, specifically Types I, III, and IV (Chakrabarti et al., 2019; Khan et al., 2022). On the other hand, Class 2 CRISPR systems rely on a single protein to perform multiple functions during the interference phase, encompassing Types II, V, and VI (Makarova et al., 2022). Among the Class 2 CRISPR systems, Cas9 is the most extensively studied and utilized protein due to its versatile capabilities. Cas9 belongs to Type II CRISPR and is responsible for various crucial tasks such as binding with the guide RNA (crRNA + tracrRNA), scanning the genome, and inducing double-strand breaks (Zhang et al., 2021).

The distinct roles and characteristics of Cas proteins in different CRISPR classes and types provide researchers with a diverse toolkit for manipulating and studying genetic material. Understanding the specific functions and mechanisms of these proteins is essential for harnessing the full potential of CRISPR technology.

Genome Editing Unlocked: The Contributions of Cas Proteins: The single guide RNA (sgRNA) serves as the instructional component for Cas endonucleases, such as Cas9, Cas12, Casx, etc., directing them to the desired location in the DNA where a double-strand break is to be introduced (Fuziwara et al., 2022). Cells possess a DNA repair system that facilitates the successful ligation of DNA, as this type of break can be highly detrimental and lead to genome loss. Gene editing techniques, including the insertion or deletion of nucleotides from the DNA sequence, are employed in this process to repair and ligate damaged DNA strands (Zhuo et al., 2021).

The two primary processes for repairing DNA double-strand breaks are non-homologous end joining (NHEJ) repair and homologous directed repair

(HDR) (Zhuo et al., 2021; Fuziwara et al., 2022).

- Non-homologous End Joining Repair (NHEJ):
  NHEJ is the primary mechanism for double-strand break repair and is effective at rejoining the broken ends. While NHEJ lacks specificity and may introduce errors, it is significant for gene disruption as it can lead to loss of gene expression or protein function through microdeletions, insertions, frameshift mutations, or premature stop codon insertions in target genes (Song et al., 2021).
- Homologous Directed Repair (HDR):
  HDR utilizes homologous DNA fragments, either exogenous or endogenous, to accurately repair double-strand breaks. HDR is less prone to errors but occurs at a lower frequency, primarily during the S-G2 phase of the cell cycle (Smirnikhina et al., 2022). Exogenous DNA templates can be introduced to provide the correct gene sequence required for gene repair through HDR, but it requires the addition of Cas9 and sgRNA.
  The frequency of HDR can be modified by various approaches, including cell synchronization, medication, or molecular manipulation, to enhance gene repair efficiency (Yoshimi et al., 2021). These strategies aim to increase the utilization of HDR for precise gene editing and repair.

Autoimmune Disorders:

Autoimmune diseases occur when the immune system mistakenly targets and attacks the body's own tissues, leading to organ damage or dysfunction (Marrack et al., 2001; Xiao et al., 2021). Maintaining immunological tolerance is a vital feature of the human immune system, as it helps prevent self-inflicted harm (Olivieri et al., 2021). However, in cases of autoimmunity, the immune system mistakenly launches an immune response against its own tissues and organs. Autoimmune disorders affect approximately 3 to 5% of the general population, and they have been identified as a leading cause of mortality among women in their 20s and 30s (Krovi and Kuchroo, 2022; Mubariki and Vadasz, 2022).

The consequences of autoimmune diseases extend beyond physical symptoms. Individuals with these conditions often experience a lower quality of life, as the chronic nature of the diseases can disrupt daily activities, limit mobility, and cause persistent pain or discomfort. Moreover, the emotional toll of living with a chronic illness should not be overlooked. Studies have indicated that individuals with autoimmune disorders are more prone to mental

health challenges, such as anxiety and depression, further impacting their overall well-being (Nadali et al., 2023).

Genetic factors, abnormal immune responses, and environmental influences are known to contribute to the development of autoimmune diseases. The intricate interplay between these factors, either individually or in combination, can disrupt the normal immune tolerance mechanisms and lead to the onset of autoimmune disorders. During the maturation of B and T cells, self-antigen-responsive cells are typically eliminated or rendered inactive (anergized) to prevent the formation of self-reactive cells (Olivieri et al., 2021; Xiao et al., 2021). In certain instances, the regulatory system responsible for eliminating self-reactive cells fails, allowing them to evade the typical checks and balances. The regulation of these cells involves various mechanisms, including the crucial role played by regulatory T cells (Marks and Rao, 2022).

Autoimmune diseases involve the production of autoantibodies by the immune system, which target the body's own tissues or organs. These autoantibodies recognize specific self-antigens and play a role in the development and advancement of various autoimmune disorders. The identification and understanding of these autoantibodies, as outlined in Table 1, are essential for accurate diagnosis, prognosis, and the development of targeted treatments for autoimmune conditions.

Table 1: Few diseases associated with autoantibodies

Autoantibody	Subtypes	Target antigens	Diseases	References
Anti-thyroid Antibody	Anti-TPO	Thyroid peroxidase (microsomal)	Graves' disease, Hashimoto's thyroiditis,	(Malandrini et al., 2022; Siddiq et al., 2023)
Anti-thrombin Ab	-	Thrombin	Systemic lupus erythematosus	(Szabó et al., 2021; Yamamoto et al., 2023)
Anti-nuclear Ab	Anti-dsDNA	Double stranded DNA	Systemic lupus erythematosus, Myasthenia	(Choi et al., 2020; Li et al., 2023)

			Gravis	
	Anti-centromere	Centromere	CREST (Calcinosis, Raynaud's phenomenon) syndrome	(Zian et al., 2020)
	Anti-SSA/Ro	Ribo-nucleoproteins	Systemic lupus erythematosus, Sjögren's syndrome	(Lin et al., 2022; Alduraibi FK et al., 2023)
	Anti-histone	histone	Systemic lupus erythematosus	(Bao S et al., 2023; Choi et al., 2023)
	Anti-ribosomal	Ribosomes	Systemic lupus erythematosus	(Bao S et al., 2023; Duca et al., 2023)
	Anti-gp210	Nuclear membrane	Primary biliary cirrhosis	(Rigopoulou and Bogdanos, 2023)
	Anti-p62	Nucleoporin 62		(Bauer et al., 2022; Tan et al., 2023)
	Anti-sp100	Sp100		(Lepri et al., 2023)
Anti-CP	-	Citrullinated peptide	Rheumatoid arthritis	(Yoshii et al., 2023)
Anti-ganglioside Ab	-	GD3/GM1/GQ1b	Guillain–Barré syndrome	(Koike and Katsuno, 2021; Zhu et al., 2023)
Anti-actin	-	Actin	Coeliac disease	(Mašić et al., 2022; Machado, 2023)
Anti-	Anti-p-	Myeloperoxidase	Microscopic	(Suwanchote et

neutrophil Ab	ANCA		polyangiitis, Eosinophilic granulomatosis with polyangiitis, Rheumatoid arthritis, Primary sclerosing cholangitis, Ulcerative colitis	al., 2018; Bianco and Allegra, 2021)
	Anti-c-ANCA	Neutrophil cytoplasm	Granulomatosis with polyangiitis, Ulcerative colitis	
Anti-Vinculin	-	Vinculin	Systemic Sclerosis	(Herrán et al., 2023)
RF	-	Fc portion of IgG	Rheumatoid arthritis	(Abdelhafiz D et al., 2023)
Anti-AChR	-	Nicotinic acetylcholine receptor	Myasthenia gravis	(Iacomino et al., 2023)

CRISPR and Rheumatoid Arthritis (RA): Several in-vitro as well as in-vivo studies have been conducted that report the importance of CRISPR application to ablate key genes responsible for the onset of different types of arthritis (Evans et al., 2023; Kumar et al., 2023). Moreover, three studies employing human cell models for rheumatoid arthritis (RA) have investigated the potential of gene therapy using CRISPR-Cas9 and identified potential targets. Notably, the *MYC1* and *FOXO1* genes have been implicated in the pathogenesis of RA (Poniewierska-Baran et al., 2023). In addition to the association of *MYC* and *FOXO1* genes with RA, the study has revealed that $CD4^+$ T-cells in RA patients exhibit

heightened autophagy. It was previously hypothesized that *MYC* regulates this pathway. The study provides evidence supporting the contribution of both *MYC* and *FOXO1* genes to RA through comprehensive analysis involving techniques such as Assay for Transposase-Accessible Chromatin with high-throughput (ATAC) sequencing, high-throughput chromosome conformation capture technique (Hi-C), Capture Hi-C, and nuclear RNA-sequencing. These methods were employed in studying activated helper T cells over a 24-hour period, further elucidating the role of these genes in the pathogenesis of RA.

In a genome-wide association study conducted by Lee et al. (2022), it was discovered that the single nucleotide polymorphism (SNP) rs6927172, located on chromosome 6q23, is a risk factor for the development of RA. The study further investigated the genes surrounding this SNP region and identified *TNFAIP3* and *OLIG3* as relevant genes. Disruption of the SNP region using CRISPR-Cas9 resulted in decreased expression of both *TNFAIP3* and *OLIG3*, indicating a significant association between rs6927172, *TNFAIP3*, *OLIG3*, and the progression of RA. These findings highlight the potential role of these genes in the pathogenesis of RA.

In studies conducted by Markovics et al. (2020) and Balchin C et al. (2023), microRNA 155 (*miR-155*) was identified as a significant pro-inflammatory component in patients with rheumatoid arthritis (RA). The researchers observed that the deletion of *miR-155* in RAW 264.7 cells resulted in the up-regulation of SHP1 and hindered the production of pro-inflammatory cytokines. Based on these findings, they propose that modifying the *miR-155* region could potentially lead to effective treatment strategies for RA. This suggests that targeting *miR-155* could have therapeutic implications for mitigating the inflammatory response associated with RA.

CRISPR and Systemic Lupus Erythematosus (SLE): In studies investigating gene therapy for systemic lupus erythematosus (SLE) using human cell culture, potential targets for CRISPR-based interventions were identified, including A20 deubiquitinase, chromosome X open reading frame 21 (*CXorf21*), transferrin receptor genes, and Semaphorin3A. Harris et al. (2019) specifically focused on the role of *CXorf21* in the development of SLE by conducting in-vitro knockdown experiments. They found that the removal of *CXorf21* led to a reduction in the expression of TNF-α and IL-6, suggesting that

CXorf21 expression, particularly in sexually dimorphic forms, may contribute to the pathogenesis of SLE. These findings highlight the potential of targeting *CXorf21* as a therapeutic strategy for SLE using CRISPR technology.

In a study by Voss et al. (2023), the role of transferrin receptor CD71 was investigated using CRISPR. The researchers found that iron uptake mediated by CD71 plays a crucial role in T cell dysfunction, contributing to the development of systemic lupus erythematosus (SLE). Additionally, Eiza et al. (2023) suggested Semaphorin3A as a potential target for CRISPR therapy. Semaphorin3A acts as a regulatory ligand for the CD72 receptor, which is involved in co-regulating B cells and is implicated in the pathogenesis of SLE. Furthermore, the expression of the *IRF5* rs4728142 SNP has also been associated with SLE, indicating its potential relevance in the disease.

CRISPR and Type-1 Coeliac Disease: In their study, Yu et al. (2023) not only analyzed the gene sequences of immunogenic epitopes, specifically α- or γ-gliadins found in gluten proteins from wheat, but they also developed CRISPR constructs to specifically target these epitopes. The study proposed that α- or γ-gliadin genes could be effective targets for CRISPR-based gene therapy and demonstrated the potential to create safe grain variants by editing these genes using CRISPR technology.

CRISPR and Multiple Sclerosis (MS): Several studies investigating gene therapy for multiple sclerosis (MS) using human cell models have identified potential targets for CRISPR therapy. These targets include the RNA for DDX39B (helicase DEAD box polypeptide 39B) as well as the genes for *IR7R*, *TNFRSF1A*, and *IL2RA*. Maier et al. (2009) described the genetic heterogeneity of the *IL2RA* in both MS and insulin-dependent diabetes mellitus (IDDM). The *IL2RA* variants were found to be independently associated with levels of soluble IL2RA and increased the risk of developing MS, suggesting that *IL2R* variants are significant risk factors for both MS and T1DM.

According to Galarza-Muñoz et al. (2017), there is an epistatic interaction in humans that increases the likelihood of developing MS. This interaction involves the RNA helicase DDX39B, which can activate exon 6 of *IL7R* and repress *IL7R* in its soluble form. Strong correlations were found between the risk of MS and the genetic variants rs6897932 in *IL7R* and rs2523506 in *DDX39B*. Additionally, the study suggests that the risk of MS is influenced by locally mutated *IL7R* as well as genetic and functional interactions involving

the *IR7R* and the rs2104286 SNP in intron 1 of *IL2RA*. These interactions contribute to the increased risk of MS.

In a study conducted by Zhao et al. (2022), it was revealed that the pathogenesis of MS is influenced by the immunogenic pathway, specifically involving IL7R and its soluble form. The research highlights the significance of rs6897932, particularly its C allele, which promotes the skipping of exon 6 in the *IL7R* gene. This variant, acting as an exon splicing silencer, is associated with MS and has the potential to affect the balance between soluble and membrane bound IL7R proteins, thereby directly impacting the risk of developing MS. In a separate study conducted by Gomez-Pinedo et al. (2022), it was demonstrated that anti-tumor necrosis factor (TNF) therapies, commonly used for the treatment of autoimmune disorders, have shown efficacy beyond MS. The research focused on the mutation in rs1800693 of the *TNFRSF1A*, which encodes TNFR1 (tumor necrosis factor receptor 1), and revealed its association with the etiology of MS. This finding suggests that the mutation in *TNFRSF1A* may contribute to the development of MS and highlights the potential of anti-TNF therapies in managing autoimmune disorders.

CRISPR and Psoriasis: Arakawa A et al. (2021) conducted a comprehensive study investigating the role of *ERAP1* (endoplasmic reticulum aminopeptidase 1) variants in the development of psoriasis. Through their research, they found that these *ERAP1* variants interact with HLA-C*06:02, a known genetic risk factor for psoriasis. This interaction suggests a critical involvement of *ERAP1* in the pathogenesis of psoriasis, potentially influencing the antigen processing and presentation pathways. The study underscores the importance of understanding the immunogenetics and immunological mechanisms underlying psoriasis, providing valuable insights for future therapeutic interventions.

In parallel, Roth-Carter et al. (2020) focused on exploring the function of Desmoglein 1 (Dsg1) in the context of psoriasis. Their investigation revealed that Dsg1 plays a regulatory role in inflammatory responses, barrier development, and epidermal differentiation. By employing Desmoglein 1 knockout mice, they demonstrated that the inhibition of Dsg1 led to barrier dysfunction and increased susceptibility to psoriatic processes. These findings shed light on the significance of Dsg1 in maintaining skin integrity and its involvement in the inflammatory cascade associated with psoriasis.

Targeting *ERAP1* and Dsg1 through CRISPR therapy holds promise for potential therapeutic interventions. By precisely editing the genetic sequences associated with these genes, it may be possible to modulate their expression or function, leading to a potential reduction in psoriatic symptoms and disease progression.

CRISPR and Insulin Dependent Diabetes Mellitus (IDDM): Zhu et al. (2019) investigated the role of *LCK* SNPs (Lymphocyte-specific protein tyrosine kinase) in Insulin-dependent diabetes. They utilized CRISPR technology to assess the activity of *LCK* SNPs in blood samples from individuals with the disease. Among the tested SNPs, rs10914542 demonstrated a significant correlation, indicating that the G allele of *LCK* rs10914542 is associated with an increased risk of Type 1 diabetes.

In a related study, Ratiu et al. (2017) used *AID* knockout mice to identify potential therapeutic targets for Insulin-dependent diabetes patients, highlighting *AID/RAD51* as a potential target. Based on these findings, both the *AID/RAD51* and the *LCK* rs10914542 are suggested as suitable targets for CRISPR therapy in the treatment of Insulin-dependent diabetes.

Limitations and Future Prospects:

One significant challenge in CRISPR/Cas9-based therapies is the potential for unintended genetic alterations in non-targeted areas (Uddin et al., 2020; Yang et al., 2021). These off-target effects can have unforeseen consequences. To address this concern, researchers are actively working on enhancing the precision of CRISPR/Cas9. Strategies such as base editing and prime editing have emerged as promising approaches. Base editing allows for precise changes in single DNA letters, reducing the risk of off-target effects (Satomura et al., 2017). Prime editing, on the other hand, offers even greater accuracy by directly rewriting DNA sequences without requiring double-strand breaks (Liu et al., 2021). These advancements hold the potential to make CRISPR/Cas9 therapies safer and more reliable.

Efficiently delivering CRISPR/Cas9 components to the specific tissues or cells that require modification is another hurdle (Salman et al., 2022). Developing safe and effective delivery methods is crucial for the success of CRISPR-based treatments. Researchers are exploring various approaches, including nanoparticle-based delivery systems and viral vectors (Sivakumar and Cherqui, 2022). Nanoparticles can protect the CRISPR cargo and deliver it precisely to the target cells (Khurana et al., 2022; Chavez et al., 2023). Viral vectors, modified

viruses, can efficiently carry CRISPR components into cells (Karimian et al., 2019). Continued progress in delivery technology is essential to ensure that CRISPR/Cas9 therapies can reach their intended destinations within the body.

The rapid advancement of CRISPR/Cas9 technologies has raised important ethical considerations. One prominent concern is the potential for unintended consequences, both in individual patients and at the societal level (Fogleman et al., 2016; Brokowski and Adli, 2019). The prospect of germline editing, where changes made to an individual's DNA could be passed on to future generations, has sparked significant debate (Schultz-Bergin, 2018; Shinwari et al., 2018). Researchers, policymakers, and the scientific community are actively engaged in discussions and regulations to address these ethical concerns (Nidhi et al., 2021). Ensuring that CRISPR/Cas9 applications adhere to strict ethical guidelines is essential to promote responsible and safe use of this powerful technology (Gostimskaya, 2022).

In a nutshell, CRISPR/Cas9 holds substantial promise for personalized therapies and cellular immunotherapy in the treatment of autoimmune disorders (Zhang, 2021). However, addressing limitations related to precision, delivery methods, and ethical considerations is crucial for realizing the full potential of CRISPR/Cas9-based treatments and ensuring their safety and ethical use in the future (Rasul et al., 2022). Researchers are committed to overcoming these challenges to benefit patients with autoimmune disorders.

Conclusion:

In our pursuit to unravel the mysteries of autoimmune disorders, we embarked on a groundbreaking exploration, fueled by the revolutionary potential of CRISPR-Cas9. Through diligent exploration, we delved into the complex genetic landscape underlying autoimmune disorders. Equipped with this knowledge, we embarked on a mission to harness the potential of precision gene editing. By targeting aberrant T cell activity and curbing inflammatory cytokines, our aim is to reshape the course of autoimmune battles. The remarkable promise of CRISPR-Cas9 as a therapeutic tool shine bright, instilling hope for those seeking relief. CRISPR-Cas9 can exhibit promising desirable effects in modulating defective genes in autoimmune disorders. Overcoming the specific challenges of CRISPR itself through precise manufacturing, we can deliberately use it to knock out defective genes and replacing it with correctly sequenced gene or can simply improvise to correct the disrupted nucleotide sequence.

While the road to clinical application may present challenges, our unwavering commitment propels us forward, envisioning a future where personalized gene therapies offer solace to those affected by autoimmune conditions. Together, we advance towards a future where the transformative potential of CRISPR-Cas9 paves the way for innovative solutions and renewed hope in the realm of autoimmune disorder treatments.

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Not Applicable.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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