Florence Nightingale | Demiroglu Science University Florence Nightingale Journal of Transplantation 2023;8(1-2):56-67
doi: 10.5606/dsufnjt.2023.13

Discovery and applications of CRISPR-Cas9 gene editing technology

Ömer Demir[1], Oytun Erbaş[2]

[1]Istanbul Medeniyet University, Faculty of Engineering and Natural Sciences, Istanbul, Türkiye
[2]Institute of Experimental Medicine, Gebze-Kocaeli, Türkiye

ABSTRACT

In the current era of science, many advancements are taking place. One of the most significant of these is clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), also known as genome editing technology, has become an exciting application in the scientific world, even earning the 2020 Nobel Prize in Chemistry. CRISPR-Cas9 serves as a genome editing tool, enabling researchers in the fields of Genetics and Medicine to add, remove, or modify deoxyribonucleic acid sequences. This technology finds applications in various fields, offering advantages such as greater speed, affordability, and higher accuracy compared to existing technologies. The purpose of this review is to offer insights into previous research on CRISPR-Cas9, a widely utilized technology across various scientific domains, with the aim of providing guidance for future studies.

Keywords: Cas9, CRISPR-Cas9, CRISPR, gene, genome editing.

The clustered regularly interspaced short palindromic repeats (CRISPR) employ guide ribonucleic acid (RNA) sequences to direct the CRISPR-associated protein 9 (Cas9) enzyme to precise locations on the deoxyribonucleic acid (DNA) that need to be edited. It consists of Cas genes, followed by the leading sequence, and subsequently, spacer and repeat sequences. Although the repeat sequences are the same for a living organism, the spacer sequences between them differ from each other. Additionally, proteins in the immune system are referred to as Cas.[1]

The CRISPR-Cas mechanism, despite emerging relatively recently, is one of the most widely used gene editing methods in our era. It has become increasingly prevalent in recent years, with researchers conducting various studies on this subject. Although it's a relatively new application, its roots can be traced back to the discovery of RNA interference (RNAi). CRISPR-Cas is the general term for the process of recognizing and removing foreign genomes entering prokaryotic and archaeal cells without making changes to their own genomes. The primary goal of the CRISPR process is to ensure that any unwanted gene regions are not present in the prokaryotic and eukaryotic genome structures before protein synthesis begins.[2] Furthermore, Cas, the general term for the proteins involved in this immune system, is essential. Cas genes are typically found in close proximity to CRISPR and encode Cas proteins. These proteins possess endonuclease, exonuclease, helicase structure, and nucleic acid-binding sites. Thanks to these characteristics, they can unwind and cleave DNA sequences.[3]

In the present day, medicine has made significant progress, leading to the development of various treatment methods for many diseases and the reinforcement of the immune system to prevent illnesses. The early 20th century witnessed a notable increase in the average lifespan of individuals due to the development of vaccines and treatment techniques, along with factors such as nutrition, hygiene, and environmental conditions. Research related to the immune system is crucial for preventing and treating diseases. While many diseases have found cures in the contemporary era, several remain untreatable, leaving numerous ailments still awaiting solutions.[4] In this context, preventing

Received: October 05, 2023
Accepted: October 11, 2023
Published online: November 07, 2023

Correspondence: Ömer Demir.
E-mail: 90omerdemir@gmail.com

Cite this article as:
Demir Ö, Erbaş O. Discovery and applications of CRISPR-Cas9 gene editing technology. D J Tx Sci 2023;8(1-2):56-67. doi: 10.5606/dsufnjt.2023.13.

and treating diseases without available cures, and enhancing the effectiveness of produced solutions, should emphasize the importance of CRISPR-Cas9 research. Researchers, research institutions, governments, and universities must provide support for these studies.

GENOME-EDITING TECHNOLOGIES

Globalization, environmental changes, climate change, reduced agricultural lands, increased diseases, and the emergence of biotic and abiotic stress factors are reducing the productivity of production. In the face of rapid population growth, global warming, and ecological balance changes, there is an increasing need for products that exhibit greater tolerance to biotic and abiotic stresses. In today's conditions, genome editing technologies can help achieve high yields in products.[5] Genome editing can prevent various diseases and treat diseases that do not yet have a cure.

For the past 20 years, a persistent goal of researchers has been to explore and discover efficient, cost-effective techniques to make precise changes in the genomes of living cells. Genome editing is one of the key elements that can achieve this. It has the potential to be used in gene therapy to remove defective genes responsible for genetic disorders in humans. On the other hand, in agriculture, altering plant DNA can contribute to increased crop productivity and better control of plant diseases.[6] Genome editing employs site-specific nucleases to create double-strand breaks in the target genome. This allows for the addition, removal, or modification of specific genes in the genome.[7]

Classification of CRISPR-Cas systems

The CRISPR-Cas systems are divided into two classes based on differences in their core Cas proteins and further divided into six types with various subtypes. Class 1 CRISPR-Cas systems operate with multi-Cas protein complexes, including endonucleases represented by Cas3, Cas10, and DinG, constituting types 1, 3, and 4, respectively. Class 2 CRISPR-Cas systems use a single Cas protein and include types 2, 5, and 4, which respectively employ Cas9, Cas12-Cas14, and Cas13 to cleave RNA-guided genetic codes. Type 1, 2, and 5 systems primarily target DNA, type 3 targets both RNA and DNA, while type 4 is exclusively involved in RNA regulation, and the function and mechanisms of type 4 systems remain unclear.[8,9] Among these systems, the type 2 system is the most well-understood, extensively researched, and holds the highest potential for adaptation to eukaryotic organisms, with the endonuclease Cas9 playing a central role in the type 2 CRISPR-Cas system.[10]

CRISPR-Cas9 is one of the gene editing or genome editing techniques, and among other popular genome editing techniques [zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), etc.], CRISPR-Cas9 is the most popular. The ease, speed, and effectiveness of the CRISPR-Cas9 technique in comparison to other genome editing techniques have generated significant interest in the scientific community.[11] CRISPR-Cas9 provides the ability to engineer the DNA found in the cells of living organisms, allowing for the addition, removal, modification, and even silencing of specific regions within a creature's genome.[12] To briefly explain how the CRISPR-Cas9 method accomplishes this: a special small RNA molecule is used to direct the Cas9 nuclease to the selected DNA sequence, and this RNA molecule induces a specific double-stranded DNA break at the chosen site. The CRISPR-Cas9 technique is derived from a natural genome editing method that occurs in the immune system of bacteria. When bacteria are infected by viruses, they capture small fragments of viral DNA and integrate them into their own DNA in a specific pattern to create segments known as CRISPR arrays.[13] These sequences enable bacteria to recognize viruses or closely related strains. When viruses infect the bacteria again, the bacteria use CRISPR arrays to recognize specific segments of the viral DNA, creating RNA segments corresponding to these recognized viral DNA portions. Then, bacteria use an enzyme like Cas9 to cut the viral DNA, rendering the virus inactive.[11] CRISPR-Cas9 is indeed a versatile technique with numerous current and potential applications. Examples of these applications include contributing significantly to understanding how cancer develops, how it can be treated, and what can be done for prevention.

CRISPR: Discovery and Mechanism

In 1987, Ishino et al.[14] from Osaka University in Japan were investigating the genes involved in

the isozyme conversion of alkaline phosphatase in *Escherichia coli* bacteria. During this research, they discovered unusual repetitive DNA clusters within the gene. However, the lack of a sufficient amount of DNA sequence data at the time left the functions of these sequences a mystery.[15] However, by the year 2005, three different studies had provided evidence that these mysterious DNA clusters played a crucial role in adaptive immunity.[16-18] In 1993, a team led by van Embden[19] revealed that various strains of *Mycobacterium tuberculosis* had different spacer sequences located among the DNA repeats. They characterized *Mycobacterium tuberculosis* strains based on these spacer sequences using a technique called Spoligotyping, and subsequently, these sequences were also identified in other archaeal and bacterial genomes. In the 2000s, Mojica et al.[20] demonstrated that spacer sequences were similar to sequences found in bacteriophages, viruses, and plasmids. They found that viruses couldn't infect prokaryotes that had homologous spacer sequences, and they suggested that these sequences played an important role in the adaptive immune system of prokaryotes. Briefly, when a virus infects a prokaryote, intermediate sequences within CRISPR arrays are copied to generate short CRISPR RNA (crRNA), which, depending on the type of CRISPR-Cas system, guides the Cas protein to cleave complementary DNA or RNA viral sequences.

In this context, CRISPR-Cas systems function as a defense mechanism to prevent repeated infections by the same virus. The role of Cas proteins as nucleases that cleave specific regions, and the comparative genomic analysis of CRISPR repeats and Cas genes were discovered by Makarova et al.[21] They hypothesized that the function of CRISPR-Cas systems is similar to RNAi, where protein complexes silence genes by degrading messenger RNA (mRNA). Some Cas proteins cleave DNA, while others cleave RNA. For example, Cas9 cleaves DNA, whereas Cas13 cleaves RNA.[15] In addition to Cas proteins, protospacer adjacent motifs (PAMs) are essential components of CRISPR-Cas systems. As the name suggests, PAMs are short sequences of two to six base pairs located adjacent to the sequences targeted by Cas nucleases in the viral genome. Cas nucleases recognize PAMs, and they cannot cleave DNA in the absence of a PAM. Protospacer adjacent motifs play a crucial role in the cleavage of foreign viral nucleic acids, not in CRISPR sequences themselves.[22] Additionally, the CRISPR-Cas9 genome editing technique is carried out in conjunction with the CRISPR system called type 2. The CRISPR genome editing method can be successfully applied not only with the Cas9 enzyme but also with other enzymes, such as the Cas13 enzyme discovered in 2016.[23,24]

Horvath and Barrangou[25] discovered CRISPR loci in cultures of *Streptococcus thermophilus*, which are important bacteria in yogurt and cheese production, providing evidence for adaptive immunity in bacteria. Additionally, they demonstrated that bacteria containing specific virus sequences in their CRISPR regions were resistant to that particular virus, and they established that CRISPR sequences, in conjunction with Cas genes, are responsible for providing protection against invading viruses.

Jinek et al.[26] designed the type 2 CRISPR system for genome editing using *Streptococcus pyogenes*. In their research, they demonstrated the general applicability of CRISPR-Cas9 for genome editing in bacteria and showed that crRNA and trans-activating CRISPR RNA come together to form a chimeric single guide RNA (sgRNA). Doudna and Charpentier indeed made groundbreaking discoveries in the field of CRISPR-Cas technology. Their research in 2012 demonstrated that the Cas9 endonuclease could be guided to specific DNA targets in the laboratory using two RNA molecules, ultimately enabling precise genome editing. This pioneering work has significantly advanced the use of CRISPR-Cas as a powerful tool for genome editing and has had a profound impact on various scientific disciplines, from molecular biology to medicine. CRISPR technology was indeed introduced to the world in 2012, and shortly after its introduction, many research articles were prepared and published to explore its vast potential.[27,28] Zhang et al.[7] successfully used CRISPR-Cas9 technology to edit the genomes of eukaryotic cells starting in 2013. This breakthrough allowed for precise genome editing in a wide range of organisms, including humans, and opened up new possibilities for both basic research and potential therapeutic applications. Cong et al.[29] were the first to demonstrate successful Cas9-based genome

editing in human cells, which was a significant milestone in the development of the CRISPR-Cas9 technology for human applications. Their work paved the way for numerous advancements in the field of genetics and potential applications in human therapeutics. Science magazine's recognition of CRISPR as one of the most significant scientific developments in 2013 highlights the rapid and substantial impact of this technology on the field of science. Additionally, awarding the 2020 Nobel Prize in Chemistry to Emmanuelle Charpentier and Jennifer Doudna for their contributions to the development of the CRISPR-Cas9 method further underscores the importance of this breakthrough in the scientific community.[1]

DEVELOPMENT AND APPLICATION OF CRISPR-CAS9

The ZFN and TALEN techniques are the most preferred techniques in genome editing.[30] Since 2013, research on the CRISPR-Cas system has contributed to the advancement of this technology, making it the most widely used technique due to its speed and cost-effectiveness compared to others. Subsequently, with the integration of the principles of genetic engineering, the CRISPR-Cas9 technology has further evolved. The CRISPR-Cas9 technique can be used for various purposes, including gene silencing or suppression through knock-out or knockdown, the creation of genome-wide loss-of-function libraries, transgenic studies with knock-in, gene therapy, chromosomal deletions and insertions, transcription control, modification of epigenetic marks, and the generation of reporter cell lines. Additionally, various research can be conducted by pulling specific DNA regions with engineered DNA-binding molecule-mediated chromatin immunoprecipitation.[31]

Applications in the food industry

Global warming, environmental pollution, and chemical and industrial waste have adversely affected arable lands. The rapid increase in the global population has also raised concerns about food security.[32] To alleviate these concerns, it is necessary to utilize molecular breeding methods and modern biotechnological tools. Agriculture is considered the beginning of civilization. From the dawn of human history, food has been of great importance, and various efforts have been made to obtain it. Today, due to the overpopulation of the world, access to food has become increasingly challenging. Approximately 795 million people, or one in every nine individuals, cannot find enough food. Particularly, the detrimental effects of fossil fuels contribute to global warming and negatively impact food security. To combat this rapidly changing situation, it is essential for crops to be more resilient, reliable, and efficient. This can be achieved through the latest breakthrough in genome editing, CRISPR-Cas9.[33]

There are harmful and beneficial microorganisms. Harmful microorganisms can cause foodborne illnesses and spoilage, while beneficial ones help preserve food for extended periods and contribute to various aspects such as maintaining a healthy digestive system in individuals.[33,34] In food engineering, applications of CRISPR systems are utilized. These include the vaccination of starter cultures against viruses, genotyping, control of acquisition and spread of antibiotic resistance genes in bacteria, and modification of probiotic cultures.[35,36] In recent times, there have been many studies related to probiotic microorganisms. Probiotics are known as live microorganisms that provide benefits to the host when administered in adequate amounts.[34,37] The success achieved in vaccinating the *Streptococcus thermophilus* starter culture used in dairy product fermentation against viruses has paved the way for the use of CRISPR-Cas systems in the food industry.[38] Furthermore, it has been reported that the CRISPR-Cas system can be used in studies aimed at controlling foodborne pathogenic microorganisms to enhance food safety and extend shelf life.[39] In a study conducted by Gomaa et al.,[40] a CRISPR-Cas system designed using the type 1-E Cas3 enzyme was used to specifically eliminate *E. coli* strains. This approach was soon tested with the use of type 2 Cas9 on *E. coli* and *Staphylococcus aureus*, evaluating the antibacterial potential of CRISPR-Cas9 systems.[41,42] In the research conducted, it was found that CRISPR-Cas9 loci were present in approximately 62.9% of the analyzed *Lactobacillus* genomes and 77% of *Bifidobacterium* genomes, which are lactic acid-producing microorganisms.[34,43,44] It has been shown that CRISPR loci can be used for genotyping purposes to distinguish different

species in products with mixed microbiota, especially those produced by fermentation.[35] One of the early studies in this field was conducted on *Lactobacillus buchneri*, which causes spoilage in pickled products, especially cucumber pickles, by altering the flavor. After identifying the formation and diversity of CRISPR-Cas systems in *L. buchneri* genomes, the use of a 36-nucleotide type 2-A CRISPR locus for identification has yielded successful results.[45] CRISPR loci have also been used for genotyping purposes in *Enterococcus faecalis*, which is commonly used in fermented meats, as well as in *Lactobacillus gasseri* and *Bifidobacterium*, known for their probiotic roles.[46]

Applications in the medical field

Gene therapy refers to techniques that repair a malfunctioning gene in a patient's genome or modify a portion of the genetic structure to eliminate the effects of a disease-causing mutant gene. In gene therapies, genetic modification methods based on homology-directed repair are often preferred for repairing mutations that cause diseases.[47,48] Today, CRISPR-Cas technology is used in research involving human stem cells or experimental animals to treat various inherited diseases. In the case of a monogenic inherited disease such as sickle cell disease (SCD), CRISPR-Cas9 technology is employed to correct the SCD mutations.[49] Cystic fibrosis (CF) is one of the most common genetic diseases caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. CRISPR-Cas9 technology has been used to repair mutations in the CFTR gene, and very promising results have been achieved.[50] Hemophilia B (HB), a hereditary disease resulting from mutations in the Factor IX (FIX) gene, leads to abnormal blood clotting. Studies conducted on animal models have shown promising results in the treatment of HB by using CRISPR-Cas9 technology to add regulatory genes to correct the mutations in the FIX gene.[51] In addition to the treatment of monogenic diseases, CRISPR-Cas systems have been used to potentially treat viral infections such as human immunodeficiency virus, hepatitis viruses, and oncogenic viruses, as well as non-viral infectious diseases caused by bacteria, fungi, and parasites. Significant potential effects have been observed in many cases.[52] The human immunodeficiency virus (HIV) infection has been found to cause acquired immunodeficiency syndrome, which continues to be one of the most serious public health issues worldwide.[53] Yin et al.[54] reported that the CRISPR-Cas9 method could potentially prevent many steps of HIV-1 infection, and various research laboratories have made significant efforts to advance the use of the CRISPR-Cas9 method for the treatment of HIV infection.[55-57] In recent years, CRISPR-Cas12 and CRISPR-Cas13 methods have been increasingly used to prevent HIV infection.[58,59]

The CRISPR-Cas9 technology has provided potential treatment capabilities for lung cancer, breast cancer, and various other types of cancer.[60-65] Furthermore, the CRISPR-Cas system has been used as a powerful tool for unbiased exploration in medicine, including the identification of new drug targets, and elucidating mechanisms leading to biomarkers and drug resistance.[66-68] In summary, CRISPR-Cas and its derivative systems, such as catalytically dead Cas9, hold tremendous potential applications due to their ability to accurately identify underlying disease causes, genetic mutation variants, immunological regulatory factors, cell signaling mediators, drug targets, as well as drug molecules and therapeutics.[69]

In our era, among the deadly diseases, cardiovascular diseases and cancer take the lead. Cancer comes second after cardiovascular diseases.[3] Cancer is a disease characterized by abnormal cell signaling, resulting from various genetic and epigenetic changes in DNA. These changes include oncogenes that promote cell proliferation and tumor suppressors that regulate cell growth and metabolism. Understanding the complex molecular mechanisms that drive tumor spread is a crucial step in advancing the development of therapeutics. Tumors often arise due to mutations in multiple genes, which complicates the development of comprehensive cancer models. Accordingly, the CRISPR system is used to create rapid *in vitro* and *in vivo* tumor models. These models enable the identification of genetic determinants and comprehensive detailing of the underlying mechanisms in tumor formation, progression, and development.[70,71]

Today, it is relatively easy and feasible to generate *in vitro* cancer models with single or multiple gene deletions in mammalian cell lines using the CRISPR-Cas technique.[72] In many

clinical applications, the CRISPR-based silencing of maternal embryonic leucine zipper kinase (MELK), which is the target of the cancer drug (OTS167), has been successfully achieved. The CRISPR-mediated inactivation of MELK renders OTS167 effective and does not affect the viability of cancer-derived cell lines.[73] In addition, CRISPR has been used to functionally introduce or disable *in vitro* drug resistance, and it allows for the rapid examination of candidate genes or specific mutations associated with drug resistance.[74] In this context, CRISPR applications have been used to detect mutations in important genes involved in therapeutic resistance that can be applied to planned drug development strategies.[75]

Application of CRISPR-Cas9 in livestock

With the increasing global population, the demand for animal-derived food is steadily rising. Therefore, there is a continuous need to carry out research and initiatives to enhance the productivity and performance of farm animals by maximizing the genetic potential within the limits of available resources and utilizing modern technologies.[32] Genome editing techniques used in animal husbandry are mainly employed to enhance disease resistance, improve the production quality and efficiency of products, and obtain animal products for biomedical purposes.[76] As an example of the application of genome editing, when vaccines prepared for porcine reproductive and respiratory syndrome virus (PRRSV), a virus affecting pig reproduction and respiratory systems, failed to effectively treat the disease, modifications were made to the cluster of differentiation 163 receptors. These changes facilitated PRRSV's entry into host cells, thereby providing immunity against the virus.[77] Another study focuses on obtaining human interferon proteins from transgenic chickens to facilitate their commercial production.[78]

In this regard, the CRISPR-Cas9 technology was used to insert the human interferon beta (hIFN-β) gene into the chicken ovalbumin locus, enabling the production of hIFN-β proteins in egg whites. Ni et al.[79] suppressed the myostatin gene to generate double-muscling. They conducted this study with goats and, with the help of the CRISPR-Cas9 system, achieved a significant increase in muscle mass, resulting in goats with enhanced muscle development. However, these goats may exhibit some undesirable conditions, such as stillbirth, early death, spinal deformities, and abnormal metabolism of fats, sugars, and proteins. In addition to the mentioned conditions, CRISPR-Cas9 technology has been observed to be potentially applicable in other breeding practices aimed at increasing animal productivity,[80,81] enhancing resistance to infectious or non-infectious diseases,[82-84] and improving the welfare of farm animals.[85,86] These applications can contribute to enhancing the adaptability and resilience of animals on the farm.[87]

Applications in plants

The rapid increase in the world's population has led to food scarcity. Innovative technologies are needed to obtain sufficient food. CRISPR-Cas9 is an effective system that allows the creation of target-specific mutations in agricultural products, offering significant potential for the development of desired new traits through breeding programs without the use of foreign genetic elements.[88]

In the past, classical breeding methods were used to increase productivity in plants. These methods involved crossbreeding and mutation breeding techniques to obtain new traits. However, these approaches have become inadequate today due to concerns about ecological imbalances. Factors such as the reliance on agricultural resources for the production of inputs in the food industry have further emphasized these limitations.[89] Classical breeding, through methods like crossbreeding and mutation breeding, used to take a significant amount of time to eliminate undesirable traits from genotypes. However, the new generation of breeding techniques offers more efficient and targeted methods.[90,91]

Especially in recent years, the popularity of the CRISPR-Cas9 technique has made it a powerful system for the successful creation of target-specific modifications.[89]

To modify plant genomes, it is sufficient to have Cas9 and sgRNA expression in the target cell. Plant-specific RNA polymerase III promoters are used to express Cas9 and guide RNA (gRNA) in plants. These promoters are named tU6 (arabidopsis), TaU6 (wheat), OsU6, or OsU3 (rice). There are several commercial vectors available

for expressing these Cas9 or Cas9 variants and gRNAs in plants. Addgene, one of these vectors, is a global, non-profit plasmid repository and currently provides over 30 empty gRNA backbones for binary vectors. These empty gRNA backbones include RNA polymerase III promoters found in plants and gRNA scaffolds to which gRNAs can be bound.[92]

Tomatoes are among the most common products where CRISPR-Cas technology is used. This plant is widely utilized in research due to its diploid inheritance, high reproductive efficiency, short growth period, and ease of genetic transformation. Studies on leaf shapes in tomatoes have demonstrated that genetic mutations created with CRISPR-Cas technology are hereditary.[93] A study conducted by Brooks et al.[94] targeted the SlAGO7 (Argonaute7) gene responsible for the typical compound and flattened appearance of tomato leaves. They reported that the deletion of this gene using CRISPR-Cas9 technology resulted in the emergence of needle- or wire-like leaves. Numerous genome editing studies have been conducted in cocoa production with the aim of obtaining varieties that are more resistant to pests, higher yielding, drought-resistant, and with improved aroma and seed quality. In one such study, the TcNPR3 gene, which suppresses the defense response, was successfully deleted using CRISPR-Cas9 technology.[95] However, the suitability of these mutant cocoa varieties obtained through genome editing for the global cocoa market, as well as their acceptability by producers and consumers, is still a subject of debate.[88]

CRISPR-Cas9 is a gene-editing technology that has caused a major breakthrough in biomedical research. This method allows for the rapid, cost-effective, and relatively simple editing of genes in cells and organisms by correcting errors in the genome. It is used in many laboratory applications, alongside fast cellular production. The rapid increase in the global population, depletion of natural resources over time, and environmental pollution, among other factors, are making it increasingly challenging for future populations to access sufficient and healthy food. To find solutions to these problems, food systems must align with the principles of sustainable nutrition. These systems should protect biodiversity and ecosystems, and be culturally accepted, accessible, safe, healthy, affordable, and economically fair. They should also provide adequate nutrients for a healthy life and make optimal use of natural resources and human labor during production. Furthermore, various alternative food sources exist to meet future food needs, including edible algae, insects, synthetic meats, and foods produced using gene-editing technologies involving CRISPR and associated Cas9 nucleases. Particularly, the CRISPR-Cas9 system has a wide range of applications, such as increasing crop yield and quality in agriculture, enhancing abiotic and biotic stress resistance, improving disease resistance and meat quality and quantity in animal husbandry, and ensuring food safety and extending the shelf life in the food industry. Gene therapy holds promise for treating various serious diseases, including cancer and genetic disorders. It is essential to find the causes of diseases and eliminate them, rather than merely treating disease symptoms.[96]

CRISPR-Cas is a versatile genome editing technique that can be adapted to different genome varieties. It is widely used in the treatment of genetic diseases, covering a range of conditions from cancer to neurological disorders. Today, animal models are employed in the research of mutated genetic diseases and to uncover unknown aspects of these conditions.[87]

The use of next-generation genome editing techniques, often referred to as the third revolution, in plant breeding is fast, cost-effective, and safe. This allows for the enhancement of yield and quality in products and the development of resistance to diseases, pests, and abiotic stresses.[97]

Successful research has been conducted in rice and wheat, both of which are important food sources for humans. In the case of rice, approximately 92% of the research is based on CRISPR-Cas9 technology.[98] Mutation studies have been targeted at some genes in wheat, such as PDS, MLO, and NAC2. It has been reported that, through the use of CRISPR-Cas9 technology in wheat, plants resistant to yellow rust disease were obtained as a result of mutations created in the MLO gene.[99-101]

Janga et al.[102] have stated that targeted gene editing in cotton is possible using the CRISPR-Cas9 system. Using the CRISPR-Cas9

system, point mutations have been created in plants, and plants carrying the mutations in the target genes were selected and developed. In a study by Chandrasekaran et al.,[103] it was reported that the developed plants showed resistance to cucumber mosaic virus and papaya ringspot virus infections. Additionally, efficient animal feeds can be obtained using CRISPR-Cas9. For example, from 2017 to the present, a total of 36 varieties of soybeans and corn have been approved and allowed for use as animal feed.[104]

In their studies on goats, Wang et al.[105] obtained goats with one or two genes altered through the injection of Cas9 mRNA and sgRNAs targeting the functional genes MSTN and FGF5 into single-cell stage embryos. Young et al.[106] applied the CRISPR-Cas9 system targeting the genes Dnaic1, Wdr63, and Ccdc63, which affect the structure of the tails of sperm in male mice. These genes are highly expressed in the testes. Their analysis revealed that the silencing of Dnaic1 and Wdr63 genes did not have an impact on male reproductive ability, but the silencing of the Ccdc63 gene resulted in male mice becoming sterile due to the shortening of their sperm tails. As a result of this study, they reported that the CRISPR-Cas9 system can easily be used for modifications in complex systems of animals.

In conclusion, despite the significant public support for CRISPR-Cas technology, ethical and safety concerns persist, and it remains one of the most debated application areas. Moreover, ethical and moral discussions regarding genome editing should not be solely under the dominion of science but should instead be conducted by an organization comprising scientists and different institutions. Regulations made in accordance with guidelines to be created with the contribution of national and international organizations from various sciences will facilitate the emergence of CRISPR technology applications in various fields. It is also believed that these regulations will contribute significantly to mitigating and eliminating potential risks.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: All authors contributed equally to the article.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biologics 2021;15:353-61. doi: 10.2147/BTT.S326422.
2. Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J 2020;18:2401-15. doi: 10.1016/j.csbj.2020.08.031.
3. Yaman G, Çini N, Altuntaş İ, Erbaş O. What does CRISPR technology provide to cancer treatments? JEB Med Sci 2021;2:41-9.
4. Rappuoli R. Twenty-first century vaccines. Philos Trans R Soc Lond B Biol Sci 2011;366:2756-8. doi: 10.1098/rstb.2011.0075.
5. Sánchez-Bermúdez M, Del Pozo JC, Pernas M. Effects of combined abiotic stresses related to climate change on root growth in crops. Front Plant Sci 2022;13:918537. doi: 10.3389/fpls.2022.918537.
6. Kozovska Z, Rajcaniova S, Munteanu P, Dzacovska S, Demkova L. CRISPR: History and perspectives to the future. Biomed Pharmacother 2021;141:111917. doi: 10.1016/j.biopha.2021.111917.
7. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell 2014;157:1262-78. doi: 10.1016/j.cell.2014.05.010.
8. Agarwal N, Gupta R. History, evolution and classification of CRISPR-Cas associated systems. Prog Mol Biol Transl Sci 2021;179:11-76. doi: 10.1016/bs.pmbts.2020.12.012.
9. Liu Z, Dong H, Cui Y, Cong L, Zhang D. Application of different types of CRISPR/Cas-based systems in bacteria. Microb Cell Fact 2020;19:172. doi: 10.1186/s12934-020-01431-z.
10. Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science 2007;315:1709-12. doi: 10.1126/science.1138140.
11. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. J Clin Invest 2014;124:4154-61. doi: 10.1172/JCI72992.
12. Zhang D, Zhang Z, Unver T, Zhang B. CRISPR/Cas: A powerful tool for gene function study and crop improvement. J Adv Res 2020;29:207-21. doi: 10.1016/j.jare.2020.10.003.

13. Wu X, Kriz AJ, Sharp PA. Target specificity of the CRISPR-Cas9 system. Quant Biol 2014;2:59-70. doi: 10.1007/s40484-014-0030-x.
14. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol 1987;169:5429-33. doi: 10.1128/jb.169.12.5429-5433.1987.
15. Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. J Bacteriol 2018;200:e00580-17. doi: 10.1128/JB.00580-17.
16. Bolotin A, Quinquis B, Sorokin A, Ehrlich SD. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology (Reading) 2005;151:2551-61. doi: 10.1099/mic.0.28048-0.
17. Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J Mol Evol 2005;60:174-82. doi: 10.1007/s00239-004-0046-3.
18. Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology (Reading) 2005;151:653-63. doi: 10.1099/mic.0.27437-0.
19. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: Recommendations for a standardized methodology. J Clin Microbiol 1993;31:406-9. doi: 10.1128/jcm.31.2.406-409.1993.
20. Mojica FJ, Díez-Villaseñor C, Soria E, Juez G. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. Mol Microbiol 2000;36:244-6. doi: 10.1046/j.1365-2958.2000.01838.x.
21. Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. A putative RNA-interference-based immune system in prokaryotes: Computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biol Direct 2006;1:7. doi: 10.1186/1745-6150-1-7.
22. Shah SA, Erdmann S, Mojica FJ, Garrett RA. Protospacer recognition motifs: Mixed identities and functional diversity. RNA Biol 2013;10:891-9. doi: 10.4161/rna.23764.
23. Anderson KM, Poosala P, Lindley SR, Anderson DM. Targeted cleavage and polyadenylation of RNA by CRISPR-Cas13. bioRxiv 531111. doi: 10.1101/531111.
24. Razzaq A, Saleem F, Kanwal M, Mustafa G, Yousaf S, Imran Arshad HM, et al. Modern trends in plant genome editing: An inclusive review of the CRISPR/Cas9 toolbox. Int J Mol Sci 2019;20:4045. doi: 10.3390/ijms20164045.
25. Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. Science 2010;327:167-70. doi: 10.1126/science.1179555.
26. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 2012;337:816-21. doi: 10.1126/science.1225829.
27. Lander ES. The heroes of CRISPR. Cell 2016;164:18-28. doi: 10.1016/j.cell.2015.12.041.
28. Doudna J. Genome-editing revolution: My whirlwind year with CRISPR. Nature 2015;528:469-71. doi: 10.1038/528469a.
29. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science 2013;339:819-23. doi: 10.1126/science.1231143.
30. Deng L, Kenchappa CS, Peng X, She Q, Garrett RA. Modulation of CRISPR locus transcription by the repeat-binding protein Cbp1 in Sulfolobus. Nucleic Acids Res 2012;40:2470-80. doi: 10.1093/nar/gkr1111.
31. Sivanandhan G, Selvaraj N, Lim YP, Ganapathi A. Targeted genome editing using site-specific nucleases, ZFNs, TALENs, and the CRISPR/Cas9 system Takashi Yamamoto (ed.). Ann Bot 2016;118:vii–viii. doi: 10.1093/aob/mcw089.
32. Bölükbaş, A, Gücükoğlu A. CRISPR/Cas9 teknolojisi ve gıda alanında kullanımı. Front Life Sci RT 2022;3:36-42. doi: 10.51753/flsrt.997899.
33. Oba Ş, Yıldırım T. Food biotechnology and food safety. IJSL 2021;3:52-64. doi: 10.38058/ijsl.855920.
34. Papadimitriou K, Pot B, Tsakalidou E. How microbes adapt to a diversity of food niches. Curr Opin Food Sci 2015;2:29-35. doi: 10.1016/j.cofs.2015.01.001.
35. Stout E, Klaenhammer T, Barrangou R. CRISPR-Cas technologies and applications in food bacteria. Annu Rev Food Sci Technol 2017;8:413-37. doi: 10.1146/annurev-food-072816-024723.
36. Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. Nat Biotechnol 2016;34:933-41. doi: 10.1038/nbt.3659.
37. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506-14. doi: 10.1038/nrgastro.2014.66.
38. Selle K, Barrangou R. CRISPR-based technologies and the future of food science. J Food Sci 2015;80:R2367-72. doi: 10.1111/1750-3841.13094.
39. Barrangou R, Notebaart RA. CRISPR-directed microbiome manipulation across the food supply

chain. Trends Microbiol 2019;27:489-96. doi: 10.1016/j.tim.2019.03.006.
40. Gomaa AA, Klumpe HE, Luo ML, Selle K, Barrangou R, Beisel CL. Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. mBio 2014;5:e00928-13. doi: 10.1128/mBio.00928-13.
41. Citorik RJ, Mimee M, Lu TK. Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. Nat Biotechnol 2014;32:1141-5. doi: 10.1038/nbt.3011.
42. Bikard D, Euler CW, Jiang W, Nussenzweig PM, Goldberg GW, Duportet X, et al. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nat Biotechnol 2014;32:1146-50. doi: 10.1038/nbt.3043.
43. Briner AE, Lugli GA, Milani C, Duranti S, Turroni F, Gueimonde M, et al. Occurrence and diversity of CRISPR-CAS systems in the genus Bifidobacterium. PLoS One 2015;10:e0133661. doi: 10.1371/journal.pone.0133661.
44. Pala HG, Erbas O, Pala EE, Artunc Ulkumen B, Akman L, Akman T, et al. The effects of sunitinib osn endometriosis. J Obstet Gynaecol 2015;35:183-7. doi: 10.3109/01443615.2014.941345.
45. Briner AE, Barrangou R. Lactobacillus buchneri genotyping on the basis of clustered regularly interspaced short palindromic repeat (CRISPR) locus diversity. Appl Environ Microbiol 2014;80:994-1001. doi: 10.1128/AEM.03015-13.
46. Hullahalli K, Rodrigues M, Schmidt BD, Li X, Bhardwaj P, Palmer KL. Comparative analysis of the orphan CRISPR2 locus in 242 Enterococcus faecalis strains. PLoS One 2015;10:e0138890. doi: 10.1371/journal.pone.0138890.
47. Li Y, Li S, Wang J, Liu G. CRISPR/Cas systems towards next-generation biosensing. Trends Biotechnol 2019;37:730-43. doi: 10.1016/j.tibtech.2018.12.005.
48. Zhang B. CRISPR/Cas gene therapy. J Cell Physiol 2021;236:2459-81. doi: 10.1002/jcp.30064.
49. Hoban MD, Lumaquin D, Kuo CY, Romero Z, Long J, Ho M, et al. CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells. Mol Ther 2016;24:1561-9. doi: 10.1038/mt.2016.148.
50. Marangi M, Pistritto G. Innovative therapeutic strategies for cystic fibrosis: Moving forward to CRISPR technique. Front Pharmacol 2018;9:396. doi: 10.3389/fphar.2018.00396.
51. Stephens CJ, Lauron EJ, Kashentseva E, Lu ZH, Yokoyama WM, Curiel DT. Long-term correction of hemophilia B using adenoviral delivery of CRISPR/Cas9. J Control Release 2019;298:128-41. doi: 10.1016/j.jconrel.2019.02.009.
52. Mohammadzadeh I, Qujeq D, Yousefi T, Ferns GA, Maniati M, Vaghari-Tabari M. CRISPR/Cas9 gene editing: A new therapeutic approach in the treatment of infection and autoimmunity. IUBMB Life 2020;72:1603-21. doi: 10.1002/iub.2296.
53. Abbar B, Veyri M, Solas C, Poizot-Martin I, Spano JP. HIV and cancer: Update 2020. Bull Cancer 2020;107:21-9. French. doi: 10.1016/j.bulcan.2020.01.001.
54. Yin L, Hu S, Mei S, Sun H, Xu F, Li J, et al. CRISPR/Cas9 inhibits multiple steps of HIV-1 infection. Hum Gene Ther 2018;29:1264-76. doi: 10.1089/hum.2018.018.
55. Sullivan NT, Allen AG, Atkins AJ, Chung CH, Dampier W, Nonnemacher MR, et al. Designing safer CRISPR/Cas9 therapeutics for HIV: Defining factors that regulate and technologies used to detect off-target editing. Front Microbiol 2020;11:1872. doi: 10.3389/fmicb.2020.01872.
56. Das AT, Binda CS, Berkhout B. Elimination of infectious HIV DNA by CRISPR-Cas9. Curr Opin Virol 2019;38:81-8. doi: 10.1016/j.coviro.2019.07.001.
57. Xiao Q, Guo D, Chen S. Application of CRISPR/Cas9-based gene editing in HIV-1/AIDS therapy. Front Cell Infect Microbiol 2019;9:69. doi: 10.3389/fcimb.2019.00069.
58. Yin L, Zhao F, Sun H, Wang Z, Huang Y, Zhu W, et al. CRISPR-Cas13a inhibits HIV-1 infection. Mol Ther Nucleic Acids 2020;21:147-55. doi: 10.1016/j.omtn.2020.05.030.
59. Gao Z, Fan M, Das AT, Herrera-Carrillo E, Berkhout B. Extinction of all infectious HIV in cell culture by the CRISPR-Cas12a system with only a single crRNA. Nucleic Acids Res 2020;48:5527-39. doi: 10.1093/nar/gkaa226.
60. Nair J, Nair A, Veerappan S, Sen D. Translatable gene therapy for lung cancer using Crispr CAS9-an exploratory review. Cancer Gene Ther 2020;27:116-24. doi: 10.1038/s41417-019-0116-8.
61. Chen Y, Zhang Y. Application of the CRISPR/Cas9 system to drug resistance in breast cancer. Adv Sci (Weinh) 2018;5:1700964. doi: 10.1002/advs.201700964.
62. Yang H, Jaeger M, Walker A, Wei D, Leiker K, Weitao T. Break breast cancer addiction by CRISPR/Cas9 genome editing. J Cancer 2018;9:219-31. doi: 10.7150/jca.22554.
63. Liu Z, Liao Z, Chen Y, Zhou L, Huangting W, Xiao H. Research on CRISPR/system in major cancers and its potential in cancer treatments. Clin Transl Oncol 2021;23:425-33. doi: 10.1007/s12094-020-02450-3.
64. Hazafa A, Mumtaz M, Farooq MF, Bilal S, Chaudhry SN, Firdous M, et al. CRISPR/Cas9: A powerful genome editing technique for the treatment of cancer cells with present challenges and future directions. Life Sci 2020;263:118525. doi: 10.1016/j.lfs.2020.118525.
65. Zhang M, Eshraghian EA, Jammal OA, Zhang Z, Zhu X. CRISPR technology: The engine that drives cancer therapy. Biomed Pharmacother 2021;133:111007. doi: 10.1016/j.biopha.2020.111007.

66. Siva N, Gupta S, Gupta A, Shukla JN, Malik B, Shukla N. Genome-editing approaches and applications: A brief review on CRISPR technology and its role in cancer. 3 Biotech 2021;11:146. doi: 10.1007/s13205-021-02680-4.
67. Xing H, Meng LH. CRISPR-cas9: A powerful tool towards precision medicine in cancer treatment. Acta Pharmacol Sin 2020;41:583-7. doi: 10.1038/s41401-019-0322-9.
68. Noorani I, Bradley A, de la Rosa J. CRISPR and transposon in vivo screens for cancer drivers and therapeutic targets. Genome Biol 2020;21:204. doi: 10.1186/s13059-020-02118-9.
69. Liu W, Li L, Jiang J, Wu M, Lin P. Applications and challenges of CRISPR-Cas gene-editing to disease treatment in clinics. Precis Clin Med 2021;4:179-91. doi: 10.1093/pcmedi/pbab014.
70. Vogelstein B, Kinzler KW. The multistep nature of cancer. Trends Genet 1993;9:138-41. doi: 10.1016/0168-9525(93)90209-z.
71. Liu Y, Hu X, Han C, Wang L, Zhang X, He X, et al. Targeting tumor suppressor genes for cancer therapy. Bioessays 2015;37:1277-86. doi: 10.1002/bies.201500093.
72. Fellmann C, Gowen BG, Lin PC, Doudna JA, Corn JE. Cornerstones of CRISPR-Cas in drug discovery and therapy. Nat Rev Drug Discov 2017;16:89-100. doi: 10.1038/nrd.2016.238.
73. Lin A, Giuliano CJ, Sayles NM, Sheltzer JM. CRISPR/Cas9 mutagenesis invalidates a putative cancer dependency targeted in on-going clinical trials. Elife 2017;6:e24179. doi: 10.7554/eLife.24179.
74. Smurnyy Y, Cai M, Wu H, McWhinnie E, Tallarico JA, Yang Y, et al. DNA sequencing and CRISPR-Cas9 gene editing for target validation in mammalian cells. Nat Chem Biol 2014;10:623-5. doi: 10.1038/nchembio.1550.
75. Rasul MF, Hussen BM, Salihi A, Ismael BS, Jalal PJ, Zanichelli A, et al. Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. Mol Cancer 2022;21:64. doi: 10.1186/s12943-021-01487-4.
76. Proudfoot C, Lillico S, Tait-Burkard C. Genome editing for disease resistance in pigs and chickens. Anim Front 2019;9:6-12. doi: 10.1093/af/vfz013.
77. Whitworth KM, Rowland RR, Ewen CL, Trible BR, Kerrigan MA, Cino-Ozuna AG, et al. Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. Nat Biotechnol 2016;34:20-2. doi: 10.1038/nbt.3434.
78. Oishi I, Yoshii K, Miyahara D, Tagami T. Efficient production of human interferon beta in the white of eggs from ovalbumin gene-targeted hens. Sci Rep 2018;8:10203. doi: 10.1038/s41598-018-28438-2.
79. Ni W, Qiao J, Hu S, Zhao X, Regouski M, Yang M, et al. Efficient gene knockout in goats using CRISPR/Cas9 system. PLoS One 2014;9:e106718. doi: 10.1371/journal.pone.0106718.
80. Crispo M, Mulet AP, Tesson L, Barrera N, Cuadro F, dos Santos-Neto PC, et al. Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes. PLoS One 2015;10:e0136690. doi: 10.1371/journal.pone.0136690.
81. Van Eenennaam AL. Application of genome editing in farm animals: Cattle. Transgenic Res 2019;28(Suppl 2):93-100. doi: 10.1007/s11248-019-00141-6.
82. Liu X, Wang Y, Tian Y, Yu Y, Gao M, Hu G, et al. Generation of mastitis resistance in cows by targeting human lysozyme gene to β-casein locus using zinc-finger nucleases. Proc Biol Sci 2014;281:20133368. doi: 10.1098/rspb.2013.3368.
83. Gao Y, Wu H, Wang Y, Liu X, Chen L, Li Q, et al. Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. Genome Biol 2017;18:13. doi: 10.1186/s13059-016-1144-4.
84. Whitworth KM, Rowland RRR, Petrovan V, Sheahan M, Cino-Ozuna AG, Fang Y, et al. Resistance to coronavirus infection in amino peptidase N-deficient pigs. Transgenic Res 2019;28:21-32. doi: 10.1007/s11248-018-0100-3.
85. Yunes MC, Teixeira DL, von Keyserlingk MAG, Hötzel MJ. Is gene editing an acceptable alternative to castration in pigs? PLoS One 2019;14:e0218176. doi: 10.1371/journal.pone.0218176.
86. Kurtz S, Petersen B. Pre-determination of sex in pigs by application of CRISPR/Cas system for genome editing. Theriogenology 2019;137:67-74. doi: 10.1016/j.theriogenology.2019.05.039.
87. Kılıç Tosun Ö, Kesmen Z. CRISPR-Cas uygulamaları, potansiyel riskler ve yasal düzenlemeler. Helal ve Etik Araştırmalar Dergisi 2022;4:11-42. doi: 10.51973/head.1209563.
88. Açar E, Kaçar YA. CRISPR/Cas9 technology and applications in plants. TURJAF 2021;9:1-6. doi: 10.24925/turjaf.v9i1.1-6.3313.
89. Chen K, Wang Y, Zhang R, Zhang H, Gao C. CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu Rev Plant Biol 2019;70:667-97. doi: 10.1146/annurev-arplant-050718-100049.
90. Demirci Y, Zhang B, Unver T. CRISPR/Cas9: An RNA-guided highly precise synthetic tool for plant genome editing. J Cell Physiol 2018;233:1844-59. doi: 10.1002/jcp.25970.
91. Yin Y, Hao H, Xu X, Shen L, Wu W, Zhang J, et al. Generation of an MC3R knock-out pig by CRSPR/Cas9 combined with somatic cell nuclear transfer (SCNT) technology. Lipids Health Dis 2019;18:122. doi: 10.1186/s12944-019-1073-9.
92. Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S, Venkataraman G. CRISPR for crop improvement: An update review. Front Plant Sci 2018;9:985. doi: 10.3389/fpls.2018.00985.

93. Wang T, Zhang H, Zhu H. CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. Hortic Res 2019;6:77. doi: 10.1038/s41438-019-0159-x.
94. Brooks C, Nekrasov V, Lippman ZB, Van Eck J. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol 2014;166:1292-7. doi: 10.1104/pp.114.247577.
95. Fister AS, Landherr L, Maximova SN, Guiltinan MJ. Transient expression of CRISPR/Cas9 machinery targeting TcNPR3 enhances defense response in theobroma cacao. Front Plant Sci 2018;9:268. doi: 10.3389/fpls.2018.00268.
96. Samsunlu ET. Gen terapisinde CRISPR-Cas9. Celal Bayar Üniversitesi Sağlık Bilimleri Enstitüsü Dergisi 2021;8:574-80.
97. Khalil AM. The genome editing revolution: Review. J Genet Eng Biotechnol 2020;18:68. doi: 10.1186/s43141-020-00078-y.
98. Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V. Plant genome editing made easy: Targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods 2013;9:39. doi: 10.1186/1746-4811-9-39.
99. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, et al. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 2014;32:947-51. doi: 10.1038/nbt.2969.
100. Ergenoglu M, Erbaş O, Akdemir A, Yeniel AÖ, Yildirim N, Oltulu F, et al. Attenuation of ischemia/reperfusion-induced ovarian damage in rats: Does edaravone offer protection? Eur Surg Res 2013;51:21-32. doi: 10.1159/000353403.
101. Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, et al. Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat Commun 2016;7:12617. doi: 10.1038/ncomms12617.
102. Janga MR, Campbell LM, Rathore KS. CRISPR/Cas9-mediated targeted mutagenesis in upland cotton (Gossypium hirsutum L.). Plant Mol Biol 2017;94:349-60. doi: 10.1007/s11103-017-0599-3.
103. Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M, et al. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. Mol Plant Pathol 2016;17:1140-53. doi: 10.1111/mpp.12375.
104. Avsar B, Sadeghi S, Turkec A, Lucas SJ. Identification and quantitation of genetically modified (GM) ingredients in maize, rice, soybean and wheat-containing retail foods and feeds in Turkey. J Food Sci Technol 2020;57:787-93. doi: 10.1007/s13197-019-04080-2.
105. Wang X, Yu H, Lei A, Zhou J, Zeng W, Zhu H, et al. Generation of gene-modified goats targeting MSTN and FGF5 via zygote injection of CRISPR/Cas9 system. Sci Rep 2015;5:13878. doi: 10.1038/srep13878.
106. Young SA, Miyata H, Satouh Y, Kato H, Nozawa K, Isotani A, et al. CRISPR/Cas9-mediated rapid generation of multiple mouse lines identified Ccdc63 as essential for spermiogenesis. Int J Mol Sci 2015;16:24732-50. doi: 10.3390/ijms161024732.