Proceedings of ICEGEE 2026 Symposium: AI-Based Medicine and Biological Data Analysis
DOI: 10.54254/2753-8818/2026.AU32101

From Mutations to Medicine: Precision Editing with CRISPR/Cas9

Mengxi Xuan

Beijing Jingshan School Caofeidian Branch, Tangshan, China
xuanmengxi2008@outlook.com

Abstract. Genetic mutation is a genetic mutation that can be inherited at the DNA level. It is not only the genetic basis of species evolution, but also the core trigger of a variety of diseases (such as genetic diseases, cancer, etc.). In recent years, the birth and development of CRISPR/Cas9 gene editing technology has provided a revolutionary tool for accurately and efficiently correcting pathogenic gene mutations. This paper first systematically explains the types of genetic mutations. Secondly, the molecular structure, working principle and application progress of CRISPR/Cas9 system in diseases are analyzed in detail. Finally, this article deeply discusses the scientific challenges such as the off-target effect, delivery system, editing efficiency, immunogenicity, etc. faced by CRISPR/Cas9 technology, as well as the profound ethical and social controversy caused by it. In the future, through AI-assisted optimisation, multi-group integration and other technological upgrades, combined with the improvement of global ethical governance guidelines, CRISPR/Cas9 technology will play a greater role in disease treatment and other fields, providing more efficient solutions to deal with problems related to genetic mutations.

Keywords: Genetic mutation, CRISPR/Cas9, Diseases

1. Introduction

The genetic information of life is stored in DNA molecules, and genetic mutation, as a genetic mutation at the genome level, runs through the whole process of growth and development of biological individuals. It may be spontaneous or induced by external factors. From the molecular mechanism, genetic mutations cover a variety of forms such as base replacement, code transfer, deletion, insertion, etc., and their impact on living things is both two-sided: on the one hand, beneficial mutations promote species to adapt to the environment and achieve evolution, while neutral mutations enrich genetic diversity; on the other hand, harmful mutations will destroy normal physiological functions. It induces genetic diseases such as sickle cell anaemia and cystic fibrosis, as well as cancers such as lung cancer and breast cancer, which seriously threaten human health and the growth of animals and plants.

With the development of molecular biology, human understanding of genetic mutations continues to deepen, and there is an urgent need for accurate and efficient technical means to deal with various problems caused by them [1]. The emergence of CRISPR/Cas9 gene editing technology has broken the limitations of traditional intervention methods, providing the possibility for targeted repair of

© 2026 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/2026.AU32101

pathogenic mutations and enhancing beneficial mutation effects [2]. This article will focus on the classification of gene mutation and its impact on diseases, focusing on the application scenarios, existing limitations and future development of CRISPR/Cas9 technology, and provide reference for relevant research and practice.

2. Classification of genetic mutations

2.1. According to nucleotide sequences

Classification gene mutations according to nucleotide sequences can be divided into two categories: small-scale mutations and large-scale mutations according to the difference in the scale of the nucleotide sequence [3]. Small-scale mutations cover types such as base substitution mutations, transcoding mutations, missing mutations and insertion mutations. Among them, base substitution mutations refer to the mutation form in which a single base pair in DNA molecules is replaced by another different base pair. Because it only involves the change of a single base site, it is also called point mutation. This mutation is further subdivided. There are two subtypes for conversion and inversion - conversion refers to the replacement between purine and purine or between pyridine and pyridine, while the inversion is the cross-replacement between pine and pyrimidine or pyridine and purine; code transfer mutation is due to the insertion or omission of one or more base pairs of non-3 multiples at a certain point in the DNA fragment, resulting in the position All coding sequences after the point occur a mutation type in which the reading framework is misaligned; deletion mutation is a mutation caused by the loss of a continuous DNA fragment in the gene DNA sequence, and insertion mutation is a mutation caused by the insertion of a foreign DNA fragment into the gene. Large-scale mutations usually cause changes in chromosome structure, manifested as amplification, deletion, gene fusion and heterozygous loss of local areas of chromosomes. Local areas of chromosomes contain a different number of coding genes, which will increase or decrease the number of copies with the amplification or deletion of regional fragments, and dyeing Structural rearrangement events such as chromosome transposition and inversion sometimes lead to abnormal splicing of different DNA fragments, thus forming fusion genes. Heterogenerous loss refers to the phenomenon of missing one of the two alleles of a gene in the somatic cell.

2.2. According to amino acid sequence

According to the influence of gene mutations on coding polypeptide chain amino acid sequences, they can be divided into homonymous mutations, malsonymous mutations and non-sense mutations [4]. Synonymic mutation means that after gene mutation, although the corresponding codon changes, the type of amino acid encoded has not changed; mismeaning mutation will cause changes in the amino acid sequence encoded by the gene after mutation, resulting in abnormalities in the spatial structure and biological function of the synthesised protein or enzyme; semantic mutation It refers to the mutation of a codon that originally encodes an amino acid into a termination codon, which terminates the synthesis of the polypeptide chain in advance, and finally produces a truncated polypeptide fragments without biological activity. Genetic mutations can occur at any time of individual growth and development. They can not only occur spontaneously in the process of DNA replication, but also be induced by toxic chemicals, X-rays and other external factors. Among them, spontaneous mutations usually occur in the interphase of cell division where DNA replication is active, including the mitosis interphase and reduction. The interphase of several divisions.

DOI: 10.54254/2753-8818/2026.AU32101

3. The relationship between genetic mutations and diseases

The core pathogenesis of sickle cell anaemia originates from the point mutation (GAG→GTG) of the haemoglobin β chain gene. This base replacement causes glutamic acid in the peptide chain to be replaced by balamine, thus changing the spatial conformation and function of haemoglobin. Some rare cases are caused by insertion mutations, which change the base Abnormal protein synthesis is caused by the reading framework. There is a deletion of 3 base pairs of CFTR genes in patients with cystic fibrosis, resulting in the deletion of phenylalanine residues in proteins, resulting in CFTR protein folding errors or abnormal functions, and eventually leading to chloride ion transport disorders and abnormal mucus secretion. KRAS is the most frequently frequency carcinogenic mutation gene in human tumours, especially in pancreatic cancer (mutation frequency >80%), colorectal cancer and lung adenocarcinoma. Its mutation frequency and mode have significant cancer specificity. For example, G12C mutation accounts for nearly 40% of lung adenocarcinoma and less than 5% in pancreatic cancer. This difference is closely related to the cell origin and microenvironmental selection pressure of different cancer species.

KRAS mutations are often accompanied by allele imbalance (such as wild-type allele deletion) and cancer-specific co-existing mutations (such as TP53 and STK11 mutations in lung adenocarcinoma, and APC and PIK3CA mutations in colorectal cancer). These mutation synergies shape unique tumour biological characteristics and genes. The expression spectrum affects the proliferation, invasion and metabolic reprogramming of tumour cells. KRAS mutation heterogeneity in the tumour and between primary foci and metastatic foci is particularly critical. This heterogeneity may lead to clonal selection and acquired resistance in the process of targeted treatment (such as anti-EGFR therapy). For example, subclones carrying KRAS G12D mutations may become dominant clones after treatment, resulting in Treatment failed [5].

The Najie team of Tsinghua University and the team of Anhui Medical University carried out cooperative research to conduct full-exon sequencing of 12 infertile women mainly manifested as sygotic development and stagnation. It was found that three of the patients carried heterosygousing mutations of the TUBA4A gene, including the newly discovered E284G misense mutation and the reported E2 84K misense mutation. TUBA4A is a key α-microtubule protein subtype that makes up cell microtubules. This gene mutation will lead to abnormal assembly of oocyte meiosis spindle body and wrong chromosome arrangement, thus blocking oocyte maturation and fertilised egg development. In order to verify this mechanism, the research team constructed a mouse model test. The results showed that more than half of the oocytes injected with mutant mRNA had meiosis block, and the embryo formation rate of fertilised eggs decreased by about 30% compared with that of the wild group; and after the injection of wild-type and mutant TUBA4A protein in a ratio of 2:1, the oocyte The proportion of normal cell meiosis has increased to more than 65%, and the blastocyst formation rate has also improved significantly. This finding not only reveals the molecular mechanism of TUBA4A mutation causing infertility, but also provides accurate diagnosis of biomarkers and potential protein supplement treatment strategies for genetic infertility, which is of important clinical guidance for embryo screening and genetic counselling in assisted reproductive technology [6].

A retrospective study on low-risk acute myeloid leukaemia (AML) focussed on molecular changes after recurrence in 31 patients. The study found that disease recurrence was often accompanied by dynamic evolution at the genetic level, which was closely related to cloning selection under treatment pressure. Among them, the most common molecular event is the new mutation of FLT3-ITD (5 cases), suggesting that the mutation may be related to disease progression and chemotherapy drug resistance; while the RAS mutation present at the initial diagnosis is prone

DOI: 10.54254/2753-8818/2026.AU32101

to expression deficit (3 cases) at the time of recurrence, which is speculated that it may be due to the cloning of RAS mutations to the treatment. Sensitive and cleared. In addition, multi-factor survival analysis showed that the DNMT3A mutation was identified as an independent risk factor affecting the overall survival of patients. The 5-year total survival rate of patients carrying the mutation was about 40% lower than that of wild patients, significantly increasing the risk of death [7].

4. Response needs of genetic mutations- CRISPR/Cas9

The targeted application of gene editing technology with CRISPR/Cas9 as the core of gene editing technology, with the ability to accurately repair pathogenic mutations, has become a key solution for targeted intervention in gene mutation-related diseases. Its targeted characteristics make it directly effective without interfering with normal gene function. In the focal gene site.

4.1. Technically basic gene editing technology

The genealogy covers zinc finger nuclease (ZFNs), transcription activator-like effector nuclease (TALENs) and CRISPR/Cas system, etc. Among them, CRISPR/Cas9 has become the most widely used mainstream technology due to its advantages of easy operation, high editing efficiency and low cost. The system originates from the acquired immune system of prokaryotes. It is the core Defence mechanism for most bacteria and archaea to resist the invasion of exogenous genetic elements such as bacteriophages or plasmids. Prokaryotes form "immune memory" by capturing exogenous DNA fragments and integrating them into their own CRISPR array, and then encounter the same external When the source genetic material is transmitted, it will be transcribed out of crRNA-guided Cas protein that complements the target sequence to cut exogenous DNA. In 1987, Japanese scientists first discovered the clustered regular interval short palindylosis repeat sequence in the flank sequence of the E. coli iap gene, but its biological function was not clarified at that time; it was not until 2002 that the Dutch scientist Jansen officially composed of repeated sequences, interval sequences and cas genome The structure is named CRISPR, and it is preliminarily revealed that the coding product of the cas gene is related to nuclease activity [8]. Its core working principle is to simulate the immune Defence mechanism of bacteria and assemble the artificially designed sgRNA (single-guided RNA, integrating the functions of crRNA and tracrRNA) and Cas9 protein into a complex. sgRNA identifies the target DNA sequence through base complementary pairing and guides C The as9 protein accurately cuts the DNA double strands like molecular scissors, and then realises the knockout, insertion or replacement of specific gene fragments through the cell's own repair mechanism.

The molecular mechanism of the CRISPR-Cas9 system can be subdivided into three stages: first is the mature process of crRNA, the transcription of the CRISPR array in the bacterial genome produces pre-crRNA containing repeated sequences and interval sequences, and the cooperation between Cas9 protein and RNaseIII Under the same effect, pre-crRNA is cut into a mature crRNA containing a single interval sequence; followed by the assembly of the effect complex. The mature crRNA and the tracrRNA generated by transcription form a double-stranded structure through base complementarity, and then recruit Cas9 protein to assemble into an active Ribonucleoprotein complex (RNP); finally, it is the identification and cutting of target DNA. RNP achieves specific identification through the complementary pairing of 20 nucleotides at the 5' end of sgRNA with the target DNA sequence. At the same time, the PAM identification domain of Cas9 protein needs to be combined with the PAM matrix at the 3' end of the target sequence (through Often NGG, N is arbitrary nucleotide) to activate the cutting activity, then the HNH domain of the Cas9 protein cuts

DOI: 10.54254/2753-8818/2026.AU32101

the complementary DNA strands with sgRNA, and the RuvC domain cuts the non-complementary chain, resulting in DNA double-strand fracture (DSB); after the DNA double-strand fracture, the cell will initiate homologous recombination. Repair (HR) or non-hogeneic terminal connection (NHEJ) mechanism - If a repair template containing homogene arms is provided, HR can realise precise gene insertion or replacement, while NHEJ is easy to produce small fragment insertion/deletion (indel), so as to achieve gene knockout [9].

4.2. Application of CRISPR/Cas9

In the field of ophthalmology, the team of the National Institute of Ophthalmology in the United States uses CRISPR/Cas9 to treat pigmented retinitis [10]. The disease is caused by more than 60 genetic mutations, which will first lead to the death of rod cells and eventually blindness. The study did not directly repair the pathogenic mutation, but inactivated the identity genes of rod cells through gene editing to induce them to be converted into cone cells that are not affected by mutation. In 3 retinal degeneration mouse models (30 mice in total), the technology successfully prevented retinal degeneration and significantly improved the visual function of mice.

4.3. Limitations of CRISPR/Cas9

Although CRISPR/Cas9 gene editing technology has great potential, it still faces multiple limitations at the technical, clinical and ethical levels in its development. The core bottleneck at the technical level is the lack of accuracy, the risk of off-target effect, and the possibility of accidentally injuring non-target genes and causing functional abnormalities, etc. [11]. At the same time, viruses and other delivery vectors have problems of low efficiency and high immunogenicity, which affect the therapeutic effect and safety. In terms of clinical application, gene therapy usually requires highly personalised customisation. The process is complex and extremely expensive, which limits its popularity, and the long-term efficacy and safety still lack sufficient evidence-based support. The challenges in the field of ethics are also prominent, especially in embryo editing, the uncertainty and potential long-term impact of technology have caused widespread controversy, and also touch on core ethical issues such as individual autonomy [12]. The previously exposed case of ethical examination and fraud further highlights the urgency of building a strict and transparent scientific research ethics supervision system. Therefore, the technology really moves towards mature and safe clinical application, and still needs to achieve systematic breakthroughs in terms of accuracy, reach and ethical norms.

4.4. Future development of CRISPR/Cas9 technology

In the future, CRISPR-Cas9 hopes to integrate technology into intelligence, such as the "Pythia" design strategy reported by Nature Biotechnology, which realises the deep integration of CRISPR-Cas9 and deep learning. With the help of the inDelphi deep learning model, the system analyses millions of gene editing data, accurately predicts the repair results of micro-hology-mediated terminal connection (MMEJ), and then designs a repair template with repeated micro-hogeneity sequences in series. This intelligent system does not need to rely on the inefficient homology guidance repair (HDR) pathway, and can guide cells to complete genetic integration according to the preset target. The verification results in cells, claw toads and mouse neurons show that the efficiency of precise integration has increased from 52.0% of a single repetition template to 62.2% of two repetitions, significantly It reduces the problem of missing sequences caused by traditional non-

DOI: 10.54254/2753-8818/2026.AU32101

hogeneic terminal connections (NHEJ), and upgrades gene editing from "random insertion" to "precise engineering integration" [13].

Moreover, the CRISPR editing and optimisation system driven by AI has become an intelligent core application. Deep CRISPR, SPROUT and other AI models can predict potential detarget sites in advance by analysing DNA sequence characteristics and Cas protein interactions and combine with CIRCLE-seq and other detection technologies to achieve risk prediction; Elevation, CRISPR-Ne T-algorithms can screen highly specific gRNA from massive data and reduce the detargeting rate from 5% to less than 0.5%. In CAR-T cell therapy, AI-optimised CRISPR editing reduces the number of off-target events from hundreds to a single digit; the Prime Design tool of Broad Research Institute significantly reduces the risk of off-targeting pilot editing through AI-assisted design, so that the safety of gene therapy can be realised.

5. Conclusion

Genetic mutation are fundamental changes in genetic material, having dual biological effects: on the one hand, they drive evolution and diversity; on the other hand, they can cause various diseases such as sickle cell anemia and cancer. Mutations can be classified by scale into small-scale ones (such as point mutations and deletions) and large-scale mutations (such as chromosomal structural variations); by functional impact, they are divided into synonymous, missense, and nonsense mutations.

The CRISPR/Cas9 gene editing technology uses guide RNA to target and cut DNA and utilizes the cell repair mechanism to achieve precise modification of specific genes. This technology has made progress in disease model research, such as through cell reprogramming to treat retinal degenerative diseases. However, its clinical application is still limited by off-target risks, delivery efficiency, and ethical controversies.

In the future, this technology is deeply integrated with artificial intelligence. AI models can predict editing results, optimize targeted designs, significantly improve editing accuracy and safety, and drive gene editing towards an intelligent and precise direction. In summary, the study of gene mutations has deepened our understanding of disease mechanisms, while the CRISPR/Cas9 technology, through continuous optimization, is expected to open up new avenues for the treatment of genetic diseases.

References

[1] Xue S, Wang S, Liu L, Zhong Q, Cheng Z, Xiao S. [Precision gene editing technologies based on CRISPR/Cas9: a review]. Sheng Wu Gong Cheng Xue Bao. 2023 Jul 25; 39(7): 2566-2578. Chinese.
[2] Chen Ming, Li Juan, Wang Hao. Accurate modification mechanism and research progress of CRISPR/Cas9 gene editing technology [J]. Journal of Bioengineering, 2024, 40(2): 412-425.
[3] Wang Jianguo. Molecular mechanism and classification research of gene mutation [J]. Journal of Genetics, 2023, 50(8): 678-689.
[4] Gene mutation.2023.https: //www.zgbk.com/ecph/words? SiteID=1& ID=129776& Type=bkzyb& SubID=208457
[5] Timar J, Kashofer K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. Cancer Metastasis Rev. 2020 Dec; 39(4): 1029-1038.
[6] Li L, Mao L, Zhang Z, Yu Y, Ding M, Zhi Y, Cao Y, Chen B, Na J. Mutations in TUBA4A lead to human zygotic arrest and early developmental failure. Life Med. August 23, 2023; 2(5): lnad032.
[7] Five. Gao Yunfei, Tan Yehui, Su Long, etc. Molecular characteristics and prognosis analysis after recurrence of low-risk acute myeloid leukaemia [J]. Chinese Journal of Experimental Haematology, 2025, 33(06): 1551-1557.
[8] Zhang Qiulang, Liu Zhifei, Zhang Beibei, etc. Research progress on CRISPR-Cas9 gene editing system and its application in insects [J/OL]. Biological Disaster Science, 1-9 [2025-12-10].

DOI: 10.54254/2753-8818/2026.AU32101

[9] Ma S, Chang J, Wang X, Liu Y, Zhang J, Lu W, Gao J, Shi R, Zhao P, Xia Q. CRISPR/Cas9 mediated multiplex genome editing and heritable mutagenesis of BmKu70 in Bombyx mori. Sci-Rep. 2014 Mar 27; 4: 4489.
[10] Editing the Code: UCSF's Mission to Cure Sickle Cell Disease with CRISPR.
[11] Guo C, Ma X, Gao F, Guo Y. Off-target effects in CRISPR/Cas9 gene editing. Front Bioeng Biotechnol. March 9, 2023; 11: 1143157.
[12] Wiley L, Cheek M, LaFar E, Ma X, Sekowski J, Tanguturi N, Iltis A. The Ethics of Human Embryo Editing via CRISPR-Cas9 Technology: A Systematic Review of Ethical Argume Nts, Reasons, and Concerns. HEC Forum. 2025 Jun; 37(2): 267-303.
[13] Khan Z, Mumtaz, Gupta S, Mehan S, Sharma T, Kumar M, Maurya PK, Sharma AK, Gupta GD, Narula AS. CRISPR-Cas9: Transforming Functional Genomics, Precision Medicine, and Drug Development - Opportunit Ies, Challenges, and Future Directions. Curr Gene Ther. 2025 Mar 21.