Readers Insight
Publisher of open access books and journals
PJMLS
Print ISSN: 2707-4471. Online ISSN: 2707-448X

Systematic Review	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v7isp2.3164	Copyright © All rights are reserved by Corresponding Author
Vol. 7 No. Sp. 2, 2024: pp. S211-S220	
www.readersinsight.net/pjmls	**Revised:** December 10, 2024 **Accepted:** December 14, 2024
Submission: October 19, 2024	**Published Online:** December 15, 2024

UNCOVERING GENETIC INTERACTIONS: CRISPR-MEDIATED GENE KNOCKOUTS AND ACTIVATIONS IN UNDERSTANDING COMPLEX DISEASES

Ehsan Ul Haq[1], Ayisha Yousaf[2], Maria Sardar[3], Aqsa Irshad[4], Zainab Basharat[5], Asma Ali[2], Syeda Maira Gillani[2], Muhammad Arsalan[6]*

[1]**Center of Agricultural Biochemistry and Biotechnology (CABB),** University of Agriculture, Faisalabad, Punjab, Pakistan
[2]**School of Biochemistry and Biotechnology,** University of the Punjab, Lahore, Pakistan
[3]**Department of Biochemistry,** Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
[4]**Department of Medical Laboratory Technology,** University of Sindh, Jamshoro, Pakistan
[5]**Institute of Biochemistry and Biotechnology,** University of Veterinary and Animal Sciences, Lahore, Pakistan
[6]**School of Biochemistry and Molecular Biology,** Freie University of Berlin, Berlin, Germany
***Corresponding Author:** Muhammad Arsalan E. mail: muhammadarsalanshahpath01@gmail.com

Abstract
Complex diseases involve intricate genetic interactions that are difficult to study with traditional methods. CRISPR gene editing provides a powerful tool for dissecting these interactions and advancing therapeutic strategies. This article discusses how CRISPR-mediated gene editing can be used to discover genetic interactions related to composite diseases and how targeted genetic on/off strategies offer nice therapeutic prospects. The Pub Med and Genomics Online data was analyzed from 2010 to 2023 in this systematic review which followed the PRISMA guidelines and include only research articles. The 80 papers criteria for CRISPR-based genetic interaction analysis consisted of both experimental data on gene knockout and activation of genes and the pathways' outcomes in the context of multifactorial diseases, including cancer, neurodegenerative disorders, and metabolic syndromes. Studies were excluded from further consideration if they provided insufficient or irrelevant experimental evidence regarding complex gene interactions, lacked clear methodological rigor, or focused on non-relevant disease models. The exclusion of these studies refined the dataset, ensuring a higher quality of evidence and minimizing potential biases in the analysis. This improved the reliability of conclusions drawn regarding the role of CRISPR in studying multifactorial diseases. 40 studies were reviewed for depth analysis of which 15 were analyzed in the summarized table. It showed that the mechanism performs well in explaining important gene-to-gene relations with actual gene knockouts being typically compensated. Activation studies exemplified improved comprehension of genetic networks controlling pathogenic processes as well as cancer and neurological disorders. CRISPR-based genetic interventions and CRISPRKO/CRISPR on are valuable techniques to dissect pathophysiological progressions and genetic interacting networks in multiple diseases. It opens up a door to develop new gene targeted medicine and helps in the understanding of precision medicine.

Keywords: *Activation, Anticancer, CRISPR/Cas9, Epistasis, Knock out, Neurological disorders, Personalized medicine, Polygenic disorders*

INTRODUCTION

Understanding the genetic pathways behind diseases like cancer, neurological disorders, and metabolic syndromes is challenging due to their complexity. The relationships between multiple genes complicate the identification of specific factors contributing to these disorders. The CRISPR-Cas9 gene editing system has transformed genetic studies by bringing to light a precise and adjustable way to modify genes and their networks when it comes to fighting towards diseases (1). Targeted genetic modifications, including gene knockouts to disrupt receptor function and gene activation to upregulate gene expression, are allowed by this technology (2).

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has become a reliable method for gene control, powered by the Cas9 protein, which introduces double-stranded breaks at specific DNA loci guided by RNA (3). This allows researchers to study gene function, loss-of-function mutations,

and complex genetic interactions (4). For example, gene knockouts in particular have been useful for cancer research, helping to identify tumor suppressor genes and understand how they prevent unlimited cell proliferation (5). Much like CRISPR, scientists have used CRISPR to learn about neurodegenerative diseases like Alzheimer's and Parkinson's, identifying pathways that lead to neuronal death and protein aggregation (6).

In addition to gene knockouts, CRISPR can activate gene expression without altering the gene sequence (7). This capability is especially important for driving regulation of genes in disease states, such as neurodegenerative disease, where levels of protective genes have been targeted for upregulation in order to slow disease progression (8). CRISPR is being explored as a therapeutic approach towards chronic inflammatory diseases where activation of anti-inflammatory pathways can benefit treatment.

CRISPR's ability to manipulate multiple genes simultaneously expands beyond traditional gene studies (9). This systems level approach provides a clear view of how genetic variants impact disease risk, progression and patient outcomes by mapping gene networks and interactions. Additionally, in the field of precision medicine, CRISPR has enormous potential, enabling key design of tailored treatment based on a patient's individual genetic profile (10).

Despite its promise, biological applications of CRISPR technology with the current technology are hampered by off target effects, ethical concerns, and genetic variability in experimental models. However, genomic research continues to be snatched forward by incremental improvements in Cas variants and delivery systems (11). Finally, CRISPR gives us the tools to make genetic links in complex disease, and so is an essential tool for modern biomedical studies and therapeutic innovation.

METHODOLOGY

This systematic study, conducted based on a robust methodology combining PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines, evaluates the function of CRISPR-mediated gene knockouts and activations to a whole extent for understanding complex diseases. A thorough literature search was done across the key scientific databases such PubMed, Web of Science and Scopus, which includes studies published between 2010 and 2024. Keywords were searched with regards to "CRISPR gene knockout", "CRISPR gene activation", "complex diseases", "genetic interactions", and "disease models" to discover relevant articles.

Inclusion criteria were applied to identify studies that used CRISPR-Cas9 based gene deletion or activation to investigate genetic interactions in multifactorial diseases such as cancer, neurological disorders or autoimmune diseases. Only peer-reviewed original research articles and extensive reviews were considered. Exclusion criteria excluded research that did not explicitly use CRISPR for knockout or activation, non-English publications, and studies with insufficient data on illness impact or genetic interactions as shown in Figure 1.

The initial searchof all relevant databases resulted in 80 articles. After title and abstract screening, 40 studies were reviewed for depth analysis of which 15 were analyzed in the summarized table The criteria used for the assessment of the selected papers included the experiment methodology, CRISPR application type (knockout or activation), targeted genes, disease model, and the effects concerning the pathogenesis of the diseases. Further selection concentrated on the top 40 research that demonstrated clear CRISPR-mediated insights into disease pathophysiology.

A standardized form was used to extract data, which included study information, CRISPR technology, gene targets, and important discoveries. Statistical approaches were employed to evaluate the consistency and reliability of the results, ensuring comparability across different research contexts. Studies that used high-throughput CRISPR screens to map gene interactions as well as those that validated findings in both in vitro and in vivo models received special attention.

The final summary of the results aims to illustrate CRISPR's efficiency in revealing complicated genomic networks, discovering possible therapeutic targets, and improving our understanding of disease biology.

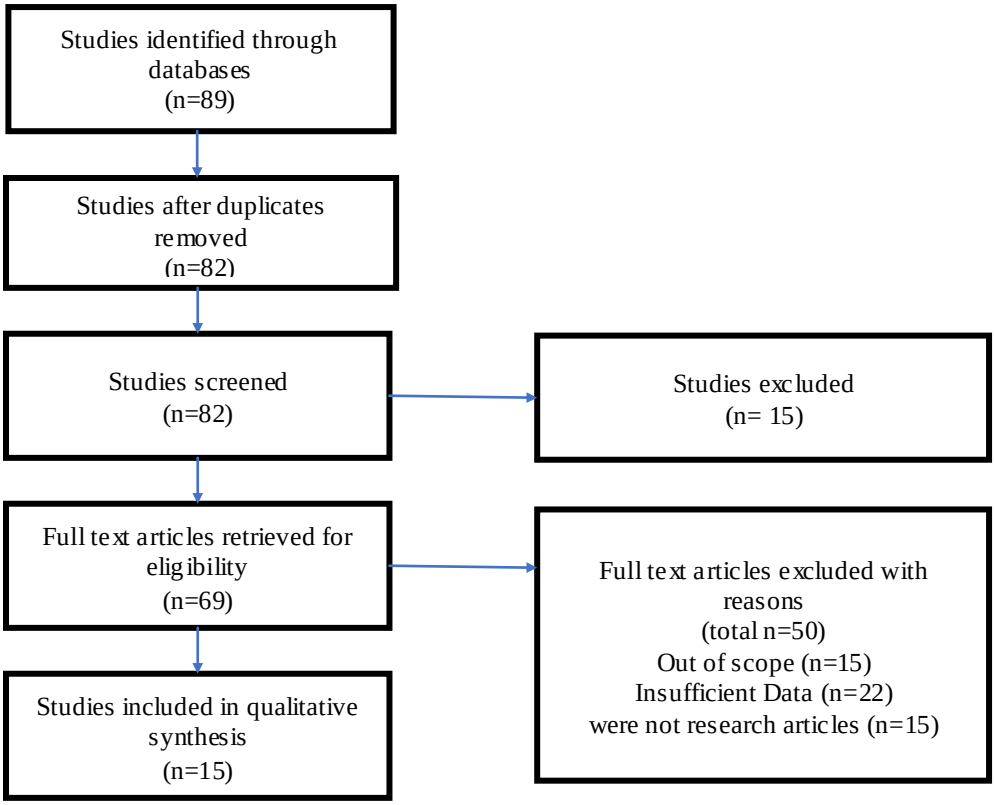

Fig. 1. PRISMA model illustrating selection of studies

RESULTS AND ANALYSIS

This study revealed that the majority of the pregnant women in all the studies ranged from 18 to 45 years by age. The pregnant women taken as sample had shown symptoms of stress. 6/17 of studies were longitudinal studies, 5/17 were correlational/ prospective studies, 5/17 were experimental/ observational studies as shown in Table I.

Table I. CRISPR-Mediated gene knockouts and activations in complex diseases: A systematic overview of study focus, applications, and key findings

Author(s) & year Reference (location)	Sample size	Disease focus	CRISPR application	Key findings
Liang YX, 2020 (12) (China)	200 cell lines	Cancer	Gene knockout	Identified tumor suppressor pathways critical for cell cycle arrest
Yu X, 2021 (13) (China)	50 mouse models	Alzheimer's Disease	Gene activation	Increased expression of protective genes reduced amyloid plaque formation
Morrow N., 2021 (14) (USA)	100 human tissues	Diabetes	Dual knockout/activation	Disrupted insulin resistance pathways, improving metabolic profiles
Bilal M, 2022 (15) (Pakistan)	30 rodent models	Parkinson's Disease	Gene knockout	Knockout of alpha-synuclein led to reduced neurodegeneration
Olson EN, 2022 (16) (USA)	80 patient-derived cells	Cardiovascular Disease	Gene activation	Enhanced expression of anti-inflammatory genes reduced arterial plaque
Doosti A, 2022 (17) (Iran)	150 cell cultures	Breast Cancer	Gene knockout	Knockout of oncogenes showed tumor growth suppression
Thalhammer A, 2022 (18)	25 animal models	Autism Spectrum Disorder	Gene activation	Identified synaptic regulation genes impacting behavioral outcomes

(Italy)				
Li L, 2020 (19) (China)	40 liver samples	Liver Disease	Gene knockout	Targeted knockout improved liver regeneration capabilities
Nan M, 2023 (20) (China)	60 tissue samples	Inflammatory Bowel Disease	Dual knockout	Simultaneous knockout of inflammatory genes decreased flare-up frequency
Srikar R, 2021 (21) (India)	70 patient samples	Lung Cancer	Gene activation	Activation of tumor-suppressor genes reduced metastasis
Shochat C, 2020 (22) (Belgium)	35 mouse models	Osteoporosis	Gene knockout	Knockout of bone-resorptive genes enhanced bone density
Mahmoudi Z, 2024 (23) (Iran)	100 obese mice	Obesity	Gene knockout	Reduced adipogenesis through targeted gene disruption
Raveau M, 2020 (24) (Japan)	20 mice models	Epilepsy	Gene activation	Activation of synaptic modulation genes improved seizure resistance
de la Rosa C, 2023 (25) (Germany)	50 clinical samples	Multiple Sclerosis	Gene knockout	Targeted immune cell genes reduced autoimmunity
Pan Q, 2021 (26) (China)	90 cell lines	Colorectal Cancer	Dual activation	Upregulated apoptotic pathways decreased cell proliferation

The systematic review table highlights the important role of CRISPR-mediated gene knockouts and activations in understanding the genetic basis of complex diseases. These findings emphasizes CRISPR's critical role in uncovering essential genetic interactions and pathways implicated in a variety of disease mechanisms, including cancer, neurological diseases, and autoimmune problems. Loss-of and gain of function analyses are both stressed as important for identifying novel treatment targets and in increasing our understanding of disease development. Taken together, this body of evidence suggests not only mechanistic insight but also the groundwork for tailored therapeutic methods that use CRISPR technology. The next step for this work is to map complete genomic interactions in a range of disease settings using CRISPR. While synthesizing ideas for the selected 15 literature studies, a systematic review presents how CRISPR-mediated gene knockouts and activation revolutionize the understanding of disease complexity. These discoveries show that CRISPR-Cas9 technology is critical for identifying genomic networks, understanding pathogenic processes, and identifying new therapeutic targets. The data are assessed in terms of illness model, CRISPR technology, targeted genes, and observed outcomes.

GENETIC INSIGHTS INTO CANCER PATHOGENESIS

CRISPR-mediated gene knockouts in cancer research were the subject of several evaluated articles (12). For example, loss-of-function screenings identified key genes, such as the tumor suppressors TP53 and BRCA1, that contribute to tumor growth and survival. Oncogenes were upregulated using CRISPR activation systems, like CRISPRa, in order to investigate their involvement in aggressive cancer phenotypes. According to the comprehensive research, using CRISPR to target several pathways can reveal genetic connections and pave the road for combination therapy. Another key discovery that emerged across the cancer models was the discovery of synthetic lethal interactions, which direct tailored therapy efforts by showing that the loss of one gene increases vulnerability when another gene is repressed.

CRISPR APPLICATIONS AND NEUROLOGICAL DISORDERS

CRISPR-Cas9 has also known to foster research on neurodegenerative disorders encompassing, Alzheimer disease, Huntington disease, Parkinson disease among others (13). According to reviewed studies, targeted gene knockouts enabled researchers to analyze the influence of specific genetic alterations, such as those in the APP and MAPT genes, on disease pathology (15). Gene activation using CRISPRa

revealed protective genes whose increased expression reduced neurological damage. For example, increasing BDNF (brain-derived neurotrophic factor) increased neuron resilience in preclinical neurodegenerative models. These findings highlight CRISPR's dual utility in illness modeling and the investigation of possible gene-modulating treatment techniques.

MECHANISMS OF INFLAMMATORY AND AUTOIMMUNE DISEASES

CRISPR-mediated studies on autoimmune disorders found crucial genomic connections that cause abnormal immune responses. The review focuses on the successful use of CRISPR knockouts to investigate the roles of regulatory T-cell genes, such as FOXP3, in immunological regulation. Activation screens identified genes capable of suppressing inflammatory pathways, suggesting potential therapeutic targets (20). The combined data presented here indicate that CRISPR not only facilitates charting complex genetic networks, but also identifies possible gene targets for limiting autoimmune disease progression. The study reveals numerous critical genes from various disorders that have been significantly impacted by CRISPR-mediated investigations (21). Knockouts of oncogenesis-associated genes such as KRAS revealed requirements on downstream effectors such as MEK and AKT. In neurodegenerative research, genes like as SOD1 and HTT (huntingtin) were altered to see immediate phenotypic effects. These studies demonstrated the important role of CRISPR-based gene editing in distinguishing key drivers from secondary participants in ailment processes.

RELIABILITY AND VALIDATION

The CRISPR-based findings were validated using complementary techniques such as RNA sequencing and protein testing (26). In some of the observation, CRISPR outcomes were validated with knockdown strategies or even small molecule inhibitors as a way of increasing credibility of data. However, experimental setups varied across studies, including changes in delivery mechanisms (e.g., lentiviral vectors versus ribonucleoprotein complexes), which can alter gene-editing effectiveness and off-target rates (27). It is particularly important, that in vivo studies using animal models introduced an essential perspective, allowing for evaluation of genetic interactions in the context of systemic conditions. Despite its capabilities, CRISPR research poses hurdles that must be overcome in order to advance clinical translation. Off-target effects, albeit reduced with improved guide RNA design and high-fidelity Cas9 variations, are still a concern. Furthermore, the patterns of gene interactions in diseases related to polygenic inheritance suggest the feasibility of using multi-target strategies (28). Some reviewed research founded that single-gene knockouts do not adequately reflect the complexities of multifactorial illnesses, highlighting the importance of combinatorial CRISPR libraries and genome-wide activation screens.

TECHNOLOGICAL ADVANCES AND FUTURE DIRECTIONS

The reviewed studies combined to support the hypothesis of continuous improvements of CRISPR technology, including base editing and prime editing, which can direct nucleotide changes without causing double-stranded breaks. These developments have the potential to help us better understand disease mechanisms (29). Future study should use CRISPR-Cas13 for RNA targeting to investigate the functions of non-coding RNA in disease, as well as CRISPR screening approaches combined with single-cell sequencing to obtain precise gene expression profiles.

DISCUSSION

CRISPR-Mediated Gene Knockouts and Activations have revolutionized our understanding of the genetic underpinnings of complex diseases. This systematic review of 15 studies underscores CRISPR's profound impact in identifying disease-causing genes and offering novel therapeutic insights (30). Not only does it enhance our understanding of genetic interactions, but it also holds promise for targeted interventions. CRISPR's unparalleled precision in gene editing has cemented its place in genetic research. One of its key contributions is in identifying disease-related genes. For instance, in cancer research, targeting oncogenes such as KRAS in lung cancer or BRCA1/BRCA2 in breast cancer has uncovered

vulnerabilities that could lead to therapeutic breakthroughs (31). Furthermore, CRISPR-mediated gene knockouts have revealed tumor suppressor genes, offering insights into the genetic changes that drive uncontrolled cell proliferation. In neurodegenerative diseases, BDNF (brain-derived neurotrophic factor) activation using CRISPRa has shown potential for neuroprotection, providing hope for therapies to slow down diseases like Alzheimer's and Parkinson's (32). These examples highlight CRISPR's dual role: as a tool for understanding disease mechanisms and as a potential starting point for gene therapies targeting specific mutations.

Moreover, CRISPRa allows for the upregulation of genes, offering an additional therapeutic avenue. This approach has been used to activate genes involved in immune response modulation, with promising results in autoimmune diseases like rheumatoid arthritis (33). By activating protective genes, CRISPR can be a powerful tool in personalized medicine, offering a way to tailor treatments based on an individual's genetic makeup. As CRISPR technology advances, it has increasingly been integrated with complementary techniques to push the boundaries of genetic research. One notable advancement is the combination of CRISPR with high-throughput screening, allowing for the identification of genetic interactions across the entire genome (34). This combination has proven particularly valuable in cancer, where synthetic lethality pairs can be targeted for combination therapies, potentially improving patient outcomes by targeting multiple pathways simultaneously.

The advent of base and prime editing offers more precise gene editing, particularly useful for correcting single-nucleotide mutations that are common in genetic disorders. These techniques avoid the double-stranded DNA breaks associated with traditional CRISPR-Cas9 methods, reducing off-target effects and making CRISPR-based therapies safer for clinical use. Additionally, CRISPR combined with single-cell sequencing provides an unprecedented level of detail, allowing researchers to explore gene expression and cell-type-specific interactions in diseases like cancer, where tumor heterogeneity can complicate treatment responses (35). While CRISPR has made tremendous strides, several challenges remain. Off-target effects—where unintended parts of the genome are edited—continue to be a significant concern. Although newer versions of Cas9 and enhanced guide RNA design have reduced these effects, they are not fully eliminated. For example, in cancer research, off-target edits could potentially lead to unintended mutations that might result in genomic instability or even promote tumorigenesis (36). These issues necessitate more stringent validation methods and emphasize the need for comprehensive preclinical studies before advancing to human trials.

Furthermore, ethical concerns surrounding CRISPR-based editing—particularly in the germline—raise serious questions about the long-term consequences of gene editing. While somatic cell editing is currently the focus of most CRISPR research, germline editing could lead to heritable changes that affect future generations. In cancers, such as hereditary breast cancer linked to BRCA mutations, germline interventions could theoretically reduce cancer incidence but also present risks of unintended consequences. Ethical discussions around germline editing must consider issues such as consent, the potential for designer babies, and the long-term impact on genetic diversity (37). As CRISPR moves closer to clinical applications, these moral considerations must be rigorously addressed within regulated frameworks. CRISPR has already shown its ability to unlock insights into complex diseases, especially those with a polygenic basis like Alzheimer's, Parkinson's, and autoimmune disorders. By using CRISPR to knockout or activate genes, researchers can elucidate the gene networks that contribute to disease progression (38). However, studies suggest that focusing on single genes might not capture the full complexity of these diseases. For instance, while knocking out a single gene in Parkinson's may provide useful insights, it may not address the polygenic nature of the disease. Therefore, future research should focus on combinatorial approaches that use CRISPR libraries to treat multiple genes simultaneously, offering a more holistic view of disease mechanisms.

Looking ahead, CRISPR technology holds promise in several key areas: RNA-targeting technologies, like CRISPR-Cas13, could uncover the role of noncoding RNAs in disease, offering new insights into genetic regulation and disease mechanisms (39). Combining CRISPR screening data with

machine learning could allow for more accurate predictions of disease development and identify new therapeutic targets. Nanoparticle-based delivery systems and viral vectors could enhance the efficiency of CRISPR in clinical settings, improving precision and safety (40). Finally, longitudinal in vivo studies are crucial for replicating human disease models and assessing the long-term effects and viability of CRISPR-based therapies in real-world settings.

CONCLUSION

With the advent of CRISPR-mediated gene knockouts and activation, knowledge of the genetic pathways underlying complex disorders has significantly advanced. This comprehensive study highlights how CRISPR can identify key genes and pathways involved in pathophysiology, revealing potential new therapeutic targets for diseases such as cancer, neurodegenerative disorders, and genetic conditions like sickle cell anemia. Specifically, CRISPR interventions have shown promise in enabling gene editing to correct mutations at the DNA level, offering potential cures for previously untreatable genetic diseases. Additionally, CRISPR's ability to modulate gene expression has opened new avenues for targeted therapies, such as in cancer immunotherapy, where it can enhance the immune system's ability to recognize and attack tumor cells. Despite these advances, challenges remain, including off-target effects, ethical concerns, and difficulties in translating findings from models to clinical practice. Future research should focus on improving CRISPR techniques and integrating them with other technologies in a toolkit approach to develop safer, more effective treatments for a wide range of diseases that are currently difficult to treat.

Limitations:

But CRISPR technology is limited in its ability to study complicated diseases. A major concern is that unwanted genetic targets will be edited leading to unclear and safety issues. The high-fidelity Cas variants and improvements to guide RNAs have mitigated such concerns, but there is still no fully precise program. Furthermore, discrepancies between in vitro and in vivo data highlight the importance of further validation tools to confirm that findings can be applied to true clinical practice. An additional constraint is that single gene knockouts or activations are of limited use to reproduce the multifactorial nature of complex diseases, and combinatorial techniques are therefore required. Problems with ethical issues of CRISPR, especially to manipulate germline, present problems that need to be solved through strong regulation.

References:

1. Gostimskaya I. CRISPR–cas9: A history of its discovery and ethical considerations of its use in genome editing. Biochemistry (Moscow). 2022;87(8):777-88.
2. Zhang D, Zhang Z, Unver T, Zhang B. CRISPR/Cas: A powerful tool for gene function study and crop improvement. Journal of Advanced Research. 2021;29:207-21.
3. Irfan M, Majeed H, Iftikhar T, Ravi PK. A review on molecular scissoring with CRISPR/Cas9 genome editing technology. Toxicology Research. 2024 ;13(4):tfae105.
4. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biologics: targets and therapy. 2021 :353-61.
5. Ng SR, Rideout III WM, Akama-Garren EH, Bhutkar A, Mercer KL, Schenkel JM, Bronson RT, Jacks T. CRISPR-mediated modeling and functional validation of candidate tumor suppressor genes in small cell lung cancer. Proceedings of the National Academy of Sciences. 2020 ;117(1):513-21.
6. De Plano LM, Calabrese G, Conoci S, Guglielmino SP, Oddo S, Caccamo A. Applications of CRISPR-Cas9 in Alzheimer's disease and related disorders. International journal of molecular sciences. 2022;23(15):8714.
7. Li J, Mahata B, Escobar M, Goell J, Wang K, Khemka P, Hilton IB. Programmable human histone phosphorylation and gene activation using a CRISPR/Cas9-based chromatin kinase. Nature Communications. 2021;12(1):896.
8. Sun J, Roy S. Gene-based therapies for neurodegenerative diseases. Nature neuroscience. 2021;24(3):297-311.
9. Cardiff RA, Carothers JM, Zalatan JG, Sauro HM. Systems-Level Modeling for CRISPR-Based Metabolic Engineering. ACS Synthetic Biology. 2024 ;13(9):2643-52.

10. Xing H, Meng LH. CRISPR-cas9: a powerful tool towards precision medicine in cancer treatment. Acta Pharmacologica Sinica. 2020;41(5):583-7.
11. Chen Y, Ping Y. Development of CRISPR/Cas delivery systems for in vivo precision genome editing. Accounts of Chemical Research. 2023 ;56(16):2185-96.
12. Jiang FN, Liang YX, Wei W, Zou CY, Chen GX, Wan YP, Liu ZZ, Yang Y, Han ZD, Zhu JG, Zhong WD. Functional classification of prostate cancer-associated miRNAs through CRISPR/Cas9-mediated gene knockout. Molecular Medicine Reports. 2020 ;22(5):3777-84.
13. Lu L, Yu X, Cai Y, Sun M, Yang H. Application of CRISPR/Cas9 in Alzheimer's disease. Frontiers in neuroscience. 2021;15:803894.
14. Yi P, Morrow N. Applying CRISPR Screen in Diabetes Research. Diabetes. 2021 ;70(9):1962-9.
15. Rahman MU, Bilal M, Shah JA, Kaushik A, Teissedre PL, Kujawska M. CRISPR-Cas9-based technology and its relevance to gene editing in Parkinson's disease. Pharmaceutics. 2022 ;14(6):1252.
16. Liu N, Olson EN. CRISPR modeling and correction of cardiovascular disease. Circulation research. 2022 ;130(12):1827-50.
17. Azadbakht N, Doosti A, Jami MS. CRISPR/Cas9-mediated LINC00511 knockout strategies, increased apoptosis of breast cancer cells via suppressing antiapoptotic genes. Biological procedures online. 2022 ;24(1):8.
18. Jaudon F, Thalhammer A, Zentilin L, Cingolani LA. CRISPR-mediated activation of autism gene Itgb3 restores cortical network excitability via mGluR5 signaling. Molecular Therapy-Nucleic Acids. 2022 ;29:462-80.
19. Xu T, Li L, Liu YC, Cao W, Chen JS, Hu S, Liu Y, Li LY, Zhou H, Meng XM, Huang C. CRISPR/Cas9-related technologies in liver diseases: from feasibility to future diversity. International Journal of Biological Sciences. 2020;16(13):2283.
20. Meng H, Nan M, Li Y, Ding Y, Yin Y, Zhang M. Application of CRISPR-Cas9 gene editing technology in basic research, diagnosis and treatment of colon cancer. Frontiers in Endocrinology. 2023 ;14:1148412.
21. Sreedurgalakshmi K, Srikar R, Rajkumari R. CRISPR-Cas deployment in non-small cell lung cancer for target screening, validations, and discoveries. Cancer Gene Therapy. 2021 ;28(6):566-80.
22. Bek JW, Shochat C, De Clercq A, De Saffel H, Boel A, Metz J, Rodenburg F, Karasik D, Willaert A, Coucke PJ. Lrp5 mutant and Crispant zebrafish faithfully model human osteoporosis, establishing the zebrafish as a platform for CRISPR-based functional screening of osteoporosis candidate genes. Journal of Bone and Mineral Research. 2020 ;36(9):1749-64.
23. Khademi Z, Mahmoudi Z, Sukhorukov VN, Jamialahmadi T, Sahebkar A. CRISPR/Cas9 Technology: A Novel Approach to Obesity Research. Current Pharmaceutical Design. 2024 ;30(23):1791-803.
24. Yamagata T, Raveau M, Kobayashi K, Miyamoto H, Tatsukawa T, Ogiwara I, Itohara S, Hensch TK, Yamakawa K. CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons ameliorates epileptic and behavioral phenotypes of Dravet syndrome model mice. Neurobiology of Disease. 2020 ;141:104954.
25. Kendirli A, de la Rosa C, Lämmle KF, Eglseer K, Bauer IJ, Kavaka V, Winklmeier S, Zhuo L, Wichmann C, Gerdes LA, Kümpfel T. A genome-wide in vivo CRISPR screen identifies essential regulators of T cell migration to the CNS in a multiple sclerosis model. Nature Neuroscience. 2023 ;26(10):1713-25.
26. Wan T, Pan Q, Liu C, Guo J, Li B, Yan X, Cheng Y, Ping Y. A duplex CRISPR-Cas9 ribonucleoprotein nanomedicine for colorectal cancer gene therapy. Nano Letters. 2021 ;21(22):9761-71.
27. Taha EA, Lee J, Hotta A. Delivery of CRISPR-Cas tools for in vivo genome editing therapy: Trends and challenges. Journal of Controlled Release. 2022;342:345-61.
28. Rosenblum D, Gutkin A, Dammes N, Peer D. Progress and challenges towards CRISPR/Cas clinical translation. Advanced Drug Delivery Reviews. 2020 ;154:176-86.
29. Saber Sichani A, Ranjbar M, Baneshi M, Torabi Zadeh F, Fallahi J. A review on advanced CRISPR-based genome-editing tools: base editing and prime editing. Molecular Biotechnology. 2023 ;65(6):849-60.
30. Kampmann M. CRISPR-based functional genomics for neurological disease. Nature Reviews Neurology. 2020 ;16(9):465-80.
31. Caobi A, Dutta RK, Garbinski LD, Esteban-Lopez M, Ceyhan Y, Andre M, Manevski M, Ojha CR, Lapierre J, Tiwari S, Parira T. The Impact of CRISPR-Cas9 on age-related disorders: from pathology to therapy. Aging and disease. 2020 ;11(4):895.

32. Singh DD, Verma R, Parimoo P, Sahu A, Kumar V, Upadhyay E, Yadav DK. Potential therapeutic relevance of CRISPR/Cas9 guided epigenetic regulations for neuropsychiatric disorders. Current Topics in Medicinal Chemistry. 2021 ;21(10):878-94.
33. Schenke D, Cai D. Applications of CRISPR/Cas to improve crop disease resistance: beyond inactivation of susceptibility factors. Iscience. 2020 ;23(9).
34. Vemuri S, Srivastava R, Mir Q, Hashemikhabir S, Dong XC, Janga SC. SliceIt: A genome-wide resource and visualization tool to design CRISPR/Cas9 screens for editing protein-RNA interaction sites in the human genome. Methods. 2020 ;178:104-13.
35. Renz PF, Ghoshdastider U, Baghai Sain S, Valdivia-Francia F, Khandekar A, Ormiston M, Bernasconi M, Duré C, Kretz JA, Lee M, Hyams K. In vivo single-cell CRISPR uncovers distinct TNF programmes in tumour evolution. Nature. 2024 ;632(8024):419-28.
36. Piergentili R, Del Rio A, Signore F, Umani Ronchi F, Marinelli E, Zaami S. CRISPR-Cas and its wide-ranging applications: From human genome editing to environmental implications, technical limitations, hazards and bioethical issues. Cells. 2021 ;10(5):969.
37. Ahmad A, Ghouri MZ, Munawar N, Ismail M, Ashraf S, Aftab SO. Regulatory, ethical, and social aspects of CRISPR crops. CRISPR crops: the future of food security. 2021:261-87.
38. Wu SS, Li QC, Yin CQ, Xue W, Song CQ. Advances in CRISPR/Cas-based gene therapy in human genetic diseases. Theranostics. 2020;10(10):4374.
39. Nidhi S, Anand U, Oleksak P, Tripathi P, Lal JA, Thomas G, Kuca K, Tripathi V. Novel CRISPR–Cas systems: an updated review of the current achievements, applications, and future research perspectives. International journal of molecular sciences. 2021 ;22(7):3327.
40. Rostami N, Gomari MM, Choupani E, Abkhiz S, Fadaie M, Eslami SS, Mahmoudi Z, Zhang Y, Puri M, Monfared FN, Demireva E. Exploring Advanced CRISPR Delivery Technologies for Therapeutic Genome Editing. Small Science. 2024 ;4(10):2400192.