IntechOpen

IntechOpen Series
Genetics, Volume 10

CRISPR Technologies

Advances in Genome Editing, Applications, and Ethical Implications

Edited by Arli Aditya Parikesit, Arif Nur Muhammad Ansori and Adhityo Wicaksono

CRISPR Technologies - Advances in Genome Editing, Applications, and Ethical Implications

Edited by Arli Aditya Parikesit, Arif Nur Muhammad Ansori and Adhityo Wicaksono

Published in London, United Kingdom

CRISPR Technologies – Advances in Genome Editing, Applications, and Ethical Implications
http://dx.doi.org/10.5772/intechopen.1008645
Edited by Arli Aditya Parikesit, Arif Nur Muhammad Ansori and Adhityo Wicaksono

Contributors
Adhityo Wicaksono, Agbesi Kwadzo Keteku, Arif Nur Muhammad Ansori, Arli Aditya Parikesit, Augustine Antwi-Boasiako, Berkay Özdemir, Dorkina Myrick, Emrah Yücesan, Francis Azumah Chimsah, Gourab Chatterjee, Harrison Kwame Dapaah, Juliet Mensah, Kusum Kumari Singh, Kwame Yaw-Kan Kojo, Kübra Telli, Philip Ghanney, Richard Dormatey, Sude Danışman

First published in London, United Kingdom, 2025 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 167-169 Great Portland Street, London, W1W 5PF, United Kingdom

For EU product safety concerns: IN TECH d.o.o., Prolaz Marije Krucifikse Kozulić 3, 51000 Rijeka, Croatia, info@intechopen.com or visit our website at intechopen.com.

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

CRISPR Technologies – Advances in Genome Editing, Applications, and Ethical Implications
Edited by Arli Aditya Parikesit, Arif Nur Muhammad Ansori and Adhityo Wicaksono
p. cm.

This title is part of the Genetics Book Series, Volume 10
Topic: Genomics
Series Editor: Kenji Ikehara
Topic Editor: Irina Vlasova-St. Louis

Print ISBN 978-1-83635-584-7
Online ISBN 978-1-83635-583-0
eBook (PDF) ISBN 978-1-83635-585-4
ISSN 3049-7094

IntechOpen Book Series

Genetics

Volume 10

Aims and Scope of the Series

"Genetics," which has been proud of its tradition since Mendel presented his research results in 1865, initially progressed quite slowly due to simple observational approaches of individuals and groups. However, the discovery of double-stranded DNA by Watson and Crick about 70 years ago triggered rapid progress in life sciences, including genetics, which was primarily conducted using Escherichia coli and bacteriophages infecting E. coli. Subsequently, genetics has achieved remarkable developments, such as understanding genetic disorders, including cancers, through research on the biogenesis and differentiation of plants and animals.

The two topics of this book series - Human Genetics, and Genomics - will address important areas of advancement in genetics.

Human Genetics: After fundamental genetics, initially studied with the main goal of revealing the functions of individual genes and proteins, genetics expanded from understanding the genetic system itself to understanding many infectious diseases caused by bacteria and viruses. Consequently, human beings are now overcoming infectious diseases by developing medicinal chemicals, including antibiotics and vaccines. However, genetic disorders remain challenging to cure up to now. Nevertheless, even the cure for them, including various cancers, is coming closer to reality due to the rapid progress of human genetics. In this way, the welfare of human life continues to improve, and even longevity, which was once a dream, has been achieved to some extent in recent years.

Genomics: On the other hand, the understanding of the comprehensive interrelationship of whole genes or whole proteins functioning in one organism has become possible now, as research has entered the era of genomics, owing to the rapid progress of base sequence analysis and bioinformatics. The development of genomics has further made it possible to understand the evolutionary processes of organisms through comparative studies among the genomes of many organisms.

This book series will discuss the findings obtained during the advancement of human genetics and genomics. It is also expected that this series will trigger the formation of a better world composed of human beings and all other organisms on Earth through discussions of research results obtained under the development of general genetics.

Meet the Series Editor

Kenji Ikehara graduated from the Department of Industrial Chemistry, Faculty of Engineering, Kyoto University in 1968. He received his B. Eng. (1968) and subsequently earned M. Eng. (1970) and D. Eng. (1976) degrees from Kyoto University. He began his career as a research associate in the Faculty of Science at the University of Tokyo before moving on to become an associate professor in the Faculty of Science at Nara Women's University. He was later promoted to professor and subsequently served as the dean of the Faculty of Science at Nara Women's University. Additionally, he held the position of director at the Nara Study Center of the Open University of Japan. For approximately 15 years, he focused his research on sporulation initiation of Bacillus subtilis. Later, he shifted his focus to the origins and evolutionary processes of microbial genes, the genetic code, proteins, and life. He has proposed several hypotheses, including the GC-NSF(a) hypothesis on the origin of genes, the GNC-SNS hypothesis on the genetic code, the protein 0th-order structure hypothesis on the origin of proteins, and the [GADV]-protein world hypothesis (GADV hypothesis) on the origin of life. Furthermore, he served as the local chair of the International Conference, Origin 2014, held in Nara in 2014.

Meet the Volume Editors

Prof. Dr. rer. nat. Arli Aditya Parikesit is a Bioinformatics Streaming Professor at the Indonesia International Institute for Life Sciences (i3L). He earned his bachelor's and master's degrees in chemistry from the University of Indonesia. Awarded by the German Academic Exchange Service (DAAD), he pursued his doctorate in bioinformatics at the University of Leipzig, Germany, with a focus on protein domain annotation across the three domains of life. Prof. Parikesit specializes in immunoinformatics, structural bioinformatics, in silico drug design, and transcriptomics. He developed pipelines for COVID-19 drug and vaccine design and is currently advancing LAMP-CRISPR diagnostics for infectious diseases, combining cutting-edge bioinformatics with practical applications in global health.

Dr. Arif Nur Muhammad Ansori is an experienced virologist and molecular biologist specializing in natural products. He earned his bachelor's and master's degrees in Biology, Vaccinology, and Immunotherapeutic Science from Universitas Airlangga, Indonesia. Currently, he is a researcher at Universitas Airlangga's Postgraduate School and a Junior Researcher at Kumamoto University's Division of Genomics and Transcriptomics in Japan. He also serves as an Adjunct Faculty Member at Uttaranchal University, India, and is affiliated with the European Virus Bioinformatics Center. Dr. Ansori has published over 100 international articles with an h-index of 22. Awarded the MEXT Scholarship 2024 and the TCID Author Award, he is an Associate Editor for multiple Frontiers journals, Founder of CV Jalan Tengah, and Director of the Virtual Research Center for Bioinformatics and Biotechnology. He is active in scientific societies and multilingual.

Adhityo Wicaksono, Ph.D., is a plant molecular biologist and bioinformatician from Indonesia, currently serving as Bioinformatics Research Fellow at Genomik Solidaritas Indonesia (GSI) Lab. He earned his Ph.D. in Chemical Engineering from Åbo Akademi University, Finland, where he studied biomechanics and bioinspired design. His research spans plant omics, molecular docking, and dynamics, with a focus on parasitic plants (Rafflesiaceae), crop plants, and synthetic biology. He has held postdoctoral positions at Chulalongkorn University, Thailand, and Institut Teknologi Bandung, Indonesia, where he worked on stress-responsive transcription factors and banana ripening omics. Dr. Wicaksono has authored over 50 peer-reviewed publications, several books, and is active in iGEM as a judge and mentor. His multidisciplinary work bridges the fields of plant science, bioinformatics, and biotechnology.

Contents

Preface

The advent of CRISPR technologies has ushered in a new era in genome editing, revolutionizing biological research and its applications. This edited book, *CRISPR Technologies – Advances in Genome Editing, Applications, and Ethical Implications,* provides a clear and detailed account of the rapidly growing developments and diverse applications of CRISPR-Cas systems. It gathers contributions that collectively reflect state-of-the-art developments, real-life applications across different domains, and the significant ethical and societal implications of being connected to such a revolutionary technology.

The initial chapters set forth background knowledge of CRISPR systems, presenting their evolution and molecular processes. The chapters demonstrate how CRISPR-Cas functions as a bacterial and archaeal adaptive immune system, and how natural processes are leveraged for targeted genome editing. It is necessary to understand this biological background to appreciate the subsequent engineering developments and technical innovations that have made CRISPR more precise and applicable in a diverse array of ways.

Acknowledging that scientific advancement must weigh innovation against responsibility, the ensuing section of the book explores the ethics, law, and societal considerations of CRISPR. This entails subtle discussions of biosafety, governance, and civic involvement. The book also addresses rising concerns regarding fair access and control of human genome editing, promoting a detailed appreciation of the responsibilities that accompany such powerful technologies.

The latter part highlights the wide-ranging applications of CRISPR technologies in various fields. Deliberation extends from therapeutic genome editing for correcting genetic diseases to its applied usage in agriculture for crop improvement and food safety. The book also discusses a wide range of CRISPR editing tools for medical and agricultural biotechnology, further expanding the tool's functionality from simple gene editing to new horizons.

Throughout the book, great emphasis is placed on interdisciplinary approaches that combine genome editing with bioinformatics, computational biology, and artificial intelligence to improve editing effectiveness and specificity. This also reflects the shifting landscape of CRISPR research, in that interdisciplinarity across fields is required to move laboratory discoveries to practical applications in everyday life.

CRISPR Technologies – Advances in Genome Editing, Applications, and Ethical Implications is intended to serve as a comprehensive reference for researchers, practitioners, teachers, policymakers, and other stakeholders. For those new to the subject or seeking deeper

insights, this volume provides a clear, authoritative, and balanced account of the prospects and challenges of CRISPR. It offers not just a recitation of the massive scientific achievements but also of the necessary conversations to frame the proper development and deployment of genome editing technologies.

Arli Aditya Parikesit
Department of Biotechnology,
Indonesia International Institute for Life Sciences,
Jakarta, Indonesia

Arif Nur Muhammad Ansori
School of Postgraduate Studies,
Universitas Airlangga,
Surabaya, Indonesia

Adhityo Wicaksono
GSI Lab,
Jakarta, Indonesia

Section 1

Fundamentals of CRISPR Technology

Chapter 1

Introductory Chapter: CRISPR Technologies – Advances in Genome Editing, Applications, and Ethical Implications

Arli Aditya Parikesit, Arif Nur Muhammad Ansori and Adhityo Wicaksono

1. Introduction

One of the most significant scientific breakthroughs of the twenty-first century is the discovery and creation of CRISPR-Cas systems, which have completely changed the genetic engineering industry. In the genomes of bacteria and archaea, what at first seemed to be a random pattern of repeating DNA sequences was really a component of an advanced adaptive immune system. Together with CRISPR-associated (Cas) proteins, these sequences—now known as Clustered Regularly Interspaced Short Palindromic Repeats, or CRISPR—allow microbes to identify and eliminate invasive viruses by severing their genetic material. Researchers have cleverly transformed this natural defensive system into a very precise and adaptable genome-editing tool.

2. From bacterial immunity to revolutionary technology

In 1987, Japanese scientist Yoshizumi Ishino and his team accidentally identified unusual repeated DNA sequences while analyzing an *Escherichia coli* gene [1]. These repetitive sequences, later termed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), remained enigmatic for years [2]. It was not until 2005 that researchers noted these sequences matched DNA from viruses that had previously infected the bacteria, suggesting a potential role in immunity [3].

The definitive breakthrough came in 2007 when Rodolphe Barrangou and colleagues at Danisco demonstrated that CRISPR, together with CRISPR-associated (Cas) proteins, functioned as an adaptive immune system in bacteria [4]. They showed that *Streptococcus thermophilus* could incorporate DNA snippets from attacking viruses into its genome as "spacers" within CRISPR arrays. These spacers served as a genetic memory, allowing bacteria to recognize and destroy the same viruses during subsequent infections [5].

This natural bacterial defense mechanism operates in three main stages: adaptation (acquisition of foreign DNA as spacers), expression (transcription of spacers into CRISPR RNA or crRNA), and interference (recognition and destruction of matching

foreign DNA) [6]. The most widely studied system, CRISPR-Cas9 from *Streptococcus pyogenes*, utilizes a single guide RNA (sgRNA) to direct the Cas9 endonuclease to specific DNA sequences, resulting in double-strand breaks at targeted sites.

The revolutionary potential of CRISPR for genome engineering was realized in 2012 when Jennifer Doudna, Emmanuelle Charpentier, and their teams demonstrated that the CRISPR-Cas9 system could be programmed to cut specific DNA sequences in vitro [7]. Shortly thereafter, in 2013, researchers showed that this system could be adapted for genome editing in mammalian cells, including human cells, opening the door to countless applications [8]. For their groundbreaking work, Doudna and Charpentier were awarded the Nobel Prize in Chemistry in 2020 [9].

3. Medical applications and clinical progress

CRISPR technology has rapidly advanced from a laboratory tool to a therapeutic platform. The medical applications are particularly promising for genetic disorders, infectious diseases, and cancer [10]. In late 2023, a significant milestone was reached with the approval of Casgevy, the first CRISPR-based medicine for treating sickle cell disease (SCD) and transfusion-dependent beta-thalassemia (TDT) [11]. Current clinical trials are exploring CRISPR-based treatments for various conditions, including leukemias, lymphomas, and urinary tract infections [12]. For cancer applications, engineered CAR-T cell therapies enhanced with CRISPR modifications are showing promising results, with some patients experiencing complete remission lasting months or even years [13]. The technology enables more precise and efficient genetic modifications compared to earlier gene-editing methods, potentially offering solutions for previously untreatable conditions [11]. Researchers from the Children's Hospital of Philadelphia and Penn Medicine developed a patient-specific adenine base editing (ABE) therapy called kayjayguran abengcemeran (k-abe). This therapy is delivered via lipid nanoparticles (LNPs) directly to the liver, enabling repeat dosing and avoiding immune responses associated with viral vectors [14].

Beyond direct therapeutic applications, CRISPR has become invaluable for creating disease models to understand pathological mechanisms and test potential treatments [15]. The ability to introduce specific mutations or correct disease-causing variants allows researchers to study disease processes with unprecedented precision [16]. The hybrid system of CRISPR-LAMP is also deployed for diagnostics purposes, for instance, in Malaria disease monitoring [17, 18]. Furthermore, CRISPR screens enable high-throughput identification of genes involved in cellular processes and disease states, accelerating drug discovery and development [19].

4. Beyond medicine: Agricultural, environmental and synthetic biology applications

CRISPR applications extend well beyond the medical field. In agriculture, CRISPR offers promising approaches for crop improvement, including enhanced yield, disease resistance, drought tolerance, and nutritional content [20]. The technology allows for precise genetic modifications without introducing foreign DNA, potentially addressing some regulatory and public acceptance concerns associated with traditional genetically modified organisms [21]. Precision genome editing now enables the

targeted alteration of regulatory elements [22], epigenetic factors [23], and non-coding RNAs that influence complex traits in plants [24].

In environmental science and conservation, researchers are exploring CRISPR for various applications, including the development of gene drives to control disease vectors and invasive species [20]. The technology also holds the potential for bioremediation, enabling the engineering of microorganisms capable of degrading environmental pollutants [25]. CRISPR multiplexing, where multiple genes are edited simultaneously, has also been deployed to improve crop quality [26]. Because of its adaptability, CRISPR can be used in industrial biotechnology to precisely genetically edit microbes for improved chemical synthesis, biofuel generation, and other processes [20].

Acknowledgements

The authors would like to thank the Department of Research and Community Service of Indonesia International Institute for Life Sciences (LPPM i3L) for their heartfelt support. This manuscript was proofread and improved with the Grammarly Premium Software.

Author details

Arli Aditya Parikesit[1*], Arif Nur Muhammad Ansori[2,3,4] and Adhityo Wicaksono[5]

1 Bioinformatics Streaming, Department of Biotechnology, School of Life Sciences, Indonesia International Institute for Life Sciences, Indonesia

2 Drug and Vaccine Innovation Research Group, Virtual Research Center for Bioinformatics and Biotechnology, Surabaya, Indonesia

3 European Virus Bioinformatics Center, Jena, Germany

4 Postgraduate School, Universitas Airlangga, Surabaya, Indonesia

5 Genomik Solidaritas Indonesia Lab (GSI Lab), Jakarta, Indonesia

*Address all correspondence to: arli.parikesit@i3l.ac.id

References

[1] Bio B. A Brief History of CRISPR-Cas9 Genome-Editing Tools. 2024. Available from: https://bitesizebio.com/47927/history-crispr/

[2] Al-Attar S, Westra ER, van der Oost J, Brouns SJJ. Clustered regularly interspaced short palindromic repeats (CRISPRs): The hallmark of an ingenious antiviral defense mechanism in prokaryotes. Biological Chemistry. 2011;**392**(4):277-289. DOI: 10.1515/BC.2011.042

[3] Li J, Wu S, Zhang K, Sun X, Lin W, Wang C, et al. Clustered regularly interspaced short palindromic repeat/CRISPR-associated protein and its utility all at sea: Status, challenges, and prospects. Microorganisms. 2024;**12**(1):118. DOI: 10.3390/microorganisms12010118

[4] 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**(5819):1709-1712. DOI: 10.1126/science.1138140

[5] Shivram H, Cress BF, Knott GJ, Doudna JA. Controlling and enhancing CRISPR systems. Nature Chemical Biology. 2021;**17**(1):10-19. DOI: 10.1038/s41589-020-00700-7

[6] Sander JD, Joung JK. A broad overview and review of CRISPR-Cas technology and its applications. Genome Biology. 2016;**17**:1-16. DOI: 10.1186/s13059-016-1012-2

[7] KoLedford H, Callaway E. Pioneers of revolutionary CRISPR gene editing win chemistry Nobel. Nature. 2020;**586**(7829):346-347. DOI: 10.1038/d41586-020-02765-9

[8] Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J. RNA-programmed genome editing in human cells. eLife. 2013;**2**:e00471. DOI: 10.7554/eLife.00471

[9] Westermann L, Neubauer B, Köttgen M. Nobel prize 2020 in chemistry honors CRISPR: A tool for rewriting the code of life. Pflügers Archiv. 2021;**473**(1):1-2. DOI: 10.1007/s00424-020-02497-9

[10] Wu S-S, Li Q-C, Yin C-Q, Xue W, Song C-Q. Advances in CRISPR/Cas-based gene therapy in human genetic diseases. Theranostics. 2020;**10**(10):4374-4382. DOI: 10.7150/thno.43360

[11] Institute, I. G. CRISPR Clinical Trials: A 2024 Update. 2024. Available from: https://innovativegenomics.org/news/crispr-clinical-trials-2024/

[12] Zhang S, Wang Y, Mao D, Wang Y, Zhang H, Pan Y, et al. Current trends of clinical trials involving CRISPR/Cas systems. Frontiers in Medicine. 2023;**10**:1292452. DOI: 10.3389/fmed.2023.1292452

[13] Developers. CRISPR Clinical Trials: A 2024 Update. Berkeley, California: Innovative Genomics Institute (IGI); 2024. Available from: https://innovativegenomics.org/news/crispr-clinical-trials-2024/

[14] Musunuru K, Grandinette SA, Wang X, Hudson TR, Briseno K, Berry AM, et al. Patient-specific in vivo gene editing to treat a rare genetic disease. The New England Journal of Medicine. Advance Online Publication. 2025;**392**(22):2235-2243. DOI: 10.1056/NEJMoa2504747. Available from: https://pubmed.ncbi.nlm.nih.gov/40373211/

[15] Azeez SS, Hamad RS, Hamad BK, Shekha MS, Bergsten P. Advances in CRISPR-Cas technology and its applications: Revolutionising precision medicine. Frontiers in Genome Editing. 2024;**6**:1509924. DOI: 10.3389/fgeed.2024.1509924

[16] Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;**157**(6):1262-1278. DOI: 10.1016/j.cell.2014.05.010

[17] Parikesit AA, Hermantara R, Kevin G, Sidhartha E. Designing hybrid CRISPR-Cas12 and LAMP detection systems for treatment-resistant plasmodium falciparum with an in silico method. Narra J. 2023;**3**(3):3. DOI: 10.52225/narra.v3i3.301

[18] Parikesit AA, Hermantara R, Sidharta E, Fugaha D, Herlie D, Aslanzadeh S. Optimizing CRISPR-Cas12-based detection methods for drug-resistant plasmodium falciparum with an advanced gene visualization pipeline. Journal of Research in Pharmacy. 2025;**29**(1):1078-1088. Available from: https://dergipark.org.tr/en/pub/jrespharm

[19] Bock C, Datlinger P, Chardon F, Coelho MA, Dong MB, Lawson KA, et al. High-content CRISPR screening. Nature Reviews Methods Primers. 2022;**2**(1):1-23. DOI: 10.1038/s43586-021-00093-4

[20] Ansori AN, Antonius Y, Susilo RJ, Hayaza S, Kharisma VD, Parikesit AA, et al. Application of CRISPR-Cas9 genome editing technology in various fields: A review. Narra J. 2023;**3**(2):2. DOI: 10.52225/narra.v3i2.184

[21] Ahmad A, Jamil A, Munawar N. GMOs or non-GMOs? The CRISPR conundrum. Frontiers in Plant Science. 2023;**14**:1232938. DOI: 10.3389/fpls.2023.1232938

[22] Wolter F, Puchta H. Application of CRISPR/Cas to understand cis- and trans-regulatory elements in plants. In: Yamaguchi N, editor. Plant Transcription Factors. Methods in Molecular Biology. Vol. 1830. New York, NY: Humana Press; 2018. DOI: 10.1007/978-1-4939-8657-6_2

[23] Jogam P, Sandhya D, Alok A, Peddaboina V, Allini VR, Zhang B. A review on CRISPR/Cas-based epigenetic regulation in plants. International Journal of Biological Macromolecules. 2022;**219**:1261-1271. DOI: 10.1016/j.ijbiomac.2022.08.182

[24] Bravo-Vázquez LA, Méndez-García A, Chamu-García V, Rodríguez AL, Bandyopadhyay A, Paul S. The applications of CRISPR/Cas-mediated microRNA and lncRNA editing in plant biology: Shaping the future of plant non-coding RNA research. Planta. 2024;**259**(2):32. DOI: 10.1007/s00425-023-04303-z

[25] Saxena S. Enhancing microbial bioremediation: The role of CRISPR-Cas9 in? Environmental restoration. International Journal for Research in Applied Science and Engineering Technology. 2025;**13**(1):1804-1813. DOI: 10.22214/ijraset.2025.66660

[26] Abdelrahman M, Wei Z, Rohila JS, Zhao K. Multiplex genome-editing technologies for revolutionizing plant biology and crop improvement. Frontiers in Plant Science. 2021;**12**:721203. DOI: 10.3389/fpls.2021.721203

Section 2

Ethical and Legal Aspects of CRISPR Technology

Chapter 2

Ethical and Legal Dimensions of Access to CRISPR Technologies in Rare Disease Therapies

Kübra Telli, Berkay Özdemir, Sude Danışman and Emrah Yücesan

Abstract

The CRISPR/Cas9 system has progressed from a prokaryotic immune defense to a foundational technology in genome engineering and precision medicine. Initially identified in *Escherichia coli* in 1987 as unexplained repetitive DNA elements, its role as an adaptive immune mechanism in bacteria and archaea was gradually elucidated over the following decades. Pivotal advances between 2002 and 2012 culminating in the demonstration by Doudna and Charpentier of RNA-guided DNA cleavage unlocked its transformative potential for targeted genome editing. Since then, CRISPR/Cas9 has enabled durable, mutation-specific interventions in monogenic disorders such as sickle cell anemia, β-thalassemia, and Leber congenital amaurosis. However, the global implementation of CRISPR-based therapies remains uneven, hindered by disparities in infrastructure, regulatory frameworks, and ethical oversight. As an intermediately developed country with a disproportionately high burden of rare genetic diseases, Türkiye presents a compelling case of both advancement and ongoing limitations. Its national rare disease strategy, evolving legal access mechanisms, and active engagement in international consortia underscore its potential to serve as a regional exemplar for equitable and ethically responsible integration of CRISPR-based therapeutics.

Keywords: CRISPR technologies, rare diseases, ethical implications, legal framework, orphan drugs

1. Introduction

1.1 CRISPR/Cas9

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a technique created by mimicking the nature. The key milestones in the evolution of CRISPR/Cas9 range from its initial discovery in prokaryotes to its adaptation as a genome editing tool (**Figure** 1). In a 1987 study in which the iap gene of *E. coli* was

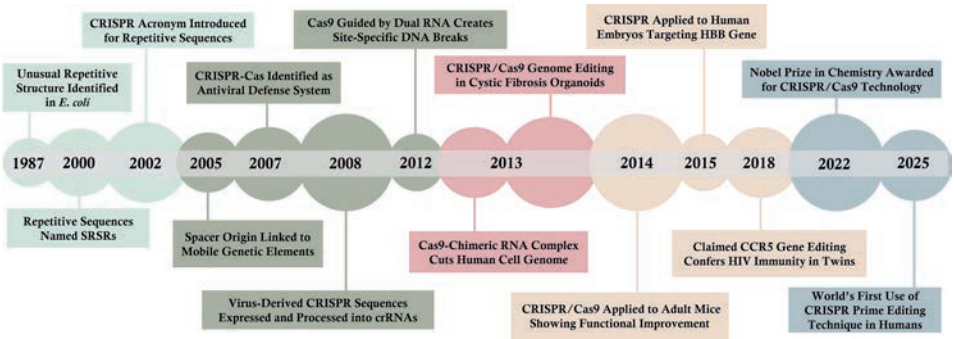

Figure 1.
Timeline of CRISPR/Cas9 discovery and development. Major milestones in the evolution of CRISPR/Cas9 systems are illustrated, beginning with the identification of repetitive DNA sequences in E. coli *in 1987, followed by the elucidation of their function as a prokaryotic adaptive immune system, and culminating in the development of such versatile genome editing tool.*

sequenced and the gene product was identified, a structure described by researchers as "unusual" was found at the 3′ end of iap. Five homologous sequences, each 29 nucleotides long, were separated by 32-nucleotide intervals. The researchers, who noted that the biological significance of this structure was unknown, had discovered the sequences we now know as CRISPR [1]. These discovered repeating sequences were named Short Regularly Spaced Repeats (SRSRs) in a MicroCorrespondence released in 2000 [2]. Two years later, the repeated sequences were referred to as CRISPR [3]. In 2005, it was shown that spacers originated from transferable genetic elements such as bacteriophages or conjugative plasmids. This finding suggested that CRISPR is related to the immune system [4]. It was discovered that CRISPR-Cas systems found in prokaryotes are a genomic defense mechanism against bacteriophage in 2007 [5]. The following year, it was shown that virus-derived CRISPR sequences are expressed and produce precursor CRISPR RNA (crRNA), which are then cleaved by Cas proteins to form mature CRISPR RNAs [6]. In 2012, Doudna, Charpentier, et al. described that the double RNA structure directs the Cas9 endonuclease to create site-specific double-strand breaks in the target DNA. They also demonstrated that the chimeric guide RNA, created by combining crRNA and tracrRNA, can be used with Cas9 to cleave a specific dsDNA sequence [7, 8]. It employs a single guide RNA (sgRNA) along with the Cas9 endonuclease to accurately locate and alter designated DNA regions of interest. The editing process initiates when the sgRNA, a fusion of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA), forms a complex with the Cas9 enzyme [9–11]. This ribonucleoprotein complex is directed to the target DNA site by the sgRNA, which ensures specificity through recognition of a short DNA sequence known as the protospacer adjacent motif (PAM). Upon binding to the correct location, Cas9 introduces double-strand breaks (DSBs) in DNA [12–14]. These breaks are subsequently repaired by cell's intrinsic repair systems, predominantly through two pathways: nonhomologous end joining (NHEJ) or HDR. NHEJ often results in insertions or deletions, thereby disrupting gene function, while HDR allows precise modifications by using an external DNA template [15–19]. In 2013, using the CRISPR-Cas system of *Streptococcus pyogenes*, it has been shown that the complex formed by the Cas9 protein and artificial chimeric RNA can cut the genome in human cells [20]. The same year, genome editing was performed using the CRISPR/Cas9 system in intestinal stem cell organoids from cystic fibrosis patients.

This study provided the first concept proof for CRISPR-mediated gene correction via homology-directed repair (HDR) in a clinically relevant single-gene disorder [21]. In 2014, the CRISPR/Cas9 method was applied directly to adult mice for the first time, resulting in functional improvement in the mouse model of tyrosinemia [22]. In the first study in which CRISPR was applied to human embryos in 2015, the focus was on the HBB gene, whose pathogenic variants cause beta-thalassemia [23]. Three years later, it was reported that twin girls born in China were immune to HIV. He Jiankui claimed that he had made modifications to the C-C chemokine receptor type 5 (CCR5) gene using the CRISPR/Cas9 method, thereby conferring immunity to the human immunodeficiency virus (HIV) virus. Developments in the CRISPR/Cas9 system have revolutionized modern biotechnology, and in 2020, Charpentier and Doudna were awarded the Nobel Prize in Chemistry. Building upon these historical milestones, the molecular intricacies of the CRISPR/Cas9 system have since been elucidated, revealing a remarkably precise genome editing mechanism grounded in microbial defense biology.

Although CRISPR/Cas9 has revolutionized gene editing, by its groundbreaking potential in correcting pathogenic gene anomalies, practical application still faces substantial limitations. A major challenge remains the efficient intracellular delivery of the Cas9/gRNA complex, which necessitates precise timing and concentration [24, 25]. Even with successful delivery, key scientific hurdles include the risk of off-target effects and persistent technical limitations in delivery and editing precision. Editing efficiency might be low, particularly for gene knock-in strategies reliant on HDR, even though recent advancements have improved HDR outcomes [26, 27]. CRISPR also faces profound ethical and societal limitations such as concerns about germline editing, enhancement, inequality, regulatory inconsistencies, and long-term safety underscore the need for cautious implementation, clear oversight, and equitable access [28–31]. Altogether, the ongoing expansion and functional diversification of CRISPR/Cas9 underscore their evolutionary adaptability and technological potential, with deeper molecular insights and advancing engineering strategies expected to further propel their evolution and drive innovation across basic research, biotechnology, and translational therapeutics [12]. While the molecular underpinnings of CRISPR/Cas9 have established its technical viability, its greatest promise lies in translational medicine particularly in addressing the unmet needs of patients with rare diseases.

2. CRISPR/Cas9 in rare diseases

Rare diseases collectively affect over 300 million individuals worldwide. A substantial proportion of these conditions are monogenic in origin, resulting from mutations in a single gene, and are typically marked by early onset, high morbidity, and limited treatment options [32]. Approximately 80% of rare diseases have a genetic basis, with nearly 70% manifesting in childhood. On average, an accurate diagnosis takes 4.8 years, and nearly 30% of affected children die before the age of five [33]. Alarmingly, around 95% of these disorders lack approved therapies. Conventional therapeutic approaches include dietary modifications, surgical interventions, pharmacological treatments, and hematopoietic stem cell transplantation (HSCT), particularly in inherited conditions [34, 35]. Pharmacological strategies range from small-molecule drugs and monoclonal antibodies to enzyme replacement therapies (ERT), stem cell-based treatments, and drug repurposing, each targeting distinct molecular mechanisms [36]. Despite regulatory and economic incentives,

drugs developed using these strategies are categorized as orphan drugs, which are neglected by pharmaceutical companies due to their high costs [37]. However, rare disease therapies face critical limitations such as insufficient mechanistic understanding, prohibitively high development costs, small patient cohorts that complicate clinical validation, and substantial challenges in therapeutic delivery especially across the blood–brain barrier [38]. Moreover, stark global disparities persist in diagnosis and access to care. In light of these challenges, CRISPR/Cas9 represents potentially transformative therapeutic platform. They offer unmatched precision, programmability, and the capacity for durable, mutation-specific genomic correction, directly addressing the root causes that conventional therapies often cannot. Recent breakthroughs have expanded their clinical promise, paving the way for more precise and personalized interventions with substantial potential to improve outcomes in complex and previously untreatable rare disorders [39–41]. Numerous diseases could be given as examples; however, below we will present a few selected ones to clarify the topic more clearly. For instance, in sickle cell anemia, abnormal hemoglobin is formed by the substitution of glutamic acid with valine at position 6 of the HBB gene. The resulting sickle hemoglobin causes deformation of erythrocytes, chronic hemolysis, vaso-occlusion, and end-organ damage [42–44]. Fetal hemoglobin can reduce the symptoms of sickle cell disease. For this reason, Exagamglogene autotemcel (exa-cel), the first drug approved by the FDA for sickle cell disease, focuses on the BCL11A transcription factor that suppresses fetal hemoglobin [44–46]. The CRISPR/Cas9 system cuts the enhancer region of the BCL11A gene to enable fetal hemoglobin production [44, 47]. A phase 3 study was conducted on 44 patients aged 12–35 with sickle cell disease. In the 2-year study, the median follow-up period for individuals was 19.3 months. As a result of the treatment, 97% of patients experienced no vaso-occlusive crises for 12 months or longer [48]. As an another example, β-thalassemia occurs due to more than 300 different mutations in the HBB gene. All of these mutations cause a quantitative decrease in β-globin protein and thus adult-type hemoglobin (HbA, $\alpha 2\beta 2$) [49]. As in sickle cell disease, fetal hemoglobin reduces the symptoms of beta-thalassemia. Therefore, the aim of exa-cel treatment is to increase fetal hemoglobin production in patients [44]. The phase 3 exa-cel study was conducted on 55 transfusion-dependent thalassemia patients aged 12 to 35 years, with a median follow-up duration of 20.4 months and a total study period of approximately two years. Transfusion independence was achieved in 91% of patients following treatment with exa-cel [50]. Lastly, Leber congenital amaurosis type 10 is a disease that shows an autosomal recessive inheritance pattern caused by the ISV26 mutation in the CEP290 gene. It manifests itself in childhood with severe visual impairment or blindness [51]. As a gene-editing approach, EDIT-101 was developed to correct mutations in the CEP290 gene by delivering *Staphylococcus aureus* Cas9 and CEP290-specific guide RNA to photoreceptor cells using AAV5 [52]. The Phase 1–2 study of EDIT-101 was conducted in individuals aged 3 years and older with homozygous or compound heterozygous IVS26 variants and CEP290-related hereditary retinal degeneration. Four non-ocular serious adverse events were reported following the treatment. One participant with a history of epilepsy experienced two seizures and a major depressive episode. Approximately 41% of adverse events were attributed to the surgical procedure itself. While some safety concerns were noted, the observed improvements in photoreceptor function suggest that EDIT-101 warrants further clinical investigation [53]. Despite these remarkable global advances, the application of CRISPR-based therapies remains uneven worldwide, with many countries facing systemic, regulatory, and infrastructural barriers that hinder equitable patient access.

3. Ethical, legal, and access challenges in CRISPR-based rare disease therapies: Cross-national perspectives

The integration of CRISPR/Cas9 into rare disease therapeutics marks a transformative advance in precision medicine. Yet, its implementation has highlighted significant cross-national differences in healthcare infrastructure, ethical oversight, and legal preparedness. These disparities emphasize the importance of coordinated international efforts to ensure equitable and responsible access to gene-based innovations. The global response to rare diseases has evolved considerably over recent decades [54]. Initiated by the National Organization for Rare Disorders (NORD) in the USA, whose advocacy led to the 1983 Orphan Drug Act, this movement has expanded through the establishment of EURORDIS, now representing nearly 900 patient organizations across more than 70 countries [55]. Similar alliances in Germany (ACHSE), Spain (FEDER), Canada (CORD), Japan (ASrid), and India (ORDI) have contributed to building a global framework for collaboration. These efforts have been further consolidated through transnational initiatives such as the International Rare Diseases Research Consortium (IRDiRC) and Orphanet, a comprehensive open-access database led by the EU and coordinated by France. Differences in ethical governance are another key area of divergence. While high-income countries such as the USA and EU members have established robust norms around informed consent, pediatric data protection, and biobank governance, implementation in low- and middle-income countries (LMICs) may be challenged by limited resources and differing legal traditions [56]. Nevertheless, such environments also present valuable opportunities to develop culturally sensitive, context-specific ethical frameworks. Greater inclusivity in global discussions particularly around data sovereignty, germline implications, and fair resource distribution can help bridge current gaps and foster more equitable innovation. As gene therapy options expand, ethical questions grow increasingly complex. Issues such as patient autonomy, risk-benefit thresholds, and therapeutic versus enhancement applications require nuanced debate [57]. These discussions extend beyond legal and medical realms, engaging deeply with philosophical dimensions. Engaging bioethicists, legal scholars, clinicians, and patient communities in open dialog will be essential to guiding policy in an inclusive and ethically grounded direction [58].

4. Opportunities and barriers for CRISPR-based rare disease therapies in Türkiye: Ethical, legal, and access considerations in an intermediately developed country

Türkiye has emerged as an active participant in global rare disease initiatives, with nine national associations affiliated with EURORDIS (https://www.eurordis.org/about-eurordis). Under the leadership of the Ministry of Health, the country is working toward deeper integration into these platforms and expanding national access, reflecting its growing commitment to international partnerships in rare disease research and policy (https://www.tuseb.gov.tr/en). Türkiye represents a particularly instructive case due to its unique demographic and genetic landscape. The country faces a high prevalence of monogenic rare diseases, partly attributable to consanguineous marriages, and an estimated five million individuals are directly or indirectly affected [59]. Recent milestones, such as the establishment of the Department of Autism, Intellectual Disabilities, and Rare Diseases in 2020 and the

publication of a national rare disease strategy in 2022, signal promising steps toward building a coherent national framework. Despite these advances, access to rare disease treatments remains a significant challenge. Of the 416 orphan drugs approved by the FDA and EMA, only 151 are available in Türkiye 76 of which are licensed, while 75 remain unlicensed. However, the Social Security Institution (SGK) has recently begun expanding reimbursement coverage for selected EMA-approved treatments, marking a gradual shift toward more inclusive healthcare policies. Legal mechanisms also play a critical role in improving access. When high-cost therapies are not listed in the Health Implementation Communiqué (SUT), patients can pursue legal remedies. This process, initiated through physician applications to the Türkiye Medicines and Medical Devices Agency (TITCK) (https://www.titck.gov.tr) and followed by judicial review, has successfully enabled coverage for numerous rare disease treatments. These legal precedents underscore the principle that constitutional rights especially the right to health can and should be safeguarded through actionable judicial pathways. However, gene therapies such as CRISPR remain in the early stages of adoption. Türkiye currently lacks the specialized application centers, reimbursement frameworks, and regulatory guidelines needed for clinical implementation. Building this infrastructure will require coordinated efforts among key government institutions, including the Ministries of Health, Treasury and Finance, Environment and Urban Planning, and the Presidency of Strategy and Budget, alongside academic and research bodies. While these plans are not yet fully reflected in national policy documents, increasing awareness and strategic investment could catalyze future progress. Given the sensitive nature of gene therapy, data protection represents a critical ethical and legal consideration. Türkiye's Law on the Protection of Personal Data (No. 6698) classifies genetic data as sensitive personal information [60]. While this legal framework ensures a strong baseline for privacy, it also highlights the need for clear and consistent ethical guidelines for data processing and informed consent particularly as gene-editing technologies evolve rapidly. In conclusion, while Türkiye still faces challenges in fully integrating CRISPR/Cas9-based therapies, it has made notable strides toward alignment with international rare disease initiatives. As an intermediately developed country with growing scientific capacity and strong constitutional guarantees for health, Türkiye is uniquely positioned to serve as a model for similarly situated nations. With sustained investment in infrastructure, regulatory clarity, and ethical oversight, the country can play a pivotal role in advancing equitable access to next-generation therapies and shaping a more inclusive global future for genomic medicine.

5. Conclusive remarks

CRISPR/Cas9 has revolutionized genomic medicine by enabling precise and durable correction of disease-causing mutations, with landmark clinical advances in monogenic rare disorders such as sickle cell anemia, β-thalassemia, and Leber congenital amaurosis. However, the global implementation of CRISPR-based therapies remains uneven, constrained by disparities in regulatory frameworks, infrastructure, reimbursement systems, and ethical oversight. Addressing these challenges necessitates coordinated international strategies to ensure safe, equitable, and ethically responsible access to gene-editing technologies. Türkiye, as an intermediately developed country with a high prevalence of rare genetic disorders, presents a compelling case of both progress and persistent challenges. It has taken notable steps through the

publication of a national rare disease strategy, the establishment of judicial pathways for accessing high-cost treatments, and active involvement in international rare disease consortia. Yet, key obstacles remain, including the absence of a formal legal definition for rare diseases, limited access to licensed orphan drugs, and a lack of authorized centers for gene therapy delivery. Despite these gaps, Türkiye's growing scientific infrastructure, evolving policy landscape, and strong constitutional commitment to health form a robust foundation for future advances. As ethical, legal, and institutional frameworks continue to mature, Türkiye is well-positioned to serve as a model for similarly situated nations seeking to integrate CRISPR-based therapies. Sustained investment in regulatory clarity, infrastructure development, and bioethical oversight will be essential for transforming CRISPR's potential into equitable, real-world benefits offering hope to millions affected by rare genetic conditions.

Conflict of interest

The authors declare no conflict of interest.

Author details

Kübra Telli[1*], Berkay Özdemir[2], Sude Danışman[3] and Emrah Yücesan[3]

1 Department of Molecular Biology and Genetics, Istanbul Kultur University, Istanbul, Turkey

2 Ankara Bar Association, Platform for Rare Diseases, Cancer, and Diabetes Law, Ankara, Turkey

3 Department of Institute of Neurological Sciences, Istanbul University Cerrahpasa, Istanbul, Turkey

*Address all correspondence to: k.telli@iku.edu.tr

References

[1] Ishino Y, Shinagawa H, Makino K, Amemura M, Nakatura A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. Journal of Bacteriology. 1987;**169**(12):5429. DOI: 10.1128/JB.169.12.5429-5433.1987

[2] Mojica FJM, Díez-Villaseñor C, Soria E, Juez G. Biological significance of a family of regularly spaced repeats in the genomes of archaea, bacteria and mitochondria. Molecular Microbiology. 2000;**36**(1):244-246. DOI: 10.1046/J.1365-2958.2000.01838.X

[3] Jansen R, Van Embden JDA, Gaastra W, Schouls LM. Identification of genes that are associated with DNA repeats in prokaryotes. Molecular Microbiology. 2002;**43**(6):1565-1575. DOI: 10.1046/J.1365-2958.2002.02839.X

[4] Mojica FJM, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. Journal of Molecular Evolution. 2005;**60**(2):174-182. DOI: 10.1007/S00239-004-0046-3

[5] 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**(5819):1709-1712. DOI: 10.1126/SCIENCE.1138140

[6] Brouns SJJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJH, Snijders APL, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science (New York, N.Y.). 2008;**321**(5891):960. DOI: 10.1126/SCIENCE.1159689

[7] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (New York, N.Y.). 2012;**337**(6096):816-821. DOI: 10.1126/science.1225829

[8] Katti A, Diaz BJ, Caragine CM, Sanjana NE, Dow LE. CRISPR in cancer biology and therapy. Nature Reviews Cancer. 2022;**22**(5):259279. DOI: 10.1038/s41568-022-00441-w

[9] Nambiar TS, Baudrier L, Billon P, Ciccia A. CRISPR-based genome editing through the lens of DNA repair. Molecular Cell. 2022;**82**(2):348388. DOI: 10.1016/j.molcel.2021.12.026

[10] Villiger L, Joung J, Koblan L, Weissman J, Abudayyeh OO, Gootenberg JS. CRISPR technologies for genome, epigenome and transcriptome editing. Nature Reviews Molecular Cell Biology. 2024;**25**(6):464487. DOI: 10.1038/s41580-023-00697-6

[11] Wilbie D, Walther J, Mastrobattista E. Delivery aspects of CRISPR/Cas for in vivo genome editing. Accounts of Chemical Research. 2019;**52**(6):1555-1564. DOI: 10.1021/acs.accounts.9b00106

[12] Zheng R, Zhang L, Parvin R, Su L, Chi J, Shi K, et al. Progress and perspective of CRISPR/Cas9 technology in translational medicine. Advanced Science. 2023;**10**(15):2300195. DOI: 10.1002/advs.202300195

[13] Yang H, Patel DJ. Structures, mechanisms and applications of RNA-centric CRISPR-Cas13. Nature Chemical Biology. 2024;**20**(6):673688. DOI: 10.1038/s41589-024-01593-6

[14] Volodina O, Smirnikhina S. The future of gene therapy: A review of

In vivo and ex vivo delivery methods for genome editing-based therapies. Molecular Biotechnology. 2025;**67**:425-437. DOI: 10.1007/s12033-024-01070-4

[15] Wang JY, Doudna JA. CRISPR technology: A decade of genome editing is only the beginning. Science (New York, N.Y.). 2023;**379**(6629):eadd8643. DOI: 10.1126/science.add8643

[16] Van Dongen JE, Berendsen JTW, Steenbergen RDM, Wolthuis RMF, Eijkel JCT, Segerink LI. Point-of-care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities. Biosensors and Bioelectronics. 2020;**166**:112445. DOI: 10.1016/j.bios.2020.112445

[17] Sharma G, Sharma AR, Bhattacharya M, Lee S-S, Chakraborty C. CRISPR/Cas9: A preclinical and clinical perspective for the treatment of human diseases. Molecular Therapy. 2021;**29**(2):571586. DOI: 10.1016/j.ymthe.2020.09.028

[18] Ghaemi A, Bagheri E, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M. CRISPR/Cas9 genome editing delivery systems for targeted cancer therapy. Life Sciences. 2021;**267**:118969. DOI: 10.1016/j.lfs.2020.118969

[19] Kim S, Ji S, Koh HR. CRISPR as a diagnostic tool. Biomolecules. 2021;**11**(8):1162. DOI: 10.3390/biom11081162

[20] Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nature Biotechnology. 2013;**31**(3):230-232. DOI: 10.1038/NBT.2507

[21] Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell. 2013;**13**(6):653-658. DOI: 10.1016/j.stem.2013.11.002

[22] Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nature Biotechnology. 2014;**32**(6):551-553. DOI: 10.1038/nbt.2884

[23] Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, et al. CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. Protein & Cell. 2015;**6**(5):363-372. DOI: 10.1007/S13238-015-0153-5

[24] Chiper M, Niederreither K, Zuber G. Transduction methods for cytosolic delivery of proteins and bioconjugates into living cells. Advanced Healthcare Materials. 2018;**7**(6):e1701040. DOI: 10.1002/adhm.201701040

[25] Yang Y, Xu J, Ge S, Lai L. CRISPR/Cas: Advances, limitations, and applications for precision cancer research. Frontiers in Medicine. 2021;**8**:649896. DOI: 10.3389/fmed.2021.649896

[26] Rasul MF, Hussen BM, Salihi A, et al. Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. Molecular Cancer. 2022;**21**:64. DOI: 10.1186/s12943-021-01487-4

[27] Pacesa M, Pelea O, Jinek M. Past, present, and future of CRISPR genome editing technologies. Cell. 2024;**187**(5):1076-1100. DOI: 10.1016/j.cell.2024.01.042

[28] Song M. The CRISPR/Cas9 system: Their delivery, in vivo and ex vivo applications and clinical development by startups. Biotechnology Progress.

2017;**33**(4):1035-1045. DOI: 10.1002/btpr.2484

[29] Behr M, Zhou J, Bing X, Zhang H. In vivo delivery of CRISPR-Cas9 therapeutics: Progress and challenges. Acta Pharmaceutica Sinica B. 2021;**11**(8):2150-2171. ISSN 2211-3835. DOI: 10.1016/j.apsb.2021.05.020

[30] Hryhorowicz M, Lipiński D, Zeyland J. Evolution of CRISPR/Cas systems for precise genome editing. International Journal of Molecular Sciences. 2023;**24**(18):14233. DOI: 10.3390/ijms241814233

[31] Schambach A, Buchholz CJ, Torres-Ruiz R, Cichutek K, Morgan M, Trapani I, et al. A new age of precision gene therapy. Lancet (London, England). 2024;**403**(10426):568-582. DOI: 10.1016/S0140-6736(23)01952-9

[32] Smith CIE, Bergman P, Hagey DW. Estimating the number of diseases—The concept of rare, ultra-rare, and hyper-rare. iScience. 2022;**25**(8):104698. DOI: 10.1016/j.isci.2022.104698

[33] Haendel M, Vasilevsky N, Unni D, Bologa C, Harris N, Rehm H, et al. How many rare diseases are there? Nature Reviews. Drug Discovery. 2020;**19**(2):77-78. DOI: 10.1038/d41573-019-00180-y

[34] Tambuyzer E, Vandendriessche B, Austin CP, Brooks PJ, Larsson K, Miller Needleman KI, et al. Therapies for rare diseases: Therapeutic modalities, progress and challenges ahead. Nature Reviews. Drug Discovery. 2020;**19**(2):93-111. DOI: 10.1038/s41573-019-0049-9

[35] Papaioannou I, Owen JS, Yáñez-Muñoz RJ. Clinical applications of gene therapy for rare diseases: A review. International Journal of Experimental Pathology. 2023;**104**(4):154-176. DOI: 10.1111/iep.12478. Epub 2023 May 13. Erratum in: Int J Exp Pathol. 2024 Jun;105(3):114. doi:10.1111/iep.12505

[36] Man A, Groeneweg GSS, Ross CJD, Carleton BC. The role of pharmacogenomics in rare diseases. Drug Safety. 2024;**47**(6):521-528. DOI: 10.1007/s40264-024-01416-6

[37] Yoo HW. Development of orphan drugs for rare diseases. Clinical and Experimental Pediatrics. 2023;**67**(7):315. DOI: 10.3345/CEP.2023.00535

[38] Larizza L, Cubellis MV. Rare diseases: Implementation of molecular diagnosis, pathogenesis insights and precision medicine treatment. International Journal of Molecular Sciences. 2023;**24**(10):9064. DOI: 10.3390/ijms24109064

[39] Roessler HI, Knoers NVAM, van Haelst MM, van Haaften G. Drug repurposing for rare diseases. Trends in Pharmacological Sciences. 2021;**42**(4):255-267. DOI: 10.1016/j.tips.2021.01.003

[40] Condò I. Rare monogenic diseases: Molecular pathophysiology and novel therapies. International Journal of Molecular Sciences. 2022;**23**(12):6525. DOI: 10.3390/ijms23126525

[41] Tesi B, Boileau C, Boycott KM, Canaud G, Caulfield M, Choukair D, et al. Precision medicine in rare diseases: What is next? Journal of Internal Medicine. 2023;**294**(4):397-412. DOI: 10.1111/joim.13655

[42] Laurent M, Geoffroy M, Pavani G, Guiraud S. CRISPR-based gene therapies: From preclinical to clinical treatments. Cells. 2024;**13**(800). DOI: 10.3390/cells13100800

[43] Brandow AM, Liem RI. Advances in the diagnosis and treatment of sickle

cell disease. Journal of Hematology & Oncology. 2022;**15**(1):20. DOI: 10.1186/S13045-022-01237-Z

[44] Frangoul H, Altshuler D, Cappellini MD, Chen Y-S, Domm J, Eustace BK, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. New England Journal of Medicine. 2021;**384**(3):252-260. DOI: 10.1056/NEJMOA2031054

[45] Bauer DE, Orkin SH. Hemoglobin switching's surprise: The versatile transcription factor BCL11A is a master repressor of fetal hemoglobin. Current Opinion in Genetics & Development. 2015;**33**:62. DOI: 10.1016/J.GDE.2015.08.001

[46] Steinberg MH. Fetal hemoglobin in sickle cell anemia. Blood. 2020;**136**(21):2392. DOI: 10.1182/BLOOD.2020007645

[47] Li Y, Glass Z, Huang M, Chen ZY, Xu Q. Ex vivo cell-based CRISPR/Cas9 genome editing for therapeutic applications. Biomaterials. 2020;**234**:119711. DOI: 10.1016/j.biomaterials.2019.119711

[48] Frangoul H, Locatelli F, Sharma A, Bhatia M, Mapara M, Molinari L, et al. Exagamglogene autotemcel for severe sickle cell disease. New England Journal of Medicine. 2024;**390**(18):1649-1662. DOI: 10.1056/NEJMOA2309676

[49] Christakopoulos GE, Telange R, Yen J, Weiss MJ. Gene therapy and gene editing for B thalassemia. Hematology/Oncology Clinics of North America. 2023;**37**(2):433. DOI: 10.1016/J.HOC.2022.12.012

[50] Locatelli F, Lang P, Wall D, Meisel R, Corbacioglu S, Li AM, et al. Exagamglogene autotemcel for transfusion-dependent β-thalassemia. New England Journal of Medicine. 2024;**390**(18):1663-1676. DOI: 10.1056/NEJMOA2309673

[51] Leroy BP, Birch DG, Duncan JL, Lam BL, Koenekoop RK, Porto FBO, et al. Leber congenital amaurosis due to Cep290 mutations-severe vision impairment with a high unmet medical need: A review. Retina. 2021;**41**(5):898-907. DOI: 10.1097/IAE.0000000000003133

[52] Maeder ML, Stefanidakis M, Wilson CJ, Baral R, Barrera LA, Bounoutas GS, et al. Development of a gene-editing approach to restore vision loss in leber congenital amaurosis type 10. Nature Medicine. 2019;**25**(2):229-233. DOI: 10.1038/s41591-018-0327-9

[53] Pierce EA, Aleman TS, Jayasundera KT, Ashimatey BS, Kim K, Rashid A, et al. Gene editing for CEP290 -associated retinal degeneration. New England Journal of Medicine. 2024;**390**(21):1972-1984. DOI: 10.1056/NEJMOA2309915

[54] Herder M. What is the purpose of the orphan drug act? PLoS Medicine. 2017;**3**(1):e1002191

[55] Wellman-Labadie O, Zhou Y. The US orphan drug act: Rare disease research stimulator or commercial opportunity? Health Policy. 2010;**95**(2-3):216-228

[56] The Lancet Global Health. The landscape for rare diseases in 2024. The Lancet. Global Health. 2024;**12**(3):e341. DOI: 10.1016/S2214-109X(24)00056-1

[57] Rothschild J. Ethical considerations of gene editing and genetic selection. Journal of General and Family Medicine. 2020;**21**(3):37-47. DOI: 10.1002/jgf2.321

[58] Rubeis G, Steger F. Risks and benefits of human germline genome editing: An ethical analysis. Asian Bioethics Review.

2018;**10**(2):133-141. DOI: 10.1007/s41649-018-0056-x

[59] Hatirnaz Ng O, Sahin I, Erbilgin Y, Ozdemir O, Yucesan E, Erturk N, et al. Obstacles and expectations of rare disease patients and their families in Türkiye: ISTisNA project survey results. Frontiers in Public Health. 2023;**10**:1049349. DOI: 10.3389/fpubh.2022.1049349

[60] Turkish Law on the Protection of Personal Data, Law No. 6698. Kişisel Verilerin Korunması Kanunu [Law on the Protection of Personal Data]. 2016. Available from: https://www.kvkk.gov.tr

Chapter 3

The Historical Evolution of *Ordre Public*, Morality, and Ethics Principles in European Union Regulation of Patented Gene Edited Materials

Dorkina Myrick

Abstract

The ability to target and selectively remove genetic sequences allows CRISPR-inspired scientific research that diagnoses, treats, or cures a host of diseases. As applications of gene editing have become more commonplace over time, *ordre public* and morality have become more problematic. International organizations such as the United Nations and World Trade Organization defer definitions of *ordre public* and morality concerning biotechnology, genetic engineering, and gene editing to individual nation-states, their domestic laws, and court systems. The European Union and its member nations must embrace a circumspect stance to ensure genetic editing is applied appropriately for medical improvement. Thus, historically, society and national institutions have influenced the passage of legislation and the progress of cases in national judicial systems through the lens of *ordre public* and morality.

Keywords: CRISPR, gene editing, ordre public, morality, law, European Union, states, genes, genetics, human rights, patent, patentability, technology

1. Introduction

Personalized medicines have exponentially increased the number of options for accessing novel therapies - particularly genetic therapies [1]. Gene editing, a type of genetic technology that encompasses techniques for creating personalized medicines, has catalyzed dynamic advances in medical technology [2]. CRISPR, an acronym for Clustered Regularly Interspaced Short Palindromic Repeats, is a type of gene editing which involves targeting, removing, and replacing palindromic genetic sequences cleaved with the assistance of Cas9, a biological enzyme [3]. The ability to target and selectively remove genetic sequences allows CRISPR-inspired scientific technologies

to aid in research toward treating or curing a host of diseases, including AIDS, muscular dystrophy, and sickle cell anemia [4, 5]. CRISPR has also been used for COVID-19 testing, diagnostics, and vaccines [4].

CRISPR technology has been the subject of numerous queries regarding ethical applications for its use [6]. Unlike the European Union, currently, *ordre public*, morality, or ethical provisions for the patentability of subject matter are not applicable in the United States. However, this was not always the case. Historically, the Moral Utility Doctrine outlined parameters for usefulness and morality in patented inventions in the United States [7]. The 1817 United States court case *Lowell v. Lewis* established the Moral Utility Doctrine in stating that "[a]ll that the law requires is, that the invention should not be frivolous or injurious to the well-being, good policy, or sound morals of society. The word 'useful', therefore, is incorporated into the act in contradistinction to mischievous or immoral. For instance, a new invention to poison people, or to promote debauchery, or to facilitate private assassination, is not a patentable invention" [8, 9]. *Bedford v. Hunt* supported the Moral Utility Doctrine in stating that "[A] useful invention, in the statute, is...such a one as may be applied to some beneficial use in society, in contradistinction to an invention, which is injurious to the morals, the health, or the good order of society...It is sufficient, that it has no obnoxious or mischievous tendency...and that the use is such as sound morals and policy do not discountenance or prohibit" [10]. Examples of early, unpatentable materials were those which facilitated gambling, as described in the 1889 case *Nat'l Automatic Device Co. v. Lloyd* [11].

The advent of the twentieth century correlated with fewer unfavorable public attitudes toward gambling [12]. What followed was the enactment of the Patent Act of 1952 (35 U.S.C. § 101), along with the adjudication of two court cases, *Webber v. Virginia* and *Juicy Whip, Inc. v. Orange Bang, Inc. and Unique Beverage Dispensers* [13]. These cases illustrated the dissociation of the concepts of morality and patent utility and signaled the demise of the Moral Utility Doctrine in the United States [13]. *Webber v. Virginia* is cited by *Juicy Whip, Inc. v. Orange Bang, Inc. and Unique Beverage Dispensers* in stating that "[c]ongress never intended that the patent laws should displace the police powers of the States, meaning by that term those powers by which the health, good order, peace and general welfare of the community are promoted" [14]. *Juicy Whip, Inc. v. Orange Bang, Inc. and Unique Beverage Dispensers* iterated that "[t]he requirement of 'utility' in patent law is not a directive to the Patent and Trademark Office or the courts to serve as arbiters of deceptive trade practices. Other agencies, such as the Federal Trade Commission and the Food and Drug Administration, are assigned the task of protecting consumers from fraud and deception in the sale of food products" [15].

Now, *ordre public*, morality, or ethical provisions for the patentability of subject matter are not applicable in the United States. According to United States law at 35 U.S.C. § 101: "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title." The word, "whoever," encompasses anyone who "invents or discovers" under the statute at 35 U.S.C. § 101. The statutory requirement at 35 U.S.C. § 101 does not contain provisions for *ordre public*, morality, or ethical circumstances. United States law further states that 35 U.S.C. § 271 (a), "whoever without authority makes, uses, offers to sell, or sells any patented invention, within the United States or imports into the United States any patented invention during the term of the patent therefor, infringes the patent." Again, as in 35 U.S.C. § 101, the statutory requirement at 35 U.S.C. § 271 (a) does not contain provisions for *ordre public*, morality, or ethical circumstances.

European Union states vary regarding laws and edicts concerning the patentability of products of gene editing [16]. Yet, in contrast with the United States, nearly all contain provisions for *ordre public* and morality [17]. The laws in several European Union (EU) nations have struggled to maintain pace with the myriad choices EU patients possess for accessing better health *via* genetic diagnostics and therapies [18]. Supporters of the position that the progress and utilization of genetic engineering can have potential negative impacts on human dignity frequently reflect upon the history and horrors of the eugenics movement [19]. *Ordre public* and morality usually have culturally relevant connotations which informally influence how patent laws are regulated [18]. As such, no international consensus exists concerning precisely what *ordre public* and morality represent [18].

Overwhelmingly, EU nations are signatories to several regional and international agreements, including the Paris Convention, Patent Cooperation Treaty, and European Patent Convention. European Union member national laws follow provisions outlined in the Trade-Related Aspects of Intellectual Property (TRIPS) Agreement pertaining to patentability and *ordre public* and morality [20]. Article 53(a) of the European Patent Convention contains similarities to Article 27.2 of the TRIPS Agreement. Some EU nations such as Finland only allow genetically engineered stem cell research for hereditary diseases, to the exclusion of other diseases [21]. Iceland's Act on Artificial Fertilization and use of Human Gametes and Embryos for Stem-Cell Research (55/1996) allows research on congenital medical diseases in embryos [22]. Yet, in Lithuania, unless research on embryos is for the purpose of treating or curing a disease, the research is not permitted [23].

Historically, *ordre public* was closely associated with domestic civil law and anchored in statute [24]. Morality can be attributed to a certain code of conduct [25]. Yet, international organizations such as the United Nations and World Trade Organization defer definitions of *ordre public* and morality concerning biotechnology, genetic engineering, and gene editing to individual nation-states, their domestic laws, and court systems [26]. As such, the European Union and sovereign nations such as Italy, France, Spain, Austria, Belgium, Denmark, Estonia, Germany, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, and Romania, have declared their positions in law concerning *ordre public* and morality in biotechnology, genetic engineering, and gene editing.

2. Italy: Progression from the birthplace of patent guilds to modern day quandaries of *ordre public* and morality

Patent guilds in the Venetian Republic – which included present-day Italy – represented the first organized associations of intellectual property protection in the modern world [27, 28]. As early as the year 1173, the government of the Venetian Republic began to regulate trade associations in which the practice of specialized fields of expertise were predecessors of present-day patented entities [27]. Codification of regulated guild activity in the year 1261 resulted in legally binding execution of exclusionary trade practices and rights sanctioned by the government of the Venetian Republic [27]. Later, the Venetian Patent Act of 1474 added a 10-year layer of legal protection for individuals and organizations whose crafts, processes, or creations were deemed patent eligible by the Venetian Republic. The Venetian Patent Act of 1474 - which resulted in the award of nearly 600 new patents between 1501 and 1600 - was the first codified, formalized patent system characterized by patent eligibility

independent of citizenship status, agency in the patent-award process, and a requirement to lend access to patent rights *via* licensing fees for all patentees [29, 30].

In the modern era Italy's Legislative Decree 10 of February 2005, n. 30, excluded "inventions concerning genetic screening protocols, the exploitation of which leads to discrimination or stigmatization of human subjects on genetic, pathological, racial, ethnic, social and economic grounds, or having eugenic and non-diagnostic purposes" from patentability. Moreover, "any technological process of cloning...whatever the technique used..." along with "[p]rocedures for modifying the germinal genetic identity of the human being" is prohibited by law in congruence with standards of *ordre public* and morality [31]. The Italian government issued an initial 5 December 2006 decree which stated patients could receive emergency experimental medical treatment for recalcitrant and grave illnesses. On 27 March 2013, legislative decree 24/2013 was issued, which prohibited patient stem cell treatments to protect public safety. The ECHR considered individual versus societal needs regarding individual rights [32]. Articles 2, 6, 8, and 14 of the European Convention of Human Rights helped ensure the preservation of such rights for private citizens.

3. France: The historic Civil Code of France guides modern *ordre public* and morality in genetics and technology

Although the modern patent system in France began in 1791, intellectual property rights in France were considered natural rights, not government-issued rights [33]. France issued patents under the *Breveté Sans Garantie Du Gouvernement* ("sans examen préalable, aux risques et perils des demandeurs, et sans garantie soit de la réalité, de la nouveauté ou du mérite,de l'invention, soit de la fidélité ou de l'exctitude de la description" or "without guarantee from the government of France") system on 5 July 1844 [34]. Since these patents did not adhere to standard government patent application and prosecution procedures, the authenticity of these patents could not be guaranteed [34]. Since the authenticity of French patents could not be assured, *Breveté Sans Garantie Du Gouvernement* patents could not be enforced [34].

The earliest modern origins of domestic *ordre public* may be found in the Civil Code of France [33, 35]. *Ordre public* in French law pertains to the protection of basic domestic values for citizens to be able to live together harmoniously [36]. As pertains to patent law in France, activities which conflict with the preservation of human dignity are in violation of the law [37]. French law does not specifically reference CRISPR gene editing and patent activity [38]. However, French law does address permissible and impermissible practices regarding genetic engineering [38]. This is particularly true if such gene patenting activity is contrary to *ordre public* and morality [37]. Article L611-18 of the Code de la propriété intellectuelle states that: "Le corps humain, aux différents stades de sa constitution et de son développement, ainsi que la simple découverte d'un de ses éléments, y compris la séquence totale ou partielle d'un gène, ne peuvent constituer des inventions brevetables" [39]. In other words, patents cannot be awarded for complete or partial sequences of genes in developing human beings [39].

Thus, certain applications of genetic engineering and technology may be deemed unethical by *ordre public* and morality. This includes specific types of activities concerning gene editing. However, patents in France may be obtained for technical applications of human cells or tissues [39]. In addition, gene patents may be obtained for diagnostic and research purposes [40]. Recently, biotechnology company Cellectis was awarded two patents for CRISPR for immunotherapeutics in cancer [41].

As such, patents for gene editing for somatic therapeutic purposes are acceptable and not generally contrary to *ordre public* and morality in France.

France is a signatory to the Paris Convention, TRIPS Agreement, Patent Cooperation Treaty, and European Patent Convention.

4. Germany: Eugenics practices lead to enforcement of the German Civil Code and robust enforcement of ethical and legal genetic technology practices

Dual goals of preserving human dignity and adhering to moral law are listed in Articles 1 and 2, respectively, of the Basic Law for the Federal Republic of Germany. Consideration of *ordre public* and morality regarding the Law means that public sentiment and opinion pertinent to lifestyles and common social practices are considered with the adoption of all German laws [42]. *Ordre public* has been noted in the German Civil Code since at least 1896 [43]. Regardless, Germany was a central figure in the eugenics movement. The passage of the German Law for the Prevention of Defective Progeny in 1933, or Law for the Prevention of Genetically Diseased Offspring (Gesetz zur Verhütung erbkranken Nachwuchses), aided in ensuring the elimination of undesirables in Germany [44]. In addition to euthanizing and exterminating individuals who failed to possess Aryan and/or Nordic human physical characteristics, many other persons in Germany were selected for euthanasia [45]. These included people with medical disabilities, physical disabilities, mental disabilities or incapacitation and psychiatric disease (including insanity, both criminal and non-criminal) [45]. In all, over 350,000 people were euthanized in Germany [44].

After World War II, the Nuremberg trials, the creation of the United Nations, the drafting of the Universal Declaration for Human Rights, and the promulgation of the Universal Declaration on the Human Genome and Human Rights, nations drastically changed their approaches to scientific and medical experimentation. The German Patent Act, Section 2, states that "(1) No patents are granted for inventions the commercial exploitation of which would be contrary to '*ordre public*' or morality; such exploitation is not deemed to be so contrary merely because it is prohibited by law or regulation." Thus, according to present law, human genes may be patented in Germany as long as violations of *ordre public* and morality do not exist [46]. Specifically, the German Patent Act, Section 2, continues, stating that "(2) patents are, in particular, not granted for 1. processes for cloning human beings; 2. processes for modifying the germ line genetic identity of human beings; 3. uses of human embryo for industrial or commercial purposes; 4. processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and animals resulting from such processes" (**Table 1**) [46, 80].

5. Spain: CRISPR-Cas9 evolution, *ordre public*, and the Oviedo Convention

The history of CRISPR-Cas9 gene editing can be traced back to Spain [81]. Francisco Mojica, of the University of Alicante in Spain, is credited with the initial discovery of what we now know as CRISPR-Cas9 in 1989 [81–83]. It is unlikely that Dr. Mojica and his colleagues anticipated the evolution of CRISPR as a catalyst for moral and ethical debate concerning CRISPR's manner of use in humans three decades after

International entity, region, or nation	National legislation	Agreement(s) for which the nations are signatories	Text of international agreement, regional convention, or national legislation
World Trade Organization	N/A	Article 27.2. Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), Apr. 15, 1994, Marrakesh Agreement Establishing the World Trade Organization, Annex 1C, 1869 U.N.T.S. 299, 33 I.L.M. 1197 (1994).	Article 27.2, TRIPS: "Members may exclude from patentability inventions, the prevention within their territory of the commercial exploitation of which is necessary to protect *ordre public* or morality, including to protect human, animal or plant life or health or to avoid serious prejudice to the environment, provided that such exclusion is not made merely because the exploitation is prohibited by their law" [47].
European Union	N/A	Article 53(a). Convention on the Grant of European Patents, October 5, 1973, 13 INT'L LEGAL MATS. 268 (1974).	European Patent Convention Article 53(a): "European patents shall not be granted in respect of: (a) inventions the commercial exploitation of which would be contrary to *ordre public* or morality, provided that the exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation in some or all of the Contracting States" [48]
Italy	Italy. Article 81-quinquies (Exclusions Codice della proprietà industriale (decreto legislativo 10 febbraio 2005, n. 30, aggiornata con le modifiche introdotte dal decreto legislativo 19 maggio 2020, n. 34)). Legislative Decree 10 February 2005, n. 30. Industrial Property Code, pursuant to article 15 of the law of 12 December 2002, n. 2	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Italy. Legislative Decree 10 February 2005, n. 30. Article 81. Excluded from patentability: "1(a) the sequence or partial sequence of a gene" [49]. "1(b) inventions whose commercial exploitation is contrary to human dignity, public order and morality, the protection of health" [49]. "1(b)(1) and 1 (b) (2) any technological process of cloning, whatever the technique used..." [49]. "Procedures for modifying the germinal genetic identity of the human being..." [49] "(4) procedures for the modification of genetic identity" [49] "(5) inventions concerning genetic screening protocols, the exploitation of which leads to discrimination or stigmatization of human subjects on genetic, pathological, racial, ethnic, social and economic grounds, or having eugenic and non-diagnostic purposes" [49].
France	Intellectual Property Code (07 August 2004) Article L.611-17 [38]. Article L611-18 of the Code de la propriété intellectuelle [39]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Article L.611-17: "Inventions whose commercial exploitation would be contrary to human dignity, public order or morality are not patentable; this contrariety cannot result from the sole fact that this exploitation is prohibited by a legislative or regulatory provision" [38]. Article L.611-18: "The human body, at the different stages of its constitution and development, as well as the simple discovery of one of its elements, including the total or partial sequence of a gene, cannot constitute patentable inventions" [39].

International entity, region, or nation	National legislation	Agreement(s) for which the nations are signatories	Text of international agreement, regional convention, or national legislation
Germany	Patent Act as published on 16 December 1980 (Federal Law Gazette 1981 I, p. 1) [46], as last amended by Article 1 of the Act of 30 August 2021 (Federal Law Gazette I, p. 4074) [46]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	The German Patent Act, Section 2, states that "(1) No patents are granted for inventions the commercial exploitation of which would be contrary to '*ordre public*' or morality; such exploitation is not deemed to be so contrary merely because it is prohibited by law or regulation." Thus, according to present law, human genes may be patented in Germany as long as violations of *ordre public* and morality do not exist [46]. Specifically, the German Patent Act, Section 2, continues, stating that "(2) patents are, in particular, not granted for 1. processes for cloning human beings; 2. processes for modifying the germ line genetic identity of human beings; 3. uses of human embryo for industrial or commercial purposes; 4. processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and animals resulting from such processes" [46].
Spain	Spain. Ley N° 24/2015, de 24 de julio de 2015, de Patentes (modificada por la Ley N° 6/2018, de 3 de julio de 2018) [50]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	"Article 5. Exceptions to patentability. The following may not be the subject of a patent… In particular, the following will not be considered patentable by virtue of the provisions of the preceding paragraph: a) Human cloning procedures. b) The procedures for modifying the germline genetic identity of the human being. c) The uses of human embryos for industrial or commercial purposes" [50] "Article 5. Exceptions to patentability… d) Procedures for modifying the genetic identity of animals that cause these suffering without substantial medical or veterinary utility for man or animal, and the animals resulting from such procedures…." [50] "Article 5. Exceptions to patentability…(d)(5). The human body in the different stages of its constitution and development, as well as the simple discovery of one of its elements, including the total or partial sequence of a gene. However, an element isolated from the human body or otherwise obtained by a technical process, including the sequence or partial sequence of a gene, may be considered as a patentable invention, even if the structure of said element is identical to that of a natural element" [51]. "Article 5. Exceptions to patentability….6. A mere deoxyribonucleic acid (DNA) sequence with no indication of any biological function" [51].

International entity, region, or nation	National legislation	Agreement(s) for which the nations are signatories	Text of international agreement, regional convention, or national legislation
Austria	Austrian Gene Technology Act of 1995	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	The Austrian Gene Technology Act of 1995 (Gentechnikgesetz, GTG) - which permits somatic applications for genetic engineering, genetic testing or analysis of genes, gene fragments, or chromosomes - is restricted to medical (diagnostic of therapeutic) or scientific research applications [52, 53]. Amendments to the Act in 2005, Änderung des Gentechnikgesetzes, aided in outlining more specific information as per the ramifications of genetic technology for therapeutics [52]. On the contrary, germline manipulation of the human genome is prohibited by the Austrian Gene Technology Act. [54]. Moreover, the Reproductive Medicine Act prohibits diagnostic testing, treatment, or alteration of embryos in a manner that would alter the human germline [55]. The Genetic Engineering Act provides further legal guidance for gene editing procedures in Austria [56].
Belgium	Belgium Patent Law (1997), Chapter II, Section1: General Provisions; Article 4 [57]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Belgium Patent Law (1997), Chapter II, Section1: General Provisions; Article 4: "2. Patents shall not be granted for inventions the exploitation of which would be contrary to public policy or morality, provided that the exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation" [57].
Denmark	Denmark Patents Act (01 January 2007) [58] Danish Patents Act (1997) [59] Denmark ratified the EU Directive 98/44/EC [60] Denmark Patents Act Consolidate Act No. 221 of 26 February 2017 [61].	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Denmark Patents Act Consolidate Act No. 221 of 26 February 2017: "1b. (1) Patents shall not be granted in respect of inventions the commercial exploitation of which would be contrary to *ordre public* or morality" [61]. Denmark Patents Act Consolidate Act No. 221 of 26 February 2017: "(2) An exploitation shall not be deemed to be contrary to *ordre public* or morality merely because the exploitation is prohibited by law or administrative regulation" [61]. Denmark Patents Act Consolidate Act No. 221 of 26 February 2017: "(3) Pursuant to subsection 1 patents may inter alia not be granted in respect of: (i) processes for cloning human beings, (ii) processes for modifying the germ line genetic identity of human beings, (iii) uses of human embryos for industrial or commercial purposes, and (iv) processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes" [61]. Denmark Patents Act Consolidate Act No. 221 of 26 February 2017: "(4) Patents shall not be granted in respect of (i) inventions the exploitation of which would be contrary to law and order or morality…." [59].

International entity, region, or nation	National legislation	Agreement(s) for which the nations are signatories	Text of international agreement, regional convention, or national legislation
Estonia	Estonia Riigi Teataja. Patents Act [62]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Estonia Riigi Teataja. Patents Act § 6: Unpatentable inventions "(1) The subject of an invention may be a device, process, material, including biological material, or a combination thereof. (2) The following, inter alia, are not regarded as the subject of inventions: 1) discoveries, including descriptions of the formation or development of the human body or sequence or partial sequence of human gene...." [62].
Latvia	Latvia. Patent Law (as amended up to January 1, 2016). law Latvijas Vēstnesis, 34 (3610), Adoption: 15.02.2007. 27.02.2007. Entry into force: 01.03.2007 [63].	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Latvia. Patent Law: "(4) A patent shall not be granted to the inventions whose utilization is in conflict with public order or the principles of morality accepted in society; however, the decision not to grant a patent shall not be taken only on the basis of the fact that such a utilization is prohibited by a regulatory or administrative enactment" [63].
Lithuania	The Republic of Lithuania. Patent Law. 1994 January 18 No. I-372. Consolidated version from 25/04/2020 to 31/12/2021. Patentų įstatymo Nr.I-372 18 Sau 1994 Patentų įstatymo Nr.I-372 18 Sau 1994 (su pakeitimais, padarytais įstatymas 2020 m. balandžio 21 d. Nr. XIII-2857) [64].	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	The Republic of Lithuania. Patent Law: "Article 5. Exceptions to patentability 1. Patents shall not be granted... 3) inventions the commercial exploitation of which would be contrary to the public interest, morals, and principles of humanity. Decisions not to grant patents cannot be taken on the sole ground that the use of such inventions is prohibited by law or regulation" [64]. The Republic of Lithuania. Patent Law: "2. On the grounds referred to in paragraph 1 (3), patents shall not be granted, inter alia: 1) methods of human cloning;" [64] The Republic of Lithuania. Patent Law: "2) methods for altering the identity of the genetic line of human gametes;" [64] The Republic of Lithuania. Patent Law: "3) the use of human embryos for industrial or commercial purposes;" [64] The Republic of Lithuania. Patent Law: "4) methods of modifying the genetic identity of animals which may cause them suffering greater medical benefit to humans or animals, and to animals produced in these ways." [64] The Republic of Lithuania. Patent Law: "3. A patent for a product containing or constituting genetic information shall confer legal protection includes all materials containing the product and genetic information performing its functions, excluding the human body or its elements, including the gene sequence or parts thereof in the natural environment" [64]

International entity, region, or nation	National legislation	Agreement(s) for which the nations are signatories	Text of international agreement, regional convention, or national legislation
Luxembourg	Luxembourg. Law of July 20, 1992, on the Changes in the System for Patents for Invention (as amended by the Law of May 24, 1998) [65]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Luxembourg. Law of July 20, 1992, on the Changes in the System for Patents for Invention: "Inventions the publication or exploitation of which would be contrary to public policy or morality, provided that the exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation" [66].
Malta	Malta. Patent and Designs Act (Chapter 417). 01 June 2002. ACT XVII of 2000, as amended by Acts IX of 2003 and XVIII of 2005; and Legal Notices 181 and 186 of 2006, and 426 of 2007 [67].	• Paris Convention • TRIPS tAgreement • Patent Cooperation Treaty • European Patent Convention	Malta. Patent and Designs Act: "A patent shall not be granted in respect of: (a) an invention the exploitation of which would be contrary to public order or morality: Provided that exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation;" [68] "A patent shall not be granted in respect of: the human body, at the various stages of its formation and development, and the simple discovery of one of its elements, including the sequence or partial sequence of a gene:" [68] Malta. Chapter IV. Patents and Patentability: "Provided that an element isolated from the human body or otherwise produced by means of a technical process, including the sequence or partial sequence of a gene, may constitute a patentable invention, even if the structure of that element is identical to that of a natural element;" [68] Malta. Chapter IV. Patents and Patentability: "(c) processes for cloning the human body, processes for modifying the germ line genetic identity of the human body and uses of the human embryo for industrial or commercial purposes;" [69] Malta. Chapter IV. Patents and Patentability: "(d) processes and products for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefits to man or animal;" [70] Malta. Chapter IV. Patents and Patentability: "(a) Where an application refers to an element isolated from the human body or otherwise produced by means of a technical process including the sequence or partial sequence of a gene, the industrial application of a sequence or a partial sequence of a gene must be disclosed in the patent application" [71]. Malta. Chapter IV. Patents and Patentability: "(b) When the application concerns a sequence or a partial sequence of a gene used to produce a protein or part of a protein, it is necessary to specify which protein or part of protein is produced or function or sequence it performs" [72].

International entity, region, or nation	National legislation	Agreement(s) for which the nations are signatories	Text of international agreement, regional convention, or national legislation
Netherlands	Netherlands. Patent Law. Rijkswet van 15 december 1994, houdende regels met betrekking tot octrooien. [73]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Netherlands. Patent Law. (3)(1)(b): "Not susceptible for patent are: the human body at various stages of its development, as well as the mere discovery of one off the parts, thereof" [74] Netherlands. Patent Law (3)(2)(a-d): "Under inventions of which the commercial exploitation in conflict would be with the public order and good customs as referred to in the first paragraph, part a, is in each case understood to mean: (a) methods for the cloning of people; (b) methods for modification of the germ identity of man; (c) the use of human embryos; (d) methods for modification of the genetic identity of animals which appropriately have this to do suffering without substantial medical benefit to man or animal to be delivered..." [74].
Poland	Poland. Act of June 30, 2000, on Industrial Property (as amended up to Act of February 13, 2020). Ustawa z dnia 30 czerwca 2000 r. Prawo własności przemysłowej (zmieniona do ustawy z dnia 13 lutego 2020) [75]	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Polish patent law defines gene editing patents contrary to public order and morality: "For biotechnological inventions the use of which would be contrary to public order or morality...or public morality, it is in particular: (1) ways of cloning people; (2) ways of modifying the genetic identity of the human germline;(3) use of human embryos for industrial or commercial purposes; (4) ways of modifying the genetic identity of animals that may cause make the suffer without any real benefit" [75]. "Patent for a product containing or having genetic information includes all materials into which the product has been incorporated and in which genetic information has been contained" [76].

International entity, region, or nation	National legislation	Agreement(s) for which the nations are signatories	Text of international agreement, regional convention, or national legislation
Romania	Law No. 64 of October 11, 1991, on Patents (as amended up to Law No. 83/2014) [77].	• Paris Convention • TRIPS Agreement • Patent Cooperation Treaty • European Patent Convention	Law No. 64 of October 11, 1991, on Patents: "(2) Inventions in the field of biotechnology shall be patentable if they relate to… d) an element isolated from the human body or otherwise produced by a technical process, including the sequence or partial sequence of a gene…" [78]. Law No. 64 of October 11, 1991, on Patents: "(1) Patents shall not be granted under this Law in respect of: a) inventions the exploitation of which would be contrary to public order or morality, including inventions harmful to the health or life of persons, animals or plants, and which are likely to seriously harm the environment…(c) the inventions having as a subject-matter the human body in its various stages of formation and development, as well as the mere discovery of one of its elements, including the sequence or partial sequence of a gene…" [78]. Law No. 64 of October 11, 1991, on Patents: "(9) The protection conferred by a patent to a product containing genetic information or consisting of genetic information shall be extended to any other material incorporating the product and wherein the genetic information is contained and exercises its function, except for the human body in the various stages of its formation or development" [79].

Table 1.
The historical evolution of ordre public*, morality, and ethics principles in European union regulation of patented gene edited materials.*

CRISPR's discovery. The Biomedical Research Act of 2007 endeavored to resolve some questions regarding appropriate, moral, ethical, and legal exercises of CRISPR-Cas9 in biomedical research in Spain [84]. Article 2 of Law 14/2007 ensures and guarantees that "[t]he protection of the dignity and identity of the human being shall be protected in relation with any research that involves interventions on human beings in the field of biomedicine, thus guaranteeing every person, without any discrimination, the respect to their integrity and to other fundamental rights and freedoms" [85]. In addition, "[t] he health, interest and wellbeing of the human being who participates in biomedical research shall prevail over the interest of society or science" [85]. As such, *ordre public* and morality in Spain regarding modification of the human germline *via* genetic intervention does not indicate enthusiastic public support for patents of this nature [86]. Specifically, Law 14/2007 states that "[t]he performance of any intervention aimed at the introduction of a modification in the genome of the offspring" is a serious infraction of the Biomedical Research Act of 2007 [87]. Therefore, the patenting of genetically modified germline products would likely be strictly prohibited. According to the Oviedo Convention, "[a]n intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants" [88]. Thus, genome editing for reproductive purposes is not permitted [89]. However, manipulation of germline DNA *via* gene editing in Spain seems to be permissible if the application of the gene editing is exclusively for basic science and clinical research purposes [90]. Accordingly, CRISPR-Cas9 gene editing is likely permissible for non-basic science and clinical research purposes if CRISPR-Cas9 gene editing is used for the purpose of medical treatment or medical diagnostics [90]. The Spanish Patents and TradeMark Office (SPTO) may refuse patents to inventors who are in violation of their respective patent laws [50]. This includes the refusal of the issue of patents for gene editing for germline gene modification and human cloning [91]. Totally and partially sequenced genes of human embryos in development are also prohibited from being patented [91].

6. Austria: Gene editing regulation *via* the Austrian Gene Technology Act

The Austrian Gene Technology Act of 1995 (Gentechnikgesetz, GTG) - which permits somatic applications for genetic engineering, genetic testing or analysis of genes, gene fragments, or chromosomes - is restricted to medical (diagnostic of therapeutic) or scientific research applications [52, 53]. Amendments to the Act in 2005, Änderung des Gentechnikgesetzes, aided in outlining more specific information as per the ramifications of genetic technology for therapeutics [52]. On the contrary, germline manipulation of the human genome is prohibited by the Austrian Gene Technology Act [54]. Moreover, the Reproductive Medicine Act prohibits diagnostic testing, treatment, or alteration of embryos in a manner that would alter the human germline [55]. The Genetic Engineering Act provides further legal guidance for gene editing procedures in Austria [56].

According to Austrian patent law, inventions contrary to *ordre public* and/or morality may not be patented [92]. However, genetic sequences procured *via* unique technical methods may be patented, including genetic sequences identical to those naturally existing within the human body [92]. Serendipitous discovery of naturally occurring genetic sequences does not, however, constitute patentable material [92]. Germline gene editing patent issue is prohibited, as well, along with the patenting of gene editing methods or products of gene editing which result in animal suffering without significant medical benefit to humans or animals [92].

7. Belgium: Belgium Patent Law and genetic ethics

Ordre public and morality provisions are written into Belgian patent law [57]. As a signatory to the Paris Convention, TRIPS Agreement, Patent Cooperation Treaty, and European Patent Convention, Belgian patent law closely follows the provisions of these documents regarding *ordre public* and morality as pertains to gene editing. Specifically, the Belgium Patent Law states in part that "[p] atents shall not be granted for inventions the exploitation of which would be contrary to public policy or morality, provided that the exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation" [57]. Gene patents may be obtained for diagnostic and research purposes [40]. However, Belgium prohibits "research or treatments of a eugenic nature, that is to say focused on the selection or amplification of non-pathological genetic characteristics of the human species" [93]. Although the formal practice of eugenics has been outlawed, questionable practices of using gene editing to manipulate the genetic code of an individual to produce specific phenotypic esthetic results such as hair or eye color would likely be prohibited.

8. Denmark: Patents Act and the Danish Act on biomedical research ethical evaluation

Denmark patent law is congruent with the Oviedo Convention as per gene editing. According to the Oviedo Convention, "[a]n intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants" [94]. After ratifying the Biotech Directive in 1998, Denmark passed a Consolidated Patents Act which addressed a variety of areas potentially influenced such as germline gene editing cloning, and the genetic manipulation of human embryos [95]. The Danish National Committee on Health and Research Ethics and a Regional Science Ethics Committee - assisted by a Ministerial Order on the Use of Tissue Registration - make primary decisions concerning applications of biotechnology for genetic engineering in compliance with the Human Tissues Act in Denmark [96]. These committees apply the guidelines as outlined in the Danish Act on Biomedical Research Ethical Evaluation on medical research projects [97]. Not all genetic material may be patented [98]. Denmark passed Patent Acts in 1997 and 2007 which prohibited the authorization of patents in conflict with *ordre public* or morality [58, 59]. Additional applicable law is found in the Act on Cloning and Modification of Animals, Environment and Genetic Engineering [99]. Denmark is a signatory to the Paris Convention, TRIPS Agreement, Patent Cooperation Treaty, and the European Patent Convention.

9. Romania: Commercial and environmental concerns with heritable gene CRISPR technologies

Romanian patent law prohibits the patenting of complete or partial human genetic sequences. According to Law No. 64 of October 11, 1991, on Patents: "(2) Inventions in the field of biotechnology shall be patentable if they relate to... d) an element isolated from the human body or otherwise produced by a technical process, including the sequence or partial sequence of a gene..." [78] Gene editing activities which violate *ordre public* and morality cannot be patented [100]. The Romanian Patent

Office enforces Romanian law's prohibition of patenting of stem cells and embryos for commercial use [100]. Article 13 of the Oviedo Convention is also a primary source of reference about patent permissibility and *ordre public* and morality, in addition to Romania's *ordre public* and morality obligations as a signatory to the Paris Convention, TRIPS agreement, Patent Cooperation Treaty, and the European Patent Convention [101, 102]. Environmental harm because of gene editing could include disruption of the human germline through genetic manipulation [103]. Specifically, "[a]n intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants" [88]. In Romania, even heritable genome research for reproductive purposes is prohibited by the Romanian law [89].

10. Netherlands: *Kingdom of the Netherlands v. European Parliament and Council*

The European Union wants its member states to comply with harmonization and adoption of the European Biotech Directive (Directive 98/44/EC) [104]. For this reason, the EU wanted a unanimous vote in order preserve unity within the European Union regarding the criteria member nations abide by in considering the law and policy of scientific and technological advancement in the area of genetic engineering [104]. The Netherlands believed that a unanimous vote on the adoption of harmonization measures of the European Biotech Directive would harm the national Netherlands scientific research and genetic engineering internal market [104]. *Kingdom of the Netherlands v. European Parliament and Council* reflects the importance of balancing member state needs vs. European Union needs in navigating regulations concerning genetic engineering. Harmonization measures are one way to "prevent the emergence of future obstacles to trade resulting from multifarious development of national laws." [105]. Thus, opponents of harmonization who wanted to renegotiate terms of the agreement so that other nations would be accommodated with regards to their individual needs cannot prevail [105]. The Court of Justice declared in *Kingdom of the Netherlands v. European Parliament and Council* that European Union law endorses a fundamental right to human dignity [106]. The Netherlands sued to annul the European Biotech Directive (Directive 98/44/EC) under Article 230 of the Treaty Establishing the European Community (now Article 263 TFEU) [107]. The CJEU denied the Netherlands' request to annul the European Biotech Directive [108]. The Court concerns itself with the preservation of the integrity of the human body and the maintenance of its human dignity in patenting inventions derived from genetically engineered means [108]. "An element of the human body may be part of a product which is patentable but it may not, in its natural environment, be appropriated. That distinction applies to work on the sequence or partial sequence of human genes" [108]. In stating this, the court reminds us that the human body cannot be patented in its natural state either in whole or in part [109]. Accordingly, genetically modified organisms may be patented with the demonstration that they show a functional improvement upon the organism's natural state.

11. Gene editing, *ordre public* and morality, and the courts

Ordre public and morality concerns frequently test the limits of human dignity and human rights regarding the ability of an individual to avail oneself of the latest

advances in genetic technology. *Kingdom of the Netherlands v. European Parliament and Council* is just one such example of a case. Several other cases which balance *ordre public* and morality concerns with the use of genetic technologies include *Brüstle Oliver v. Greenpeace,* German case *T 1285/13 (miR-1/Max-Planck-Gesellschaft) of 20.10.2016*, and *Durisotto v. Italy*.

In *Brüstle Oliver v. Greenpeace*, Oliver Brüstle, a German citizen, filed a patent for a specific type of neural cell. Since the 1998 European Directive did not expound upon the definition of a human embryo concerning age, Brüstle did not anticipate that the neural stem cells' age violated the law [110]. Brüstle insisted that medically, embryos develop fourteen days after an egg is fertilized [110]. Brüstle extracted neurally-differentiated five-to-six-day-old human embryonic stem cells for the purpose of conducting scientific research [110]. Brüstle's patent did not comply with the terms of the European Biotechnology Directive [110]. Thus, Brüstle violated both the European Directive and several European Court of Human Rights (ECHR) provisions, namely right to life, prohibition of discrimination, and right to private life [110]. A European Court of Justice (ECJ) ruling was requested [110]. The Court of Justice of the European Union (CJEU) disputed Brüstle's statements concerning the age of the embryonic stem cells, stating that an embryo forms at egg fertilization and deserves both dignity and "fundamental rights." Precisely, "any human ovum must, as soon as fertilised, be regarded as a 'human embryo' if that fertilisation is such as to commence the process of development of a human being" [110]. According to the Biotechnology Directive, "an unfertilised human ovum whose division and further development have been stimulated by parthenogenesis does not constitute a 'human embryo' under the condition that 'it does not, in itself, have the inherent capacity of developing into a human being'" [111]. Additionally, Article 6(2)(c) of Directive 98/44 precludes patentability... "where the technical teaching which is the subject-matter of the patent application requires the prior destruction of human embryos..." [111].

Ordre public and morality is examined in the European Patent Office in *T 1285/13 (miR-1/Max-Planck-Gesellschaft) of 20.10.2016.* According to the case in the Patent Board of Appeals, Max-Planck-Gesellschaft submitted a main request for a patent, which was initially rejected by the European Patent Office [112, 113]. The main request for a patent from the European Patent Office was for a nucleic acid molecule with genetic modifications of miRNAs for staging (assessing the age and development) of *Drosophila* (fruit fly) embryos [113–115]. As a model biological system for genetic manipulation, engineering, and modification, *Drosophila melanogaster* (fruit fly) can aid scientists and inventors with patents in determining ethical issues in patentability [116–118]. The European Patent Office fails to formally discuss issues of *ordre public* and morality in this case. However, since generations of fruit flies multiply relatively quickly, researchers may quickly gain facility with assessing generational changes in the fruit flies within relatively short amounts of time, in contrast with mammalian systems (mice, rabbits, monkeys, and humans), in which comparable types of research may take much longer [118]. In addition, similar types of research may be restricted by law, depending upon jurisdiction, due to what is allowed morally and ethically with regard to research conducted in invertebrate vs. vertebrate organisms [119].

The genetic material, miRNAs – or microRNAs - in *Drosophila* in *T 1285/13* do not code for specific proteins [115]. However, miRNAs in *Drosophila* in *T 1285/13* regulate gene expression by silencing, expressing, enhancing, or activation gene activity [115]. The activity in *Drosophila* was indicative of an industrial application for a patent for the genetic material [113, 120]. The invention in The invention in *T 1285/13 (miR-1/*

Max-Planck-Gesellschaft) of 20.10.2016 was found to be patentable due to its suitability for industrial applications as per Article 57 of the European Patent Convention [113, 120]. The industrial applicability for patent eligibility in the genetically modified *Drosophila* in *T 1285/13* is reminiscent of the industrial applicability of the genetically-modified bacteria which aid in cleaning oil spills in the United States case *Diamond v. Chakrabarty*. However, distinct differences between the two cases include the fact that the *Drosophila* in *T 1285/13* concern modifications in miRNAs while *Diamond v. Chakrabarty* addressed genetic modification of DNA [121]. One argument was directed toward industrial applicability in Article 57 of the European Patent Convention while *Diamond v. Chakrabarty,* while useful industrially, was primarily concerned with whether DNA could be modified and patented at all [113, 121].

Durisotto v. Italy (2014) was a decision regarding permission to use experimental stem cell therapies. The case involved an appeal from the European Court of Human Rights in which Durisotto's daughter suffered from a degenerative brain disease. Durisotto enrolled his daughter in a clinical trial entitled, "Stamina," which involved a stem cell therapy at an Italian university. Durisotto's daughter was denied the treatment. Consequently, Durisotto sought a court order on 8 April 2013 to coerce an Italian hospital to treat his daughter with the "Stamina" stem cell therapy protocol. Mr. Durisotto proclaimed that his daughter's right to life as per Article 2 of the European Convention of Human Rights was being denied through lack of access to the treatment [122]. Mr. Durisotto also claimed that his daughter's rights were forfeited as per the European Convention of Human Rights, Article 6, in that he did not believe his daughter was fully granted a fair trial in receiving a proper appeal. Moreover, Mr. Durisotto stated that his daughter's Article 8 rights under the European Convention of Human Rights were violated in that the government failed to honor her right to private life. Even more so, Mr. Durisotto claimed that his daughter's rights under Article 14 of the European Convention of Human Rights, which dissuaded discrimination, had been violated.

On 3 May 2013, the Brescia, Italy, hospital requested dismissal of Durisotto's court case because the Brescia hospital claimed Durisotto's daughter was ineligible for the experimental therapy as per the treatment criteria. Furthermore, the hospital in Brescia, Italy, claimed that Durisotto's daughter did not begin "Stamina" stem cell treatments until after the 27 March 2013 legislative decree. On 30 August 2013, Durisotto's Italian case was dismissed. The court cited the 27 March 2013 enactment date of the Legislative Decree as a reason for the dismissal of the case [123]. The Legislative Decree was relevant only to treatment ordered prior to entry into force of the Legislative Decree [124].

The European Court of Human Rights received the case on 20 September 2013. One issue in the case was whether Italy had overstepped its mark in refusing Durisotto's daughter the experimental therapy he requested. Related questions involved whether Italy's refusal to grant Durisotto's daughter the "Stamina" therapy was an act of violation of Durisotto's daughter's rights under Articles 2, 6(1), 8, or 14 of the European Convention of Human Rights. The court consensus was that the "Stamina" stem cell therapy had not been scientifically proven. As such, the court decided that the lower court decision to deny Durisotto's daughter the therapy was not discriminatory and upheld prohibition on stem cell therapy. Thus, Durisotto's daughter's rights under Articles 2, 6(1), 8, and 14 of the European Convention of Human Rights had not been violated through Italy's decision to deny Durisotto's daughter the therapy she sought. The Court did not believe that Durisotto's daughter had been denied her right to life *via* the treatment refusal [124].

Durisotto wanted to engage in experimental therapy for the sake of his daughter. However, the chosen stem cell therapy was not deemed safe for use by regulatory authorities. The father's needs regarding obtaining an appropriate therapy for his daughter failed to coincide with societal needs of ensuring that available experimental therapies are safe for public use. Although requests such as Durisotto's can be considered on a case-by-case basis, ethical issues loom once permission for use of experimental therapy is granted for some but not for others. The European Court of Justice, in adjudicating these types of cases, helps ensure fairness to all for those who reside in European Union member states. Equanimity in decision-making is particularly important with the advent of genetic engineering technology and expanded use of stem cell research. Additionally, some scholars believe that the Durisotto ruling is a missed opportunity to assess and more rigorously scrutinize legal criteria for compassionate care in the context of experimental stem cell therapies [125].

12. The role of national institutions in regulating gene editing through the lens of *ordre public* and morality

National institutions may act as a policymakers and liaisons between scientific experts and citizens [126]. For example, best practices for Austrian diagnostic and therapeutic applications of CRISPR gene editing are promulgated by the Austrian Academy of Sciences [127]. Implementation and promulgation of rules concerning gene editing in Austria, along with enforcement and penalty recommendations for legal violations pertinent to the use of gene editing, are led by several scientific committees, along with the Advisory Board on Gene Technology (Gentechnikkommission) [128]. The Austrian Bioethics Commission monitors utilization of CRISPR technology in Austria [129]. In this manner, the Austrian Bioethics Commission serves in an advisory capacity to the Federal Chancellor of Austria on *ordre public*, ethical and morality issues pertinent to CRISPR gene editing and other forms of genetic engineering [130]. The Austrian Ministry of Social Affairs supports the deliberations and advice of the Austrian Bioethics Commission given to the Federal Chancellor of Austria [130].

The French Institut national de la santé et de la recherche médicale (Inserm) - a collaborative institution of over 15,000 scientific and technical experts - engages in innovative decision-making that ultimately influences public policy and may serve as a catalyst for pertinent legislation [131]. Currently, Inserm retains 1650 biotechnology patents [132]. These biotechnology patents also include patents for genetic inventions for rare diseases such as Friedreich's ataxia [133, 134]. Inserm is keenly interested in the development of therapeutics for chimeric antigen receptor cells (CAR-T cells) (for generating immunogenic responses in the treatment of cancer) and sickle cell anemia, with over 104 drugs currently in development [135]. Most recently, the European Medicines Agency authorized treatment of spinal muscle atrophy with Zolgensma (onasasemnogene abeparvovec), a gene therapy that replaces abnormal copies of genes responsible for spinal muscle atrophy, with healthy genes [136].

Inserm convened an advisory panel of scientists, clinicians, ethicists, and stakeholders from several different European universities and institutions in September 2017 [137]. This meeting proposed ethical recommendations for the use of gene editing, examine the biomedical ethics of gene editing, and promulgate recommendations for state legislators to consider [138]. The Inserm meeting occurred 2 years after the 2015 National Academy of Medicine meeting in the United States in which the

ethics of appropriate uses of gene editing *via* CRISPR were examined. Specifically, Inserm recommended the formation of a European Steering Committee (ESC) to further evaluate deleterious effects of gene editing such as off-target effects [138]. Additionally, the ESC would study epigenetic effects and mosaicism, and the long-term impact of gene applications on humans and biodiversity [138]. Inserm recommended further study of threats to national security *via* the utilization of gene editing technology.

13. Conclusions

Gene editing patentability and *ordre public* and morality in European Union states will continue to be relevant issues as long as genetic technology continues to increase in sophistication. Therapeutic uses for gene editing have raised questions regarding the possible use of elective gene editing which could potentially select for eye color, stature, or other physical characteristics, not excluding traits such as intelligence or athletic ability. Still, it is important to ensure that EU nations maintain compliance with the EU Directive. The European Union and its member nations must guard against the arbitrary application of genetic engineering for medical improvement. Myriad choices regarding the limits of morality in the use of gene editing technologies will persist in vexing humanity. Regardless, scientific progress in this area must not cease.

Author details

Dorkina Myrick[1,2,3,4,5,6]

1 University of Oxford, England

2 University of Turin/World Intellectual Property Organization/ITCILO, Italy

3 Université Paris II Panthéon-Assas, France

4 Boston University School of Law, United States

5 Brown University School of Medicine, United States

6 National Institutes of Health/National Cancer Institute Anatomic Pathology Residency Program, Turin, Italy

*Address all correspondence to: dcm776@gmail.com

References

[1] Marks P, Witten C. Toward a new framework for the development of individualized therapies. Gene Therapy. 2021;**28**:615-617

[2] Musunuru K, Grandinette SA, Wang X, Hudson TR, Briseno K, Berry AM, et al. Patient-specific *in vivo* gene editing to treat a rare genetic disease. New England Journal of Medicine. 15 May 2025;**392**(22):2235-2222

[3] Ghaemi A, Bagheri E, Abnous K, Taghdisi SM, Ramezani M, Alibolandi M. CRISPR-Cas9 genome editing delivery systems for targeted cancer therapy. Life Sciences. 2021;**267**:118969

[4] Li T, Yang Y, Qi H, Cui W, Zhang L, Fu X, et al. CRISPR/Cas9 therapeutics: Progress and prospects. Signal Transduction and Targeted Therapy. 16 Jan 2023;**8**(1):1-23

[5] Adashi EY, Gruppuso PA, Cohen IG. CRISPR therapy of sickle cell disease: The dawning of the gene editing era. The American Journal of Medicine. 4 Jan 2024;**137**(5)

[6] Brokowski C, Adli M. CRISPR ethics: Moral considerations for applications of a powerful tool. Journal of Molecular Biology. 4 Jan 2019;**431**(1):88-101

[7] *Lowell v. Lewis*, 15 F. Cas. 1018 (C.C.D. Mass. 1817) (No. 8568)

[8] 15 F. Cas. 1018, 1019 (C.C.D. Mass. 1817) (No. 8568); Enerson BD; Protecting Society from Patently Offensive Inventions: The Risk of Reviving the Moral Utility Doctrine. Cornell Law Review. 2004; 89: 685

[9] Myrick D. The decline of *ordre public* and morality in the regulation of patented genetic materials in the United States. In: Global Bioethics - Current Challenges, New Developments, and Future Directions [Working Title]. 2025. Available from: https://www.intechopen.com/online-first/1217783

[10] 3 F. Cas. 37 (C.C.D. Mass. 1817) (No. 1216); Spiel P, Deceptive Patents: *Deconstructing Juicy Whip*. BYU Law Review. 2017;743 (2018)

[11] 40 F. 89, 90 (C.C.N.D. Ill. 1889)

[12] Spiel P. Deceptive patents: *Deconstructing juicy whip*, 2017. BYU Law Review. 2018:743-778

[13] 185 F.3d 1364,1367, 1368 (Fed. Cir. 1999); 103 U.S. (13 Otto) 344, 347-48, 26 L.Ed. 565 (1880)

[14] 185 F.3d 1364,1367, 1368 (Fed. Cir. 1999); *quoting* 103 U.S. (13 Otto) 344, 347-48, 26 L.Ed. 565 (1880)

[15] 185 F.3d 1364,1368 (Fed. Cir. 1999)

[16] Sterckx S, Cockbain J, Exclusions from Patentability. How Far Has the European Patent Office Eroded Boundaries? Cambridge University Press; 2012. pp. 243-308

[17] Coutts S. The evolution of public order and public policy in EU law: Europeanisation of essential state functions and the Union's public order. European Papers. 2024;**9**(3):1474-1485

[18] Mahalatchimy A, Lau PL, Li P, Flear ML. Framing and legitimating EU legal regulation of human gene-editing technologies: Key facets and functions of an imaginary. Journal of Law and the Biosciences. 2021;**8**(2)

[19] Segers S, Mertes H. Does human genome editing reinforce or violate human dignity? Bioethics. 2020;**34**:33-40

[20] Myrick D. Perspective chapter: Ethical implications and international human rights protections of gene editing of DNA sequences. London, UK: IntechOpen eBooks; 2025. Available from: https://www.intechopen.com/online-first/1216879

[21] Finland: Medical Research Act (488/1999, 295/2004, 794/2010)

[22] Act on Artificial Fertilisation and Use of Human Gametes and Embryos for Stem-Cell Research (55/1996)

[23] Law on Ethics of Biomedical Research (VIII-1679/2000, amended 2007)

[24] Breger MJ. *Ordre public* and the first amendment. The Review of Faith and International Affairs. 2 Jan 2015;**13**(1):39-50

[25] Gert B, Gert J. The definition of morality (Stanford encyclopedia of philosophy). Stanford Encyclopedia of Philosophy. 2002 Available from: https://plato.stanford.edu/entries/morality-definition/

[26] Cassidy EK. Restricting rights? The public order and public morality limitations on free speech and religious liberty in UN human rights institutions. The Review of Faith and International Affairs. 2015;**13**(1):5-12

[27] Sichelman TM, O'Connor SM. Patents as promoters of competition: The guild origins of patent law in the venetian republic. San Diego Law Review. 2012;**1267**

[28] Lemley MA. Property, intellectual property, and free riding. 83 Texas Law Review. 2005:1031-1075

[29] Comino S, Galasso A, Graziano C. The diffusion of new institutions: Evidence from renaissance Venice's patent system. SSRN Electronic Journal. 2017. Available from: https://www.nber.org/system/files/working_papers/w24118/w24118.pdf

[30] Roberto B. Inventori Stranieri a Venezia (1474-1797). Importazione di tecnologia e circolazione di tecnici artigiani inventori. Venezia: Istituto Veneto di Scienze; 1995

[31] *Diamond v. Chakrabarty*, 447 US 303, 309, 310 (1980)

[32] *Durisotto v. Italy* [appeal n. 62804/13]

[33] Galvez-Behar G. The patent system during the French industrial revolution: Institutional change and economic effects. Jahrbuch für Wirtschaftsgeschichte / Economic History Yearbook. 2019;**60**(1):31-56

[34] Adams S. Information Sources in Patents. DeGruyter; 2020. p. 128

[35] French Civil Code Article 1965; French Civil Code Article 6

[36] Martin Gebauer, Felix Berner. *Ordre Public* (Public Policy). Oxford Public International Law. Article last updated: August 2019. Available from: https://opil.ouplaw.com/view/10.1093/law:epil/9780199231690/law-9780199231690-e1448

[37] France. Intellectual Property Code (07 August 2004). Article L611-17

[38] France. Intellectual Property Code (07 August 2004). Article L611-17; Article L611-18 of the Code de la propriété intellectuelle

[39] France. Article L611-18 of the Code de la propriété intellectuelle

[40] Robert Cook-Deegan. Gene Patents. Hastings Center Bioethics Briefings. The Hastings Center. September 23, 2015

[41] France's Cellectis Wins U.S Patents for Gene Editing Technology. Reuters Staff. Reuters. February 13, 2018, 12:02 AM. Available from: Https://Www.Reuters.Com/Article/Us-Cellectis-Gene/Frances-Cellectis-Wins-U-S-Patents-For-Gene-Editing-Technology-Iduskbn1fx0eo

[42] Murphy K. The traditional view of public policy and *ordre public* in private international law. The Georgia Journal of International and Comparative Law. 1981;**11**:591

[43] Introductory Act, Article 30. German Civil Code. 1896

[44] Eugenics Sterilization Laws. Available from: http://www.eugenicsarchive.org/html/eugenics/essay8text.html

[45] Euthanasia Program. Holocaust Encyclopedia. United States Holocaust Memorial Museum. https://encyclopedia.ushmm.org/content/en/article/euthanasia-program

[46] Patent Act as published on 16 December 1980 (Federal Law Gazette 1981 I, p. 1), as last amended by Article 1 of the Act of 30 August 2021 (Federal Law Gazette I, p. 4074)

[47] Article 27.2. Agreement on Trade-Related Aspects of Intellectual Property Rights, Apr. 15, 1994, Marrakesh Agreement Establishing the World Trade Organization, Annex 1C, 1869 U.N.T.S. 299, 33 I.L.M. 1197 (1994)

[48] Article 53(a). Convention on the Grant of European Patents, October 5, 1973, 13 INT'L LEGAL MATS. 268 (1974)

[49] Italy. Article 81-quinquies ((Exclusions Codice della proprietà industriale (decreto legislativo 10 febbraio 2005, n. 30, aggiornata con le modifiche introdotte dal decreto legislativo 19 maggio 2020, n. 34)). Legislative Decree 10 February 2005, n. 30. Industrial Property Code, pursuant to article 15 of the Law of 12 December 2002, n. 273

[50] Spain. Patent Law. Ley N° 24/2015, de 24 de julio de 2015, de Patentes (modificada por la Ley N° 6/2018, de 3 de julio de 2018)

[51] Spain. Patent Law. Article 5(d)(5). Exceptions to Patentability. Ley N° 24/2015, de 24 de julio de 2015, de Patentes (modificada por la Ley N° 6/2018, de 3 de julio de 2018)

[52] Austria. Gene Technology Act of 1995

[53] OECD. Genetic Testing: A Survey of Quality Assurance and Proficiency Standards. OECD Publishing; 2007

[54] Regulatory Developments in Genetic Testing in Austria. Organisation for Economic Co-operation and Development (OECD). Available from: https://www.oecd.org/sti/emerging-tech/regulatorydevelopmentsingeneticte stinginaustria.htm [Accessed: August 11, 2021]

[55] Austria: Reproductive Medicine Act - FmedG (1992 as amended 2018); Reproductive Medicine Act (Fortpflanzungsmedizingesetz) of 2004

[56] Austria: Genetic Engineering Act, Section 2 para 2 subpara 4

[57] Belgium Patent Law. Chapter II, Section1: General Provisions; Article 4, as last amended on January 28, 1997. Entry Into Force: April 14, 1997

[58] Denmark Patents Act Consolidate Act No. 221 of 26 February 2017. Enter into force on 1 January 2017

[59] Denmark. The Consolidated Patents Act (Consolidated Act No. 824 of September 13, 1996, Part 1, Section 1 (4), as amended by Act No. 972 of December 17, 1997)

[60] Genetic Inventions, Intellectual Property, Rights and Licensing Practices. Evidence and Policies. Organisation for Economic Co-operation and Development (OECD). 2002

[61] Denmark Patents Act Consolidate Act No. 221 of 26 February 2017. Entered into force on 1 January 2017

[62] Estonia Riigi Teataja. Patents Act. Chapter II. Patent Protection and Patentability. Passed 16.03.1994. RT I 1994, 25, 406. Entry into force 23.05.1994

[63] Latvia. Patent Law (as amended up to January 1, 2016). law Latvijas Vēstnesis, 34 (3610), Adoption: 15.02.2007. 27.02.2007. Entry into force: 01.03.2007

[64] The Republic of Lithuania. Patent Law. Article 5. Exceptions to patentability. 1994 January 18 No. I-372. Consolidated version from 25/04/2020 to 31/12/2021. Patentų įstatymo Nr.I-372 18 Sau 1994 Patentų įstatymo Nr.I-372 18 Sau 1994 (su pakeitimais, padarytais įstatymas 2020 m. balandžio 21 d. Nr. XIII-2857)

[65] Luxembourg. Law of July 20, 1992, on the Changes in the System for Patents for Invention (as amended by the Law of May 24, 1998)

[66] Luxembourg. Article 5 (1) Exceptions to patentability. Law of July 20, 1992, on the Changes in the System for Patents for Invention (as amended by the Law of May 24, 1998)

[67] Malta. Patent and Designs Act (Chapter 417). 01 June 2002. ACT XVII of 2000, as amended by Acts IX of 2003 and XVIII of 2005; and Legal Notices 181 and 186 of 2006, and 426 of 2007

[68] Malta. Chapter IV. Patents and Patentability, 4 (5)(a). Patent and Designs Act (Chapter 417). 01 June 2002. ACT XVII of 2000, as amended by Acts IX of 2003 and XVIII of 2005; and Legal Notices 181 and 186 of 2006, and 426 of 2007

[69] Malta. Chapter IV. Patents and Patentability, 4 (5)(c). Patent and Designs Act (Chapter 417). 01 June 2002. ACT XVII of 2000, as amended by Acts IX of 2003 and XVIII of 2005; and Legal Notices 181 and 186 of 2006, and 426 of 2007

[70] Malta. Chapter IV. Patents and Patentability, 4 (5)(d). Patent and Designs Act (Chapter 417). 01 June 2002. ACT XVII of 2000, as amended by Acts IX of 2003 and XVIII of 2005; and Legal Notices 181 and 186 of 2006, and 426 of 2007

[71] Malta. Chapter IV. Patents and Patentability, 15 (2)(a). Patent and Designs Act (Chapter 417). 01 June 2002. ACT XVII of 2000, as amended by Acts IX of 2003 and XVIII of 2005; and Legal Notices 181 and 186 of 2006, and 426 of 2007

[72] Malta. Chapter IV. Patents and Patentability, 15 (2)(b). Patent and Designs Act (Chapter 417). 01 June 2002. ACT XVII of 2000, as amended by Acts IX of 2003 and XVIII of 2005; and Legal Notices 181 and 186 of 2006, and 426 of 2007

[73] Netherlands. Patent Law. Rijkswet van 15 december 1994, houdende regels met betrekking tot octrooien

[74] Netherlands. Article 3(1)(b). Patent Law. Rijkswet van 15 december 1994,

houdende regels met betrekking tot octrooien

[75] Poland. Act of June 30, 2000, on Industrial Property (as amended up to Act of February 13, 2020). Ustawa z dnia 30 czerwca 2000 r. Prawo własności przemysłowej (zmieniona do ustawy z dnia 13 lutego 2020)

[76] Poland. Article 93 (4.3). Act of June 30, 2000, on Industrial Property (as amended up to Act of February 13, 2020). Ustawa z dnia 30 czerwca 2000 r. Prawo własności przemysłowej (zmieniona do ustawy z dnia 13 lutego 2020)

[77] Romania. Law No. 64 of October 11, 1991, on Patents (as amended up to Law No. 83/2014)

[78] Romania. Chapter II. Patentable Inventions. Article 6(2)(d) - Law No. 64 of October 11, 1991, on Patents (as amended up to Law No. 83/2014)

[79] Romania. Chapter IV. Rights and Obligations. Article 31 (9) - Law No. 64 of October 11, 1991, on Patents (as Amended up to Law No. 83/2014)

[80] Germany. Patent Act, Section 1(a)

[81] Mojica FJM, Rodríguez-Valera F. The discovery of CRISPR in archaea and bacteria. The FEBS Journal. 2016;**283**(17):3162-3169

[82] Mojica F, Ferrer C, Juez G, Rodríguez-Valera F. Long stretches of short tandem repeats are present in the largest replicons of the *Archaea Haloferax mediterranei* and *Haloferax volcanii* and could be involved in replicon partitioning. Molecular Microbiology. 1995;**17**:85-93

[83] Ruud. Jansen, Jan. D. A. van Embden, Wim. Gaastra, and Leo. M. Schouls. Identification of genes that are associated with DNA repeats in prokaryotes. Molecular Microbiology. 2002;**43**(6):1565-1575

[84] Law 14/2007, of 3 July, on Biomedical Research. Spain

[85] Article 2(a) of Law 14/2007 of 3 July, on Biomedical Research. Spain

[86] Human Germline Modification and the Right to Science: A Comparative Study of National Laws and Policies. Editors: Andrea Boggio, Cesare P. R. Romano, Jessica Almqvist. Cambridge University Press, 2020. p. 595

[87] TITLE VI. Offenses, Penalties and Compensation for Damages. Article 74. Offenses. (2)(a) Biomedical Research Act (2007). Spain

[88] Oviedo Convention Article 13 – CRISPR Cannot Be Used to Alter Human Germline Cells. Oviedo Convention and Its Protocols. Council of Europe Portal. Bioethics. 2020. See also https://www.coe.int/en/web/bioethics/oviedo-convention. Council of Europe (2017) Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine. Available from: https://www.coe.int/en/web/conventions/full-list/-/conventions/rms/090000168007cf98

[89] Baylis F, Darnovsky M, Hasson K, Krahn TM. Human germ line and heritable genome editing: The global policy landscape. The CRISPR Journal. Oct 2020;**3**(5):365-377; HumanGenomeEditingPolicies_AllRegions.docx [Internet]. docs.google.com. Available from: https://docs.google.com/document/d/e/2PACX-1vSm2ck_5PMArmzxH5dHZJ5Atumlmx_

jn4dIoUNxvOBde6tufQpgnqxkIrmf xmq0sA/pub

[90] Human Germline Modification and the Right to Science: A Comparative Study of National Laws and Policies. Editors: Andrea Boggio, Cesare P. R. Romano, Jessica Almqvist. Cambridge University Press, Jan 9, 2020. p. 375

[91] Spain. Patent Law. Article 5 (a)(b), de Ley N° 24/2015, de 24 de julio de 2015, de Patentes (modificada por la Ley N° 6/2018, de 3 de julio de 2018)

[92] Austria Patent Law. Patentgesetz 1970 (zuletzt geändert durch das Bundesgesetz BGB1. I. Nr. 37/2018). § 2 (1) 1

[93] Belgium. Law on Research Involving In Vitro Embryos (2003 as amended 2014)

[94] Article 13, Oviedo Convention

[95] Denmark Consolidated Patents Act

[96] Denmark: Ministerial Order on the Use of Tissue Registration. *See also* Denmark: Regional Science Ethics Committee, and The Danish National Committee on Health and Research Ethics (National Science Ethics Committee)

[97] Denmark: Act on Biomedical Research Ethical Evaluation of Medical Research Projects

[98] Denmark Patent Act No. 221 of 26/2/2017

[99] Act on Cloning and Modification of Animals, Environment and Genetic Engineering

[100] Law No. 64 of October 11, 1991, on Patents (as amended up to Law No. 83/2014)

[101] Article 13 of the Oviedo Convention

[102] Rule 28(c) of the European Patent Convention

[103] Romania. Chapter II. Patentable Inventions. Article 8 (1)(a)(c) - Law No. 64 of October 11, 1991, on Patents (as Amended up to Law No. 83/2014)

[104] Kokott J, Diehn T. Kingdom of the Netherlands v. European Parliament and Council of the European Union. American Journal of International Law. 2002;**96**(4):950-955

[105] Case C-377/98, Kingdom of the Netherlands v. European Parliament, 2001 ECR 1

[106] Case C-377/98 Netherlands v European Parliament and Council [2001] ECR I-7079. 9 October 2001. Official Journal of the European Union C 303/17 - 14.12.2007

[107] European Biotech Directive (Directive 98/44/EC)

[108] Kingdom of the Netherlands v European Parliament and Council of the European Union. Case C-3 77/98. (Annulment — Directive 98/44/EC — Legal protection of biotechnological inventions — Legal basis — Article 100a of the EC Treaty (now, after amendment, Article 95 EC). Summary of the Judgment. http://curia.europa.eu/juris/showPdf.jsf?docid=101839&doclang=EN. *See also* Article 5(3) of the Directive, European Biotech Directive (Directive 98/44/EC)

[109] Kingdom of the Netherlands v European Parliament and Council of the European Union. Case C-3 77/98. (Annulment — Directive 98/44/EC — Legal protection of biotechnological inventions — Legal

basis — Article 100a of the EC Treaty (now, after amendment, Article 95 EC). Summary of the Judgment. http://curia.europa.eu/juris/showPdf.jsf?docid=101839&doclang=EN; *See also* Article 5(3) of the Directive, European Biotech Directive (Directive 98/44/EC); *Also see Diamond v. Chakrabarty*, 447 U.S. 303 (1980) (where bacteria were genetically modified to clean oil spills; bacteria in their natural state cannot be patented)

[110] Case C-34/10. *Brüstle Oliver v. Greenpeace*

[111] Article 6(2)(c) of the European Biotech Directive (Directive 98/44/EC) of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnological inventions OJ 1998 L213 (Biotech Directive)

[112] Myrick D. The Impact of *Ordre Public* and Morality on the Regulation of Gene Editing Patents in the United States and the European Union. 3 Feb 2023. DOI: 10.2139/ssrn.4347343. Available from: https://ssrn.com/abstract=4347343

[113] *T 1285/13 (miR-1/Max-Planck-Gesellschaft) of 20.10.2016*

[114] Wessels HH, Lebedeva S, Hirsekorn A, et al. Global identification of functional microRNA-mRNA interactions in *Drosophila*. Nature Communications. 2019;**10**:1626

[115] O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Frontiers in Endocrinology (Lausanne). 3 Aug 2018;**9**:402

[116] Cook-Deegan R, Heaney C. Patents in genomics and human genetics. Annual Review of Genomics and Human Genetics. 2010;**11**:383-425

[117] Kohler RE. Lords of the Fly: Drosophila Genetics and the Experimental Life. Chicago: University of Chicago Press; 1994

[118] Jennings BH. Drosophila – A versatile model in Biology and Medicine. Materials Today. 2011;**14**(5):190-195

[119] Baenas N, Wagner AE. *Drosophila melanogaster* As an alternative model organism in nutrigenomics. Genes and Nutrition. 2019;**14**:14

[120] Article 57 of the European Patent Convention

[121] *Diamond v. Chakrabarty*

[122] *Durisotto v. Italy* (application no. 62804/13). 2014. Global Health and Human Rights Database. Available from: https://www.globalhealthrights.org/pdf.php?ID=15249 [Accessed: August 29, 2021]

[123] Legislative decree 24/2013

[124] *Durisotto v. Italy* (application no. 62804/13). Global Health and Human Rights database. Available from: https://www.globalhealthrights.org/pdf.php?ID=15249 [Accessed: August 30, 2021]

[125] Rial-Sebbag E, Blasimme A. The European court of human rights' ruling on unproven stem cell therapies: A missed opportunity? Stem Cells and Development. 2014;**23**(Suppl 1):39-43

[126] Austrian Academy of Sciences Position on Gene Editing in the Light of Recent Reports about Gene Edited Babies. 2021. Available from: https://www.oeaw.ac.at/fileadmin/NEWS/2019/PDF/OeAW-Position-on-genome-editing.pdf; INSERM. Republique Francaise. About INSERM. Missions, https://www.inserm.fr/en/about-us/missions/

[127] Austrian Academy of Sciences Position on Gene Editing in the Light of Recent Reports about Gene Edited Babies. Available from: https://www.oeaw.ac.at/fileadmin/NEWS/2019/PDF/OeAW-Position-on-genome-editing.pdf

[128] Austria. Gene Technology Act of 1995; Description of Austria Regulations on Genetic Engineering, https://www.verbrauchergesundheit.gv.at/gentechnik/gentDescription.html [Accessed: August 12, 2021]

[129] "Ethical Aspects of the Development and Use of Assistive Technologies," Opinion of the Austrian Bioethics Commission, 13 July 2009, Bunderkanzleramt Österriech, Austrian Bioethics Commission, at http://www.bka.gv.at/DocView.axd?CobId=39411 [Accessed: July 27, 2011]

[130] Federal Chancellery Republic of Austria. Bioethics Commission. Bundesrechenzentrum. Federal Chancellery of Austria. 2019. Available from: https://www.bundeskanzleramt.gv.at/en/topics/bioethics-commission.html [Accessed: August 12, 2021]

[131] INSERM. Republique Francaise. About INSERM. Missions. Available from: https://www.inserm.fr/en/about-us/missions/ [Accessed: July 07, 2021]

[132] Value Creation and Transfer of New Discoveries. INSERM. 2021. Available from: https://www.inserm.fr/en/our-research/value-creation-and-transfer-of-new-discoveries/.

[133] Key Patent Granted for AAVLife's Gene-Therapy Program to Treat Cardiomyopathy in Friedreich's Ataxia. Business Wire. 05 August 2015 08:05 AM EDT. Available from: https://www.businesswire.com/news/home/20150805005812/en/Key-Patent-Granted-For-AAVLife%E2%80%99s-Gene-Therapy-Program-to-Treat-Cardiomyopathy-in-Friedreich%E2%80%99s-Ataxia

[134] Perdomini M, Belbellaa B, Monassier L, Reutenauer L, Messaddeq N, Cartier N, et al. Prevention and reversal of severe mitochondrial cardiomyopathy by gene therapy in a mouse model of Friedreich's ataxia. Nature Medicine. 2014;**20**:542-547

[135] Picanço-Castro V et al. Emerging patent landscape for non-viral vectors used for gene therapy. Nature Biotechnology. 2020;**38**(2):151-157

[136] New Gene Therapy to Treat Spinal Muscular Atrophy (Corrected). Press Release 27/03/2020. European Medicines Agency. Available from: https://www.ema.europa.eu/en/news/new-gene-therapy-treat-spinal-muscular-atrophy-corrected

[137] Hervé Chneiweiss, Francois Hirsch, Lluis Montoliu, Albrecht Müller, Solveig Fenet, et al. Fostering Responsible Research with Genome Editing Technologies: A European Perspective. Transgenic Research, Springer Verlag, 2017. The universities and institutions included the University of Vienna (Austria), Catholic University of Louvain (Belgium), Vrije Universiteit Brussel (Belgium), European Medicines Agency, University Panthéon-Assas (France), German National Academy of Sciences Leopoldina (Germany), Institute of Medical Radiology and Cell Research, University of Würzburg (Germany), National University of Ireland, Galway (Ireland), the National Centre for Biotechnology (CNB-CSIC), CSIC Ethics Committee and CIBERER-ISCIII, Madrid (Spain), University of Geneva (Switzerland), University of Malta (Malta), University of Warsaw (Poland), Universidade Católica Portuguesa,

Instituto de Bioética, and Portuguese National Council for Ethics in Life Sciences (Portugal), and UNESCO

[138] Chneiweiss H, Hirsch F, Montoliu L, Müller A, Fenet S, et al. Fostering Responsible Research with Genome Editing Technologies: A European Perspective. Transgenic Research: Springer Verlag; 2017

Chapter 4

Editing the Future: Ethical Challenges of Therapeutic Use, Germline Modification and Human Enhancement with CRISPR

Gourab Chatterjee and Kusum Kumari Singh

Abstract

The advent of genome editing technologies traces back to the discovery of Type IIS restriction enzymes, especially FokI, which laid the foundation for early genetic manipulation. Over the decades, various attempts were made to develop affordable and precise gene-editing tools. This vision became reality with the emergence of CRISPR-Cas systems, which have revolutionised modern biology by enabling targeted, efficient and cost-effective genome editing. However, with transformative power comes significant ethical responsibility. This chapter digs into the major ethical dilemmas CRISPR technologies pose, particularly the ambivalent issue of germline genome editing. Concerns over unintended off-target effects, questions about the adequacy and scope of informed consent, especially in cases involving embryos or future generations, and the potential rise of 'designer babies' have sparked critical discourse among scientists, ethicists and policymakers. This chapter will provide a comprehensive overview of CRISPR's ethical landscape through a balanced analysis of scientific milestones, moral frameworks, real-world controversies and global regulatory responses. It aims to foster a deeper understanding of how society can navigate the power and pitfalls of genome editing while upholding principles of justice, equity and responsibility.

Keywords: genome editing, CRISPR-cas9, germline editing, off-targets, ethics

1. Introduction

For many years, the *holy grail* of biotechnological advancement was the ability to modify organisms genetically. The race to develop genome editing tools began with the discovery of the FokI nuclease, a Type IIS restriction enzyme [1]. In 1994, Chandrasegaran engineered the first artificial nuclease capable of targeted genome editing by fusing the Ubx homeodomain of *Drosophila* with the nuclease domain of FokI [2]. This strategy laid the foundation for developing zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) [3, 4].

Although these tools were practical for targeted genome modification and could theoretically be customised for specific sequences, they were technically challenging to engineer and difficult to deliver into cells.

The emergence of CRISPR-Cas systems revolutionised the field by making targeted sequence editing more accessible and efficient. CRISPR was first observed in *Escherichia coli* by Ishino *et al.* at Osaka University in Japan, during their research to identify the gene product of the *iap* gene [5]. They found five highly homologous sequences of 29 nucleotides arranged in direct repeats with a spacing of 32 nucleotides. Though this kind of architecture in the genome of *E. coli* was unusual, they failed to identify what the potential function could be.

Later, it was discovered that these repetitive sequences function as an adaptive immune system in bacteria, enabling them to survive viral infections by recognising and cleaving foreign genetic material. Initially, the CRISPR system was believed to operate through RNA interference. However, Marraffini and Sontheimer provided experimental evidence that these genomic structures, associated with CRISPR-associated (Cas) genes, targeted DNA directly, thereby highlighting their potential for gene editing [6].

On August 17, 2012, Jennifer Doudna and Emmanuelle Charpentier published their groundbreaking findings on the components of the CRISPR-Cas9 system and how it could be optimised to edit virtually any DNA sequence [7]. Their work earned them the Nobel Prize in Chemistry in 2020 and marked a transformative moment in the field of genome engineering.

This breakthrough triggered an intense global race to harness the power of CRISPR-Cas9 for targeted genetic modification. In January 2013, five independent research groups published protocols enabling the genetic editing of mammalian cells, bringing humanity one step closer to the possibility of engineering organisms at will [8].

While this achievement was hailed as a dream come true, promising cures for genetic disorders, cancer treatments and the development of genetically enhanced plants and animals, it also raised serious ethical concerns. Discussions around germline editing, designer babies and potential military applications of CRISPR emerged, serving as a stark warning of the consequences of unregulated use. This led to the 'International Summit on Human Gene Editing' [9].

The ethical implications of CRISPR continue to be a topic of active debate, and the question of how to regulate its use remains unresolved. In this chapter, we aim to explore the key ethical questions that have generated remarkable dilemmas within the scientific community regarding the conscientious application of CRISPR technology. We also examine the broader social and cultural perspectives on genome editing, whether in the context of medical treatments or the development of genetically modified organisms.

2. The evolution of CRISPR

2.1 A brief history of CRISPR

The CRISPR-Cas9 system represents the most recent and significant advancement in genome editing. It is arguably the most user-friendly and accessible method of targeted genome modification, enabling precise editing without altering the amino acid sequence to target specific genomic loci.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a naturally occurring adaptive immune system found in bacteria and archaea that provides protection against viral infections. This elegant system enables microorganisms to 'remember' past infections by storing fragments of viral DNA and using them to mount a faster response upon reinfection [10]. Depending on the mechanisms involved and the associated *cas* genes, CRISPR systems are categorised into several types [11]. This section focuses on giving a brief overview of the CRISPR system and how it functions as an adaptive immune system in bacteria.

2.1.1 Adaptation (spacer acquisition)

To understand the mechanism, we must first examine the CRISPR locus. A typical locus consists of three components: a leader sequence, a CRISPR array and *cas* genes. The leader is an AT-rich region located upstream of the array. The CRISPR array is composed of short palindromic repeats interspersed with unique spacer sequences, which are derived from foreign genetic material, serving as molecular 'memories' of past infections. The *cas* genes encode proteins responsible for the three stages of the CRISPR mechanism [12].

During infection, Cas1 and Cas2 proteins cleave invading foreign DNA. The resulting fragments, known as protospacers, are incorporated into the CRISPR array as new spacers. This acquisition process is not random; it is PAM-dependent, meaning a short sequence called the Protospacer Adjacent Motif (PAM) is required for spacer integration [13, 14].

2.1.2 crRNA biogenesis

Upon a subsequent infection, the CRISPR array is transcribed into a long precursor crRNA (pre-crRNA). This transcript is processed into multiple mature crRNAs, each containing a unique spacer sequence corresponding to a specific invader [14]. In Type II systems, crRNA pairs with a trans-activating CRISPR RNA (tracrRNA), which is transcribed from a nearby gene. Together, they form a duplex that is processed by host RNase III into a functional crRNA-tracrRNA complex [15]. This complex is then loaded onto Cas9 for the next phase.

2.1.3 Target interference

In the interference stage, the mature crRNA guides Cas9 to the target DNA sequence, where it binds *via* base pairing and cleaves the foreign DNA. To avoid attacking its own DNA, the system distinguishes self from non-self by recognising PAM sequences, which are absent in the host genome. Depending on the CRISPR type, the PAM may be located upstream or downstream of the protospacer [16–19].

This bacterial defence mechanism was repurposed by Jennifer Doudna and Emmanuelle Charpentier for targeted genome editing in eukaryotic cells, an achievement that earned them the Nobel Prize in 2020. By combining Cas9 with a synthetic single guide RNA (sgRNA) – a fusion of crRNA and tracrRNA – they demonstrated programmable double-stranded breaks at specific genomic locations [20]. These breaks can be repaired *via* Non-Homologous End Joining (NHEJ) for gene disruption or Homology-Directed Repair (HDR) for gene insertion (**Figure 1**).

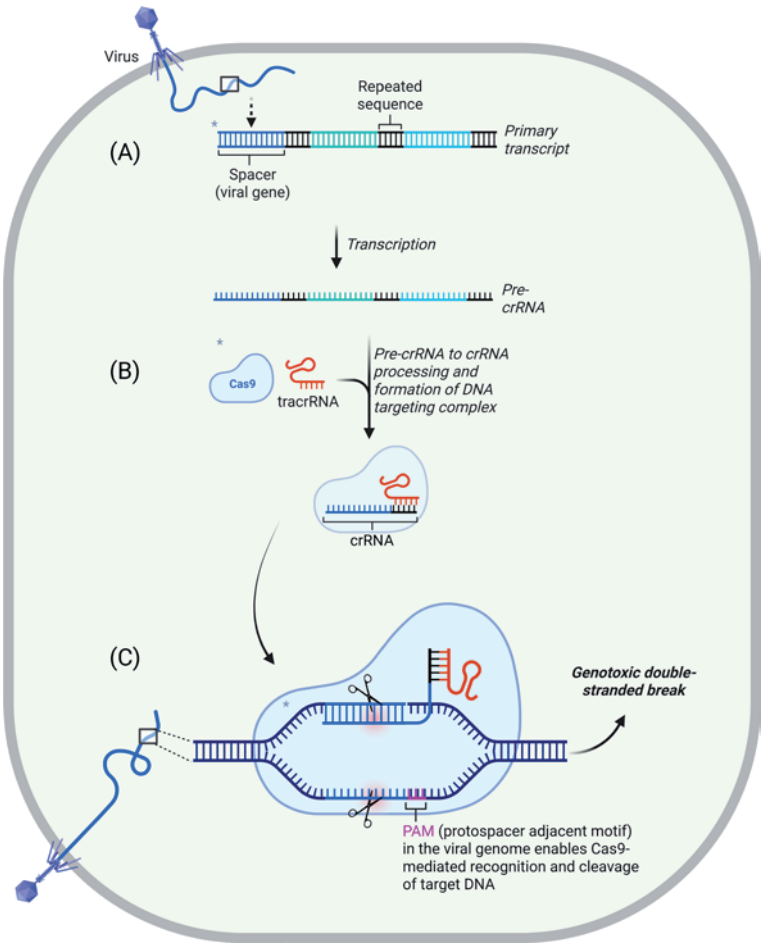

Figure 1.
The process of (A) spacer adaptation, (B) crRNA biogenesis and (C) Target interference for CRISPR-Cas9 complex. Created in BioRender. Chatterjee, G. (2025). https://BioRender.com/d0d201v.

2.2 Landmark events in the post-2012 era

2.2.1 The race towards genome editing

In January 2013, five research articles from independent groups were published, marking a pivotal moment in the advancement of genome editing. It was like an unspoken race had begun, with each group striving to contribute and remain relevant in the rapidly evolving CRISPR landscape. These publications reported successfully establishing protocols for CRISPR-mediated genetic editing in mammalian cells. Among the most influential studies were those from the laboratories of George Church (Harvard University, USA), Jennifer Doudna (University of California, Berkeley, USA) and Feng Zhang (Broad Institute, USA). Their work demonstrated that several key optimisations were required to adapt the CRISPR-Cas9 system for effective genome editing [21–23]. These included codon optimisation of the Cas9 gene, the addition of a nuclear localisation signal (NLS), determination of the optimal length of the single guide RNA (sgRNA) and the incorporation of homologous

DNA templates to promote the homology-directed repair (HDR) pathway over the error-prone non-homologous end joining (NHEJ) pathway. In the same month, J. Keith Joung's group at Harvard Medical School became the first to use CRISPR *in vivo*, applying the system to zebrafish embryos [24]. This marked the first instance of germline editing using CRISPR technology.

2.2.2 The patent dispute

Between May and December 2012, two patents were filed concerning the CRISPR-Cas9 system. Jennifer Doudna and Emmanuelle Charpentier submitted the first [25], supported by their respective institutions, while Feng Zhang and the Broad Institute filed the second [26]. In April 2014, Zhang's patent was granted before that of Doudna and Charpentier, prompting Doudna to challenge the decision. The case was referred to the U.S. Patent and Trademark Office's Patent Trial and Appeal Board (PTAB). Following a prolonged legal dispute in 2018, Doudna and Charpentier were awarded their patent, while Zhang's patent remained valid. This dual-patent scenario created complications, as some companies obtained licences from Zhang and the Broad Institute, while others licenced the technology from Doudna and Charpentier. In 2022, the patent tribunal revisited the case to determine the rightful ownership of the foundational CRISPR-Cas9 intellectual property. While it was acknowledged that Doudna and Charpentier were pioneers in the field, their early data lacked clear evidence of successful genome editing in animal models such as zebrafish. In contrast, Zhang's 2012 work provided conclusive evidence of CRISPR-based genome editing in human and mouse cells. As a result, the tribunal ruled in favour of Zhang. However, the dispute has not ended. The University of California, on behalf of Doudna and Charpentier, has recently reopened the challenge, keeping the legal battle over CRISPR patents ongoing [27].

2.2.3 International summit on human gene editing

As the race to dominate the CRISPR landscape intensified, ethical concerns remained ever-present. To define the boundaries of research and reach a global consensus on the responsible use of CRISPR, several leading scientific bodies, including the U.S. National Academy of Sciences, the U.S. National Academy of Medicine, the Royal Society and the Chinese Academy of Sciences, organised the first International Summit on Human Gene Editing in December 2015. David Baltimore, chair of the summit, captured the significance of the moment in his opening remarks:

> *'We could be on the cusp of a new era in human history. Today, we sense that we are close to being able to alter human heredity. Now we must face the questions that arise. How, if at all, do we as a society want to use this capability? This is the question that has motivated this meeting' [9].*

Indeed, it marked the beginning of an era of unprecedented advancement in the application of CRISPR. But alongside this progress came hard-earned lessons and growing awareness of the ethical boundaries this technology must respect. The 2015 summit was the first formal international platform where scientists, ethicists and policymakers could collectively address the questions surrounding the aftermath of the CRISPR revolution. It offered an opportunity to voice ethical and social concerns globally. Among the most pressing issues raised was germline editing—modifying

heritable genetic traits. A stark division emerged within the scientific community. While some called for an outright ban on germline editing, others advocated for refining the technique, believing it could one day contribute to human health and even evolutionary advancement. Following the success of the inaugural summit, second and third summits were convened, with the most recent held in London in 2023 [28, 29]. Together, these summits have laid the groundwork for the international coordination of governance, oversight and regulation of human genome editing.

2.2.4 CRISPR'd babies

A day before the second International Summit on Human Genome Editing, a sensational revelation shocked the world: Chinese biophysicist He Jiankui had edited the germline of human embryos, resulting in the birth of non-identical twins in October. Until then, human CRISPR research had been restricted to non-viable embryos not intended for implantation. However, He had begun experiments involving CRISPR-edited embryos suitable for implantation as early as 2017 [30].

The project aimed to delete 32 base pairs in the *CCR5* gene on chromosome 3, mimicking the naturally occurring *CCR5Δ32* mutation (**Figure 2**). Individuals with this mutation lack functional CCR5 receptors on T-cells, which prevents HIV from binding and entering these immune cells, conferring resistance to infection (**Figure 3**). Although He's stated intention was to protect future children from HIV, his approach was widely condemned as reckless and ethically irresponsible, sparking one of the most significant controversies in modern science.

Since the transferrable embryos were edited using a CRISPR construct, this process led to germline editing. This means the changes influenced by the editing process were heritable and changed the heritable pattern of the offspring from these edited embryos.

He experimented with a private organisation in Beijing and recruited couples who met a specific criterion: HIV-positive fathers and HIV-negative mothers. Eight couples agreed to participate; one later withdrew. Among the remaining seven women, 13

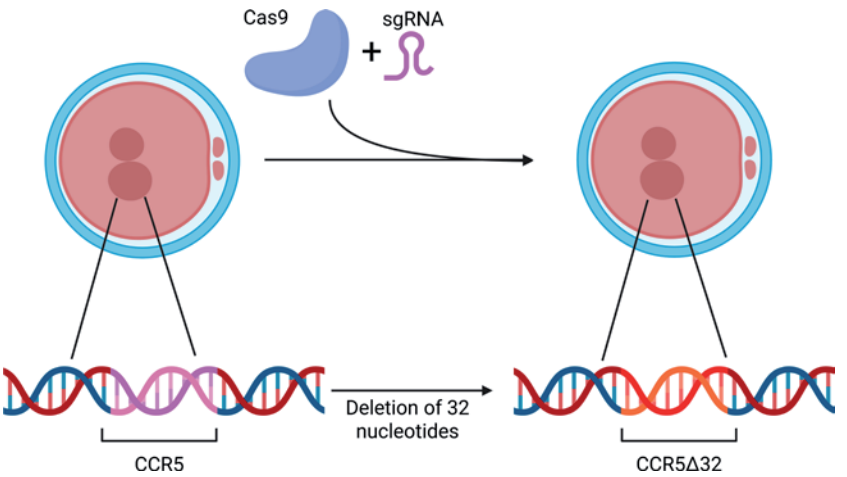

Figure 2.
The strategy adopted by He Jiankui to create the first CRISPR babies. He injected fertilised zygotes with Cas9, sgRNA targeting CCR5 gene. This strategy was supposed to create a 32 bp deletion in the CCR5 gene mimicking the CCR5Δ32 mutation. Created in BioRender. Chatterjee, G. (2025). https://BioRender.com/7xgpsm0.

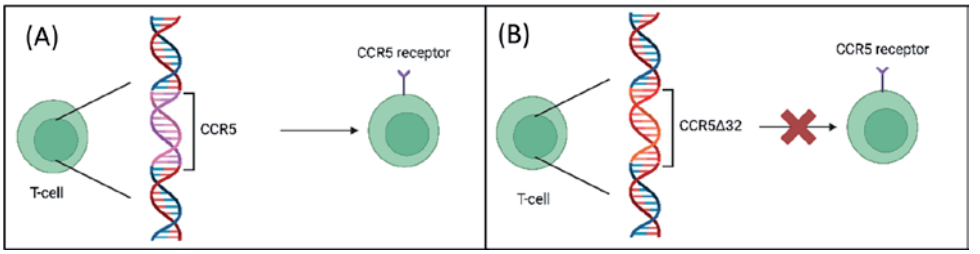

Figure 3.
(A) The CCR5 gene produces the CCR5 receptor, which is expressed on the cell surface of T cells. (B) Upon deletion of 32 nucleotides, there is no more expression of the CCR5 receptor on the T-cell surface.

embryos were transferred, resulting in two pregnancies. One of the participants gave birth prematurely to non-identical twin girls, who were assigned the pseudonyms Nana and Lulu.

He performed deep sequencing and Sanger sequencing to analyse the genetic edits. The results showed that while both twins had modifications in the *CCR5* gene, the intended *CCR5Δ32* deletion was not achieved. One twin was heterozygous – possessing one edited allele and one wild-type allele – while the other had a homozygous edit, but not of the precise type intended. A significant issue was the presence of genetic mosaicism: not all cells in either child carried the same genetic modification. This meant that only a subset of their cells had edited versions of *CCR5*, raising serious concerns about the consistency and predictability of the edits. Moreover, while the resulting proteins appeared non-functional, the mutations were novel and had never been observed in humans; therefore, their safety could not be guaranteed. One positive outcome was the absence of detectable off-target effects in other genes [31].

Even before the news became public, He had been invited to speak at the second summit. Despite facing international condemnation, he presented his findings. After his talk, David Baltimore responded strongly, reminding attendees that the first summit had concluded with a consensus that CRISPR should not be used clinically until its safety was assured [32]. He declared:

> *'I think there has been a failure of self-regulation by the scientific community because of a lack of transparency' [33].*

Several scientists posed difficult questions. David Liu from the Broad Institute challenged the rationale behind choosing *CCR5*, noting that very little is known about its role beyond HIV susceptibility. He also questioned the medical necessity of the intervention, as sperm washing, a standard IVF procedure, can prevent HIV transmission from father to child, making He's intervention unnecessary and ethically indefensible.

The 'CRISPR babies' scandal dominated headlines across both scientific forums and mainstream media. In 2019, He Jiankui was convicted of forging ethical review documents and misleading physicians to implant genetically modified embryos. He was sentenced to 3 years for violating domestic and international laws [20]. In 2023, after serving his sentence, he returned to research, this time working on non-human model systems [34].

This incident was a stern reminder to the global scientific community: reckless and premature clinical use of CRISPR technology will not be tolerated. The path forward must be paved with caution, rigorous oversight and a commitment to transparency, safety and ethics.

3. Recent advances

Following the success of the CRISPR/Cas9 system in gene editing, several Cas variants have been discovered or engineered to possess unique features that enhance the editing process. One such advancement is the discovery of Cas12, which enables the use of more extended guide RNA sequences, thereby increasing the specificity of target recognition. Unlike Cas9, Cas12 generates sticky ends at the cleavage site, which can be advantageous for DNA repair via either the non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways [16]. While the catalogue of Cas variants has grown rapidly in recent years, their efficacy and precision in somatic and germline editing remain under investigation.

Researchers engineered the Cas9 nickase (Cas9n), which creates single-strand breaks (SSBs) on adjacent DNA strands at the target site to reduce off-target effects, thereby improving specificity and minimising unintended edits [35]. Another notable development is the creation of 'dead' Cas9 (dCas9), in which the nuclease domains are mutated and inactivated. Although dCas9 lacks cutting ability, it can target specific DNA sequences for regulatory or epigenetic modifications by fusing it with other functional domains [36].

DNA base editing is a significant breakthrough in precision editing, which allows for converting a single nucleotide in the target gene without inducing double-strand breaks. This technique holds great promise for treating diseases such as cystic fibrosis with high accuracy. Building on this, a more versatile method known as prime editing has been developed. Prime editing enables the insertion of at least 44 nucleotides or deleting up to 80 nucleotides at a specific site in the genome.

In DNA base editing, a Cas9 nickase is fused with the *Apolipoprotein B mRNA Editing Enzyme Catalytic Subunit 1* (APOBEC1) and a *Uracil Glycosylase Inhibitor* (UGI), which together mediate precise base conversions. In contrast, prime editing involves fusing Cas9n with an engineered reverse transcriptase and using a specially designed guide RNA called pegRNA (prime editing guide RNA). The pegRNA contains a ~ 30-nucleotide extension that encodes the desired edit. This extension anneals to the nicked target DNA strand and acts as a template for the reverse transcriptase to synthesise the edited sequence.

4. Core ethical dilemmas

Genome editing using CRISPR remains a relatively new and rapidly evolving technology, and global regulations governing its use are still under development. While CRISPR offers unprecedented potential for precise and effective genetic modification, it also raises profound ethical, social and practical concerns that must be addressed.

Key questions remain unanswered: Is it ethically justifiable to edit the genome of human embryos? How can informed consent be obtained for individuals who have not yet been born? Will genome editing become a luxury available only to the wealthy, exacerbating social inequality?

This chapter explores these concerns in depth, examining the origins of these ethical dilemmas and why they continue to challenge scientists, ethicists and policy-makers alike.

4.1 Somatic editing Vs germline editing

One of the most pressing questions the scientific community poses is whether genome editing – particularly somatic or germline editing – is safe. To assess the risks, it is essential to understand the differences between these two approaches.

4.1.1 Somatic cell gene editing

Somatic cells (also known as vegetative cells) comprise the body's various tissues and organs, excluding gametes, gametocytes and undifferentiated stem cells. Editing the genome of somatic cells does not result in heritable changes, meaning that any edits made will not be passed on to future generations. This form of genome editing, *somatic cell gene editing*, is considered more ethically acceptable and safer than germline editing [37].

Recent advances in CRISPR technology have enabled researchers to edit somatic cells to treat and sometimes prevent diseases. The underlying strategy often mirrors the techniques used in animal models. Typically, the CRISPR system, including the single guide RNA (sgRNA) and Cas9 nuclease, is packaged into viral vectors. These vectors are then used to infect target cells within the host. The host expresses the sgRNA and Cas9 upon infection, resulting in the intended genomic edits (**Figure 4**) [38].

A landmark moment occurred in March 2020, when the first patient received *in vivo* CRISPR-Cas9 therapy to treat a form of inherited blindness caused by a point mutation in the *CEP290* gene (centrosomal protein of 290 kDa). The results were

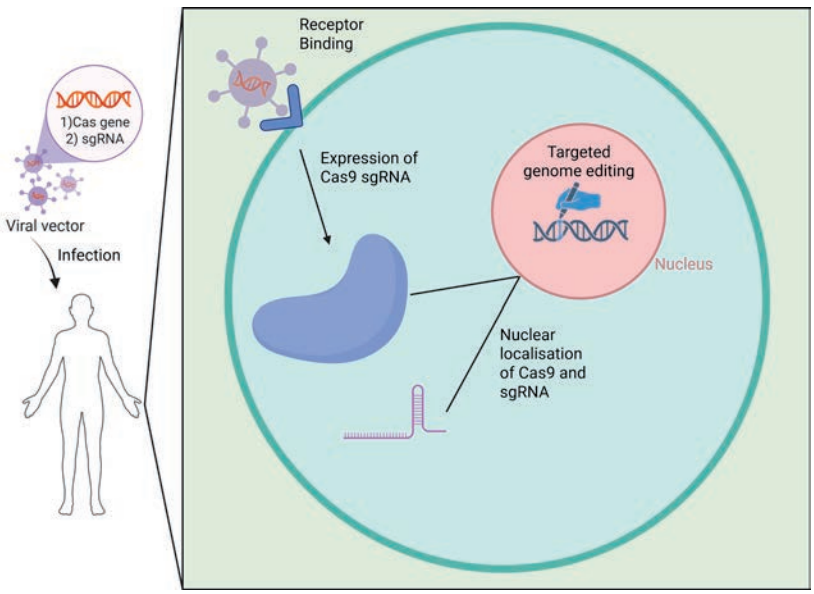

Figure 4.
Somatic gene editing strategy; the Cas gene, sgRNA and ssDNA are packaged into a non-integrative viral vector, and the patients are infected with the vector. This allows the expression of all the components inside the cell, thereby making in vivo genome editing a possibility. Created in BioRender. Chatterjee, G. (2025). https://BioRender.com/4r7c1dz.

promising; no off-target effects or adverse neurological symptoms were observed, and the patient's vision improved [39].

One of the notable advantages of somatic cell editing is that it can be performed *ex vivo* [40]. In this approach, somatic cells are extracted from the patient, edited outside the body, and only those with the desired edits, free from off-target effects, are reintroduced. This method has shown significant success, especially in treating haematological disorders like beta-thalassaemia [41]. It has dramatically advanced the development of more efficient CAR T-cell therapies for cancer treatment.

The somatic cell gene editing avoids the ethical conundrums and therefore is much easier to perform. To accelerate the development of such therapeutic strategies, the Somatic Cell Genome Editing consortium was formed.

On November 16, 2023, the UK's Medicines and Healthcare Products Regulatory Agency (MHRA) approved Casgevy, a CRISPR-based therapy, for treating sickle cell disease and beta-thalassaemia. Less than a month later, on December 8, 2023, the U.S. Food and Drug Administration (FDA) approved Casgevy and Lyfgenia for treating sickle cell disease. Since then, several other CRISPR-based therapies have entered clinical trials, signalling that we are truly entering the age of CRISPR-enabled medicine [42].

4.1.2 Germline editing

Germline cells pass genetic information from one generation to the next. These include gametes—sperm and ova—as well as their precursors. Germline editing is the intentional modification of DNA in these cells or early-stage embryos, resulting in heritable changes passed on to future generations [37].

A notable example of germline editing is the controversial case of He Jiankui, who used CRISPR to edit the CCR5 gene in human embryos that later developed into two children, nicknamed Lulu and Nana. The aim was to confer resistance to HIV infection; however, the experiment sparked international outrage due to its premature application, lack of transparency and ethical violations.

Germline editing is often viewed negatively because of its irreversible and heritable nature and the current lack of precision and understanding of long-term consequences. However, it is essential to acknowledge that germline editing also holds potential for eliminating severe inherited diseases. Conditions like cystic fibrosis, Tay–Sachs disease and certain forms of muscular dystrophy could, in theory, be prevented in future generations through germline modifications.

That said, significant scientific and ethical concerns remain unresolved. One major issue highlighted in the Lulu and Nana case was *mosaicism* – a condition where different cells within the same individual have distinct genetic makeups. This indicates that the genome editing was not uniformly successful across all embryonic cells, creating uncertainty about both efficacy and safety.

Furthermore, there is no current method to guarantee complete precision in editing, or to prevent unintended *de novo* mutations – changes that have not been previously observed in human genetics. These unanticipated edits could result in unforeseen and potentially harmful consequences.

Following the First International Summit on Human Genome Editing, a global consensus emerged that germline genome editing is premature for clinical use [9]. He Jiankui's actions violated this consensus and, as a result, drew severe criticism from both the scientific and bioethics communities.

It is worth noting, however, that human embryo genome editing is not entirely novel. Researchers have long experimented with *tripronuclear (3PN)* embryos formed

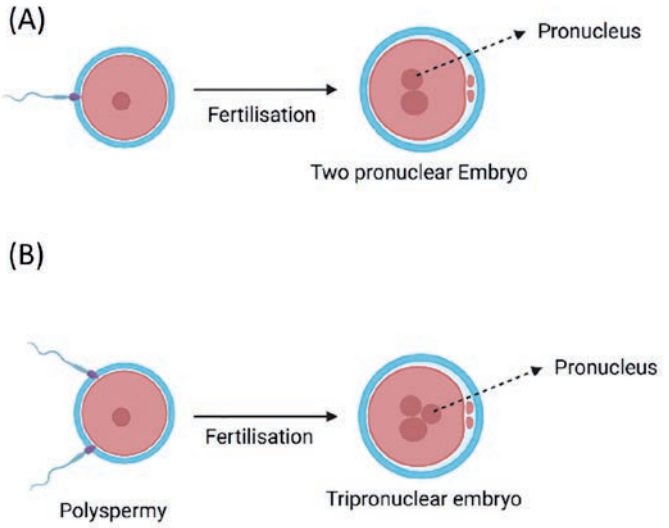

Figure 5.
During IVF if polyspermy occurs it results in a (B) tripronuclear embryo (3PN) which are developmentally challenged and therefore discarded. The (A) Two pronuclear embyos are used for implantation. Germline studies of 2PN embryos are banned in several countries while 3PN embryos are used for the same due to their incapability to be implanted. Created in BioRender. Chatterjee, G. (2025). https://BioRender.com/7gn2588.

with three pronuclei due to polyspermy during in vitro fertilisation (IVF) (**Figure 5**). These embryos have lower developmental potential and are therefore considered to carry fewer ethical concerns, making them useful for proof-of-concept experiments.

There are two main approaches for performing germline genome editing. The first involves microinjecting the guide RNA (gRNA), Cas9 protein and a single-stranded DNA (ssDNA) repair template into zygotes approximately 18 hours post-fertilisation, during the S phase of the embryonic cell cycle [43]. This method typically results in the formation of blastomeres at the 4- to 8-cell stage. The second approach is to co-inject the Cas9-gRNA complex along with the ssDNA template and sperm directly into a metaphase II (M phase) oocyte at the time of fertilisation, using intracytoplasmic sperm injection (ICSI). The latter showed an increase in the number of edited embryos, but the off-target mutations and unintended deletions still made a major contribution to the sample set.

While recent advancements have improved the specificity and reduced off-target effects of CRISPR tools, significant technical hurdles remain. Large-scale chromosomal rearrangements, for instance, are still frequently observed [44, 45] Emerging gene-editing platforms, such as Cas9 nickases, base editors (BE) and prime editors (PE), may offer improved precision, but their safety and efficiency in embryonic systems remain under investigation.

Mosaicism remains a significant challenge in germline genome editing, indicating a need for better kinetic control and delivery methods during early embryogenesis.

4.2 Boundaries of informed consent

In modern research trials, informed consent is not merely a regulatory requirement but an ethical imperative. One of the earliest examples underscoring its

importance is the Nuremberg Code. Informed consent enables individuals to understand the potential benefits and risks of the study in which they are invited to participate and to make an autonomous decision about whether or not to proceed. Conversely, it provides researchers the opportunity, and responsibility, to thoroughly educate participants about the research's purpose, procedures, risks and potential outcomes.

A significant challenge with informed consent can be illustrated through a commonly encountered scenario: software agreements. When installing new software, users must often accept the terms and conditions. Yet most people do so without reading a single line. This analogy reflects a similar problem in research participation. Participants may be unable to fully comprehend complex study protocols, while researchers may find it challenging to explain intricate scientific details in a digestible manner. In some cases, researchers may even omit crucial information – intentionally or inadvertently – resulting in a participant agreeing to something fundamentally different from what is conducted [46].

Moreover, participants may sometimes sign consent documents without reading them carefully, especially when under perceived pressure or authority influence. This compromises the principle of voluntary participation.

Further complicating the matter is a phenomenon known as therapeutic misconception, the mistaken belief that participation in research is equivalent to receiving personalised clinical care [47]. This misconception blurs the lines between clinical treatment and experimentation, potentially rendering the informed consent process null or ethically invalid.

A notable example of a flawed consent process is seen in the case of Dr. He Jiankui. As discussed earlier, his experiment involving germline genome editing drew global criticism. The consent forms used in his study were found to be misleading and, in some cases, forged. As a result, Dr. He was sentenced to 3 years in prison by the Chinese government. However, this high-profile case triggered a global response, leading to collaborative efforts to standardise and strengthen informed consent protocols – especially for genome editing research. This is particularly relevant today, as CRISPR-based therapeutics move into clinical trial stages.

Researchers must assess participants' life circumstances and potential vulnerabilities to create an ethically sound informed consent process. The International Conference on Harmonisation (ICH) defines vulnerable individuals as those whose willingness to participate in a clinical trial may be unduly influenced by perceived benefits – whether real or not. These include patients with incurable diseases, minors and individuals incapable of providing consent [48].

In embryo genome editing, vulnerability manifests in more complex ways. For instance, parents may be overly eager to ensure their offspring are free from devastating genetic diseases – diseases they may suffer from. This eagerness may compromise their ability to evaluate the risks critically. Meanwhile, the future child, the actual subject of the intervention, is unable to consent altogether. This raises the profound ethical question: can such consent be considered informed? While the intervention may yield significant health benefits, it distinguishes between medical progress and coercive genetic enhancement.

Returning to Dr. He's study, the recruited couples involved HIV-positive male partners. He offered them IVF and sperm-washing techniques to prevent HIV transmission – services that were not readily available in China. This lack of access likely heightened their willingness to participate in the experimental genome-editing study, further exacerbating the ethical concerns around undue inducement [49].

To mitigate such risks, Institutional Review Boards (IRBs) or Ethics Committees may recommend that early genome-editing trials include only participants with alternatives available for achieving the same outcome – such as having a healthy child – without relying solely on genome editing. Presenting multiple options enables prospective participants to weigh pros and cons more rationally, reducing the coercive nature of their consent.

It is important to note that Dr. He's research lacked approval from an IRB, as ICH guidelines require. Court records later confirmed that his consent documents were forged and that he engaged in 'illegal medical practices' [20].

Furthermore, consent forms must be written in clear, accessible language. They should avoid excessive technical jargon and provide concise yet complete research explanations. Research aims to generate *generalisable knowledge*, and it is often unclear whether participants will personally benefit. Therefore, consent forms must avoid guaranteeing direct benefits. For instance, Dr. He's claim that 'this research project will likely help you produce HIV-resistant infants' was not only overly optimistic but also ethically coercive. It is of great importance to mention that even though the CCR5 mutation can possibly produce HIV-resistant offspring, it also increases the susceptibility to West Nile disease [50].

The debate on designing an ethically robust and truly informed consent process continues. The rise of CRISPR-based therapeutics offers hope as well as underscores the urgent need for global ethical standards, mainly when interventions affect not just current participants but future generations. This leads us to the central question: can germline editing ever be ethically justified, and if so, how can consent be truly informed for a child yet unborn?

4.3 Genetic enhancement vs. therapeutic uses

To understand the ethical debates surrounding genome editing, it is important to distinguish between genetic therapy and genetic enhancement. A widely accepted definition of medical *therapy* stems from Norman Daniels' formulation of the standard medical model. In this model, *therapy* refers to interventions designed to maintain or restore normal bodily organisation and function, defined relative to typical states for one's species, age and sex [51]. According to Daniels, society has an obligation to provide such treatment only when there is a medical need, that is, a deviation from normal functioning.

Enhancement, by contrast, refers to interventions intended to improve upon typical or 'normal' traits, whether in appearance, health or performance. Examples include the use of anabolic steroids to build muscle mass, undergoing elective rhinoplasty for cosmetic reasons or altering gametes to increase a child's likelihood of possessing above-average musical ability [52].

A key example often discussed is the controversial case of 'CRISPR babies', in which prospective parents might select traits they desire in their children, such as blue eyes, increased muscularity or other characteristics aligned with prevailing beauty or performance ideals. Another possibility is the enhancement of physical prowess in military personnel, reminiscent of fictional concepts like Captain America's 'super serum'.

However, it is crucial to recognise that such enhancements would need to be implemented at the embryonic stage, requiring germline editing. This means that the alterations would not only affect the individual but also be inherited by future generations. As a result, such interventions raise fundamental questions about the

long-term consequences of human-directed evolution. Could germline editing alter the natural evolutionary trajectory of our species? This was pointed out by John Harris from the University of Manchester in the first summit, where he said gene editing provides a means of evolving, which is much quicker, rational and biased than Darwinian evolution [28].

Another major concern lies in the issue of access. If genetic enhancement ever becomes permissible, will it be available to everyone or only to those who can afford it? The fear is that these interventions could deepen existing inequalities. As Faith Lagay points out in her commentary in the *AMA Journal of Ethics*, 'The rich will not only have more money than the rest of us—they'll be taller, smarter, and better looking too' [52].

4.4 Access equity and justice

A major question in the field of genome editing is how CRISPR/Cas9 can be made maximally beneficial to all communities. For this technology to truly mitigate, rather than exacerbate existing healthcare disparities, equitable opportunities for participation in and benefit from research are essential. This section outlines several key barriers to equity in CRISPR-related research and proposes opportunities to address them.

One of the foremost barriers is mistrust towards research participation, particularly among marginalised or historically exploited communities. This mistrust stems from past unethical practices, such as inadequate informed consent or even coerced participation in clinical studies [53, 54]. The He Jiankui case serves as a critical example: through coercive language and a lack of transparency in his study involving genome-edited embryos, he violated ethical boundaries, ultimately leading to imprisonment. Incidents like this erode public trust and create long-lasting scepticism about participating in genetic research.

Moreover, disparities in healthcare access further reinforce this mistrust. Minority communities often receive inferior healthcare compared to wealthy or non-minority populations [55, 56]. This raises pressing ethical concerns about whether CRISPR-based therapies, and potential future enhancements, will be equitably available, or if they will become tools of privilege confined to the affluent few.

Another major barrier is the underrepresentation of diverse ethnic groups in genomic research. Although humanity's genetic diversity is vast, participation in studies remains skewed. For instance, only about 3% of participants in Genome-Wide Association Studies (GWAS), as reported in the GWAS catalogue, are of African descent [57]. This is troubling, given that genetic variation differs significantly across populations, and failing to include underrepresented groups makes it harder to develop therapies that are safe and effective for everyone. CRISPR-based interventions, like other forms of precision medicine, risk being less applicable, or even ineffective, for populations not adequately represented in research.

Finally, disparities in access to the benefits of scientific research have been a concern since the early days of bioethics. If CRISPR is to fulfil its potential as a transformative biomedical technology, the benefits of related research must be made equitably accessible across all communities. Legal and policy frameworks should be developed to ensure fair distribution of therapeutic advancements, especially for historically underserved populations.

Addressing these barriers – by rebuilding trust, increasing diversity in research participation and ensuring equitable access – will be critical to shaping a more inclusive and ethical future for CRISPR-based therapeutics.

4.5 Social and cultural perspectives

Although the rapid progress of CRISPR technology has raised hopes for transformative public-health benefits, its cultural and social acceptance remains a major concern.

At the first International Summit on Human Gene Editing, participants warned that genome editing could inadvertently revive eugenic thinking. Early-twentieth-century eugenics promoted the idea that traits such as 'criminality', 'feeble-mindedness' and 'pauperism' were heritable in the same way as eye colour. These beliefs justified forced sterilisation and discriminatory immigration policies until the atrocities of Nazi Germany discredited the movement. As Daniel Kevles observed, some of the social forces that once fuelled eugenics – fear of "genetic decline," pursuit of perceived perfection – still linger today. CRISPR's power to edit the germline therefore demands heightened vigilance against any resurgence of coercive or discriminatory practices [9].

Policymakers must also respect diverse religious and philosophical views when crafting regulations:

- Islamic bioethics – Mohammed Ghaly (Hamad Bin Khalifa University) noted at the second summit that *somatic* gene therapy is generally permissible because it treats disease, has a limited scopeand affects only the treated individual. *Germline* editing, however, is far more controversial: it is seen as overstepping human authority granted by God, since humans are 'trustees, not owners' of their bodies [28].

- Japanese philosophy – Satoshi Kodama (Kyoto University) explained that in Japanese thought, embryos are regarded as 'sprouts of human life' that deserve protection to uphold human dignity. Germline modification therefore raises profound ethical reservations [28].

These examples illustrate a broader consensus emerging at successive summits: governance frameworks for CRISPR must integrate philosophical, spiritual and cultural insights, not merely scientific risk-benefit analyses. Only by engaging multiple world-views can global policy strike a balance between scientific progress and respect for deeply held values.

5. Global regulatory landscape

Since the discovery of CRISPR, the scientific community has engaged in ongoing debate about the ethical and regulatory frameworks required for its responsible use. Scientists around the world have called for internationally accepted limitations on the application of genome editing, with regulatory guidelines already in place in countries such as the UK, US, China and Sweden [58–60].

In India, the Indian Council of Medical Research (ICMR) has prohibited human germline editing and reproductive cloning, as stated in the *National Guidelines for Stem Cell Research*. However, these guidelines have not yet been translated into binding legislation, leaving a regulatory gap that could be exploited.

At the global level, the United Nations Educational, Scientific and Cultural Organisation (UNESCO) has strongly condemned germline genome editing,

emphasising the importance of preserving human dignity and shared ethical values [61]. In response to growing ethical concerns, the National Academies of Sciences, Engineering, and Medicine (NASEM) issued guiding principles in 2017. These principles stress the need to protect social well-being, fairness and transparency in human genome editing. While germline editing is generally discouraged, NASEM allows for its use in rare and exceptional circumstances, subject to strict oversight and ethical review [62].

In 2021, the World Health Organisation (WHO) released its own set of recommendations on genome editing. These included a strong warning against unauthorised clinics offering unproven stem cell therapies, referencing cases like that of He Jiankui, whose unethical experiment on human embryos caused global uproar. The WHO also emphasised the importance of public education and engagement to ensure that society understands the risks, benefits and ethical dimensions of genome editing. Only with informed public participation can we determine the social acceptability and desirability of such powerful technologies [37].

Before the Third International Summit on Human Gene Editing, a group of national collaborators assessed the governance rules of CRISPR-based tools in their own countries, which showed the current scenario of germline genome editing. These countries included Brazil, China, India, Mexico, Singapore, South Africa, Uganda and Ukraine. In countries like Brazil, China, India, Singapore and Uganda, germline genome editing was banned. While in all the countries, some form of law exists for somatic editing and somatic cell gene therapy, in countries like South Africa, Mexico and Ukraine, it was pointed out that no specific oversight or governance measure was found. Though at the time of writing this chapter, this might have changed [63].

6. Conclusions

The debate surrounding the regulation of CRISPR technologies continues to evolve, delicately balancing the promise of scientific progress with deeply rooted ethical considerations. While perspectives often vary across cultural and individual lines, one aspect has gradually achieved broad consensus: the acceptance of somatic gene therapy. This growing approval is reflected in the sharp increase in clinical trials over recent years. As of February 2025, approximately 250 clinical trials involving gene-editing therapeutic candidates have been registered, with over 150 currently active [64]. These numbers not only underscore the transformative potential of CRISPR but also highlight its expanding global footprint in the realm of modern medicine.

In contrast, germline editing remains highly controversial and is explicitly prohibited in many countries. This widespread resistance underscores a collective hesitation to embrace heritable genetic modifications – a boundary that society is not yet prepared to cross. The primary concerns stem from profound ethical implications, particularly regarding how such technologies might deepen existing socio-economic divides. If access to germline editing were to be dictated by wealth or privilege, it could exacerbate inequities and reinforce systemic disparities, raising troubling questions about fairness, consent and the future of human diversity.

Acknowledgements

I would like to sincerely acknowledge the support and intellectual environment provided by all members of our RBPs laboratory, as well as the Department of

Biosciences and Bioengineering at IIT Guwahati, for their valuable discussions during the development of this chapter.

Conflict of interest

The authors declare no conflicts of interest regarding this book chapter.

Author details

Gourab Chatterjee and Kusum Kumari Singh*
Department of Biosciences and Bioengineering, IIT Guwahati, India

*Address all correspondence to: kusumsingh@iitg.ac.in

References

[1] Sugisaki H, Kanazawa S. New restriction endonucleases from Flavobacterium okeanokoites (FokI) and Micrococcus luteus (MluI). Gene. Dec 1981;**16**(1-3):73-78

[2] Kim YG, Chandrasegaran S. Chimeric restriction endonuclease. Proceedings of the National Academy of Sciences of the United States of America. 1 Feb 1994;**91**(3):883-887

[3] Klug A. The discovery of zinc fingers and their applications in gene regulation and genome manipulation. Annual Review of Biochemistry. 2010;**79**(1):213-231

[4] Sun N, Zhao H. Transcription activator-like effector nucleases (TALENs): A highly efficient and versatile tool for genome editing. Biotechnology and Bioengineering. 2013;**110**(7):1811-1821

[5] 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. Journal of Bacteriology. Dec 1987;**169**(12):5429-5433

[6] Marraffini LA, Sontheimer EJ. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science (1979). 2008;**322**(5909):1843-1845

[7] Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. Available from: http://science.sciencemag.org/

[8] Gostimskaya I. CRISPR–Cas9: A history of its discovery and ethical considerations of its use in genome editing. Biochemistry (Moscow). 2022;**87**:777-788

[9] Olson S, editor. International Summit on Human Gene Editing. Washington, D.C.: National Academies Press; 2016. Available from: http://www.nap.edu/catalog/21913

[10] Bhaya D, Davison M, Barrangou R. CRISPR-cas systems in bacteria and archaea: Versatile small RNAs for adaptive defense and regulation. Annual Review of Genetics. 2011;**45**:273-297

[11] Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ, et al. An updated evolutionary classification of CRISPR–Cas systems. Nature Reviews Microbiology. 2015;**13**(11):722-736

[12] Jiang F, Doudna JA. The structural biology of CRISPR-Cas systems. Current Opinion in Structural Biology. 2015;**30**:100-111

[13] Swarts DC, Mosterd C, van Passel MWJ, Brouns SJJ. CRISPR interference directs strand specific spacer acquisition. PLoS One. 2012;**7**(4):e35888

[14] Haurwitz RE, Jinek M, Wiedenheft B, Zhou K, Doudna JA. Sequence- and structure-specific RNA processing by a CRISPR endonuclease. Science (1979). 2010;**329**(5997):1355-1358

[15] Liao C, Beisel CL. The tracrRNA in CRISPR biology and technologies. Annual Review of Genetics. 2021;**55**(1):161-181

[16] Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM,

Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;**163**(3):759-771

[17] Westra ER, Semenova E, Datsenko KA, Jackson RN, Wiedenheft B, Severinov K, et al. Type I-E CRISPR-Cas systems discriminate target from non-target DNA through base pairing-independent PAM recognition. PLoS Genetics. 2013;**9**(9):e1003742

[18] Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nature Biotechnology. 2013;**31**(3):233-239

[19] Deveau H, Barrangou R, Garneau JE, Labonté J, Fremaux C, Boyaval P, et al. Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus*. Journal of Bacteriology. 2008;**190**(4):1390-1400

[20] Dennis Normile. Chinese scientist who produced genetically altered babies sentenced to 3 years in jail. AAAS Articles DO Group. 30 Dec 2019

[21] Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, et al. RNA-guided human genome engineering via Cas9. Science (1979). 2013;**339**(6121):823-826

[22] Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. Science (1979). 2013;**339**(6121):819-823

[23] Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J. RNA-programmed genome editing in human cells. eLife. 2013;**2**:e00471

[24] Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature Biotechnology. 2013;**31**(3):227-229

[25] Doudna JA, Jinek M, Chylinski K, Charpentier E. Methods and Compositions for RNA-Directed Target DNA Modification and for RNA-Directed Modulation of Transcription. United States; US10113167B2; 2016

[26] Zhang F. CRISPR-Cas systems and methods for altering expression of gene products. United States of America; US8697359B1; 2013

[27] Court reignites CRISPR patent dispute. Nature Biotechnology. 2025;**43**(6):835-835

[28] Olson S, editor. Second International Summit on Human Genome Editing: Continuing the Global Discussion. Washington, D.C.: National Academies Press; 2019. Available from: https://www.nap.edu/catalog/25343

[29] Olson S, editor. Third International Summit on Human Genome Editing. Washington, D.C.: National Academies Press; 2023

[30] Greely HT. CRISPR'd babies: Human germline genome editing in the "He Jiankui affair". Journal of Law and the Biosciences. 2019;**6**(1):111-183

[31] Jiankui H. Available from: https://www.youtube.com/watch?v=pcGALqX_YD8&t=2450s. Hong Kong: Youtube; 2018

[32] Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, et al. Biotechnology. A prudent path forward for genomic engineering and germline gene modification. Science. 2015;**348**(6230):36-38

[33] Cohen J. Inside the circle of trust. Science (1979). 2019;**365**(6452):430-437

[34] McCurry J. Scientist who gene-edited babies is back in lab and 'proud' of past work despite jailing. The Guardian. 2024. Available from: https://www.theguardian.com/science/2024/apr/01/crispr-cas9-he-jiankui-genome-gene-editing-babies-scientist-back-in-lab

[35] Guerra-Resendez RS, Hilton IB. Harnessing CRISPR-Cas9 for epigenetic engineering. Methods in Molecular Biology. 2022;**2518**:237-251

[36] Shen B, Zhang W, Zhang J, Zhou J, Wang J, Chen L, et al. Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. Nature Methods. 2014;**11**(4):399-402

[37] National Academies of Sciences, Engineering, and Medicine, et al. Human Genome Editing: Science, Ethics, and Governance. US: National Academies Press; 14 Feb 2017

[38] Athanasopoulos T, Munye MM, Yáñez-Muñoz RJ. Nonintegrating gene therapy vectors. Hematology/Oncology Clinics of North America. 2017;**31**(5):753-770

[39] Maeder ML, Stefanidakis M, Wilson CJ, Baral R, Barrera LA, Bounoutas GS, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nature Medicine. 2019;**25**(2):229-233

[40] Kan MJ, Doudna JA. Treatment of genetic diseases with CRISPR genome editing. Journal of the American Medical Association. 2022;**328**(10):980-981

[41] Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. The New England Journal of Medicine. 2021;**384**(3):252-260

[42] FDA. FDA Approves First Gene Therapies to Treat Patients with Sickle Cell Disease. 2023. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-gene-therapies-treat-patients-sickle-cell-disease

[43] Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;**548**(7668):413-419

[44] Boutin J, Rosier J, Cappellen D, Prat F, Toutain J, Pennamen P, et al. CRISPR-Cas9 globin editing can induce megabase-scale copy-neutral losses of heterozygosity in hematopoietic cells. Nature Communications. 2021;**12**(1):4922

[45] Adikusuma F, Piltz S, Corbett MA, Turvey M, McColl SR, Helbig KJ, et al. Large deletions induced by Cas9 cleavage. Nature. 2018;**560**(7717):E8-E9

[46] Shaw D. The consent form in the Chinese CRISPR study: In search of ethical gene editing. Journal of Bioethical Inquiry. 2020;**17**(1):5-10

[47] Appelbaum PS, Roth LH, Lidz CW, Benson P, Winslade W. False hopes and best data: Consent to research and the therapeutic misconception. The Hastings Center Report. 1987;**17**(2):20

[48] Jonlin EC. Informed consent for human embryo genome editing. Stem Cell Reports. 2020;**14**(4):530-537

[49] Krimsky S. Ten ways in which He Jiankui violated ethics. Nature Biotechnology. 2019;**37**(1):19-20

[50] Lim JK, McDermott DH, Lisco A, Foster GA, Krysztof D, Follmann D, et al.

CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus infection but not for viral transmission. The Journal of Infectious Diseases. 2010;**201**(2):178-185

[51] Murray TH, Rothstein MA, Murray RF Jr. The Human Genome Project and the Distribution of Scarce Medical Resources. Bloomington and Indianapolis, IN, United States: Indiana University Press; 1996

[52] Gene therapy or genetic enhancement: Does it make a difference? AMA Journal of Ethics. 2001;**3**(2):37-39

[53] Shivayogi P. Vulnerable population and methods for their safeguard. Perspectives in Clinical Research. 2013;**4**(1):53

[54] Corbie-Smith G, Thomas SB, Williams MV, Moody-Ayers S. Attitudes and beliefs of African Americans toward participation in medical research. Journal of General Internal Medicine. 1999;**14**(9):537-546

[55] Benz JK, Espinosa O, Welsh V, Fontes A. Awareness of racial and ethnic health disparities has improved only modestly over a decade. Health Affairs. 2011;**30**(10):1860-1867

[56] Hunt KA, Gaba A, Lavizzo-Mourey R. Racial and ethnic disparities and perceptions of health care: Does health plan type matter? Health Services Research. 2005;**40**(2):551-576

[57] Popejoy AB, Fullerton SM. Genomics is failing on diversity. Nature. 2016;**538**(7624):161-164

[58] Zou Y, Li Y, Tao Y. Regulatory framework of human germline and heritable genome editing in China: A comparison with the United States and the United Kingdom. Journal of Law and the Biosciences. 13 May 2025;**12**(1):lsaf007

[59] Ishii T. Germ line genome editing in clinics: The approaches, objectives and global society. Briefings in Functional Genomics. 2017;**16**(1):46-56

[60] Ishii T, Araki M. A future scenario of the global regulatory landscape regarding genome-edited crops. GM Crops & Food. 2017;**8**(1):44-56

[61] Dickenson D, Darnovsky M. Did a permissive scientific culture encourage the 'CRISPR babies' experiment? Nature Biotechnology. 2019;**37**(4):355-357

[62] Coller BS. Ethics of human genome editing. Annual Review of Medicine. 2019;**70**(1):289-305

[63] Millett P, Naik S, Otim G, Piven O, Roca MM, Shozi B, et al. Somatic Genome Editing Governance Approaches and Regulatory Capacity in Different Countries. 28 Feb 2023

[64] CRISPR. Medicine News. Available from: https://crisprmedicinenews.com/clinical-trials/

Section 3

Application of CRISPR Technology

Chapter 5

Applications of CRISPR in Crop Improvement and Global Food Security

Richard Dormatey, Augustine Antwi-Boasiako, Francis Azumah Chimsah, Kwame Yaw-Kan Kojo, Juliet Mensah, Agbesi Kwadzo Keteku, Philip Ghanney and Harrison Kwame Dapaah

Abstract

In view of the growing world population, climate change, and shrinking fertile land, climate-smart, and high-yielding crops are urgently needed. Agriculture must continue to supply food, feed, and raw materials to sustain the world, in spite of all the challenges arising from both the biotic and abiotic stress factors. The use of conventional breeding techniques to cope with stress factors remains a challenge. However, genome editing based on CRISPR-Cas9 provides a revolutionary answer. This chapter offers a comprehensive overview of the role of CRISPR-Cas9 in improving crop traits to ensure global food security. Moreover, it highlights the value of bioinformatics in CRISPR applications and delves into the regulations governing the application of CRISPR and the ethical concerns, and consumer acceptance of the products. Using contemporary sequencing tools, scientists can accurately, and rapidly modify crop genes to confer traits such as nutritional quality, disease resistance, and stress tolerance. Feeding a growing global population, achieving sustainability targets, and potentially lessening the consequences of climate change, CRISPR-based genome editing offers tremendous agricultural opportunities for breeding crops across the food supply chain that could benefit larger segments of the population. In addition, the CRISPR-Cas9 system has been applied in the regulation of genes, the domestication of plants, and the reduction of pesticide usage in crop production.

Keywords: genome editing, CRISPR, innovation, sustainability, food security and nutrition

1. Introduction

The global population is anticipated to reach 9.8 billion in 2050. This presents agriculture with the crucial constraint of meeting the growing demand for food while

mitigating the impacts of climate change on the environment [1, 2]. Conventional plant breeding has historically been effective, but has struggled with problems such as delayed progress, the possibility of undesirable trait associations, and reliance on subjective observations [3]. Although genetically modified (GM) crops can increase agricultural production and improve food security, there are several challenges and concerns regarding their use, including environmental consequences, public opinion, and ethical considerations [4]. Concerns include the risk of unexpected consequences for organisms that were not targeted and for the environment, as well as major social and ethical disputes over corporate control of the food supply. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas technology is a gene-editing device that has transformed agriculture, providing precise, and effective techniques to improve plant traits and agricultural practices [5, 6]. It can be applied to produce herbicide-resistant plants, increase yields, improve quality, and increase resistance to diseases and pests. Additionally, CRISPR-Cas enables the creation of plant varieties that are tolerant to drought and salinity, and more resilient and sustainable in several agricultural systems [7]. It has been successfully applied in improving crop quality. For instance, modification of crops can contain less allergens. CRISPR has been utilized to generate gluten-free wheat strands that are suitable for people with coeliac disease [8]. This is a positive sign that the efficiency and productivity of agriculture can also be increased when CRISPR application is embraced.

Rapid advances in sequencing technologies are making more genomic data available on a growing number of crop species [9], and genome editing techniques can be utilized to precisely alter genes, opening up new avenues for crop improvement. The terms "CRISPR" and "CRISPR-Cas9" are frequently used interchangeably in the context of genome engineering to refer to the various CRISPR-Cas9, CRISPR-Cpf1, CRISPR-Cas12a, etc. Researchers can use these techniques to permanently alter genes in living cells, tissues, organs, or organisms. Approximately 20 crop species have so far harnessed the CRISPR-Cas9 gene-editing technique to achieve a range of traits, such as higher yields and the ability to cope with various biotic and abiotic stress [10]. For instance, the CRISPR-Cas9 system has been utilized in knocking out precise genes critically to biotic or abiotic stress tolerance systems [11]. To ensure food security, sustainable crop development initiatives are needed in the face of increasing intensity of biotic and abiotic stress factors. Abiotic stress has become a serious challenge for agriculture in the context of advancing climate change, leading to production losses that affect the global food supply [12]. According to Rogo et al. [13], plants are able to adapt to normal fluctuations in their environment and continue to grow and develop. The abiotic stress occurs when a plant's responsiveness is seriously affected by non-biological environmental factors such as salt, heat, water scarcity or nutrient imbalances (toxicity or deficiency) [14]. When these environmental conditions become severe or persist for long periods of time, it has detrimental effects on plants. Abiotic stress has become a serious challenge for agriculture within the framework of advancing climate change, leading to production losses that affect the global food supply [12]. The production of disease-resistant crops is severely hindered by biotic stress caused by pathogenic microorganisms. This also contributes to 15% of global food production losses and over 42% of potential yield losses [15]. For instance, in soybeans, 200 pathogens are known to cause destruction to the plants [16]. To ensure food security, the increasing intensity of biotic and abiotic stress factors calls for sustainable crop development initiatives.

CRISPR technology allows for accurate and effective genome editing. Hence, it offers a substantial advantage for improving food security in light of the growing

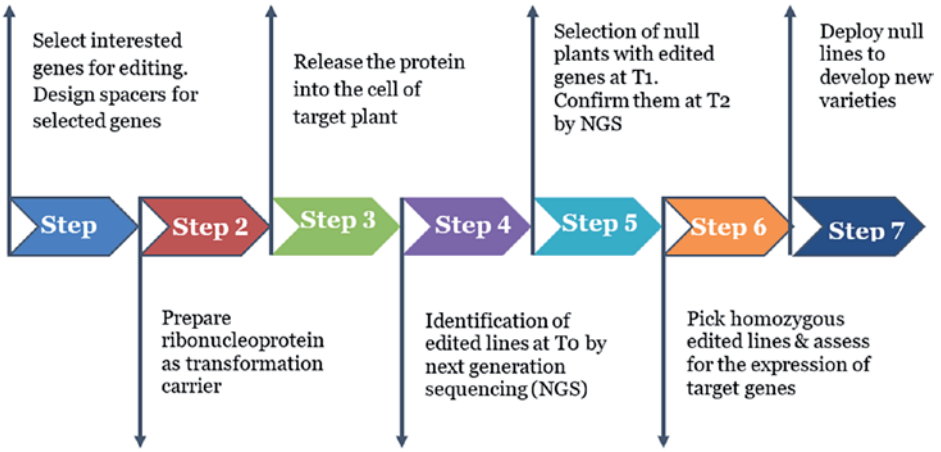

Figure 1.
CRISPR gene-editing workflow [18].

world population and changing climate [17]. Crop yields, nutritional value, and plant resistance to environmental stressors like drought, salt, and pests can all be enhanced when CRISPR-Cas9 technology is applied. More dependable food supplies and higher agricultural output may result from this. Several researchers agree that applications of CRISPR-Cas9 technology in agriculture result in crop improvement [18, 19]. For instance, Li et al. [20] employed CRISPR-Cas9 to selectively delete genes related to grain size regulation to increase the yield of rice grains [21]. The capacity to precisely modify plant genomes makes it possible to improve desired characteristics, including stress tolerance, yield, disease resistance, and nutritional value. For instance, Shan et al. successfully applied CRISPR-Cas9 in rice, resulting in increased resistance to bacterial blight [22]. In addition, CRISPR-Cas9 has shown the potential to speed up the breeding process [23]. With conventional breeding methods, it often takes several generations to achieve the desired traits incorporated in a breeding material. By introducing targeted genetic changes directly into the plant's germline, CRISPR-Cas9 can drastically reduce the time required for breeding, thereby contributing to solving the problem of global food security. Moreover, this technique offers the possibility of growing nutrient-rich plants that can help prevent malnutrition and improve human health [20, 21]. By examining current limitations and upcoming projections, this chapter aims to demonstrate how CRISPR-CAS technology can be utilized to develop resilient, high-yielding, and nutrient-rich varieties that will ultimately contribute to agricultural sustainability and food security for generations to come. The overview of the CRISPR gene-editing workflow, detailing each critical step from design to product, is illustrated in **Figure 1**.

2. CRISPR for crop yield improvement and stress resilience

CRISPR technology has transformed agricultural biotechnology internationally [24]. Because of its precise genome editing capacity, it can target genes that govern essential agronomic properties such as crop production potential, biotic resistance, and abiotic stress tolerance. Climate change and rising food demand necessitate the use of the CRISPR-Cas system as a long-term solution for effective photosynthesis

improvement, biomass production, and resilience to environmental hazards such as heat, cold, salinity, and drought [25, 26]. CRISPR technology has great potential for increasing crop productivity and tolerance to diverse pressures, making it an effective tool for agricultural enhancement [26, 27].

2.1 Development of photosynthetic efficiency and biomass production with CRISPR

CRISPR-Cas9 technology offers great potential for improving photosynthesis and biomass production in crops [28]. This allows researchers to focus on genes related to light yield, CO_2 fixation, and other photosynthetic processes, resulting in increased yields and enhanced efficiency. The productivity and photosynthetic efficiency of plants can be improved by CRISPR-Cas9 technology [29, 30]. CRISPR-Cas9 is a gene-editing technique that enables genetic engineers to disable or alter the function of certain genes in the photosynthetic pathway. According to Li et al. [31], photosynthesis is a sequence of complex reactions that can be optimized by editing critical genes that regulate these activities. Light is necessary for photosynthesis. Improving a plant's ability to utilize light can increase photosynthetic efficiency and crop yield [32]. Genes involved in light uptake, such as the Light Harvesting Complex Protein B1 (Lhcb1), can be modified to improve the absorption of certain wavelengths. The Lhcb1 genes encode important components of the protein complexes that bind chlorophyll and collect light in the thylakoid membrane, which are critical for light uptake during photosynthesis [33, 34]. Targeted modifications of these proteins can improve their ability to convert light energy into chemical energy. This has been shown to promote plant growth and is particularly useful in locations where plants grow in low light or under controlled conditions.

Rubisco is an important enzyme in the Calvin cycle, although it is inefficient because it is often converted to oxygen instead of carbon dioxide. This process of oxygen fixation, known as photorespiration, leads to a decrease in energy efficiency. Biotechnology can be utilized in various ways to reduce photorespiration: Scientists have applied CRISPR-Cas9 to modify genes such as RBCS (RuBisCo small subunit genes) to improve the selectivity of Rubisco for carbon dioxide or alter its active site to increase catalytic efficiency [35, 36]. To overcome the inefficiency of RuBisCO, CRISPR-Cas9 can be employed to improve the carbon dioxide-concentrating processes of C4 plants, for example, by modifying genes such as PEPC (phosphoenolpyruvate carboxylase). These changes increase the concentration of carbon dioxide near RuBisCO and reduce oxygen competition and photorespiration [37]. CRISPR-Cas9 has been shown to alter genes involved in the photorespiratory system, such as GOX1 (glycolate oxidase), reducing energy waste [38]. Alternatively, genes that enable more efficient recycling of photorespiratory waste can be optimized to direct them into productive metabolic pathways. The stomata of a plant regulate the loss of water and the uptake of carbon dioxide. There are several genes (e.g., *EPF1* and *EPF2*) that regulate stomata density of a which could increase water efficiency while maintaining photosynthesis [39]. The approaches listed above are just a few examples of how biotechnology can be used to modify photosynthesis. Crop yield is the outcome of a comprehensive system. Photosynthesis supplies the carbon and energy essential for the entire system; however, this interaction is nonlinear and influenced by various factors, including growth, leaf, and canopy shape and design, source-sink dynamics, and the impact of photosynthesis within a field crop. In summary, photosynthesis is key to crop

yield and any gene involved in photosynthesis can be altered with CRISPR-Cas9 to improve its efficiency, leading to improved photosynthesis and food production.

2.2 Application of CRISPR technology to alleviate abiotic stress

The response to abiotic stress is a complicated quantitative trait that is difficult to control since it is regulated by several genes and is therefore difficult to manage [40, 41]. In the last few years, the application of CRISPR-Cas9 technology for beleaguered gene editing in plants has developed dramatically. CRISPR-P, among the most recent technologies, is a web-based platform that enables the creation of sgRNAs in over 20 diverse plant species [42]. Furthermore, the creation of multiple vectors and instruments for gene editing of plants with CRISPR-Cas9 has increased accessibility for useful research [43]. CRISPR-Cas9 type II technology has facilitated accurate site-specific modifications in various plant species, including major plants. For example, CRISPR-Cas9 technology targeting *OsDERF1*, *OsERF922*, and *OsRMC* in rice has shown the ability to generate stable lines with enhanced resistance to abiotic stress, especially drought [44]. In soybean, the application of CRISPR/Cas9 to the *GmMYB118*, *GmDrbza*, and *GmDrbzb* genes has been planned to generate genome-edited varieties that exhibit increased drought and salt tolerance [45, 46]. In wheat, genes such as *TaDREB2*, *TaDREB3*, *TaHAG1*, and *TaALs* are key targets for the creation of varieties with increased tolerance to drought, salinity, and herbicide resistance [47]. Furthermore, the creation of several vectors and tools for CRISPR-Cas9-based gene editing of plants has increased accessibility for useful research [43]. These developments have validated CRISPR-Cas9 technology for genetic modification, transcriptome control, the development of stress-tolerant plants, and molecular exploration of response to multigenic stresses. Additionally, Wu et al. [48] pointed out that Brassica napus can be modified using CRISPR/Cas9 technology to increase drought and herbicide tolerance by modifying the genes *BnaA6.RGA*, and BnAls. Genes like *ZmARGOS8*, *ZmALS1*, *ZmALSZ*, and *ZmTMS5* have been used to create genome-edited lines of maize that are more tolerant to drought, herbicides, and extremely high temperatures [49, 50].

Sardar [51], CRISPR-Cas and other genome editing tools have been used in many plant species like cereals, vegetables, and fruit crops to make them more tolerant to abiotic stress by targeting their sensitivity genes. B-amylase (BMY) genes contribute to the cold tolerance of rice. They control the breakdown of starch and the build-up of maltose to protect against cold stress. The function of BMY is affected by the association of the cold-responsive R2R3 MYB transcription factor *OsMYB30* and *OsJAZ9* with the promoter regions of the BMY genes. Scientists created cold-tolerant rice variants by disrupting *OsMYB30* with CRISPR-Cas [52]. Moreover, CRISPR/Cas9 technology can be utilized in *Brassica napus* to modify the BnaA6.RGA and BnAls genes to improve both drought and herbicide resistance [48]. Genes like *ZmARGOS8*, *ZmALS1*, *ZmALSZ*, and *ZmTMS5* have been used in maize to create genome-edited lines that are more resistant to drought, herbicides, and extremely high temperatures [50, 53].

Chen et al. [54] and Tran et al. [55] used CRISPR-Cas9 technology in vegetable crops to create drought-tolerant tomato varieties by targeting the following genes: *SlHyPRP1*, *SlBZR1*, *SlcBF1*, *SlARF4,* and *SlEPSPS*. The tomato varieties exhibited increased tolerance to drought and other stress factors such as heat, salinity, cold, and herbicides. This research shows how CRISPR-Cas9 technology can be utilized to improve plant traits and produce plants that can withstand stress. Another example

is that the ability of rice to cope with cold is influenced by *B-amylase* (BMY) genes. To protect against cold stress, these genes regulate the accumulation of maltose and the interruption of starch. The cold-responsive R2R3-type MYB transcription factor *OsMYB30* and *OsJAZ9* bind to the promoter regions of *BMY* genes and thus impair BMY function. Researchers created cold-tolerant rice varieties by disrupting *OsMYB30* using CRISPR-Cas [52]. The CRISPR-Cas9 technique was used to modify the salt and drought tolerance gene (DST) in the indica rice cv. MTU1010. As a result of increased leaf area and decreased stomatal density, water storage, and light consumption are enhanced; the mutant lines with a 366-bp deletion in the coding sequence were tolerant to salt, osmotic, and drought stressors [56]. Researchers have increased drought tolerance in maize by using CRISPR-Cas9 to interrupt the auxin-regulated gene Involved in Organ Size 8 (*ARGOS8*) [57]. Sensitivity of tomato to heat stress is increased by CRISPR-based editing of mitogen-activated protein kinase 3 (*SlMAPK3*) and agamous-like 6 (*SlAGL6*) orthologs, while sensitivity to salt stress is increased by ADP-ribosylation factor 4 (*SlARF4*). According to Bouzroud et al. [58], these CRISPR-edited mutants showed enhanced agronomic characteristics and tolerance to abiotic stress factors. This demonstrates the potential of CRISPR-Cas in contributing to food security in reducing stresses imposed by drought, salinity, among others.

3. Improving disease resistance and pest control through CRISPR technology

Plant pathogens, including bacteria, fungi, and viruses, trigger several plant diseases that severely affected production, while herbivores like insects further decrease production through direct damage and as vectors for disease [59, 60]. The capacity of plants to cope with various biotic stress factors will be negatively impacted by climate change. Direct crop losses due to biotic stress factors are expected to be 20–40% [61, 62]. Consequently, R gene methods do not necessarily lead to long-lasting broad-spectrum resistance and instead tend to encourage the development of resistance in the target pathogen [63]. An alternative strategy is to use CRISPR-Cas to target susceptibility genes in a plant's genome [64]. But in most cases, R genes are pathogen-specific and unnecessary avirulence genes. Hence, R gene strategies do not necessarily lead to long-lasting broad-spectrum resistance and instead tend to promote the development of resistance in the target pathogen [63]. The use of CRISPR-Cas to target susceptibility genes in a plant's genome is an alternative tactic [64].

The pathogen (*Magnaporthe oryzae*) produces blast disease, which affects rice production worldwide. By deleting specific susceptible genes in rice like *OsERF92231* and Pi21, rice genotypes resistant to rice hypertrophy have been generated [59]. Likewise, the use of CRISPR-Cas to target the Mildew Locus O (MLO) gene has improved the resistance of wheat to powdery mildew, a disease caused by the fungus *Podosphaera xanthii* [65]. According to Zaidi, Mukhtar, and Mansoor [64], targeting susceptible genes encoding sugar transporters is another strategy to improve disease resistance. Bacterial late blight, caused by *Xanthomonas oryzae* pv oryzae, leads to substantial yield losses in rice under favorable conditions. During infection, the bacterial pathogen produces transcription activator-like effectors (TALEs) that promote the transcription of genes coding for sugar transporters (e.g., *OsSWEET11*, *OsSWEET13*, and *OsSWEET14*). SWEET genes are known susceptibility genes for bacterial blight, as they promote the growth of bacteria in plant tissues. Researchers have created

rice varieties with a broad spectrum of resistance to bacterial blight by successfully disrupting the link between TALEs and SWEET genes using the CRISPR-Cas system [66]. In *OsSWEET11* (Xa13) [49], *OsSWEET13* [36], and *OsSWEET14* [67], the TALE binding site was altered for this purpose. The resulting plants exhibited increased resistance to the bacterial pathogen. In addition, genes that are sensitive to viruses are enhanced to protect plants from them [68]. For example, rice production is seriously threatened by rice tungro disease, which is produced by the interaction of the bacilliform rice tungro virus and the spherical rice tungro virus. The gene for the translation initiation factor 4 gamma (eIF4G) controls resistance to rice tungro disease, which is caused by the rice tungro sphere virus. By using CRISPR-Cas to disrupt eIF4G, transgene-free rice lines resistant to tungro disease have been created [69].

Crop losses caused by insect infestation have catastrophic effects on civilization, including poverty and famine. Immediate invention and the use of rigorous and integrated agricultural practices are required due to the combined effects of the resurgence of insect pests and the rapidly growing human population [70]. With the ability to precisely modify insect genomes to increase resistance to pests and potentially reduce the need for chemical pesticides, CRISPR-Cas9 technology is an effective tool for insect management in agriculture. Using this approach, traits such as reproductive ability, disease susceptibility, and insecticide tolerance can be altered by targeting genes in insects, leading to more environmentally friendly and sustainable farming practices [70]. We explore the new opportunities offered by the CRISPR/Cas9 system and how it is attracting great interest due to its unique advantages in controlling insect pests, which in turn ensures high agricultural production and food security. Various tephritids, mosquitoes, and *Drosophila melanogaster* meigens have already been subjected to a mixture of gene-editing tools and insect manipulation techniques that have provided answers to several fundamental problems in insect biology. This has been shown to be an effective method of pest control [71, 72] and has recently been used to develop new pest control tactics [73, 74]. Through the creation of genetically modified insects, advances in genome editing techniques have opened the door for creative pest control strategies. CRISPR/Cas technology continues to evolve as it is extremely beneficial for effective gene editing and customization. Plasmid DNA, RNA, or a ribonucleoprotein (RNP) complex can be used to introduce the components of the CRISPR-Cas tool—gRNA and the Cas9 protein—into the target organism [75].

3.1 Biofortification of crops using CRISPR

Enriching food crops with nutrients and enhancing the nutritional value of food are the safest, most effective, and sustainable approaches to comprehensively address protracted hidden hunger among the majority of the population in the least developed countries [76]. Various mechanisms and approaches have been developed, adopted, and deployed to enrich the nutritional content of globally prevalent staple foods such as maize, sweet potatoes, wheat, rice, and beans with essential micronutrients like zinc, iron, and vitamins (**Figure 2**). Meanwhile, these basic steps have the economic viability to ensure the bioavailability of adequate nutrients, particularly the essential micronutrients that are in limited supply from crops in their natural state [77]. Some of these fortification techniques include agronomic improvement of crop quality through proper soil nutrient management, conventional breeding by crossing desirable individuals to transfer and improve quality, and genetic engineering through gene editing, gene knock-in and knockout, and other sophisticated genetic manipulation systems.

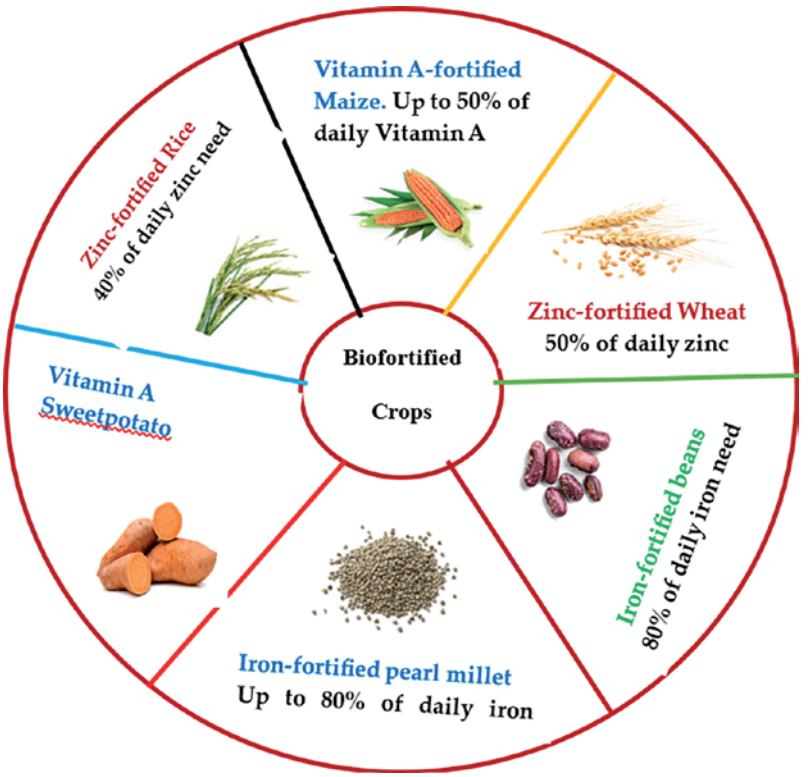

Figure 2.
Some food crops biofortified with essential nutrients.

Furthermore, agronomists' production-oriented approach of enriching crops by adding micronutrients during growth in the form of fertilizers and even conventional selection of high-nutrient-density varieties through conventional breeding is not very effective in ensuring complete safety and overall sustainability in the face of rapid radical climate change. However, the advent of genetic engineering, with its associated cutting-edge techniques and high-throughput tools, has brought additional benefits in the precise modification of specific genes of interest to precisely improve a target nutrient [78], as in the case of the famous vitamin A-rich 'golden rice'. The use of transgenic methods to enrich crops with nutrients involves the use of genetic engineering to directly modify genes for enhanced nutrient profiles. This advanced strategy entails special selection and the transfer of favorable alleles for a particular nutrient trait of interest from one plant variety to another to develop a new plant type with an improved value of the target nutrient. Transgenic tools like gene editing and genetic engineering are used much faster and more precisely than conventional breeding schemes, which sometimes have a tendency to produce untargeted and undesirable traits due to unintended errors in manual manipulation or crop pollination [79]. It is clear that even certain nutrients or genes that are difficult to predict and improve using conventional breeding methods can potentially be manipulated and improved using genetic engineering tools such as CRISPR. From an economic point of view, the biofortification of crops requires a one-off investment that brings major long-term benefits. Nutrient-enriched crops are therefore naturally able to pass on these improved nutritional properties to subsequent generations without the need for

further genetic manipulation or special treatments other than proven routine agronomic practices. Their medium- to long-term benefits become easily affordable and accessible to most resource-limited farmers [78].

The use of genetic tools in breeding has become a new force that is revolutionizing the field of plant improvement. In addition to the crops shown in the figure above, there are other types of crops that have been biofortified with various micronutrients to help address the widespread micronutrient deficiencies worldwide and combat malnutrition among an estimated 2 billion people facing hidden hunger worldwide [77], most of whom are women and children. Thanks to the introduction and use of advanced molecular breeding techniques such as CRISPR, it has shown promise in addressing these nutritional anomalies: vitamin A-enriched golden rice to combat vitamin A deficiency, iron-enriched maize, and vitamin A-enriched cassava to improve people's bioavailability and access to these limiting but essential nutrients. Peroni et al. [80] have reported a worrying rate of 20% deaths in children worldwide due to iron, iodine, zinc, and vitamin A deficiencies. The application of CRISPR to crops follows a series of workflows that ensure a high level of precision and accuracy. As shown in the figure below, these form the basic and summarized protocols underlying the use of the CRISPR system in crop improvement.

3.2 Reducing antinutritional factors and allergies in food crops using CRISPR

Antinutritive factors are secondary metabolites of plants that are described as co-factors with toxic properties and therefore limit the nutrient availability of food. Some of these antinutritive compounds, such as cyanide, tannins, oxalate, nitrate, phenols, and phytate, are widely distributed in plants and have detrimental effects on the absorption of macro- and micronutrients in humans and other animals (**Table 1**) [87]. Tannins, lectins, saponins, phytic acid, protease and amylase inhibitors, for example, significantly limit the availability of nutrients in food and have a strong growth inhibitory effect [88]. However, the integrity of these antinutrients can be reduced to a safe consumption rate in food by various techniques, depending on the type, chemical nature, and structure of the antinutrient compound in question [89]. The most common attempt to reduce antinutrient factors over the years has been the application of heat using devices such as microwaves and autoclaves, which have been shown to be very effective in reducing antinutrient factors in leafy vegetables. Reportedly, heat can break down the cell walls, allowing these secondary metabolites to be released [88]. These toxic metabolites in plants can also be reduced by soaking, cooking, fermentation, and the use of genetic engineering to manipulate the genes that control such compounds (**Table 1**).

Allergens are substances associated with food and nutrients that trigger allergic reactions to varying degrees depending on the individual's sensitivity. Vomiting and runny stools are common symptoms of food allergies. CRISPR has been utilized to combat allergens in a number of crops (peanut, durum, soybean, and sesame) (**Figure 3**). Studies have shown that high molecular weight proteins have a high potential to trigger allergic reactions when consuming foods containing them [94]. Histamine and its derivatives have been shown to counteract the defense antigens of allergens in the body. In taro, for example, protease inhibitors and calcium oxalate are known to trigger allergic reactions that lead to swelling of the throat and lips due to the pungency of taro. In particular, foods with a high oxalate content have a reduced bioavailability of calcium ions and tend to form kidney stones [95]. The genetic manipulation technique RNAi was successfully used to mutate and silence the Ara

ANF	Plant	Mode of action	Intervention	Reference
Phytic acid	Cereals	Inhibits nutrient absorption. It can cause anemia.	Soaking, germination, and fermentation	[81]
Lectins	Seeds of legumes	Limits the absorption of end products of digestion. Coagulates red blood cells.	Soaking, boiling, heating, and fermentation	[82]
Tannin	Cocoa, apples	Bond to nutrient elements and limit their absorption.	Soaking and boiling	[83]
Protease inhibitors	Potato, pineapple	Inhibits the activities of trypsin, pepsin, and chymotrypsin. Limits protein digestion and absorption.	Soaking, sprouting, and boiling	[84]
Oxalate	Rhubarb	Inhibit peptic digestion by forming complexes with proteins. Limit magnesium and calcium absorption.	Soaking and boiling	[85]
Goitrogens	Soybean and groundnuts	Inhibits the functions of the thyroid glands.	Steaming and boiling	[86]

ANF = antinutritional factor.

Table 1.
Antinutritional factors in some crops, their mechanisms, and ways to reduce their concentrations.

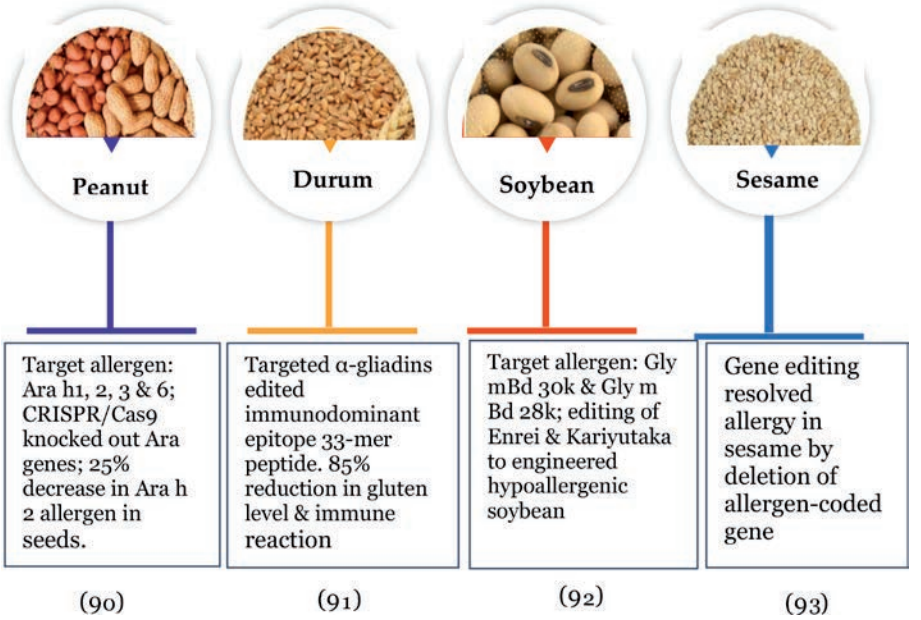

Figure 3.
Some crops in which CRISPR has been used to combat allergens [90–93].

h 2 glycoprotein, an allergen in peanuts [96]. This attempt at genetic modification led to the development of a hypoallergenic peanut with a low Ara h 2 content and significantly lower allergenicity. This milestone in the fight against peanut-associated allergies was achieved transgenically by Agrobacterium-induced modification through intron splicing of Ara h 2 RNAi formation using the CaMV 35S promoter gene

(**Figure 3**). In addition, studies have also discovered allergic proteins such as Lyc e 1, Lyc e 2 and Lyc c 3, which are present in tomato and induce oral allergic reactions. Therefore, Le et al. [97] suppressed the expression of the tomato allergens Lyc e 1.02 and Lyc e 1.01 in a transgenic experiment using an RNAi technique. The potential of CRISPR in the development of biofortified crops is highly capable of developing plants with reduced or no allergens by specifically removing genes that regulate such traits to obtain allergen-free crops. These allergens are genetically determined. Therefore, the application of the high-precision CRISPR tool can switch off the encoded genes in different crops to develop transgenic allergen-free crop varieties.

3.3 Enhancing shelf-life and storage quality of crops using CRISPR

Improving the shelf life and storage quality of agricultural products is a major challenge due to various factors. Over the years, various mechanisms have been tried to maintain the quality of plant products, especially during storage. Nevertheless, despite the successes achieved, there is still a significant level of persistent losses associated with poor shelf life and deterioration in quality during storage under the influence of several complex interactive factors [98]. This has necessitated a continuous search for the most efficient approach to mitigate this ever-present threat to food safety.

Among the numerous methods that have been developed, the application of first- and second-generation gene editing technologies stands out, with CRISPR being recently recognized as a highly precise and effective tool of genetic modification to improve the shelf life and storage quality of crops [99, 100]. The preconditioning internal factors that lead to deterioration in storage quality and affect storability in many crop species have been targeted with CRISPR to improve storability. Moreover, the factors affecting potato shelf life and storage quality, such as cold-induced sugar accumulation and enzymatic browning during postharvest, were targeted, and the responsible genes were modified to ensure the storage quality of potato tubers. The bio-induced browning of tubers is triggered in particular by the activities of polyphenol oxidase 2 (PPO2) during mechanical damage during postharvest processes [101]. Cold-induced sugar accumulation, which is favored by abiotic stress, promotes the hydrolysis of sucrose to glucose and fructose by the enzyme vacuolar invertase, which reduces the quality of French fries [102]. In response to this challenge, genome editing was used to alter and remove the function of the gene controlling vacuolar invertase to develop a new potato with enhanced chip quality under cold storage conditions [103, 104]. Likewise, simultaneous editing of the genes for asparagine synthetase and vacuolar invertase by the CRISPR/Cas9 system resulted in an 80% reduction in acrylamide content [105]. Simultaneous editing of the genes for polyphenol oxidase 2 and vacuolar invertase in the potato cultivar Spuntan also led to a significant improvement in reduced enzymatic browning and sugar synthesis during cold storage [106]. In addition, the gene encoding the enzymes VLNV, which are associated with cold-mediated accumulation of sugars, was mutated using the TALEN gene-editing technique to develop a cold-tolerant potato [106]. It is reported that polyphenol oxidase 2, which contributes to 55% of PPO activities in potato tubers during storage, was genetically modified by using sgRNAs and RNP-dispatching CRISPR/Cas9 to form protoplasts and achieved a reduction in enzymatic browning [107]. In addition, mutation of the gene FIS1 resulted in increased synthesis of the cuticle in the pericarp of the tomato fruit to increase firmness and protection, leading to a longer shelf life of tomato fruit during storage [108]. The FS8.1 gene was subjected to single-gene KO editing using the CRISPR-Cas9 system and underwent a mutation of over 65%, resulting

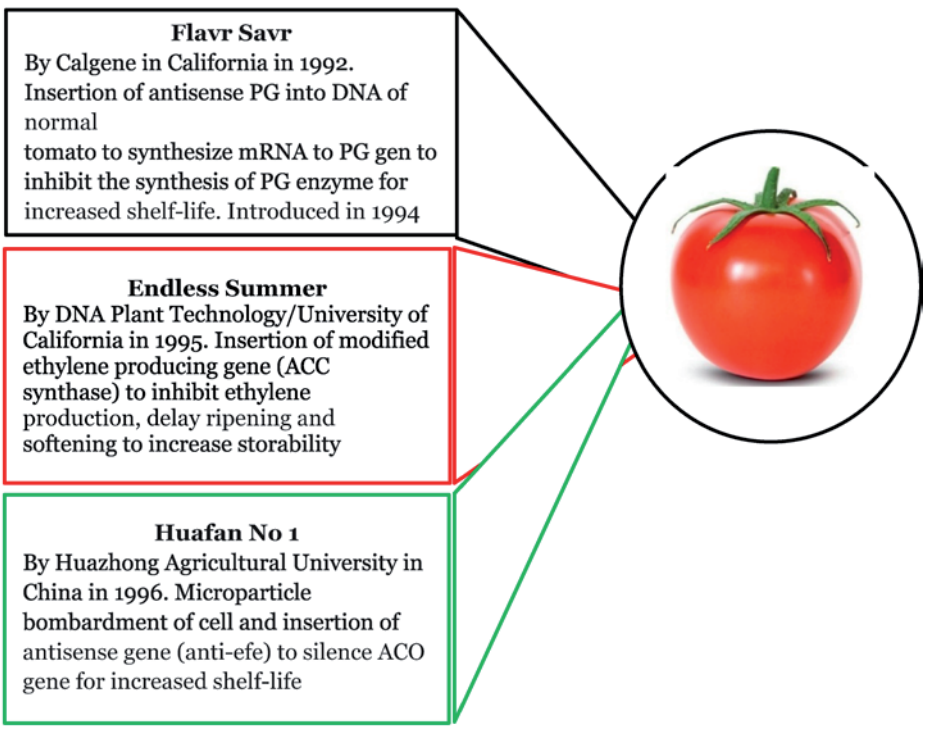

Figure 4.
Premier CRISPR-engineered tomatoes with enhanced shelf-life [115].

in a longer shelf life of tomato fruits [109]. Similar editing of the CYC-B gene with CRISPR-Cas9 led to a significant improvement in the shelf life of tomato fruits [110]. Extended shelf life was observed after editing the ALC (NOR) gene in tomato using the CRISPR-Cas9 technique [111]. Similarly, Chen et al. [112] reported a reduction in fruit water loss and an associated longer shelf life when CRISPR-Cas9 was used to edit the gene CNR. The reduction of volatile organic compounds with increased shelf life in tomato fruits was also achieved by modifying the target gene RIN with CRISPR-Cas9 [53]. In other studies, the *SlExp1*, *SlCel2*, *NOR-like1,* and *SlBES1* genes were edited in Ailsa Craig tomato to increase shelf life [113, 114]. The shelf life of the Micro-Tom tomato was also significantly improved by editing the SlMBP3 gene using the CRISPR-Cas9 method (**Figure 4**) [116].

4. Regulatory, ethical, and societal perspectives on CRISPR in agriculture

The emergence of bioengineering techniques, such as CRISPR and the associated processes, as well as their continued use in agriculture, has not been without local, regional, cultural, ethical, and socio-economic reservations. Different perspectives from different walks of life have been associated with these new technologies since they first emerged in people's minds and left the laboratories. Some of these serious concerns are related to the fragile trust in their socio-economic prospects, their cultural suitability, and the trustworthiness of regulatory mechanisms to tightly control intrusions. Moreover, careful consideration of some of these concerns has led

to the creation of regulatory frameworks for genetically modified crops and foods in many countries around the world. However, these legal frameworks vary considerably from country to country in terms of integrity and scope of capacity. Over the years, these differences have hindered straightforward approval and distribution of biotech crops and foods in the open market [117]. It is also reported that viable policy commitments for structuring and funding local frameworks and evaluation mechanisms are essential to promote widespread adoption and large-scale introduction of biofortified crops and associated foods, as some countries lack functioning seed banks and seed quality standards, as well as a real-time approval system in line with global best practice [118].

Regardless, the establishment of robust regulatory frameworks and highly accurate real-time assessment mechanisms will be crucial in changing the story of the CRISPR system and crops engineered with it globally in the future [119]. Finally, using the example of biofortified soybeans with increased methionine content, it is highly recommended and imperative to conduct thorough allergic reaction assessments and include them in packaging labels to achieve an optimal balance between safety measures and innovation to promptly control potential risks in biofortified crops and increase consumer confidence. Although the development of enriched crops using CRISPR technology is undeniable from an economic perspective compared to the conventional breeding approach, there is also growing resistance, including the uncertainty and risks of unintended gene modification and the resulting unquantified consequences [120]. According to Naeem and Alkhnbashi [121], some individuals and groups of people who favor the CRISPR system over other, earlier-developed gene-editing techniques still choose not to use biofortified crops, despite the scientific community repeatedly publicly pointing out the advances in CRISPR, which bring a higher degree of precision and a drastic reduction in unintended editing of non-target genes, on the grounds of unhealthiness. From an ethical perspective, proponents of genetic modification have expressed concerns about the wider use of genome editing in crops, further raising the barrier to the general public's acceptance of genetically engineered crops.

5. Role of bioinformatics in CRISPR-based crop improvement

Bioinformatics has contributed significantly to the establishment and further development of CRISPR-Cas as a gene-editing method. Bioinformatics tools are used for guide RNA design, off-target prediction, and data analysis, which has promoted the application of CRISPR-Cas9. For instance, CRISPR-Cas bioinformatics tools such as CASPERpam, E-CRISP, CRISPRtarget and CRISPRmap are used for specific tasks related to RNA structure maintenance, target efficiency, and low off-target activity [122]. Also, numerous bioinformatics tools (TIDE, CRISPR-GA, Cas-Analyzer, and CRISPResso2) are currently being developed for the investigation of off-target effects following experiments [121]. Hence, bioinformatics in CRISPR-based crop improvement aids researchers in understanding plant genomes, identifying genes linked to beneficial traits, and enhancing CRISPR-based editing for accurate modifications aimed at facilitating CRISPR/Cas9 studies [121, 123]. Choosing the appropriate bioinformatic tool for a particular CRISPR experiment is crucial and is contingent upon the species utilized for the CRISPR editing test, as well as the compatibility of the tool and Cas nuclease enzyme with that organism. The key role of bioinformatics in CRISPR research highlights the necessity for cutting-edge and standardized platforms.

6. Limitations of CRISPR applications in crop improvement

Although the genetic modification of crops with CRISPR has many advantages, there are also several disadvantages. The high rate of off-target mutations, which can be as high as 50%, is a major drawback of CRISPR technology compared to other genome editing techniques [124]. Getting the CRISPR system into target cells has proven to be the biggest challenge so far. Since off-target effects are well documented and common in genome editing, they occur less frequently in plants than in animals. Although off-target effects are not as problematic in CRISPR/Cas applications in plants, they can still lead to undesirable results, which is why their elimination is always necessary in genome editing. Even though CRISPR has a comparatively high accuracy, further efforts are needed to further develop the methods. Manghwar et al. [125] claim that CRISPR/Cas9 can cause off-target mutations in plants, which could lead to chromosomal rearrangements, cause damage to inappropriate genomic loci, and limit the use of GE for beneficial purposes. According to Manghwar et al. [125], off-target effects can also lead to loss of functional gene activity, which can cause a variety of physiological or signaling problems. Fortunately, whole genome sequencing has recently been used to identify cleavage at off-target sites by the Cas9 or Cas12a system nucleases in rice [126], cotton [127], and *Arabidopsis thaliana* [128]. Systemic evolution of ligands by exponential amplification (SELEX), integrase-deficient lentivirus (IDLV) capture, Cas-OFFinder (http://www.rgenome.net/cas-offinder/), DISCOVER-Seq [129], CCTop (https://crispr.cos.uniheidelberg.de), high-throughput genomic translocation sequencing (HTGTS), and other bioinformatics tools have been developed to address this problem [130].

Plants have successfully undergone homologous recombination (knock-in/gene replacement) by CRISPR/Cas, but the effectiveness of editing is modest [131, 132]. Therefore, effective knock-in of genes in plants by CRISPR/Cas-mediated homologous recombination is still a long way off. By using the novel genomics technique to identify more susceptible genes (S-genes) in a plant genome, CRISPR systems can eliminate undesirable traits [133]. Conversely, an improved CRISPR-Cas system must clone additional resistant genes (R genes) and introduce them into the plant genome *via* a DNA repair pathway mediated by homologous recombination. Without plant tissue culture, the Cas9-Cas12a proteins cannot be introduced directly into plant cells with viral vectors due to their relatively high molecular weight. Since numerous genes affect plant stress tolerance, scientists need to develop multiple sgRNAs in a single vector to target multiple genes. Because genetic modification of crops using CRISPR is a relatively new and rapidly developing field of research, it faces legal and regulatory obstacles as laws and regulations have not kept pace with scientific advances [134]. In addition, rigorous proof of safety and efficacy is required for approval.

7. Conclusions

The aim of using numerous techniques to develop safe and affordable crops to satisfy the world's growing food needs can be fraught with difficulties. A critical component will be the application of modern crop improvement methods. Compared to traditional breeding techniques, scientists can use sophisticated breeding techniques to quickly modify genes and introduce the required gene into the genome. CRISPR technology is a potent tool for gene editing with great promise for improving crops and addressing challenges relating to global food security. The CRISPR-Cas9

technique has been refined in recent decades as a flexible, targeted technique for modifying genes found in many species, including plants. Application of this method to improve the quality, productivity, and disease resistance of crops could therefore be an important area of research in the future. By improving pest, disease, and nutritional traits, this technology increases the resilience of crops to environmental stressors and climate change. It offers the opportunity to achieve high yields, reduce dependence on fertilizers, and increase the resilience of plants. In the last decade, it has been used widely in a variety of crops to conduct useful research, counteract stress-related responses, and improve key agronomic traits. Through knockdown, knock-in, replacement, point mutation, gene regulation, base editing, and other locus-specific modifications, the CRISPR/Cas9 technique has enhanced fundamental research in both plant science and the agricultural sector. Moreover, improving the specificity and accuracy of CRISPR-Cas9 editing is also an important area for future studies. Efforts are being made to reduce unwanted genomic alterations, improve the effectiveness of homology-directed repair (HDR), and reduce the consequences of off-target interventions.

Author details

Richard Dormatey[1*†], Augustine Antwi-Boasiako[1*†], Francis Azumah Chimsah[2†], Kwame Yaw-Kan Kojo[1†], Juliet Mensah[3†], Agbesi Kwadzo Keteku[1†], Philip Ghanney[1†] and Harrison Kwame Dapaah[4†]

1 Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI), Fumesua, Kumasi, Ghana

2 Faculty of Agriculture, Food and Consumer Science, University for Development Studies, Tamale, Ghana

3 Department of Public Health Education, Akenten Appiah-Menka University of Skill Training and Entrepreneurial Development (AAMUSTED), Ashanti, Ghana

4 Department of Crop and Soil Science Education, Akenten Appiah-Menka University of Skill Training and Entrepreneurial Development (AAMUSTED), Mampong, Ghana

*Address all correspondence to: rmddormatey@gmail.com and bbbantwi@yahoo.com

† All authors contribute equally to this chapter.

References

[1] Wijerathna-Yapa A, Pathirana R. Sustainable agro-food systems for addressing climate change and food security. Agriculture. 2022;**12**(10):1554

[2] Mrabet R. Sustainable agriculture for food and nutritional security. In: Sustainable Agriculture and the Environment. United States: Academic Press; 2023. pp. 25-90

[3] Akhatar J, Kaur H, Kumar H. Conventional plant breeding to modern biotechnological approaches in crop improvement. In: Technologies in Plant Biotechnology and Breeding of Field Crops. Springer; 2022. pp. 1-21

[4] Sadikiel Mmbando G. The adoption of genetically modified crops in Africa: The public's current perception, the regulatory obstacles, and ethical challenges. GM Crops and Food. 2024;**15**:185-199

[5] Guo Y, Zhao G, Gao X, Zhang L, Zhang Y, Cai X, et al. CRISPR/Cas9 gene-editing technology: A precise and efficient tool for crop quality improvement. Planta. 2023;**258**:36

[6] Das D, Singha DL, Paswan RR, Chowdhury N, Sharma M, Reddy PS, et al. Recent advancements in CRISPR/Cas technology for accelerated crop improvement. Planta. 2022;**255**:109

[7] Shelake RM, Kadam US, Kumar R, Pramanik D, Singh AK, Kim J-Y. Engineering drought and salinity tolerance traits in crops through CRISPR-mediated genome editing: Targets, tools, challenges, and perspectives. Plant Communications. 2022;**3**:34

[8] Jouanin A, Gilissen LJ, Schaart JG, Leigh FJ, Cockram J, Wallington EJ, et al. CRISPR/Cas9 gene editing of gluten in wheat to reduce gluten content and exposure—Reviewing methods to screen for coeliac safety. Frontiers in Nutrition. 2020;**7**:51

[9] Sun Y, Shang L, Zhu Q-H, Fan L, Guo L. Twenty years of plant genome sequencing: Achievements and challenges. Trends in Plant Science. 2022;**27**:391-401

[10] Ricroch A, Clairand P, Harwood W. Use of CRISPR systems in plant genome editing: Toward new opportunities in agriculture. Emerging Topics in Life Sciences. 2017;**1**:169-182

[11] Razzaq MK, Akhter M, Ahmad RM, Cheema KL, Hina A, Karikari B, et al. CRISPR-Cas9 based stress tolerance: New hope for abiotic stress tolerance in chickpea (*Cicer arietinum*). Molecular Biology Reports. 2022;**49**:8977-8985

[12] Kopecká R, Kameniarová M, Černý M, Brzobohatý B, Novák J. Abiotic stress in crop production. International Journal of Molecular Sciences. 2023;**24**:6603

[13] Rogo U, Viviani A, Pugliesi C, Fambrini M, Usai G, Castellacci M, et al. Improving crop tolerance to abiotic stress for sustainable agriculture: Progress in manipulating ascorbic acid metabolism via genome editing. Sustainability. 2025;**17**(2):2071-1050

[14] El-Ramady H, Prokisch J, Mansour H, Bayoumi YA, Shalaby TA, Veres S, et al. Review of crop response to soil salinity stress: Possible approaches from leaching to nano-management. Soil Systems. 2024;**8**:11

[15] Bainsla NK, Meena H. Breeding for resistance to biotic stresses in

plants. Recent Advances in Plant Stress Physiology. 2016;**379**:411

[16] Lin F, Chhapekar SS, Vieira CC, Da Silva MP, Rojas A, Lee D, et al. Breeding for disease resistance in soybean: A global perspective. Theoretical and Applied Genetics. 2022;**135**:3773-3872

[17] Rasheed A, Gill RA, Hassan MU, Mahmood A, Qari S, Zaman QU, et al. A critical review: Recent advancements in the use of CRISPR/Cas9 technology to enhance crops and alleviate global food crises. Current Issues in Molecular Biology. 2021;**43**:1950-1976

[18] Liu Q, Yang F, Zhang J, Liu H, Rahman S, Islam S, et al. Application of CRISPR/Cas9 in crop quality improvement. International Journal of Molecular Sciences. 2021;**22**:4206

[19] Liu H, Chen W, Li Y, Sun L, Chai Y, Chen H, et al. CRISPR/Cas9 technology and its utility for crop improvement. International Journal of Molecular Sciences. 2022;**23**:10442

[20] Li T, Liu B, Spalding MH, Weeks DP, Yang B. High-efficiency TALEN-based gene editing produces disease-resistant rice. Nature Biotechnology. 2012;**30**:390-392

[21] Usman B, Zhao N, Nawaz G, Qin B, Liu F, Liu Y, et al. CRISPR/Cas9 guided mutagenesis of grain size 3 confers increased rice (*Oryza sativa* L.) grain length by regulating cysteine proteinase inhibitor and ubiquitin-related proteins. International Journal of Molecular Sciences. 2021;**22**:3225

[22] Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, et al. Targeted genome modification of crop plants using a CRISPR-Cas system. Nature Biotechnology. 2013;**31**:686-688

[23] Ahmar S, Gill RA, Jung K-H, Faheem A, Qasim MU, Mubeen M, et al. Conventional and molecular techniques from simple breeding to speed breeding in crop plants: Recent advances and future outlook. International journal of molecular sciences. 2020;**21**:2590

[24] Zhu H, Li C, Gao C. Applications of CRISPR–Cas in agriculture and plant biotechnology. Nature Reviews Molecular Cell Biology. 2020;**21**:661-677

[25] KhokharVoytas A, Shahbaz M, Maqsood MF, Zulfiqar U, Naz N, Iqbal UZ, et al. Genetic modification strategies for enhancing plant resilience to abiotic stresses in the context of climate change. Functional and Integrative Genomics. 2023;**23**:283

[26] Rai GK, Khanday DM, Kumar P, Magotra I, Choudhary SM, Kosser R, et al. Enhancing crop resilience to drought stress through CRISPR-Cas9 genome editing. Plants. 2023;**12**:2306

[27] Sampath V, Rangarajan N, Sharanappa C, Deori M, Veeraragavan M, Ghodake B, et al. Advancing crop improvement through CRISPR technology in precision agriculture trends-a review. International Journal of Environment and Climate Change. 2023;**13**:4683-4694

[28] Arshad S, Qadir ML, Hussain N, Ali Q, Han S, Ali D. Advances in CRISPR/Cas9 technology: Shaping the future of photosynthetic microorganisms for biofuel production. Functional Plant Biology. 2025;**52**(2):FP24255. DOI: 10.1071/FP24255

[29] Bukhat S, Rasul S, Athar H-U-R, Manzoor H. Genome editing in improving photosynthesis in crop plants. In: Genome Editing for Crop Improvement: Theory and Methodology.

United Kingdom: CABI GB; 2025. pp. 217-246

[30] Anbarasan S, Ramesh S. Photosynthesis eficiency: Advances and challenges in improving crop yield. Plant Science Archives. 2022;**19**:21

[31] Li R, He Y, Chen J, Zheng S, Zhuang C. Research progress in improving photosynthetic efficiency. International Journal of Molecular Sciences. 2023;**24**:9286

[32] Simkin AJ, Kapoor L, Doss CGP, Hofmann TA, Lawson T, Ramamoorthy S. The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. Photosynthesis Research. 2022;**152**:23-42

[33] Wang L, Wei J, Shi X, Qian W, Mehmood J, Yin Y, et al. Identification of the light-harvesting chlorophyll a/b binding protein gene family in peach (Prunus persica L.) and their expression under drought stress. Genes. 2023;**14**:1475

[34] Schmid V. Light-harvesting complexes of vascular plants. Cellular and Molecular Life Sciences. 2008;**65**:3619-3639

[35] Gionfriddo M, Rhodes T, Whitney SM. Perspectives on improving crop Rubisco by directed evolution. In: Proceedings of the Seminars in Cell & Developmental Biology. Amsterdam, Netherlands: Elsevier; 2024. pp. 37-47

[36] Zhou J, Peng Z, Long J, Sosso D, Liu B, Eom JS, et al. Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. The Plant Journal. 2015;**82**:632-643

[37] Majumder S, Datta K, Datta SK. Perspective of evolution of the C 4 plants to develop climate designer C 4 Rice as a strategy for abiotic stress management. Global Climate Change and Plant Stress Management. 2023;**1**:113-124. DOI: 10.1002/9781119858553.ch10

[38] Molina-Moya E, Rodríguez-González A, Peláez-Vico MA, Sandalio LM, Romero-Puertas MC. Peroxisomal-dependent signalling and dynamics modulate plant stress responses: Reactive oxygen and nitrogen species as key molecules. Journal of Experimental Botany. 2025:eraf072

[39] Jia M, Wang Y, Jin H, Li J, Song T, Chen Y, et al. Comparative genomics analysis of the Populus epidermal pattern factor (EPF) family revealed their regulatory effects in Populus euphratica stomatal development. International Journal of Molecular Sciences. 2024;**25**:10052

[40] Pereira A. Plant Abiotic Stress Challenges from the Changing Environment. 2016;**7**:1123

[41] Sasano Y, Nagasawa K, Kaboli S, Sugiyama M, Harashima S. CRISPR-PCS: A powerful new approach to inducing multiple chromosome splitting in Saccharomyces cerevisiae. Scientific Reports. 2016;*6*:30278

[42] Jain M. Function Genomics of Abiotic Stress Tolerance in Plants: A CRISPR Approach. 2015;**6**:375

[43] Muntazir Mushtaq MM, Bhat J, Mir Z, Afreen Sakina AS, Sajad Ali SA, Singh A, et al. A new way of looking at plant-abiotic interactions. Journal of Plant Physiology. 2018;**224**:156-162

[44] Debbarma J, Sarki YN, Saikia B, Boruah HPD, Singha DL, Chikkaputtaiah C. Ethylene response factor (ERF) family proteins in abiotic stresses and CRISPR–Cas9 genome

editing of ERFs for multiple abiotic stress tolerance in crop plants: A review. Molecular Biotechnology. 2019;**61**:153-172

[45] Curtin SJ, Xiong Y, Michno JM, Campbell BW, Stec AO, Čermák T, et al. Crispr/cas9 and talen s generate heritable mutations for genes involved in small rna processing of glycine max and medicago truncatula. Plant Biotechnology Journal. 2018;**16**:1125-1137

[46] Du Y-T, Zhao M-J, Wang C-T, Gao Y, Wang Y-X, Liu Y-W, et al. Identification and characterization of GmMYB118 responses to drought and salt stress. BMC Plant Biology. 2018;**18**:1-18

[47] Zhang X, Wang Z, Jiang C, Xu J-R. Regulation of biotic interactions and responses to abiotic stresses by MAP kinase pathways in plant pathogenic fungi. Stress Biology. 2021;**1**:1-19

[48] Wu J, Yan G, Duan Z, Wang Z, Kang C, Guo L, et al. Roles of the Brassica napus DELLA protein BnaA6. RGA, in modulating drought tolerance by interacting with the ABA signaling component BnaA10. ABF2. Frontiers in Plant Science. 2020;**11**:577

[49] Li ChangYan LC, Li Wei LW, Zhou ZaiHui ZZ, Chen Hao CH, Xie CongHua XC, Lin YongJun LY. A new rice breeding method: CRISPR/Cas9 system editing of the Xa13 promoter to cultivate transgene-free bacterial blight-resistant rice. Plant Biotechnology Journal. 2020;**18**(2):313-315

[50] Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM. Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiology. 2015;**169**:931-945

[51] Sardar A. Genetic amelioration of fruit and vegetable crops to increase biotic and abiotic stress resistance through CRISPR genome editing. Frontiers in Plant Science. 2023;**14**:1260102

[52] Zeng Y, Wen J, Zhao W, Wang Q, Huang W. Rational improvement of rice yield and cold tolerance by editing the three genes OsPIN5b, GS3, and OsMYB30 with the CRISPR–Cas9 system. Frontiers in Plant Science. 2020;**10**:1663

[53] Li R, Liu C, Zhao R, Wang L, Chen L, Yu W, et al. CRISPR/Cas9-mediated SlNPR1 mutagenesis reduces tomato plant drought tolerance. BMC Plant Biology. 2019;**19**:1-13

[54] Chen M, Zhu X, Liu X, Wu C, Yu C, Hu G, et al. Knockout of auxin response factor SlARF4 improves tomato resistance to water deficit. International Journal of Molecular Sciences. 2021;**22**:3347

[55] Tran MT, Doan DTH, Kim J, Song YJ, Sung YW, Das S, et al. CRISPR/Cas9-based precise excision of SlHyPRP1 domain (s) to obtain salt stress-tolerant tomato. Plant Cell Reports. 2021;**40**:999-1011

[56] Santosh Kumar V, Verma RK, Yadav SK, Yadav P, Watts A, Rao M, et al. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. Physiology and Molecular Biology of Plants. 2020;**26**:1099-1110

[57] Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, et al. ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnology Journal. 2017;**15**:207-216

[58] Bouzroud S, Gasparini K, Hu G, Barbosa MAM, Rosa BL, Fahr M, et al. Down regulation and loss of

auxin response factor 4 function using CRISPR/Cas9 alters plant growth, stomatal function and improves tomato tolerance to salinity and osmotic stress. Genes. 2020;**11**:272

[59] Nazarov PA, Baleev DN, Ivanova MI, Sokolova LM, Karakozova MV. Infectious plant diseases: Etiology, current status, problems and prospects in plant protection. Acta Naturae. 2020;**12**:46

[60] Myers JH, Sarfraz RM. Impacts of insect herbivores on plant populations. Annual Review of Entomology. 2017;**62**:207-230

[61] Chattopadhyay C, Birah A, Jalali BL. Climate change: Impact on biotic stresses afflicting crop plants. In: Natural Resource Management: Ecological Perspectives. Cham, Switzerland: Springer; 2019. pp. 133-146

[62] Pocketbook FS. World Food and Agriculture. Rome Italy: FAO; 2017

[63] Consortium R. Combining selective pressures to enhance the durability of disease resistance genes. Frontiers in Plant Science. 2016;**7**:1916

[64] Zaidi SS-E-A, Mukhtar MS, Mansoor S. Genome editing: Targeting susceptibility genes for plant disease resistance. Trends in Biotechnology. 2018;**36**:898-906

[65] Bui TP, Le H, Ta DT, Nguyen CX, Le NT, Tran TT, et al. Enhancing powdery mildew resistance in soybean by targeted mutation of MLO genes using the CRISPR/Cas9 system. BMC Plant Biology. 2023;**23**:533

[66] Oliva R, Ji C, Atienza-Grande G, Huguet-Tapia JC, Perez-Quintero A, Li T, et al. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nature Biotechnology. 2019;**37**:1344-1350

[67] Zeng X, Luo Y, Vu NTQ, Shen S, Xia K, Zhang M. CRISPR/Cas9-mediated mutation of OsSWEET14 in rice cv. Zhonghua11 confers resistance to Xanthomonas oryzae pv. Oryzae without yield penalty. BMC Plant Biology. 2020;**20**:1-11

[68] Cao Y, Zhou H, Zhou X, Li F. Control of plant viruses by CRISPR/Cas system-mediated adaptive immunity. Frontiers in Microbiology. 2020;**11**:593700

[69] Macovei A, Sevilla NR, Cantos C, Jonson GB, Slamet-Loedin I, Čermák T, et al. Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. Plant Biotechnology Journal. 2018;**16**:1918-1927

[70] Singh S, Rahangdale S, Pandita S, Saxena G, Upadhyay SK, Mishra G, et al. CRISPR/Cas9 for insect pests management: A comprehensive review of advances and applications. Agriculture. 2022;**12**:1896

[71] Schetelig MF, Handler AM. Strategy for enhanced transgenic strain development for embryonic conditional lethality in Anastrepha suspensa. Proceedings of the National Academy of Sciences. 2012;**109**:9348-9353

[72] Schetelig M, Targovska A, Meza J, Bourtzis K, Handler A. Tetracycline-suppressible female lethality and sterility in the Mexican fruit fly, *Anastrepha ludens*. Insect Molecular Biology. 2016;**25**:500-508

[73] Mir A, Alterman JF, Hassler MR, Debacker AJ, Hudgens E, Echeverria D, et al. Heavily and fully modified RNAs guide efficient SpyCas9-mediated

genome editing. Nature Communications. 2018;**9**:2641

[74] Handler AM. Prospects for using genetic transformation for improved SIT and new biocontrol methods. Genetica. 2002;**116**:137-149

[75] Ogaugwu CE, Schetelig MF, Wimmer EA. Transgenic sexing system for *Ceratitis capitata* (Diptera: Tephritidae) based on female-specific embryonic lethality. Insect Biochemistry and Molecular Biology. 2013;**43**:1-8

[76] Yilmaz H, Yilmaz A. Hidden hunger in the age of abundance: The nutritional pitfalls of modern staple crops. Food Science and Nutrition. 2025;**13**(2):e4610. DOI: 10.1002/fsn3.4610

[77] Jangra A, Tiwari S. Empowering nutrition: Biofortification to combat hidden hunger. In: Advances in Agri-Food Systems: Volume II. Singapore: Springer Nature Singapore; 2025. pp. 285-305

[78] Panda D, Mishra SS, Behera PK. Improvement of rice quality: The new revolution. In: Rice Research for Quality Improvement: Genomics and Genetic Engineering: Volume 1: Breeding Techniques and Abiotic Stress Tolerance. Singapore: Springer Singapore; 2020. pp. 87-108

[79] Begna T. Conventional breeding methods widely used to improve self-pollinated crops. International Journal of Research. 2021;**7**:1-16

[80] Peroni DG, Hufnagl K, Comberiati P, Roth-Walter F. Lack of iron, zinc, and vitamins as a contributor to the etiology of atopic diseases. Frontiers in Nutrition. 2023;**9**:1032481

[81] Bloot APM, Kalschne DL, Amaral JAS, Baraldi IJ, Canan C. A review of phytic acid sources, obtention, and applications. Food Reviews International. 2023;**39**:73-92

[82] Sauvion N, Nardon C, Febvay G, Gatehouse AM, Rahbé Y. Binding of the insecticidal lectin Concanavalin a in pea aphid, *Acyrthosiphon pisum* (Harris) and induced effects on the structure of midgut epithelial cells. Journal of Insect Physiology. 2004;**50**:1137-1150

[83] Kumari M, Jain S. Tannins: An antinutrient with positive effect to manage diabetes. Research Journal of Recent Sciences. 2012;**2277**:2502

[84] Svensson B, Fukuda K, Nielsen PK, Bønsager BC. Proteinaceous α-amylase inhibitors. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2004;**1696**(2):145-156

[85] Bsc SN, Bsc GS. Oxalate content of foods and its effect on humans. Asia Pacific Journal of Clinical Nutrition. 1999;**8**:64-74

[86] Manikantan M, Sharma R, Yadav D, Gupta R. Selection of process parameters for producing high quality defatted sesame flour at pilot scale. Journal of Food Science and Technology. 2015;**52**:1778-1783

[87] Abdolkhani D, Mohammadi B, Fakhari AA. Antinutritional compounds in food and their targeting methods to ensure nutritional security. In: International Conference on Food Industry Science, Organic Agriculture and Food Security, Organized by IOAS. Iran (Tehran); 2024. pp. 1-9

[88] Samtiya M, Aluko RE, Dhewa T. Plant food anti-nutritional factors and their reduction strategies: An overview. Food Production, Processing and Nutrition. 2020;**2**:1-14

[89] Faizal F, Ahmad N, Yaacob J, Halim-Lim SA, Rahim MA. Food processing to reduce antinutrients in plant-based foods. International Food Research Journal. 2023;**30**:25-45

[90] Dodo H. SBIR Phase II: Development of an Allergen-Free Peanut Using Genome Editing Technology. Alexandria, VA: National Science Foundation; 2021

[91] Sugano S, Hirose A, Kanazashi Y, Adachi K, Hibara M, Itoh T, et al. Simultaneous induction of mutant alleles of two allergenic genes in soybean by using site-directed mutagenesis. BMC Plant Biology. 2020;**20**:1-15

[92] Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, et al. Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. Plant Biotechnology Journal. 2018;**16**:902-910

[93] You J, Li D, Yang L, Dossou SSK, Zhou R, Zhang Y, et al. CRISPR/Cas9-mediated efficient targeted mutagenesis in sesame (*Sesamum indicum L.*). Frontiers in Plant Science. 2022;**13**:935825

[94] Gunal-Koroglu D, Karabulut G, Ozkan G, Yılmaz H, Gültekin-Subaşı B, Capanoglu E. Allergenicity of alternative proteins: Reduction mechanisms and processing strategies. Journal of Agricultural and Food Chemistry. 2025;**73**(13):7522-7546

[95] Fufa TW, Oselebe HO, Abetw WG, Amadi CO. Review of acridity in taro [*Colocasia esculenta (L) Schott*]: Its health and economic impact, and possible reduction mechanisms. Journal of Agricultural Science and Practice. 2021;**6**:10.31248

[96] Dodo HW, Konan KN, Chen FC, Egnin M, Viquez OM. Alleviating peanut allergy using genetic engineering: The silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity. Plant Biotechnology Journal. 2008;**6**:135-145

[97] Le LQ, Mahler V, Lorenz Y, Scheurer S, Biemelt S, Vieths S, et al. Reduced allergenicity of tomato fruits harvested from Lyc e 1–silenced transgenic tomato plants. Journal of Allergy and Clinical Immunology. 2006;**118**:1176-1183

[98] Moschopoulou E, Moatsou G, Syrokou M, Paramithiotis S, Drosinos E. Food quality changes during shelf life. In: Food Quality and Shelf Life. United States: Academic Press; 2019. pp. 1-31

[99] Tuncel A, Pan C, Sprink T, Wilhelm R, Barrangou R, Li L, et al. Genome-edited foods. Nature Reviews Bioengineering. 2023;**1**:799-816

[100] Miladinovic D, Antunes D, Yildirim K, Bakhsh A, Cvejić S, Kondić-Špika A, et al. Targeted plant improvement through genome editing: From laboratory to field. Plant Cell Reports. 2021;**40**:935-951

[101] Mayer AM. Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry. 2006;**67**:2318-2331

[102] Ohara-Takada A, Matsuura-Endo C, Chuda Y, Ono H, Yada H, Yoshida M, et al. Change in content of sugars and free amino acids in potato tubers under short-term storage at low temperature and the effect on acrylamide level after frying. Bioscience, Biotechnology, and Biochemistry. 2005;**69**:1232-1238

[103] Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, et al. Improving cold storage and processing traits in potato through targeted gene

knockout. Plant Biotechnology Journal. 2016;**14**:169-176

[104] Yasmeen A, Shakoor S, Azam S, Bakhsh A, Shahid N, Latif A, et al. CRISPR/Cas-mediated knockdown of vacuolar invertase gene expression lowers the cold-induced sweetening in potatoes. Planta. 2022;**256**:107

[105] Ly DNP, Iqbal S, Fosu-Nyarko J, Milroy S, Jones MG. Multiplex CRISPR-Cas9 gene-editing can deliver potato cultivars with reduced browning and acrylamide. Plants. 2023;**12**:379

[106] Massa GA, Décima Oneto CA, González MN, Poulsen Hornum A, Arizmendi A, Sucar S, et al. CRISPR/Cas9-mediated development of potato varieties with Long-term cold storage and bruising resistance. Biology. 2025;**14**:445

[107] González MN, Massa GA, Andersson M, Turesson H, Olsson N, Fält A-S, et al. Reduced enzymatic browning in potato tubers by specific editing of a polyphenol oxidase gene via ribonucleoprotein complexes delivery of the CRISPR/Cas9 system. Frontiers in Plant Science. 2020;**10**:497481

[108] Li R, Sun S, Wang H, Wang K, Yu H, Zhou Z, et al. FIS1 encodes a GA2-oxidase that regulates fruit firmness in tomato. Nature Communications. 2020;**11**:5844

[109] Zhu Q, Deng L, Chen J, Rodríguez GR, Sun C, Chang Z, et al. Redesigning the tomato fruit shape for mechanized production. Nature Plants. 2023;**9**:1659-1674

[110] Arruabarrena A, Lado J, González-Arcos M, Vidal S. Targeted disruption of tomato chromoplast-specific lycopene β-cyclase (CYC-B) gene promotes early accumulation of lycopene in fruits and enhanced postharvest cold tolerance. Plant Biotechnology Journal. 2023;**21**:2420

[111] Yu Q-H, Wang B, Li N, Tang Y, Yang S, Yang T, et al. CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. Scientific Reports. 2017;**7**:11874

[112] Chen D, Wang T, Huang H, Zhang Q, Chen X, Sun Z, et al. SlCNR regulates postharvest water loss and wax accumulation in tomato fruit and directly represses the transcription of very-long-chain (VLC) alkane biosynthesis-related genes SlCER1-2 and SlCER6. Postharvest Biology and Technology. 2024;**208**:112641

[113] Su G, Lin Y, Wang C, Lu J, Liu Z, He Z, et al. Expansin SlExp1 and endoglucanase SlCel2 synergistically promote fruit softening and cell wall disassembly in tomato. The Plant Cell. 2024;**36**:709-726

[114] Liu H, Liu L, Liang D, Zhang M, Jia C, Qi M, et al. SlBES1 promotes tomato fruit softening through transcriptional inhibition of PMEU1. Iscience. 2021;**24**(8):102926. DOI: 10.1016/j.isci.2021.102926

[115] Lobato-Gómez M, Hewitt S, Capell T, Christou P, Dhingra A, Girón-Calva PS. Transgenic and genome-edited fruits: Background, constraints, benefits, and commercial opportunities. Horticulture Research. 2021;**8**:166. DOI: 10.1038/s41438-021-00601-3

[116] Huang B, Hu G, Wang K, Frasse P, Maza E, Djari A, et al. Interaction of two MADS-box genes leads to growth phenotype divergence of all-flesh type of tomatoes. Nature Communications. 2021;**12**(1):6892

[117] Kojo KY-K, Adoma NF, Akpatsu IB, Aggrey H, Dormatey RM, Issah N, et al. Cross sectional view of genetically modified organisms (Gmos) in Africa. Malaysian Animal Husbandry Journal (MAHJ). 2024;**4**(2):43-48

[118] Mitra-Ganguli T, Pfeiffer WH, Walton J. The global regulatory framework for the commercialization of nutrient enriched biofortified foods. Annals of the New York Academy of Sciences. 2022;**1517**:154-166

[119] Jones MG, Iqbal S, Fosu-Nyarko J. Regulation of CRISPR-edited plants in Australia and New Zealand. In: Global Regulatory Outlook for CRISPRized Plants. United States: Elsevier; 2024. pp. 281-292

[120] Guo C, Ma X, Gao F, Guo Y. Off-target effects in CRISPR/Cas9 gene editing. Frontiers in Bioengineering and Biotechnology. 2023;**11**:1143157

[121] Naeem M, Alkhnbashi OS. Current bioinformatics tools to optimize CRISPR/Cas9 experiments to reduce off-target effects. International Journal of Molecular Sciences. 2023;**24**:6261

[122] Alkhnbashi OS, Meier T, Mitrofanov A, Backofen R, Voss B. CRISPR-Cas bioinformatics. Methods. 2020;**172**:3-11

[123] Jasieniecka A, Domingues I. CRISPR-Cas9 and its bioinformatics tools: A systematic review. Current Issues in Molecular Biology. 2025;**47**:307

[124] Zhang XH, Tee LY, Wang XG, Huang QS, Yang SH. Off-target effects in CRISPR/Cas9-mediated genome engineering. Molecular Therapy Nucleic Acids. 2015;**4**:e264

[125] Manghwar H, Li B, Ding X, Hussain A, Lindsey K, Zhang X, et al. CRISPR/Cas systems in genome editing: Methodologies and tools for sgRNA design, off-target evaluation, and strategies to mitigate off-target effects. Advanced Science. 2020;**7**:1902312

[126] Tang X, Liu G, Zhou J, Ren Q, You Q, Tian L, et al. A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice. Genome Biology. 2018;**19**:1-13

[127] Li J, Manghwar H, Sun L, Wang P, Wang G, Sheng H, et al. Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants. Plant Biotechnology Journal. 2019;**17**:858-868

[128] Feng Z, Mao Y, Xu N, Zhang B, Wei P, Yang D-L, et al. Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas-induced gene modifications in Arabidopsis. National Academy of Sciences of the United States of America. 2014;**111**:4632-4637

[129] Wienert B, Wyman SK, Richardson CD, Yeh CD, Akcakaya P, Porritt MJ, et al. Unbiased detection of CRISPR off-targets in vivo using DISCOVER-Seq. Science. 2019;**364**:286-289

[130] Koo T, Lee J, Kim J-S. Measuring and reducing off-target activities of programmable nucleases including CRISPR-Cas9. Molecules and Cells. 2015;**38**:475-481

[131] Puchta H, Fauser F. Synthetic nucleases for genome engineering in plants: Prospects for a bright future. The Plant Journal. 2014;**78**:727-741

[132] Lu Y, Tian Y, Shen R, Yao Q, Wang M, Chen M, et al. Targeted,

efficient sequence insertion and replacement in rice. Nature Biotechnology. 2020;**38**:1402-1407

[133] Santillán Martínez MI, Bracuto V, Koseoglou E, Appiano M, Jacobsen E, Visser RG, et al. CRISPR/Cas9-targeted mutagenesis of the tomato susceptibility gene PMR4 for resistance against powdery mildew. BMC Plant Biology. 2020;**20**:1-13

[134] Gan WC, Ling AP. CRISPR/Cas9 in plant biotechnology: Applications and challenges. Biotechnologia. 2022;**103**:81

Edited by Arli Aditya Parikesit, Arif Nur Muhammad Ansori and Adhityo Wicaksono

CRISPR Technologies – Advances in Genome Editing, Applications, and Ethical Implications provides a comprehensive overview of one of the most revolutionary tools in contemporary biotechnology. In this volume, we explore the latest developments in CRISPR-Cas systems, highlighting their diverse applications, ranging from gene therapy and precise genome editing to agriculture and synthetic biology. It carefully considers the ethical, societal, and regulatory issues that arise from the rapid development of technology and promotes thoughtful discourse on the responsibility for innovation. The reader will gain insight into the molecular basis of CRISPR, its transformative role in disease therapy, and its potential to revolutionize fields such as bioinformatics and drug design in the future. With a focus on the applied aspects, the volume presents how CRISPR-assisted diagnosis and therapy lead to the fast-tracking of personalized medicine and move closer to understanding genetic diseases. It elaborates on integrating CRISPR with novel technologies, such as artificial intelligence and computational biology, to enhance the efficiency and accuracy of genome editing. By striking a balance between scientific treatment and readable description, this volume emerges as a priceless source for researchers, students, and professionals seeking to learn about the hopes and prospects of 21st-century genome editing technologies. Due to its detailed coverage and prescient vision, it is an essential read for all with an interest in the future directions of genetics and biotechnology.

Kenji Ikehara, Genetics Series Editor

ISSN 3049-7094
ISBN 978-1-83635-584-7
9 781836 355847

Published in London, UK

IntechOpen