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Review Article

Ethics Section

A Scoping Review on the Ethical Issues in the Use of CRISPR-Cas9 in the Creation of Human Disease Models

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ABSTRACT

Introduction: The remarkable advances in molecular science and technology have dramatically changed the landscape of Deoxyribonucleic Acid (DNA). With the rapid pace of new gene editing technologies like Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 (CRISPR-Cas9), human disease models can be created to reduce the burden of morbidity and mortality caused by genetic defects and congenital malformations. However, despite its potential to advance human health and well-being, the use of CRISPR-Cas9 technology raises numerous ethical concerns, including the lack of a well-defined regulatory framework.

Aim: To outline the ethical concerns that arise in the creation of human disease models using CRISPR-Cas9 technology and to design a conceptual framework to identify the ethical challenges and address these concerns.

Materials and Methods: The data on ethical issues in the use of CRISPR-Cas9 in the creation of human disease models were obtained by reviewing 530 articles retrieved from scientific databases such as Google Scholar, PubMed, Scopus, and Excerpte Medica dataBASE (EMBASE) from the year 2015. Based on the eligibility criteria, 24 publications from 56 full-text articles that were screened were included in this study. The selection process was conducted in three phases-screening of the title, abstract, and full text. The articles selected after full-text screening were analysed, and the data was scrutinised independently. Tables, charts, figures, and graphs were used to organise and illustrate the obtained data. The entire paper was drafted using the Preferred Repoting Items for Systematic Reviews and Meta-analyses (PRISMA) extension for scoping review reporting criteria.

Results: The present study included 24 articles for review after the screening process. The articles emphasised the bioethical issues related to CRISPR-Cas9 technology and gene editing while also shedding light on the current level of research in the field. The studies included different countries, with the maximum number of papers from the United States of America (USA), followed by the United Kingdom (UK), China, Turkey, Spain, Canada, Pakistan, Australia, Italy, France, Korea, and Sri Lanka. These articles were published between 2015 and 2021. The disease for which models were created was not mentioned in the majority of articles, while a few investigated the application of CRISPR-Cas9 in genetic disorders, cardiovascular diseases, neurodegenerative diseases, and eye disorders. The major ethical concerns identified included safety, efficacy, unintended consequences, harm to the environment, off-target effects, obtaining informed consent, and the risk of misuse.

Conclusion: The use of CRISPR-Cas9 technology in creating human disease models has raised many ethical concerns. One of the primary ethical issues is the potential for unintended consequences, which could have serious long-term effects on individuals and their offspring. To address these ethical issues, it is important to develop ethical guidelines and best practices, as well as to support ongoing research to investigate the long-term effects of gene modifications.

Keywords: Ethical concerns, Framework, Genetic editing, Gene modifications, Human diseases, Research, Technology

INTRODUCTION

Genes are the basic units of inheritance that influence the fundamental existence of all forms of life [1]. Alteration of the genome of an individual resulting in mutations is known to cause over 10,000 different types of genetic disorders, impacting the lives of 80 million people worldwide [2]. Nevertheless, the phenomenal advancements in molecular science and technology have remarkably changed the destiny of our Deoxyribo Nucleic Acid (DNA), with ground breaking technological applications that can completely renovate the genetic makeup of an individual and reduce the burden of morbidity and mortality due to genetic diseases and congenital abnormalities [3]. Gene editing is one such promising genome engineering technique that has accelerated the quantum leap in novel discoveries of disease modeling, gene therapy, drug development, and molecular treatment strategies [4].

The CRISPR-Cas9 has emerged as the most influential and outstanding technique of genome editing in recent years. This "Clustered Regularly Interspaced Short Palindromic Repeats-Associated Protein 9" system is a marvel of life sciences that gives mankind the power to resculpt DNA and potentially 'erase' and/or 'alter' genetic sequences that can cause life-threatening diseases [5]. This CRISPR-Cas9 system in the bacterial genome, which serves as a self-protective tool against invading viruses, can be applied to cleave foreign genetic material using Ribonucleic Acid (RNA)-guided endonucleases, enabling desired alterations to be made in the human genome [6-8].

The creation of genetically altered animal and cellular models for various human illnesses, such as mutagenesis models with site-direction, gene knockin and knockout models, constitutes the most significant use of CRISPR-Cas9 systems in medicine [9]. However, despite its ability to make groundbreaking changes in the field of science and technology, there are many ethical challenges concerning the application of CRISPR-Cas9. This issue raises questions about the protection of subsequent generations at risk for non Mendelian (single gene) disorders, as well as challenges that this technology may pose regarding changes in societal values, socioeconomic background, personality, inequities, and affordability [10].

As many ethical challenges arise in the use of CRISPR-Cas9 in the creation of human disease models, proper guidelines and frameworks are essential for the successful implementation of such gene editing technology. Keeping these concerns in mind, the authors intend to analyse the ethical issues associated with CRISPR-Cas9 technology by scrutinising this technology and its intended uses.

Gaps in Literature

The creation of human disease models using CRISPR-Cas9 technology poses significant ethical questions that have been covered in the literature. However, there are still some gaps in the literature concerning these moral concerns, including disagreements over the standards for choosing target diseases, ambiguity regarding the security and effectiveness of CRISPR-Cas9, uncertainty surrounding the informed consent procedure, and issues with the commercialisation of CRISPR-Cas9 technology. Hence, research focusing on creating policies and best practices for the ethical use of this technology in the healthcare environment is warranted. This study provides an overview of ethical concerns in using CRISPR-Cas9 technology for creating human disease models and a conceptual framework emphasising the need for well-defined ethical regulations and frameworks.

MATERIALS AND METHODS

From the search for relevant papers to the analysis and reporting of the study's conclusions, the present scoping study was conducted following the five-stage systematic approach designed by Arksey H and O'Malley L [11]. The information was organised to meet the criteria of the PRISMA-ScR Checklist, which is an extension of PRISMA (PRISMA reporting items for systematic reviews and meta-analyses) [12].

Identification of Relevant Studies

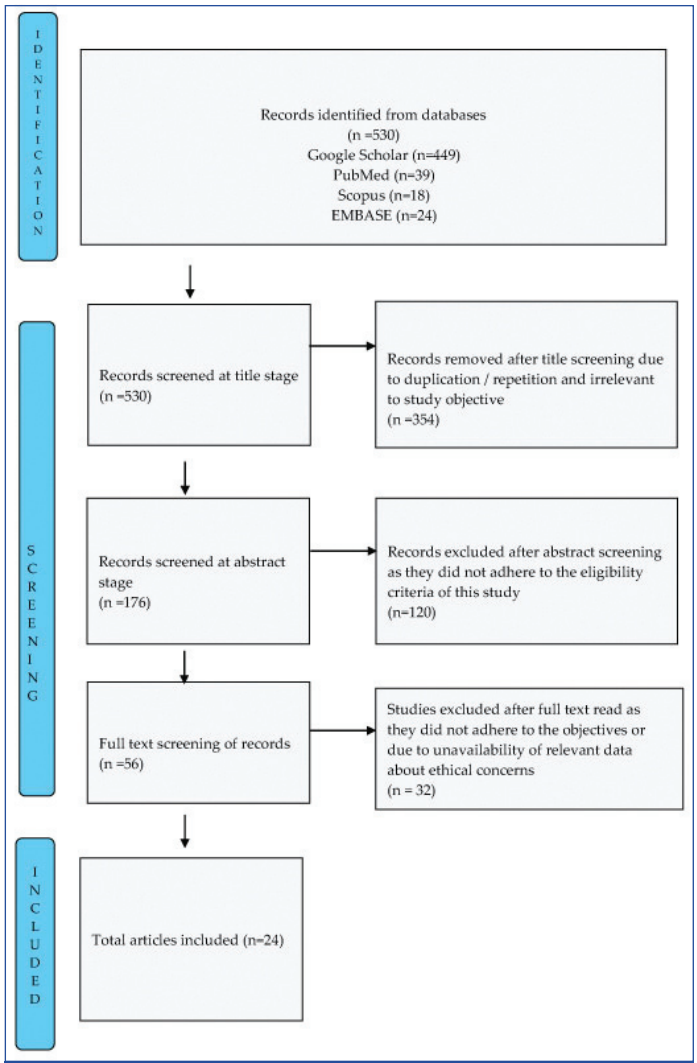
The relevant papers for the present study were searched on online databases such as Google Scholar, PubMed, Scopus, and EMBASE. The primary keywords and Medical Subject Headings (MeSH) used were: 'CRISPR-Cas9'; 'ethics'; 'gene editing'; 'human disease models'; 'bioethics'; 'ethical issues'. The present scoping review was conducted from November 2022 to March 2023.

Selection of Studies

The relevant publications for the present review were chosen based on the inclusion criteria listed in [Table/Fig-1]. Publications and papers that did not meet the objectives of the present study were excluded based on the criteria indicated in [Table/Fig-1]. In the initial search for "ethical issues in the use of CRISPR-Cas9 in human disease models" and using BOOLEAN logic of "AND," "OR," and "NOT" in PubMed, 530 full-text publications were obtained from the databases and were first screened by title. The initial search resulted in 39 papers from PubMed, 449 articles from Google Scholar, 18 from Scopus, and 24 from EMBASE, covering the years 2015 to 2023. Brief abstracts, conference abstracts, books, and cross-sectional studies were not included. 356 papers were excluded due to duplication, redundancy, and unrelated information. A total of 176 papers were included for abstract screening, but 120 were rejected as they did not meet the inclusion requirements. Out of the 56 papers that were included for full-text screening, 24 research articles were chosen [13-36]. [Table/Fig-2] displays the PRISMA selection criteria for publications.

Variables	Inclusion criteria	Exclusion criteria
Type of study	Scoping reviews, systematic reviews, narrative reviews, meta-analysis, commentaries, editorials, correspondence, letter to editor, reports	Cross-sectional, books, conference abstracts
Publication status	Peer reviewed articles	Grey literature, Preprints and other non peer reviewed articles
Text availability	Full text	Unavailable full texts
Language	English	Any other
Disease modelling	Human	Animal

[Table/Fig-1]: Eligibility criteria.



[Table/Fig-2]: PRISMA flowchart for identification and selection of articles.

Data Charting

All the selected publications were screened and scrutinised before plotting the data for additional analysis. Each article was thoroughly examined in the initial round of examination. The findings from the second phase of the study were cross-checked to resolve any differences in data extraction or graphing.

Collating, Reporting and Summarising the Findings

Every article was assigned a study Identity Document (ID), which was used to represent the studies in the results. Each publication was reviewed for title, abstract, and full text. In the initial stage of analysis, each paper was assessed and then validated with the findings following the second round of review. Any discrepancies in the data extraction in the first round were cross-checked in the second. The information is categorised as follows: Study ID, year of study, study design, country, keywords, ethical issues, disease under study, and conclusion or recommendations. Extracted data is presented using graphs, charts, and figures.

RESULTS

Data Extraction and Graphing

The results of the rigorous data extraction and graphing process are presented in [Table/Fig-3,4] [13-36].

Study ID	Name of the author	Year of study	Country of study	Study design	Keywords used
1 [13]	Xu Y and Li Z	2020	China	Review	CRISPR-Cas9, Genome editing, Human disease models, Rabbit, Gene therapy, Off-target effects
2 [14]	Ayanoğlu FB et al.,	2020	Turkey	Review	Genome editing, CRISPR-Cas9 technology, bioethical issues, bioethics
3 [15]	Martinez-Lage M, et al.,	2017	Spain	Review	CRISPR Cas 9applications, activation, repression, disease model, genome engineering, gene editing
4 [16]	Kang XJ et al.,	2017	China	Commentary	Not mentioned
5 [17]	Janet R	2018	Canada	Review	CRISPR Cas 9, ethics, gene editing, pluripotent stem cell
6 [18]	Shinwari ZK et al.,	2018	Pakistan	Review	Gene editing technology, CRISPR Cas 9, ethics
7 [19]	Cribbs AP and Perera SMW	2017	UK	Review	CRISPR, Cas9, genome editing, bioethics
8 [20]	Xu M	2020	USA	Review	CRISPR, Cas9, genome editing, Biomedical technologies, In-vitro Fertilisation (IVF)
9 [21]	Caplan AL et al.,	2015	USA	Report	Not mentioned
10 [22]	Carolyn B and Mazhar A	2019	USA	Review	Human genome editing, genomic engineering, CRISPR Cas 9
11 [23]	Megan M and Christopher G	2018	Australia	Opinion paper	Not mentioned
12 [24]	Piergentili R et al.,	2021	Italy	Review	CRISPR-Cas; germline genome editing; human embryo; bioethics; biosecurity
13 [25]	Guttinger S	2017	UK	Review	CRISPR Cas 9, recombinant DNA technology, ethics, genome editing, human germline cells
14 [26]	Foulkes AL et al.,	2020	UK	Review	CRISPR Cas 9, psychiatry, ethical, legal issues
15 [27]	Motta BM et al.,	2017	Italy	Review	CRISPR Cas 9, ethics, gene editing, disease modelling
16 [28]	Brokowski C, et al.,	2015	USA	Review	Bioengineering, bioethics, gene editing, genomic engineering, germline editing, medical ethics, research ethics, research involving human subjects
17 [29]	Vermersch E et al.,	2020	France	Review	Genome, editing, CRISPR/Cas9, genetics, cardiomyopathy, disease modelling
18 [30]	Kim EJ et al.,	2017	Korea	Review	Clustered regularly interspaced short palindromic repeats-Cas9, Gene editing, Induced pluripotent stem cells, Genetic therapy
19 [31]	de Lecuona I et al.,	2017	Spain	Review	Gene editing, ethics, CRISPR, responsible research
20 [32]	Kotagama OW et al.,	2019	Sri Lanka	Review	Genomic medical, human diseases, ethics, CRISPR
21 [33]	Yang W et al.,	2016	China	Review	Genetic diseases, CRISPR Cas 9, disease modelling, neurodegenerative diseases
22 [34]	Lanphier E et al.,	2015	USA	Commentary	Not mentioned
23 [35]	Razzouk S	2018	USA	Review	Gene editing, ethics, CRISPR Cas 9, precision medicine
24 [36]	Hung SSC et al.,	2016	USA	Review	Genome engineering, ophthalmology, clustered Regularly Interspaced Short Palindromic Repeat and associated protein (Cas) system, CRISPR Cas 9, ethical implications

[Table/Fig-3]: Extracted data on author, year, country, study design and keywords [13-36].

Study ID	Ethical issues in the use of CRISPR Cas 9	Disease under study	Recommendations
1 [13]	Safety	Monogenic diseases, Infectious diseases, cancer	The practical uses of CRISPR-Cas systems still have significant obstacles to overcome, and much work has to be done to assess their long-term usefulness and safety.
2 [14]	Unwanted alterations in the genetic material, source for obtaining informed consent, the breeding of humans and their impact on the environment, agriculture, and animals	Not mentioned	Global law should be drafted in order to ensure the safe use of CRISPR-Cas9 everywhere and to address any possible problems. It should take into consideration the views of social and life scientists, policymakers, and all other sector stakeholders.
3 [15]	Safety, misuse, privacy, consent	Not mentioned	In the near future, the application of CRISPR/Cas9-based genome engineering technologies will boost the authors comprehension of disease progression and management, even if many ethical dilemmas still need to be resolved.
4 [16]	Misuse, concerns about designer babies, unnatural features	Not mentioned	Clear guidelines must be put in place as advice to distinguish between the morally right and socially acceptable uses of gene editing and their abuse in order to prevent the misuse of CRISPR/Cas9 technology.
5 [17]	Safety, confidentiality, misuse, against nature	Not mentioned	Gene editing as a methodological approach is progressing in embryos of humans, other primates, and embryoids that are derived from stem cells, despite technical and concerns about safety suggesting that this strategy still stands far from clinical use.
6 [18]	Safety, harm to environment, consent	Not mentioned	Although there are many legal and ethical barriers to CRISPR genome modification, the potential applications cannot be disregarded. The genetic basis of disorders may now be better understood thanks to CRISPR, which also opens up new avenues for therapeutic development and other research uses.
7 [19]	Safety, informed consent	Not mentioned	The cost and yield of genomic research have decreased because of genome editing techniques, notably those involving CRISPR-Cas9. Yet, there are some significant ethical and moral problems with the way the technology is delivered, the development of new human population varieties, and the possibility for unanticipated harmful effects.
8 [20]	Safety, fidelity, future health, acceptance	Not mentioned	Numerous ethical and moral queries surrounding CRISPR-Cas9 technique that need to be addressed with a clear ethical framework since they might have unintended consequences for gene editing.
9 [21]	Safety, misuse, bioterrorism, unintended consequences	Not mentioned	Applications of CRISPR Cas 9 tool to alter human somatic and germ line cells present unique regulatory and ethical challenges. Additionally, CRISPR might be used for malevolent intents like bio war and bioterrorism. Owing to CRISPR Cas 9 being simple and effective, there is a risk that anyone with the appropriate tools could create an harmful species or a flu virus that is immune to vaccines.

10 [22]	Safety, informed consent, unintended consequences	Not mentioned	These cutting-edge tools like CRISPR Cas 9 have countless potential advantages. But there are risks involved with it that raise ethical questions. Thus explicit regulatory frameworks are essential to warrant advancement in human health and science with least harmful consequences.
11 [23]	Safety, acceptance	Not mentioned	The requirement for a workable rationale to support the application is a common thread running through all of the aforementioned ethical concerns. Therefore, clear regulatory frameworks are necessary to ensure advancement in human health and knowledge while causing the fewest negative effects and upholding all ethical principles.
12 [24]	Safety, risk-benefit ratio, environmental harm	Not mentioned	Genome editing is certain to provide difficult ethical dilemmas, just like all possible revamping technologies that appear prepared to question the fundamental beliefs ingrained in communities. In the face of incredibly difficult conditions, it is necessary to address ethical difficulties and aspects that appear to be incompatible through fair legal and regulatory procedures designed to safeguard the rights of all concerned and also to support public health.
13 [25]	Safety, uncertainty, trust, unintended consequences	Not mentioned	The success of genome editing depends on whether researchers and decision-makers are headed in the correct direction, as this direction and its likelihood of success are the key factors that should help to maintain consumer confidence in technology.
14 [26]	Safety, risk-benefit ratio, stigma, vulnerability	Psychiatric disorders	Trials using CRISPR in the field of psychology may have an impact on future generations. Scientists must think about ethical implications of perhaps removing genes linked to mental disorders from the genetic makeup of future generations.
15 [27]	Safety, trust, unintended consequences	Cardiovascular diseases	New cellular and human models of cardiovascular illnesses are being created in the cardiovascular area using genome editing technologies. The therapeutic benefits of gene editing is yet to be free from biological and technological obstacles, but most crucially, it is constrained by ethical concerns regarding the application of CRISPR technology to people.
16 [28]	Unjust eugenic future, contrary to nature	Not mentioned	The system could be unable to realise concrete therapeutic effects that seem feasible ex ante if testing on human subjects is made more widely available. Participants might also be exposed to significant risk and potentially detrimental outcomes. The inherent ambiguity of CRISPR usage should not result in reflexive, preventative restrictions, but rather in morally rigorous and carefully monitored exploration.
17 [29]	Safety, off-target effects, unfavourable outcomes, acceptability	Cardiovascular diseases	Although germline gene alteration appears to be highly efficient and practical, it could also raise moral concerns, particularly when it is suggested for use in human embryos. Even if further research is needed to align the fundamentals of CRISPR/Cas9 genome editing more better, with cardiovascular specifications, the field of gene editing has been strikingly characterised by a rapid technical advancement that makes possible what was before unthinkable.
18 [30]	Safety. Efficacy, unfavourable outcomes	Not mentioned	The CRISPR-Cas9 toolbox offers a number of benefits and can be utilised to treat a variety of systemic or congenital abnormalities, including as cystic fibrosis and Huntington's disease. It's still not apparent how to draw lines defining which human characteristics may be edited, though. Few would contest the fact that CRISPR-Cas9 still raises a number of safety and effectiveness questions.
19 [31]	Safety, unfavourable outcomes, informed consent	Not mentioned	There is a consensus that CRISPR has both advantages and disadvantages and that further study is necessary before applying it because of the growing interest in the biological evolution of CRISPR and its societal influence. Regarding the ethical and legal context in which this study should be conducted and the situations in which this approach should be used, there are differing views.
20 [32]	Safety, off-target effects, efficacy, acceptability	Monogenic disorders, Huntington's disease, thalassemia, Duchene's muscular dystrophy, sickle cell anemia, , cystic fibrosis, haemophilia A, chronic granulomatous disease, cancer, diabetes, cardiology	The rising worry over altering the genetic makeup of humans must be taken into account for any discussion of the ethical implications to be complete. With more number of scientists firmly thinking that mankind is not yet ready to experiment with embryos of humans, latest advancements indicate that a substantial thought has to be made in this regard.
21 [33]	Safety, unintended consequences, efficacy	Neurodegenerative disorders	Using CRISPR Cas 9 to treat human diseases is also important, despite the fact that it has been largely employed in creating several cellular or animal models of human diseases. However, there is a dire need to consider the ethical and legal aspects associated for full-fledged implementation.
22 [34]	Safety, off-target effects, acceptability	HIV/AIDS, haemophilia, sickle-cell anaemia and cancer	A potent method of treating numerous human diseases, such as HIV/AIDS, bleeding disorders, haemoglobinopathies, and certain cancers, may be made possible by genome-editing technology. However, there are serious issues with the ethical and security ramifications of this study. There is also concern about the detrimental effects it could have on crucial research involving the application of genome-editing methods in somatic cells.
23 [35]	Safety, off-target effects, informed consent	Genetic disorders	The rapid advancement of genome editing has had significant positive effects on both scientific and applied research. The ethical constraints for somatic cell gene editing are less stringent and are more widely recognised in the scientific community. In contrast, there is much debate on germline editing. As a result, significant efforts are being made to develop ethical standards and laws to address these problems. To oversee the implementation of the system and reduce its misuse, there is an urgent need for global and international regulations by governmental entities.
24 [36]	Safety, consent, unintentional mutations	Eye diseases	It is vital to set clear criteria and comprehend how the general public views the technology's uses as it moves from preclinical laboratory research to a main medicinal solution.

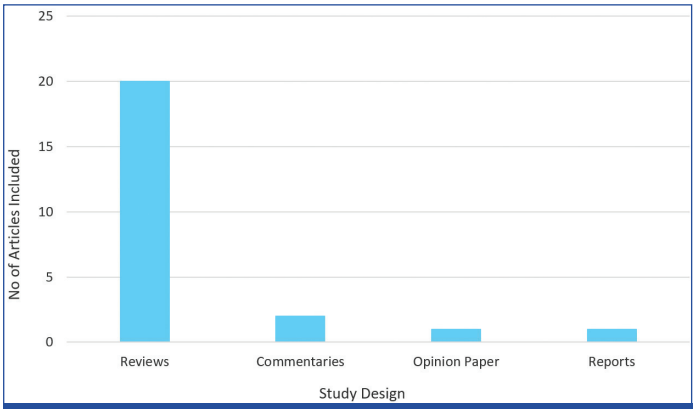
[Table/Fig-4]: Extracted data on ethical issues, disease and recommendations [13-36].

Characteristics of Charted Data

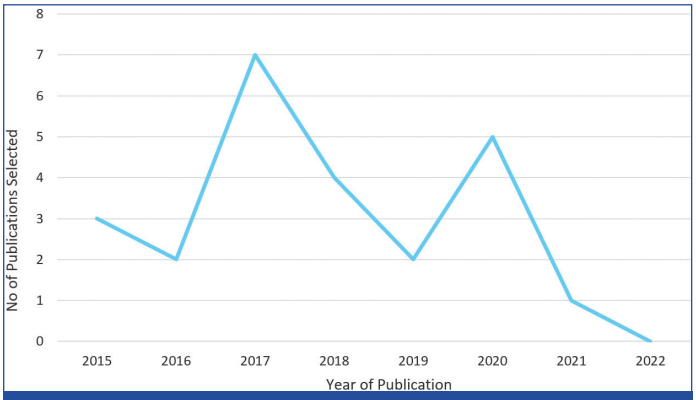
The studies included in the present scoping review provide a broad outline of the current state of research and ethical considerations related to CRISPR-Cas9 and gene editing. They cover various aspects such as human disease models, bioethics, responsible research, and precision medicine [Table/Fig-3] lists 24 studies on

the topic of CRISPR-Cas9 and gene editing, conducted in different countries and published in different years.

Out of these 24 studies, there are 14 reviews, three commentaries, one report, one opinion paper, and five studies without specified study designs. The study design is presented diagrammatically in [Table/Fig-5]. In terms of the year of study, there were three studies published in 2015, two in 2016, seven in 2017, four in 2018, two in 2019, five in 2020, and one in 2021. This distribution is represented in [Table/Fig-6].

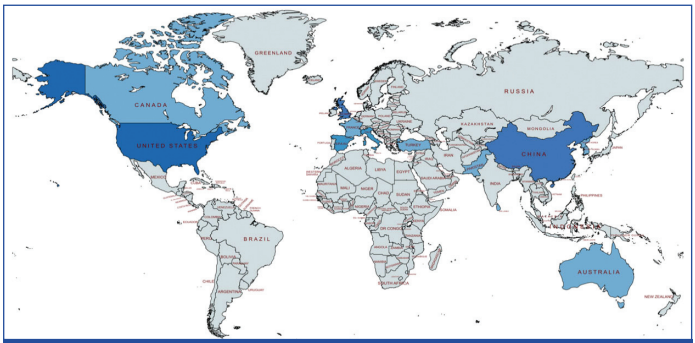


[Table/Fig-5]: Study design of selected articles.



[Table/Fig-6]: Year of study of selected articles.

The countries where the studies were conducted include China [13,16,33], Turkey [14], Spain [15,31], Canada [17], Pakistan [18], the UK [19,25,26], the USA [20-22,28,34-36], Australia [23], Italy [24,27], France [29], Korea [30], and Sri Lanka [32]. The highest number of studies came from the USA (n=7) [20-22,28,34-36], followed by three each from China [13,16,33] and the UK [19,25,26]. The geographical distribution of the selected articles is depicted in [Table/Fig-7].



[Table/Fig-7]: Geographical distribution of selected articles-Darker shades of blue represent countries with more studies (USA, n=7), intermediate shade represents the next highest number, (China and UK with n=3 each) while lighter shades indicate fewer numbers (n=1).

Additionally, the most common keywords used in these studies include CRISPR-Cas9, gene editing, ethics, disease modelling, and genome engineering. [Table/Fig-4] depicts the list of ethical challenges concerning the use of CRISPR-Cas9, along with the diseases under study and the conclusions or recommendations. The table includes various ethical dilemmas and challenges that arise when using CRISPR-Cas9 technology in different disease contexts.

Safety is a major concern across all disease areas, with specific attention given to issues such as unwanted alterations in the genome, off-target effects, and unintended consequences. Informed consent is also highlighted as an important ethical consideration. Other issues include the potential for misuse, concerns about designer babies and unnatural features, harm to the environment, and the breeding of humans and their impact on animals and agriculture.

Some studies [16,20,23,25,27] address the issue of trust and the future implications of the technology, while others discuss the potential for unjust eugenic futures [21,28]. Specific disease areas addressed include monogenic diseases, infectious diseases, cancer, psychiatric disorders, cardiovascular diseases, neurodegenerative diseases, eye diseases, and genetic disorders. This suggests that while the potential applications of CRISPR-Cas9 are vast, there are significant ethical and moral issues that must be addressed.

The development of clear ethical frameworks, legal procedures, and regulatory guidelines is essential to ensure that advancements in human health and knowledge continue with minimal ethical concerns while upholding ethical principles. The use of CRISPR-Cas systems to alter genes has the potential to transform the way illnesses are treated and advance our knowledge of genetic diseases. However, to ensure the ethical and safe use of this technology, certain serious issues must be resolved.

To distinguish between ethically appropriate and socially acceptable uses of gene editing and to prevent its abuse, clear regulatory frameworks must be put in place. It is important to consider the probability of unforeseen negative consequences as well as the risk of abuse, such as for bioterrorism or biowarfare. Genome editing will only be successful if decision-makers and researchers move in the right direction and uphold ethical standards. The moral ramifications of altering the genetic composition of future generations to remove genes associated with mental problems must be considered in CRISPR psychology trials.

There is a consensus that CRISPR has benefits and drawbacks, and further research is required to prevent unethical practices. The circumstances under which this technique should be employed and the ethical and legal framework are yet to be fully discussed. The development of a therapeutic tool using CRISPR-Cas9 for treating human illnesses is essential, but the ethical and legal ramifications must be taken into account. Drafting international legislation to protect the safety of genome editing technology is vital to promote this tool as a therapeutic intervention.

DISCUSSION

The present scoping review has outlined the major issues concerning the use of CRISPR-Cas9 technology in the creation of human disease models. The majority of the studies reviewed were conducted in the USA [20-22,28,34-36], followed by three each from China [13,16,33] and the UK [19,25,26]. Safety, informed consent, and unintended harm are the major ethical issues that need to be considered when using CRISPR-Cas9 technology. However, CRISPR-Cas9 remains an invaluable tool in gene editing that should not be compromised due to the ethical and legal challenges associated with its use.

The rapid adoption of CRISPR-Cas9 reflects its usefulness, simplicity, and effectiveness. It has revolutionised biomedical sciences by enabling precise genome alterations in various cell types and organisms [37]. The CRISPR-Cas9 system holds great therapeutic promise for treating diseases with a known genetic cause, as well as for researching these diseases through the creation of human disease models [38]. CRISPR-Cas9 offers several advantages over other gene editing methods, allowing scientists to develop human

disease models using knockin, knock-out, insertion, or deletion mutation strategies for a wide range of conditions, including cancer, cardiovascular disease, Huntington's disease, cystic fibrosis, Duchenne muscular dystrophy, haematological disorders, and viral disorders. Continued advancements in CRISPR-Cas9 technology will facilitate the creation of important human disease models for the discovery of new drugs and gene therapies [39,40].

Employing CRISPR-Cas9 technology for creating human disease models poses several ethical challenges. The most prominent ethical dilemmas are safety and efficacy concerns, as these methods may not be as accurate as anticipated [41,42]. Another issue is the safety concern regarding off-target effects. Despite ongoing efforts to improve the accuracy of CRISPR-Cas9 technology, it has been found to have more off-target complications compared to other gene editing tools [43]. Therefore, it is crucial to advance the current understanding of genetic and epigenetic impacts so that it will be possible to identify and anticipate the phenotypic consequences of genetic editing. This will help prevent any negative effects and off-target issues associated with CRISPR-Cas9 [44].

Another ethical dilemma arises from the use of germ cells for disease modelling. The use of somatic or embryonal cells does not pose many ethical conflicts, as they are generally not inherited and any masked effects are not at risk of being exposed in subsequent generations [45]. However, the use of germ cells could potentially affect the off-spring, as the DNA changes have a higher chance of being inheritable. Some scientists argue that using human embryos for research is unethical, as it involves dealing with living entities that have personhood, the right to live, and dignity [46]. However, the "European Society of Human Reproduction and Embryology" (ESHRE) and the "European Society of Human Genetics" (ESHG) have no objections to using leftover or wasted embryos in research. They believe that the moral "status" of an embryo is lower than that of a foetus, who in turn has a lower moral "status" than a newborn or an adult [47].

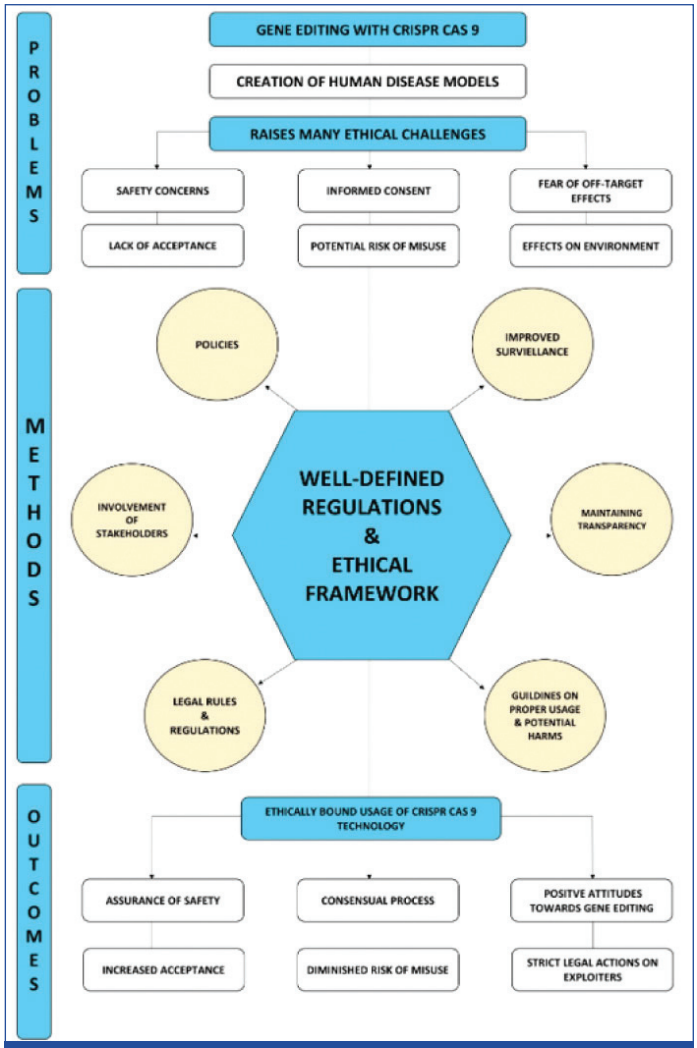
These arguments, particularly when utilising germ cells to create human disease models, expand the potential for disease elimination and treatment, thereby improving the health and well-being of humans in accordance with the United Nations Sustainable Development Goals [48]. However, this places a greater ethical burden on the use of CRISPR-Cas9 technology, despite its undeniable therapeutic potential for the treatment of various diseases and the construction of human disease models.

Conceptual Framework

The conceptual framework, which emphasises the need for a well-defined ethical framework for the usage of CRISPR-Cas9 in the creation of human disease models and highlights their advantages over conventional methods, is illustrated in [Table/Fig-8].

Problem: The use of CRISPR-Cas9 technology for editing the genome and creating human disease models has raised numerous ethical challenges, particularly regarding safety, off-target effects, and potential harm to the environment. Obtaining informed consent for the use of germ cells or other somatic cells in the gene editing process is also a major concern. Additionally, the acceptance of the process or its results may vary among individuals, as this technique is seen by many as contradicting nature. Moreover, the high reproducibility of this technology poses potential risks for misuse or exploitation of human disease models.

Methods: These ethical challenges can be addressed through the establishment of a well-defined ethical framework for the implementation of CRISPR-Cas9 in the creation of human disease models. This framework should be developed based on existing policies and laws that govern the ethical use of novel tools like CRISPR-Cas9. It should involve stakeholders and provide explicit guidelines on the appropriate use of the CRISPR-Cas9 technique, while ensuring that users are well-informed about the potential harms.



[Table/Fig-8]: A conceptual framework for ethical use of CRISPR-Cas9 technology.

The framework should also prioritise transparency and enhance surveillance of every procedure and its results, involving all stakeholders such as policymakers, funders, governmental or private organisations, and healthcare providers. By involving all relevant parties and implementing clear guidelines, this ethical framework can help ensure responsible and ethical use of CRISPR-Cas9 in the creation of human disease models.

Intended outcomes: With the implementation of such a framework, it will be possible to establish ethically and legally bound usage of CRISPR-Cas9 technology for creating human disease models. By following the guidelines provided in the framework, there will be a clear understanding of the process, potential results, and implications by both the public and the scientific community, leading to increased acceptance and consensus.

The availability of clear strategies for managing potential harm or off-target effects will help alleviate concerns about safety and other complications. Additionally, adherence to these guidelines can demonstrate that CRISPR-Cas9 poses minimal to no threat to the environment when used with caution.

Finally, with well-defined rules and regulations in place, the misuse or exploitation of this technology can be controlled by taking strict action against unlawful or unethical practices.

Directions for Future Research

The development of ethical guidelines and best practices, research into the long-term effects of genetic modifications, examination of

the social and cultural implications of CRISPR-Cas9 technology, investigation into the ethical ramifications of commercialisation, and enhancement of informed consent procedures should be the main objectives of future research concerning the ethical challenges in using CRISPR-Cas9 technology for creating human disease models. To ensure that these recommendations consider the viewpoints of all stakeholders, cooperation between researchers, physicians, patients, and ethicists is required.

Limitation(s)

The present scoping review has certain limitations. Firstly, authors did not assess the quality of individual studies included in this review, which makes it challenging to determine the strength of evidence and reliability of the findings. Additionally, the review employed a broad search strategy, and it is possible that not all relevant studies were captured.

CONCLUSION(S)

The creation of human disease models using CRISPR-Cas9 technology has raised profound ethical questions that require thorough exploration. While this technology holds the potential to revolutionise the treatment of genetic diseases, it also presents risks and challenges that need to be addressed. One of the primary ethical concerns with CRISPR-Cas9 technology is the possibility of unintended consequences, which could have negative long-term effects on individuals and their future generations. To address these ethical concerns, it is crucial to establish robust ethical standards and best practices for the use of CRISPR-Cas9 technology in developing human disease models. Additionally, funding should be allocated to continuous studies that examine the social and cultural implications of this technology and investigate the long-term consequences of genetic alterations.

REFERENCES

[1] Khalil AM. The genome editing revolution: A review. J Genet Eng Biotechnol. 2020;18:68. Doi: 10.1186/s43141-020-00078-y.
[2] Impact of Genetic Diseases. Accessed on 20.03.23. https://www.thegenehome.com/basics-of-genetics/disease-examples.
[3] New research estimates the overall disease burden of genetic risk factors. Accessed on 20.03.23. https://www.sciencedaily.com/releases/2022/09/220913110344.htm.
[4] Bak RO, Gomez-Ospina N, Porteus MH. Gene editing is on centre stage. Trends Genet. 2018;34(8):600-11.
[5] Jiang F, Doudna JA. CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys. 2017;46:505-29. Doi: 10.1146/annurev-biophys-062215-010822.
[6] Hsu PD, Lander ES, Zhang F. Development and Applications of CRISPR-Cas9 for Genome Engineering. Cell. 2014;157(6):1262-78. Doi: 10.1016/j.cell.2014.05.010.
[7] Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096. Doi: 10.1126/science.1258096.
[8] Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 2013;8:2281-308. Doi: 10.1038/nprot.2013.143.
[9] Dow LE. Modelling disease in vivo with CRISPR/Cas9. Trends Mol Med. 2015;21(10):609-21.
[10] Orlov S. Public Health Achievements and Ethical Dilemmas Associated with CRISPR-Cas9, A Gene Editing Technology. Undergrad J Public Health. 2021;5. Doi: 10.3998/ujph.17872072.0005.016.
[11] Arksey H, O'Malley L. Scoping studies: Towards a methodological framework. Int J Soci Res Methodol. 2005;8(1):19-32. Doi: 10.1080/1364557032000119616.
[12] Tricco AC, Lillie E, Zarin W, O'Brien KK, Colquhoun H, Levac D, et al. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and explanation. Ann Intern Med. 2018;169:467-73. Doi: 10.7326/M18-0850.
[13] Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J. 2020;18:2401-15. Doi: 10.1016/j.csbj.2020.08.031.
[14] Ayanoğlu FB, Elçin AE, Elçin YM. Bioethical issues in genome editing by CRISPR-Cas9 technology. Turk J Biol. 2020;44(2):110-20. Doi: 10.3906/biy-1912-52.
[15] Martinez-Lage M, Torres-Ruiz R, Rodriguez-Perales S. CRISPR/Cas9 Technology: Applications and Human Disease Modeling. Prog Mol Biol Transl Sci. 2017;152:23-48. Doi: 10.1016/bs.pmbts.2017.09.002.
[16] Kang XJ, Caparas CIN, Soh BS, Fan Y. Addressing challenges in the clinical applications associated with CRISPR/Cas9 technology and ethical questions to prevent its misuse. Protein Cell. 2017;8(10):791-95. Doi: 10.1007/s13238-017-0477-4.
[17] Janet R. Gene editing in human development: Ethical concerns and practical applications. Development. 2018;145(16):dev150888. Doi: 10.1242/dev.150888.
[18] Shinwari ZK, Tanveer F, Khalil AT. Ethical issues regarding CRISPR Mediated Genome Editing. Curr Issues Mol Biol. 2018;26(1):103-10. Doi: 10.21775/cimb.026.103.
[19] Cribbs AP, Perera SMW. Science and bioethics of CRISPR-Cas9 gene editing: An analysis towards separating facts and fiction. Yale J Biol Med. 2017;90(4):625-34. PMID: 29259526; PMCID: PMC5733851.
[20] Xu M. CCR5-Δ32 biology, gene editing, and warnings for the future of CRISPR-Cas9 as a human and humane gene editing tool. Cell Biosci. 2020;10:48. Doi: 10.1186/s13578-020-00410-6.
[21] Caplan AL, Parent B, Shen M, Plunkett C. No time to waste-the ethical challenges created by CRISPR. EMBO Rep. 2015;16(11):1421-26. Doi: 10.15252/embr.201541337.
[22] Carolyn B, Mazhar A. CRISPR Ethics: Moral considerations for applications of a powerful tool. J Mol Biol. 2018;431(1):88-101. Doi: 10.1016/j.jmb.2018.05.044.
[23] Megan M, Christopher G. Ethical issues in genetic modification and why application matters. Curr Opin Genet Dev. 2018;52:07-12. Doi: 10.1016/j.gde.2018.05.002.
[24] Piergentili R, Del Rio A, Signore F, Umani Ronchi F, Marinelli E, Zaami S. CRISPR-Cas and its wide-ranging applications: From human genome editing to environmental implications, technical limitations, hazards and bioethical issues. Cells. 2021;10(5):969. Doi: 10.3390/cells10050969.
[25] Guttinger S. Trust in science: CRISPR-Cas9 and the ban on human germline editing. Sci Eng Ethics. 2018;24:1077-96. Doi: 10.1007/s11948-017-9931-1.
[26] Foulkes AL, Soda T, Farrell M, Giusti-Rodríguez P, Lázaro-Muñoz G. Legal and ethical implications of CRISPR applications in psychiatry. North Carol Law Rev. 2018;97(5):1359-98. PMID: 31871365; PMCID: PMC6927684.
[27] Motta BM, Pramstaller PP, Hicks AA, Rossini A. The impact of CRISPR/Cas9 technology on cardiac research: From disease modelling to therapeutic approaches. Stem Cells Int. 2017;2017:8960236. Doi: 10.1155/2017/8960236.
[28] Brokowski C, Pollack M, Pollack R. Cutting eugenics out of CRISPR-Cas9. Ethics Biol Eng Med Int J. 2015;6(3-4):263-79.
[29] Vermersch E, Jouve C, Hulot JS. CRISPR/Cas9 gene-editing strategies in cardiovascular cells. Cardiovasc Res. 2020;116(5):894-907. Doi: 10.1093/cvr/cvz250.
[30] Kim EJ, Kang KH, Ju JH. CRISPR-Cas9: A promising tool for gene editing on induced pluripotent stem cells. Korean J Intern Med. 2017;32(1):42-61. Doi: 10.3904/kjim.2016.198.
[31] de Lecuona I, Casado M, Marfany G, Lopez Baroni M, Escarrabill M. Gene editing in humans: Towards a global and inclusive debate for responsible research. Yale J Biol Med. 2019;90(4):673-81. PMID: 29259532;
[32] Kotagama OW, Jayasinghe CD, Abeysinghe T. Era of genomic medicine: A narrative review on crispr technology as a potential therapeutic tool for human diseases. Bio Med Res Int. 2019;2019:1369682. Doi: 10.1155/2019/1369682.
[33] Yang W, Tu Z, Sun Q, Li XJ. CRISPR/Cas9: Implications for modeling and therapy of neurodegenerative diseases. Front Mol Neurosci. 2016;9:30. Doi: 10.3389/fnmol.2016.00030.
[34] Lanphier E, Urnov F, Haecker S, Werner M, Smolenski J. Don't edit the human germ line. Nature. 2015;519(7544):410-11. Doi: 10.1038/519410a.
[35] Razzouk S. CRISPR-Cas9: A Cornerstone for the evolution of precision medicine. Ann Hum Genet. 2021;82(6):331-57. Doi: 10.1111/ahg.12271.
[36] Hung SSC, McCaughey T, Swann O, Pébay A, Hewitt AW. Genome engineering in ophthalmology: Application of CRISPR/Cas to the treatment of eye disease. Prog Retin Eye Res. 2016;53:01-20. Doi: 10.1016/j.preteyeres.2016.05.001.
[37] Torres-Ruiz R, Rodriguez-Perales S. CRISPR-Cas9 technology: Applications and human disease modelling. Brief Funct Genomics. 2017;16(1):04-12. Doi: 10.1093/bfgp/elw025.
[38] Rodríguez Rodríguez DR, Ramírez Solís R, Garza Elizondo MA, Garza Rodríguez MD, Barrera Saldaña HA. Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases. Int J Mol Med. 2019;43(4):1559-74. Doi: 10.3892/ijmm.2019.4112.
[39] Zarei A, Razban V, Hosseini SE, Tabei SMB. Creating cell and animal models of human disease by genome editing using CRISPR/Cas9. J Gene Med. 2019;21(4):e3082. Doi: 10.1002/jgm.3082.
[40] Sharma G, Sharma AR, Bhattacharya M, Lee SS, Chakraborty C. CRISPR-Cas9: A preclinical and clinical perspective for the treatment of human diseases. Mol Ther. 2021;29(2):571-86. Doi: 10.1016/j.ymthe.2020.09.028.
[41] Gostimskaya I. CRISPR-Cas9: A history of its discovery and ethical considerations of its use in genome editing. Biochemistry Moscow. 2022;87(8):777-88. Doi: 10.1134/S0006297922080090.
[42] Rath J. Safety and security risks of CRISPR/Cas9. Ethics dumping: Case studies from north-south research collaborations. 2018:107-13.
[43] Doench J, Fusi N, Sullender M, Hegde M, Vaimberg EW, Donovan KF, et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nat Biotechnol. 2016;34(2):184-91. Doi: 10.1038/nbt.3437.
[44] Tauxe W. 4 big questions. Nature. 2015;528(7580):S17-S17. Doi: 10.1038/528S17aReturn to ref 15 in the article.
[45] Lorenzo D, Esquerda M, Palau F, Cambra FJ, Grup Investigació en Bioética. Ethics and genomic editing using the Crispr-Cas9 technique: Challenges and conflicts. Nanoethics. 2022;16:313-21. Doi: 10.1007/s11569-022-00425-y.
[46] Plaza A, Lanner F. Towards a CRISPR view of early human development: Applications, limitations and ethical concerns of genome editing in human embryos. Development. 2017;144(1):03-07. Doi: 10.1242/dev.139683.

[47] de Wert G, Pennings G, Clarke A, Eichenlaub-Ritter U, van El CG, Forzano F, et al. Human germline gene editing: Recommendations of ESHG and ESHRE. Eur J Hum Genet. 2018;26(4):445-49. Doi: 10.1038/s41431-017-0076-0.

[48] United Nations. The 2030 agenda and the sustainable development goals: An opportunity for Latin America and the Caribbean. United Nations Publication; Santiago, Chile: 2018. LC/G.2681-P/Rev.3.

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