MEGA JOURNAL OF CASE REPORTS

OPEN ACCESS

Review Article

Compiled Date: September 09, 2024

To What Extent Will Genome Editing Become an Available Cure for Genetic Disease?

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Abstract

Genome editing has many potential applications for the treatment of genetic disorders. Following a review of the literature, an overview of genome editing technologies, ZFNs, TAL-ENs and CRISPR-Cas9, is presented. The use of CRISPR-Cas9 systems to develop treatments for hemoglobinopathies, including sickle cell, is outlined as well as the potential for the technology to target more diseases such as muscle dystrophy. This type of treatment and the ethical considerations that come with introducing genome-altering technologies for clinical use are explored in this article. A judgment is then drawn that it is likely genome editing will be an available cure for genetic diseases, however, restrictions and regulations against the misuse of this valuable remedy must be put in place to ensure the conservation of the human genome.

Keywords: Genome editing; CRISPR; Ethics; Sickle cell disease; Duchenne muscular dystrophy

1. Introduction

The genetic code in any given organism is exposed to many modifications which are repaired by naturally occurring mechanisms. Gene editing is the process of targeting and modifying a selected gene to induce these repair mechanisms to effectively rewrite the genetic code. With the turn of the century, the progress of the relatively modern field of genomics, aided by technological advancements, allowed research to test the viability of genome editing in a medical setting to cure genetic diseases. Although successful models have proved the potential of genome editing as a curative treatment for otherwise lingering and persistent illnesses [1,2], a plethora of technical issues that may put patient safety at risk present significant hurdles in the adoption of this family of remedies as a clinically available route of treatment. Aside from these concerns, other research stipulates social, legal and ethical dilemmas that may arise from the adoption of genome editing tools in a clinical setting [3].

1.1. A Note on Terminology

Before beginning to evaluate the question posed in the title, the language of discourse within this topic must be addressed to minimise ambiguity and exemplify hidden subtexts that lie in idioms commonly used to describe altering DNA. For example, phrases such as 'gene-editing' conjure a depiction of precise alterations in the 'genetic alphabet', almost ignoring the multitude of various inaccuracies with the process; these range from the lack of control over epigenetic factors, imprecise changes in genes and unintended effects of editing a gene. Dismissal of these prominent issues though this euphemistic language in the common vernacular discomfort's researchers from the Nuffield Bioethics committee **[4]** who preferred the phrase 'genome editing' as a suitable alternative as it holistically encompasses changes made to DNA on a molecular level to alter characteristics of an organism. The diction used also allows for the description of alterations in introns which would not qualify under the title of 'gene-editing'; this is because introns are defined as gaps in the gene as they do not code for proteins. For these reasons, this dissertation uses the phrasing of genome editing where applicable to avoid misinterpretation.

1.2. Advancing Bio-technologies

Current comprehension of genome editing is on an upward trajectory as cheaper, easier and more efficient technologies are being discovered and researched. The recent rapid advancements in bio-chemistry have led to, as explored in a review by Gaj et al. **[5]**, the development of families of molecules utilised in genome editing; Transcription Activator-Like Effector Nucleases (TALEN), Zinc Finger Nucleases (ZFN) and CRISPR/Cas systems. At first, research **[6]** focused on ZFNs and improving their efficiency, however, after the discovery of the applications of CRISPR Cas proteins in genome editing by research **[7,8]**, the scientific effort shifted towards utilising these potent systems. Major breakthroughs with CRISPR Cas systems were achieved, for example, by the discovery of single guide RNA (sgRNA) molecules, which can be synthesised cheaply and easily in the lab or *in vitro* from a DNA template, and their role in forming effector complexes with Cas proteins **[9]**. This highlights the benefits of CRISPR Cas genome editing mechanisms compared to ZFNs and TALENs as both of these require complex arrays of either zinc fingers or TALEs to be arranged and engineered, which provides a significant cost hurdle **[5]**. Moreover, sgRNA engineering has been advanced to be more beneficial to genome editing, by reducing the number of off-target edits for example, by research over the years **[10]**. With regards to efficiency, CRISPR prevails as its derivatives were found by Cui et al. **[11]** to be more specific, have fewer off-target edits and are generally safer than TALENs or ZFNs. In brief, the progression of the microbiological toolkit for genome editing is ongoing and advancements are rampant in the field which in turn enhances the prospects of genome editing **(Figure 1)**.

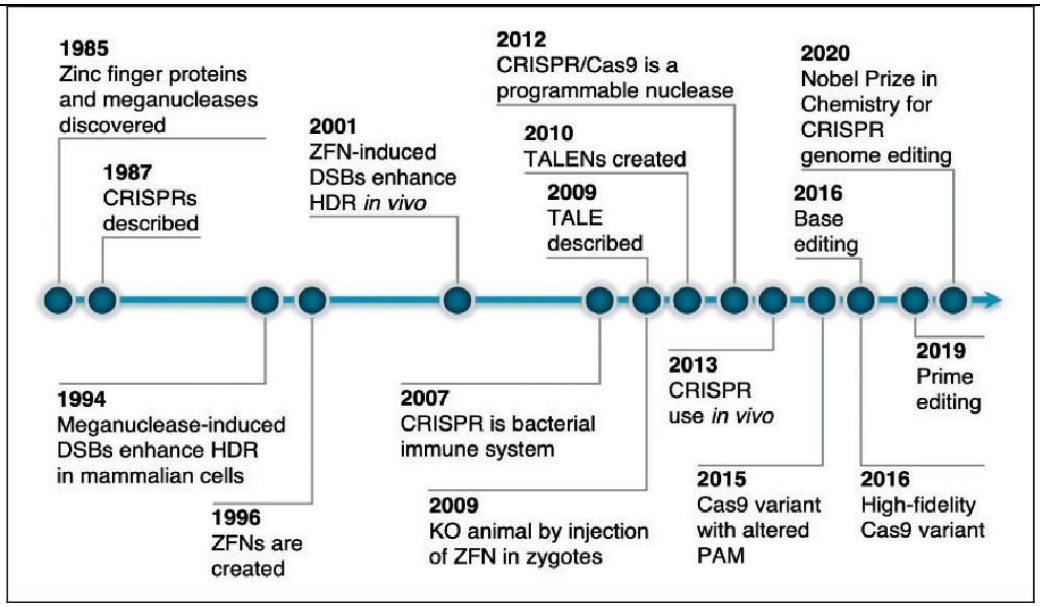

Figure 1: Timeline of genome editing technologies by Troder and Zevnik **[12]**.

1.3. Genome Editing in the Clinic

Although applications of scientific leaps, with regards to genome editing, range widely, this dissertation focuses on the medical prospects and the ethical and legal implications of such use of this technology in the clinical setting. The double effect philosophy, as depicted by medical practitioners **[13]**, is used to evaluate the permissibility of treatments that may adversely affect patients. For genome editing to be adopted as a clinically viable treatment option, issues with genome editing techniques must be addressed to minimise harm, thus adhering to the medical pillar of non-maleficence as described by medical ethicists **[14]**. Prominent hurdles of genome editing highlighted by research **[15]** range from off-target mutagenesis, large deletions and unintended effects. Despite this, research into genome editing as curative treatment for various genetic diseases, as explored by Dobner et al. **[16]** in their review, provides promising results; notable studies have been made into the use of genome editing in the treatment and diagnosis of cancer **[17]**, Duchenne Muscle Dystrophy (DMD) **[18]**, Sickle Cell Disease (SCD) or Beta Thalassemia (BT) **[19]**, Wilson's disease (WD) **[2]** and many more genetic disorders.

1.4. Genome Editing in Society

While harbouring potential as a curative remedy for previously uncurable diseases, genome editing critics highlight the socioeconomic divides such technologies would cause to arise in society. Effectively, it is argued, genetic disease will be eradicated among upper echelons of society whereas less financially able individuals might suffer. The aforementioned ethical pillars of medicine would deem this immoral as justice is broken; it must be noted, however, that research **[20]** has shown the cost-effectiveness of genome editing therapies. Another issue posed by genome editing is the social effect of providing such treatments, the concern of many is that as genomics advances those with genetic defects feel unnatural or ostracised by society **[4]**. Moral and ethical dilemmas arise when the discussion of Human Germline Genome Editing (hGGE) is brought up.

2. Review of Literature

A myriad of sources exist on genome editing for medical and therapeutic purposes, and

attempts at raising, resolving or diffusing pending moral evaluations due to the emergence of such technologies are abundant in contemporary research. Various online sources were cited, ranging from articles on Google Scholar to YouTube videos. Search terms included "Gene-editing", "CRISPR-Cas9", "ethics of gene editing" and "challenges of gene editing". Sources were then separated into one of two distinct categories; the clinical research on the use of genome editing therapies for the amelioration of human health, which contrasts sources discussing regulations, ethical evaluations and any proposed socioeconomic implications of putting such treatments to use. These groups of sources are used in conjunction with one another, which allows for a more educated and nuanced evaluation of the question posed in the title of this dissertation.

As discussed by Rees et al. **[21]**, curative treatments for SCD in the past relied on implantation from a marrow stem cell donor; where this was not possible, patients usually underwent lifelong blood transfusions requiring regular visits to the hospital. Similar treatments exist for beta-thalassemia as explored by Galanello and Origa **[22]** in their report on the disease. Both of the above sources are parts of their respective journals which require extensive peer review to ensure the validity and accuracy of the information presented, further adding to the reliability of the data referenced in the discussion. When used together with sources on genome editing to treat these conditions, these reviews become vital tools that allow the evaluation of genome editing therapies against the pre-existing treatments of the diseases.

Relatively recently, Frangoul et al. **[19]** carried out a study aimed at treating SCD and TDT using genome editing. Although only being published less than four years ago, this study has been cited over a thousand times making it one of the core pieces of literature in the field of therapeutic genome editing. A short documentary by Video **[23]** was released which expresses many of the key aims and methodologies covered in the formerly mentioned paper **[19]**. Although it is a short video, it features qualified experts in this area of study such as Ambroise Wonkam, the director of genetics at John Hopkins School of Medicine and president of the African Society of Human Genetics, and Haydar Frangoul, one of the leading names in genome editing to treat SCD. This, coupled with the fact that the publisher is Nature, a renowned scientific journal, accentuates the credibility of both the video and the study. Not only this, but also this study laid out prospects for the implementation of a genome editing therapy called Casgevy which recently gained approval by both the FDA in America **[24]** and the MHRA in the UK **[25]**. The approval of the treatment by these recognised and credible national healthcare bodies further adds to the reliability of the conclusions made by these sources. All in all, the work of researchers on treating SCD is a critical point for genome editing therapies as it led to clinical trials across the world.

Another important case of disease which has the potential to be resolved by genome therapy is muscular dystrophy (MD), an affliction which the NHS **[26]** provides information. Founded in the after math of the Second World War, the NHS is the UK's national healthcare body. To spread and make available comprehensive care for patients throughout Britain, the NHS must provide accurate health communication to the public, increasing their reliability. The NHS provides daily healthcare interactions to an estimated 1.6 million patients which further adds to the validity of the statistics and details presented by this source. However, the limitation of the NHS is that it only provides information relevant to a British audience rather than a representation of global health. Another

source for information on MD, specifically a type of MD called Duchenne MD (DMD), is a paper by Duan et al. [27] from a certified journal which makes it a scientifically reliable source. These sources will complement each other to provide domestic and global figures on the disease as well as give insight into currently available treatment options for patients in the UK compared to elsewhere.

In their extensive report, Zhang et al. [17] covered the ways genome editing can be used to ameliorate DMD. The report draws on conclusions made by other research in mouse models [1,28] and *in vitro* studies [18,29,30] which were successful in alleviating or relieving muscular dystrophy. These sources are all published by respected journals which require thorough and rigorous peer-review before any article is published, thus deeming these sources credible. Not only this but also the authors of these studies are not isolated in that they often co-author each other's paper as these familiar names work in conjunction with one another and build on the work of previous literature. This is also because these are experts in their field who have many publications with an incredible number of citations to their name which further adds to the credibility of these studies as this fact entails that this research is at the heart of the academic literature surrounding the topic; for example, Chengzu Long has over six thousand citations under his name. To be able to accurately evaluate the use of genome editing in the clinic, it is essential to comprehend the speed of the developments made by researchers to produce more accurate genome editing technologies. Various distinct families of genome editing molecular tools are outlined by researchers in academia [5,16,31]. In these reviews, researchers describe the contemporary metaphoric toolbox for genome editing and give an idea of the resource's scientists have been able to discover and manipulate to alter the gene. However, CRISPR has taken the spotlight through research into curative therapies for diseases because of its advantages detailed by Hsu et al. [32]. This journalistic paper can be used to supplement the previous ones, almost refining the focus and narrowing it down to explore one possible family of technologies whilst acknowledging the existence of a wider scope of research into other genome editing tools. In addition to this, research [33] has been made into epigenetic editing: which could be thought of as editing which genes are expressed and which are repressed. Nonetheless, these promising results should be taken into consideration as there are a plethora of concerns to do with the implementation of genome editing. Researchers [15] have identified many potential errors with the process of genome editing which may pose a significant risk to patients undertaking treatments. Off-target editing, specifically, is an infamous limitation of genome editing outlined by Fu et al. [34] and Liang et al. [35]; these peer-reviewed journal articles are cited a total of over five thousand times and are at the heart of literature surrounding genome editing. Furthermore, another issue is the potential malignancy promotion by gene therapy in SCD patients identified by recent research [36].

At its heart, the argument posed requires a concise and coherent philosophical approach to either illustrating the merits in criticisms of the technology or dismissing weak arguments in opposition to the use of genome editing in the clinic. In two exhaustive reports made by Nuffield Bioethics [4,37], a multitude of social and ethical concerns about genome editing were highlighted. Having dedicated working parties consisting of the top British bioethicists, these studies give credible reflections on the attitudes, fears and expectations of genome editing. Although not part of an

academic journal, these reports were made by a respected council of bioethicists and their publication ignited conversation among researchers; this was especially apparent in the critical response to these reports by Gyngell et al. [38]. In another article [3], the focus was on the legal aspects rather than the ethics of genome editing, although both are heavily interlinked. However, the authors declared affiliations with the Centre for Synthetic Biology which may affect their views causing them to espouse and portray genome editing more positively; it could be argued, however, that this possible bias may be subdued by the article being a part of a reputable legal journal subject to exhaustive peer reviews before publication. A set of twins were born as a result of hGGE, carried out by He Jiankui, which sparked global controversy and condemnation from various scientists and organisations. Nonetheless, this event has been a landmark in the conversation about genome editing as explained by Greely [39]. This extensive article outlining the details of He's scandal and its implications on bioethics stems from the Journal of Law and the Biosciences, a prestigious journal which has thorough peer review standards upholding the credibility of this article. One of the many issues identified by bioethicists surrounding hGGE is the "non-identity problem" which was put into context with the case of the Chinese twins by Alonso and Savulescu [40]. This philosophical argument poses the valid point that, were it not for genome editing, the Chinese twins would not have been born, thus it would be in the best interest of Lulu and Nana, the twins, that the genome editing treatment occurred as this gave them a chance to exist rather than harming them: although their lives were risked as a result of this treatment. The paper also delves into various ways such arguments could be dismantled such as the counter that a "better world", as Alonso and Savulescu [40] describes, could have existed with different non-edited twins were it not for He's affair. In summary, there are a multitude of factors which play a significant role in the argument stated in the title. As a result, this interdisciplinary review of literature sets the scene for current contention and movement in research, both scientific and bioethical. Over time, the focus of the literature on genome editing shifted from the scientific aspects to bioethical considerations and legal restrictions as a reaction to events such as Jiankui's scandal. Recently, however, more organisations have adopted genome editing therapies and clinical trials are due to be complete soon. It must be noted that this field has quickly progressed during the past decades and is projected to continue at this rate, prompting the consideration of contemporary discoveries when discussing the matter. At its current state, the literature on the state of genome editing has primarily been tackling issues which pose significant hurdles, such as off-target mutagenesis. This is in contrast to ethical literature on genome editing for therapeutic purposes, which remains a point of contention in research.

3. Discussion

3.1. Setting the Scene: The Toolbox

Scientists have harnessed an extensive arsenal of biological molecules and techniques to be able to deliberately alter genes. Fundamentally, many of these techniques rely on the action of nucleases at a specific site which stimulates the natural DNA repair mechanisms to alter DNA at the target site. Double strand breaks (DSB) in DNA caused by nucleases induce natural repair mechanisms such as Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR): the latter of these results in more predictable mutagenesis, provided a template, while the former tends to produce indels and other inaccuracies.

With these pathways, precise insertion and/or deletion of bases can be achieved by using both targeted nucleases as well as a vector containing DNA homologous to the break site to mediate HDR, as highlighted by research [41]. Having easily programmable binding domains allows zinc finger nucleases to act as suitable genetic engineering tools as detailed by research [6,42,43]. This is done by joining a nuclease with arranged and engineered zinc fingers which will only bind to a specific sequence of DNA. However, zinc fingers aligned in an array present unpredictable behaviour which complicates the design and modification of zinc finger proteins and reduces the precision of genetic modifications [31]. TALENs rely on TALE proteins from bacteria, which can each recognize a single nucleotide, fused with a FokI endonuclease as described by Szczesna [44] (Figure 2). TALENs proved advantageous to ZFNs as they were more easily produced to make precise changes in the DNA by linking TALE proteins.

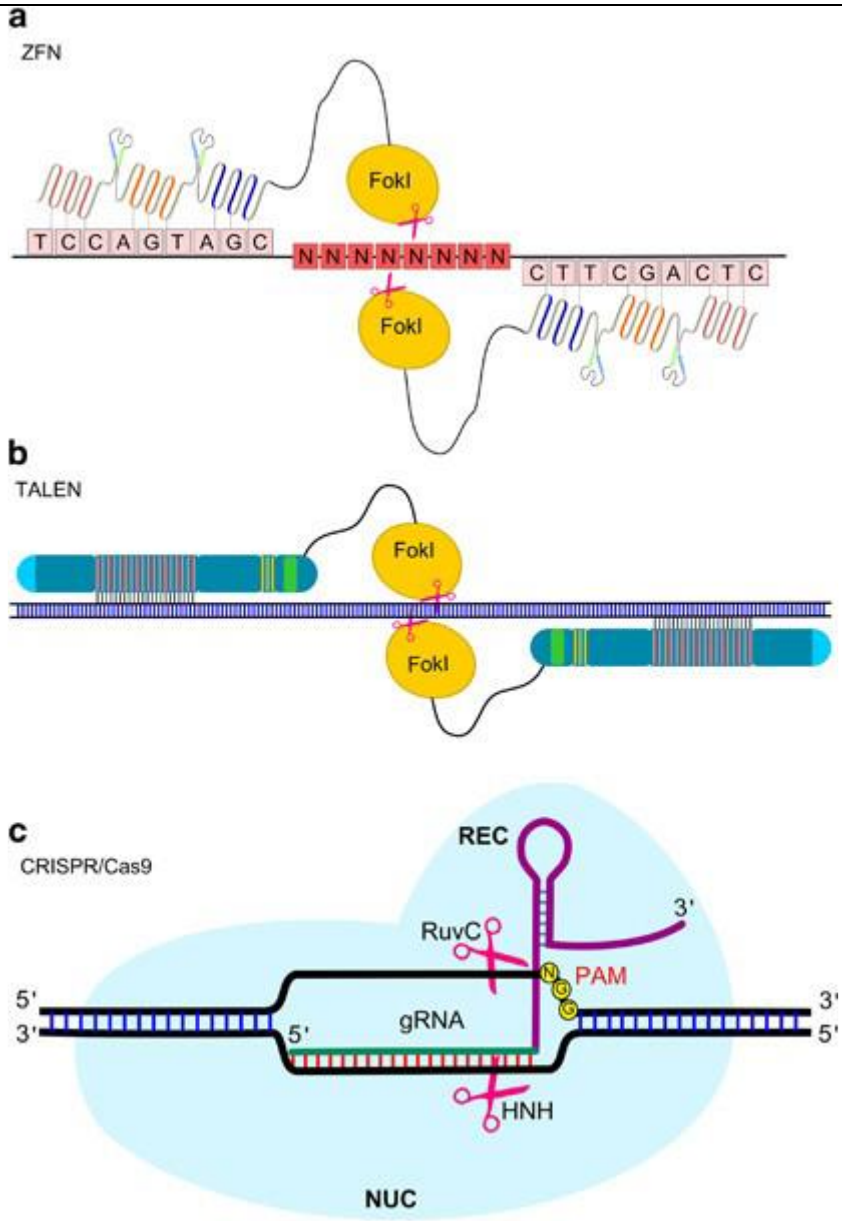

Figure 2: Outline of mechanisms of action of different genome editing tools [45].

A hurdle to the use of ZFNs and TALENs in genome editing, however, was the fact that the process of producing, building and testing the correct ZFNs and TALENs for each DNA sequence made their practical, widespread use difficult [46]. As important of a breakthrough as ZFNs and TALENs seemed at the time, new and more accurate tools for rewriting the gene were emerging in research; these advancements were made by observing the behaviour of bacteria.

3.2. Learning from prokaryotes

As it stands, the most potent gene-editing tool in the arsenal acquired and identified by research attempts to mimic mechanisms found in nature: by prokaryotes which have evolved over an astronomical number of generations to protect themselves from foreign genetic material. Clustered Repeating Interspaced Palindromic Repeats (CRISPR) in bacteria were first recognised as sequences repeating DNA with variable spacers embedded between which contained foreign DNA by Ishino et al. [47], the spacers were later identified to be viral DNA; the bacteria were keeping a history of their previous viral attacks. A Cas (CRISPR associated) protein can then form effector complexes such that if the cell encounters the viral DNA once again, the nuclease enzyme coordinates and causes its breakage, neutralising the virus [48]. The Cas protein, as discovered by Jinek et al. [7], could be manipulated to target other DNA sites by programming a single guide RNA molecule with a complementary sequence of bases to the one found on the target locus to initiate DSB and thus insertion or deletion of nucleotides. CRISPR provides many advantages compared to other genome editing technologies such as those seen in **Figure 3**. Additional advancements have been made by research to ameliorate the process of CRISPR genome editing as well as other methods. For example, the discovery of genome-wide, unbiased identification of DSBs enabled by sequencing (GUIDE-seq) enabled researchers to more easily screen and detect off-target mutagenesis Tsai et al. [49]. This demonstrates the trajectory of the field as genome editing technologies are being improved by the day.

	ZFN	*TALEN*	*CRISPR/Cas9*
Construction	Protein engineering for every single target	Protein engineering for every single target	20-Nucleotide sequence of sgRNA
Targeting	Protein–DNA interaction, less predictable	Protein–DNA interaction, less predictable	DNA–RNA interactions, highly predictable
Delivery	Two ZFNs around the target sequence are required	Two TALENs around the target sequence are required	sgRNA complementary to the target sequence with Cas9 protein
Multiplexing	Challenging	Challenging	Highly feasible
Feasibility of library construction and transformation for genome-wide screens	Technically challenging	Technically challenging	Highly feasible
Affordability	Resource intensive and time consuming	Affordable but time consuming	Highly affordable

Abbreviations: CRISPR/Cas9, clustered regularly interspaced palindromic repeats/CRISPR-associated-9; sgRNA, single-guide RNA; TALEN, transcription activator-like effector nuclease; ZFN, zinc-finger nuclease.

Figure 3: Comparison of genome editing platforms Eid and Mahfouz [45].

3.3. Disease and Genome editing

Curative applications of CRISPR genome editing systems were immediately visible and foreseeable, initiating a wave of research into novel approaches to treating diseases [46]. Genome editing allows for transplants to be made from the patient themselves, thus overcoming the challenge of finding a close match donor who presents similar Human Leukocyte Antigens (HLA). This is particularly useful to treat hematopoietic diseases due to the high supply of Hematopoietic Stem and Progenitor Cell (HSPC) found in bone marrow. As a result, the reason that the first genome editing treatments to be approved by national health organisations are therapeutic curatives to blood disorders becomes evidently clear [24,25].

3.3.1. Sickle cell disease: Sickle Cell Disease (SCD) is a family of disorders which affect the shape of Haemoglobin (Hb), causing malformed erythrocyte and reticulocyte structure [21]. More specifically, when the structure of the subunit β of Haemoglobin (HBB) is altered due to a single nucleotide alteration in the gene encoding it, known as a point mutation, the resultant mutant HBB protein contains a substituted amino acid causing the sickle Hb (HbS) allele to be presented [50]. Amount of Foetal Haemoglobin (HbF), the predominant form of Hb in a human foetus as seen in **Figure 4**, demonstrates correlation with decreased severity of symptoms and effects of SCD in patients as outlined in a landmark study by Platt OS [51]. Changes in the shape of red blood cells lead to complications such as capillary blockages, which are the reason behind pain episodes experienced by SCD patients, and an overall low supply of oxygen to the body's cells, inducing anaemia, fatigue and drowsiness and other symptoms as detailed by the NHS [52]. Current treatments for SCD include hydroxycarbamide which enhances the expression of the HbF allele, however not every patient responds to this treatment, frequent healthy erythrocyte transfusions to replace the sickle-shaped red blood cells made by the patient and haematopoietic stem cell transplantation from a close donor. Downsides with these treatments are evident, such as the impracticality of requiring multiple hospital visits for blood transfusions or the plethora of complications transplantation has on patients: requires lifelong immunosuppression and must find a closely matched donor who presents similar HLA which may be difficult for certain people. According to Gragert et al. [53], around 16% of North and South Americans of African descent have an optimal HSPC donor, a disparity that a genome editing curative can resolve.

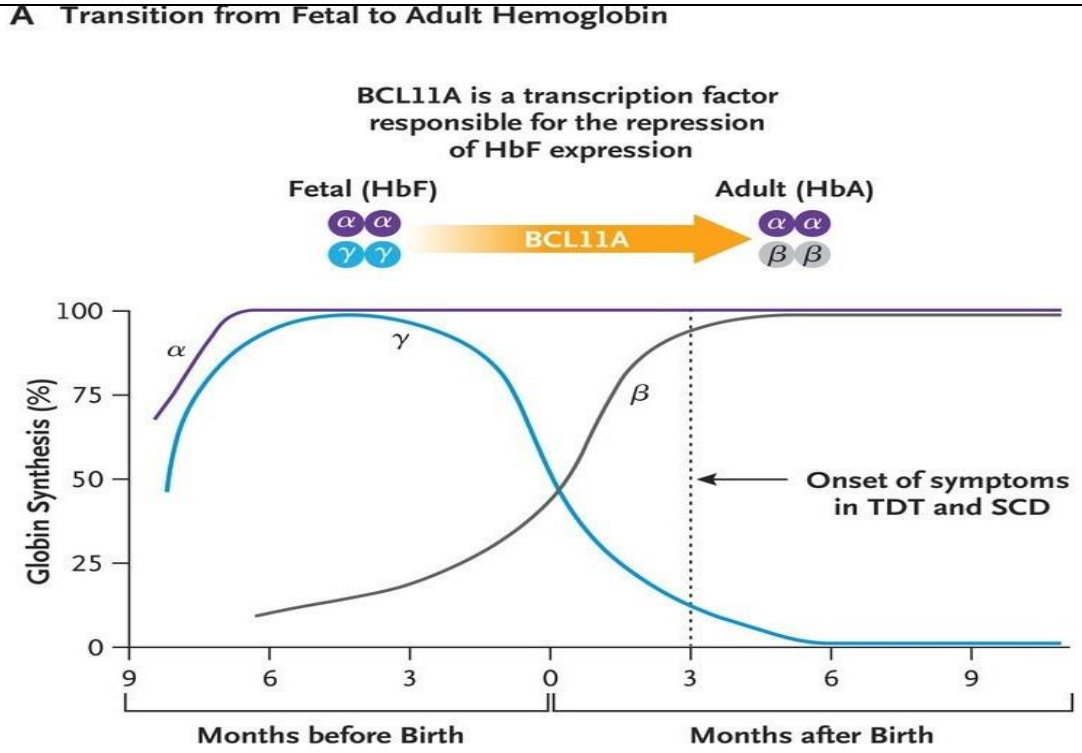

Figure 4: Foetal haemoglobin (HbF) levels falling soon after birth and being replaced by adult haemoglobin (HbA) **[19]**.

3.3.2. Beta-Thalassemia: Another disease that bears much resemblance to SCD is Beta-Thalassemia (BT), although it is rarer and often more severe. In this family of diseases, HBB is partially or fully deficient resulting in free alpha globin (HBA) chains in red blood cells which causes hemolysis, either in bone marrow or extra vascularly, leading to jaundice and hypoxia as well as other symptoms **[54]**. Production of HbF reduces the effects of BT because HBA binds to gamma globin subunits to constitute HbF, thus reducing the number of free HBA chains in red blood cells. Given that BT is an autosomal recessive phenotype, the severity of symptoms ranges depending on the alleles the patient has; the combinations of the alleles β+ and β0 determine whether the patient has BT minor, intermediate or major. Treatments for BT are similar to those of SCD; regular blood transfusions, stem cell transplantation from a close donor and hydroxycarbamide all have benefits for BT patients, however the same downsides are present as for SCD patients **[22]**.

The ability of HbF to reduce the severity of symptoms while also being naturally in the human genome prompted research to find a way to enhance the expression of the HbF allele in HSPC to increase HbF levels and therefore treat many of the chronic symptoms of BT and SCD: such as anaemia and the pain crises in SCD patients. The transcription factor which represses HbF and promotes HbA is found in the gene BCL11A **[55]**. Research has found, using GUIDE-seq profiling, that CRISPR-Cas9 mediated genome editing to find that around four out of five of the alleles at the BCL11A locus were successfully modified with no evidence of off-target mutagenesis **[19]**. This study followed two patients, one suffering from BT and the other from SCD, for a year following gene therapy treatment with promising results which illustrated restored HbF production and, for the patient with SCD, the termination of pain episodes.

This treatment, under the market name Casgevy, was also recently approved by national healthcare organisations, the Federal Drug Agency (FDA) and the Medicines and Healthcare products Regulatory Agency (MHRA), as a treatment for these diseases [24,25].

3.3.3. Duchenne muscle dystrophy: A third example of a genetic disease which genome editing has been proven to treat is MD. In this family of diseases, the protein dystrophin is affected by mutagenesis, either not produced at all, causing Duchenne muscle dystrophy, or being produced in an abnormal structure, leading to other types of MD such as Becker MD [57]. Responsible for stabilising the bridge between intracellular, cytoskeletal actin to the extracellular matrix, dystrophin is a structural protein that plays a key role in holding in place the sarcolemma and preventing them from wilting [57]. Wilting of the sarcolemma causes the cell to lose vital chemicals such as kinases and allows for the entrance of toxic substances like calcium ions into the cell, leading to cell death and, in the long term, muscle atrophy as intuitively presented in a video by from Elsevier [54] on MD. As it stands, current medical treatment offers no remedy for muscular dystrophy which reverses the underlying processes causing the disease, instead patients can receive physical therapy and conditioning to improve quality of life; this however does not address the core issue of patients. Alternatively, glucocorticoids can slow down atrophy and degradation of muscle tissue, however, this has side effects such as excessive weight gain and again does not address the lack of healthy dystrophin in muscle cells (Figure 5).

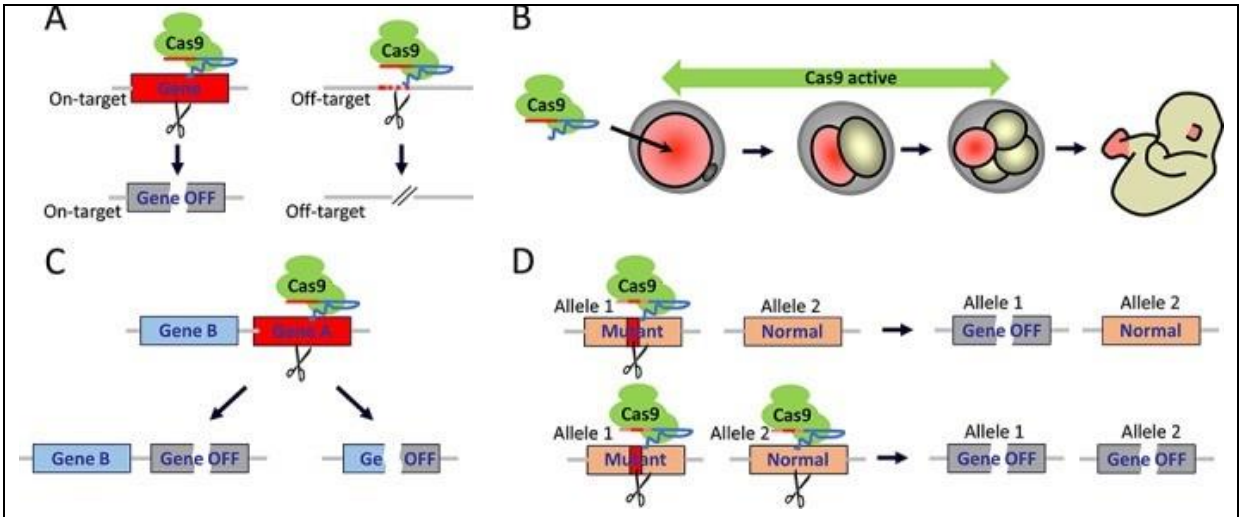

Figure 5: Technical difficulties of CRISPR-Cas9 genome editing. A The Cas9 complexes to the intended gene site in the left panel as well as off-target edits simultaneously occurring as seen on the right side B Activity of CRISPR-Cas9 within an embryo can cause mosaicism which describes different mutations in different parts of the same individual C Large deletions which cause neighbouring genes to be affected as well as the intended edit site D On-sight damage can occur if the Cas9 protein cannot discriminate between the normal and mutant alleles and acts on both [15].

Having an atypically large gene, dystrophin is coded by 2.4 million base pairs with 79 exons which makes it more susceptible to mutagenesis with deletions spanning exons being the most common mutations [58,59]. Research has been made into the restoration of dystrophin production by muscle in DMD murine models by CRISPR-Cas9 editing with results showing 2-100% expression of the edited allele and with exceeding phenotypic muscle rescue [1,28]. Other techniques have been successfully adopted by scientists to mediate genome editing's of the dystrophin gene, such as base editing and prime editing [18]. Results for genome editing to treat DMD are promising, however, issues arise when it comes to the long-term stability of the muscles and response to injuries.

3.4. The Drawbacks

Having stated the positive effects of genome editing treatments, there are a multitude of criticisms concerning its efficacy and adverse effects. On a molecular, short-term level, all genome editing tools share the risk of inducing off-target mutagenesis at closely matched sequences which may result in unintended consequences [34]. A recent study by Chapman et al. [36] exalted evidence that HSPC genome therapy, used to treat SCD and BT, increases the risk of cancer. This displays another disadvantage of genome editing: the uncertainty of long-term effects, whether that be to treat DMD, SCD, BT or any other genetic disease. Increased oncogenesis and other long-term risks associated with CRISPR-Cas9 genome editing have also been outlined by research [60,61]. This is because if the CRISPR-Cas9 system inadvertently inserts a gene or fragment into a region that activates an oncogene, it could increase the risk of cancer. Moreover, an abundance of additional issues is prominent such as unintended large deletions, mosaicism and on-sight damage as a result of unintended mutagenesis where the nuclease is unable to differentiate between the mutated and normal allele [15].

Mosaicism is when the same person has cells with different genotypes. When germline cells or zygotes are exposed to CRISPR-Cas9 systems, perpetual targeting of genes at different stages of embryonic growth occurs which leads to mosaicism [62,63]. This poses a significant challenge to the acceptance of human germline genome editing; the risk to the future embryo coupled with the ethical concerns with hGGE and the fact that alterations in embryos can make their way into the human genome has cast a well-founded stigma on the use of genome editing on germline cells or embryos. However, research [64] stipulates strategies to reduce mosaicism such as speeding up the genome editing process and shortening the lifespan of CRISPR-Cas9 systems *in vivo*. More complex methods include relying on new biochemical advancements such as Easi-CRISPR (Efficient additions with single strand DNA inserts-CRISPR) and C-CRISPR (CRISPR-Cas complexes which rely on multiple sgRNA molecules) which can increase the efficiency of genome editing, thus reducing mosaicism [65,66].

3.5. The Chinese Twins

During a conference on genomics in November 2018, Dr He Jiankui disclosed that a set of twins were born and that he had edited their embryos. The twins were edited to not produce CCR5 which is a protein T-cells present and is thought to be required to contract AIDS; however, research has stipulated that this claim may not be accurate [39]. A scientific wave of condemnation immediately followed Dr He's announcement due to the dangers associated with his methods as well as the lack of necessity and the concerns about the potential implications of his work on the girls. In addition, changing the genome of an embryo causes

issues in that alterations will be carried down generations and into the human gene pool, where the full consequences are not yet fully understood. However, Dr He's affair was a crucial milestone in the progression of regulations on genome editing, which had been lagging behind the rapid advancements in academia; effectively, the scandal was a wake-up call for regulators to determine whether the future of genome editing will be guided by beneficence or malice. Current reports about the health status of the gene-edited twins, Lulu and Nana, indicate they are doing well. However, their privacy is being upheld and not all information is public [67]. There remain concerns regarding the effects of unintended modifications and their long-term effects. Dr He was released from prison in 2022 after serving his sentence for genetically altering the twins without authorisation [68]. He has expressed regret in undertaking the project and vowed to follow up on the health of the twins. Overall, although the twins, fortunately, seem to be in good health, there may be unforeseen impacts because of Dr He's experiment.

3.6. Medical Ethics

Physicians are regularly faced with a plethora of moral dilemmas and so have built a rigid system of ethical rules underpinning many clinical decisions and it is based upon four pillars: non-maleficence, beneficence, autonomy and justice. Genome editing allows for the prevention of a multitude of diseases but may have unintended consequences, this could be argued to be permissible due to the double effect ideology which states that a treatment with adverse effects may be acceptable if the effects are unintended and provide more benefit than harm [69]; this is the ethical thinking which allowed cancer therapies to enter the clinic. The shift from more sudden deaths to a gradual decline in the health of an ageing population as a consequence of modern medicine has also had a significant impact on the image of medicine in society. This often slow and almost torturous process is a result of long-term chronic illnesses, such as SCD and DMD, which in themselves do not cause death but debilitate health. Genome editing allows for increased life expectancy as seen with the cases of SCD, BT and, especially, DMD; this would suggest that medical professionals must strive to support introducing genome editing into the clinic. However, hGGE may not have as straightforward of an answer as genome therapy due to the nuances and complex nature of the human condition. It is unknown what cascading effects it may or may not have on the human genome.

3.7. Ethical Opinions and Schools of Thought

Human Germline Genome Editing (hGGE) prompts poignant and emotive thoughts in many due to its divisiveness; this could be attributed to fear of new technology, the stigma surrounding similar subjects such as GMOs and, most importantly, the fear of the valid adverse effects of this potent biotechnology on both an individual and a societal level. As discussed previously, mosaicism is an apparent risk of hGGE as well as having a permanent mark on the human genome (see section 3.4). Aside from the technical risks associated with hGGE, there are many socioeconomic concerns as well. Society may view hGGE as a way to alter the genes of offspring, for example, to evade predisposition to diseases. However, if this practice were to be adopted on a wide scale, it may, some researchers claim [38], lend its way to eugenics; a society that handpicks genetic characteristics for its offspring ought to believe that those who conserve their "defective" genome are inferior, researchers argue. Not only this but it was also stipulated by research [4] that in a society where hGGE is normalised, those with diseases may feel ostracised by society. An

emblematic case of this is the societal pressure on prospective parents of a child with a chromosomal abnormality, such as Down syndrome, to terminate their pregnancy; it is not the choice of the parents to screen or terminate their pregnancy which is criticised but rather the societal pressure for the action to be taken is the issue. However, it is also equally true that genome editing allows amelioration of health in many cases and, given appropriate regulations, can build a healthy society. A hypothetical society where hGGE is available publicly would raise concerns about social justice; the demographics of those affected by genome editing would be vastly different. For example, philosophers criticise hGGE due to the social divide it would cause as those in the upper echelons of society have available expensive genetic treatments whereas the rest of society may suffer [37]. This disparity exists in today's world, where third-world countries suffer from diseases that have almost been eradicated from the Western world, however, hGGE is argued to exacerbate this divide. A sensible solution to this would be restricting access to genome editing treatments to only the most vulnerable groups.

Bioconservatism is a movement which seeks to maintain the natural order and rejects the imposition of technologies onto natural processes. A common example of this point of view is the rejection of GMOs by some which has had a spillover effect onto genome therapy and treatments. This illustrates the split between scientists and the broader public, of whom some may believe genome editing and other such concepts demonstrate the overstepping of science beyond its capabilities and scientists' infiltration of systems beyond their understanding where potential consequences are dire. Another ideological movement is welfarism, which is the belief that utility should be measured following what brings the most well-being to society. According to this school of thought, hGGE could be considered to be ethical it would be thought of as a moral obligation to maximise wellbeing [4].

4. Conclusion

Genome editing is a potent tool for the amelioration of health and its potential to treat and cure disease must be exploited by research. However, this must be achieved through careful regulation and under strict rules since errors in this field can cause fatal consequences, the detriments of which are not yet known and are unpredictable. One would hope that future generations look back at SCD, BT, DMD and other diseases like how contemporary society views smallpox; a relic of the past that no longer poses a threat to global health. The recent advances by curative research and clinical trials are exemplifications of the leaps research has allowed us to take and the lagging regulations are catching up, as shown by the approval of Casgevy by MHRA [25] and Incorporated [24]. He Jiankui pre-emptively disrupted the world of genome editing which could be argued to have had positive effects since this highlighted the need for and escalated the ethical, moral and legal evaluations needed for genome editing to become more accessible. With regards to the technical issues of genome editing, it is important to note that knowledge and research are ameliorating by the day and the current state of genome editing is the worst it will ever be as new methodologies are being developed by researchers. In an email correspondence with Frangoul [70], he expressed his optimism towards the overcoming of these issues by researchers stating the following:

[Currently] there is interest in curative therapies for patients who have no options. The field is progressing and I see others overcoming some of the issues in gene-editing [70]. Frangoul's remarks

are supported by promising escalations in the field made by Ma et al. [71] who were able to use a novel method to modify genes with minimal off-target mutagenesis occurring; this, however, proved difficult to reproduce with other heterozygous mutations. More recently, researchers [72] were able to increase the frequency of HDR, this ensures the alterations made in genes are more precise. This accentuates the need for research to better engineer biochemical tools that allow genome editing to become a safe and viable treatment option. It is evident that as the use of the previously mentioned treatments progresses, avenues for research will open up. For example, focus can shift increasing efficiency of genome editing to reduce off-target edits or other consequences. Patients undertaking the curative treatment must be followed-up in long-term studies to explore possible effects of genome editing. Simultaneously, progress may be made toward developing treatments for other families of genetic disorders. This would require a more proficient understanding of the genome and more extensive studies on the roles of human genes, especially those which lead to monogenic diseases. In conclusion, it is not improbable that genome editing will make its way into the clinic in the decades to come as a cure for genetic diseases. As time progresses, so too will the knowledge of genome editing leading to the development of cures for a greater variety of genetic disorders. Advancements in today's research pave the way for lifesaving treatments that may be introduced in the future. On the other hand, hGGE will likely face a multitude of ethical and legal challenges, and public condemnation and may remain restricted by legislation due to the risks it presents to both its recipients and the society as a whole.

Acknowledgement

It is without a doubt that much gratitude is due to Dr Amal Aly, MD., PhD., FACMG, for her indispensable support throughout the review process. Without her guidance, the process would have been much more difficult. I am also grateful to Dr Ahmed Shaheen who has given valuable feedback critical for refining writing and structure of the review.

Appendix

Glossary

beta-thalassemia a disease caused by deformation in the shape of haemoglobin in erythrocytes. 5, 8

Cas CRISPR-associated proteins that can be manipulated to act on human genes. 4, 7, 10

CRISPR Clustered Regularly Interspaced Short Palindromic Repeats are sequences of DNA found in many prokaryotes which allow for a primitive immune response against viral attacks. 4, 7, 8

Duchenne muscle dystrophy a disease caused by the loss of production of the protein dystrophin which leads to paralysis, heart failure and death. 9

foetal haemoglobin haemoglobin present at birth which stops being produced after around 4 months. 8, 9

hematopoietic stem and progenitor cell stem cells found in the bone marrow which can differentiate into all the blood cell types. 8

human germline genome editing the use of genome editing tools on germline or embryonic cells. 10, 11

human leukocyte antigens a system of genes which produce proteins on the surface of cell membranes to allow the immune system to identify between domestic and foreign cells. 8

non-homologous end joining a method of DNA repair which does not rely on the presence of undamaged DNA, but ends up deleting a section of DNA. 6

sarcolemma cell surface membrane of muscles. 9

sickle cell disease a family of illnesses which are caused by a malformation in haemoglobin causing red blood cells to be shaped like sickles. 8

single guide RNA an engineered RNA molecule which is used to stimulate Cas9 action on the intended DNA site. 4, 7

transcription activator-like effector nucleases similar to ZFN, however, does not require complex arrays of protein. 4

zinc finger nucleases a nuclease enzyme which can be engineered in arrays to attack specific genes and DNA sites and induce DNA repair. 4, 6

References

1. C Long, L. Amoasii, M. A. A, M. J. R, H. Li, E. Sanchez-Ortiz, S. Bhattacharyya, S. J. M, R. Bassel-Duby, and O. E. N. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science. 2016;351(6271):400-3.
2. Murillo, D. M. Luqui, C. Gazquez, D. Martinez-Espartosa, I. Navarro-Blasco, J. I. Monreal, et al. Long- term metabolic correction of wilson's disease in a murine model by gene therapy. J Hepatol. 2016;64(2):419-426.
3. Nordberg, T. Minssen, S. Holm, M. Horst, K. Mortensen, and B. L. Møller. Cutting edges and weaving threads in the gene editing (r)evolution: Reconciling scientific progress with legal, ethical, and social concerns. J Law Biosci. 2018;5(1):35-83.
4. Greenfield, T. Perry, C. Watson, D. Lawrence, C. Thompson, J. Dupre, et al. Genome editing: an ethical review. 2016.
5. Gaj, C. A. Gersbach, and C. F. Barbas. Zfn, talen, and crispr/cas-based methods for genome engineering. Trends Biotechnol. 2013;31(7):397-405.
6. M. Bibikova. Enhancing gene targeting with designed zinc finger nucleases. Science. 2003;300(5620):764.
7. M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, D. J. A, and E. Charpentier. A programmable dual-rna-guided dna endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-21.
8. M. Jinek, A. East, A. Cheng, S. Lin, E. Ma, and J. Doudna. Rna-programmed genome editing in human cells. Elife. 2013:2:e00471.
9. A. Doudna and E. Charpentier. The new frontier of genome engineering with crispr-cas9. Science. 2014;346(6213):1258096.
10. Cui, J. Xu, M. Cheng, X. Liao, and S. Peng. Review of crispr/cas9 sgrna design tools. Interdiscip Sci. 2018;10(2):455-465.
11. Cui, H. Liu, H. Zhang, Z. Huang, R. Tian, L. Li, W. et al. The comparison of zfns, talens, and spcas9 by guide-seq in hpv-targeted gene therapy. Molecular Therapy. Mol Ther Nucleic Acids. 2021:26:1466-1478.
12. E. Tro¨der and B. Zevnik. History of genome editing: From meganucleases to crispr. Lab Anim. 2022;56(1):60-68.
13. McIntyre. Doctrine of double effect (stanford encyclopedia of philosophy), 2018.
14. Varkey B. Principles of clinical ethics and their application to practice. Med Princ Pract. 2021;30(1):17-28.
15. Davies B. The technical risks of human gene editing. Hum Reprod. 2019;34(11):2104-2111.

16. Dobner, H. Ramachandran, and A. Rossi. Genome editing in translational medicine: an inventory. Front Biosci (Landmark Ed). 2022;27(8):241.
17. Zhang, C. Qin, C. An, X. Zheng, S. Wen, W. Chen, X. et al. Application of the crispr/cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Mol Cancer. 2021;20(1):126.
18. Chemello, C. A. C, H. Li, C. Rodriguez-Caycedo, E. Sanchez-Ortiz, A. Atmanli, M. A. A, N. Liu, R. Bassel-Duby, and O. E. N. Precise correction of duchenne muscular dystrophy exon deletion mutations by base and prime editing. Sci Adv. 2021;7(18):eabg4910.
19. Frangoul, D. Altshuler, C. M. Domenica, Y.-S. Chen, J. Domm, B. K. Eustace, J. et al. Crispr-cas9 gene editing for sickle cell disease and beta thalassemia. N Engl J Med. 2021;384(3):252-60.
20. Salcedo, J. Bulovic, and C. M. Young. Cost-effectiveness of a hypothetical cell or gene therapy cure for sickle cell disease. Sci Rep. 2021;11(1):10838.
21. Rees, T. Williams, and M. Gladwin. Lancet. 2010;376(9757):2018-31.
22. Galanello and R. Origa. Beta-thalassemia. Orphanet J Rare Dis. 2010:5:11.
23. Video. Can crispr cure sickle-cell disease? 2021.
24. P. Incorporated. Vertex and crispr therapeutics announce us fda approval of casgevy™ (exagamglogene autotemcel) for the treatment of sickle cell disease. 2023.
25. MHRA. Mhra authorises world-first gene therapy that aims to cure sickle-cell disease and transfusion-dependent beta – thalassemia. 2023.
26. NHS. Overview-muscular dystrophy. 2021.
27. Duan, N. Goemans, S. Takeda, E. Mercuri, and A. Aartsma-Rus. Duchenne muscular dystrophy. Nat Rev Dis Primers. 2021;7(1):13.
28. Long, M. J. R, S. J. M, M. A. A, R. Bassel-Duby, and O. E. N. Prevention of muscular dystrophy in mice by crispr/cas9-mediated editing of germline dna. Science. 2014;345(6201):1184-1188.
29. Maggio, J. Liu, J. M. Janssen, X. Chen, and M. Adenoviral vectors encoding crispr/cas9 multiplexes rescue dystrophin synthesis in unselected populations of dmd muscle cells. Sci Rep. 2016:6:37051.
30. Maggio, L. Stefanucci, J. M. Janssen, J. Liu, X. Chen, V. Mouly, et al. Selection-free gene repair after adenoviral vector transduction of designer nucleases: Rescue of dystrophin synthesis in dmd muscle cell populations. Nucleic Acids Res. 2016;44(3):1449-70.
31. L. Maeder, S. Thibodeau-Beganny, A. Osiak, D. A. Wright, R. M. Anthony, M. Eichtinger, et al. Rapid open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell. 2008;31(2):294-301.
32. D. Hsu, E. S. Lander, and F. Zhang. Development and applications of crispr-cas9 for genome engineering. Cell. 2014;157(6):1262-1278.
33. I. Thakore, J. B. Black, I. B. Hilton, and C. A. Gersbach. Editing the epigenome: technologies for programmable transcription and epigenetic modulation. Nat Methods. 2016;13(2):127-37.

34. Fu, J. A. Foden, C. Khayter, M. L. Maeder, D. Reyon, J. J. Keith, and J. D. Sander. High-frequency off-target mutagenesis induced by crispr-cas nucleases in human cells. Nat Biotechnol. 2013;31(9):822-6.
35. Liang, Y. Xu, X. Zhang, C. Ding, R. Huang, Z. Zhang, J. et al. Crispr/cas9-mediated gene editing in human tripronuclear zygotes. Protein Cell. 2015;6(5):363-372.
36. Chapman, A. H. Cull, M. F. Ciuculescu, E. B. Esrick, E. Mitchell, H. Jung, et al. Clonal selection of hematopoietic stem cells after gene therapy for sickle cell disease. Nat Med. 2023;29(12):3175-3183.
37. Yeung, R. Ashcroft, N. Haites, J. Harper, J. Hitchcock, J. L. Scully, T. Perry, and C. Watson. Genome editing and human reproduction, 2018.
38. Gyngell, H. Bowman-Smart, and J. Savulescu. Moral reasons to edit the human genome: Picking up from the nuffield report. J Med Ethics. 2019;45(8):514-523.
39. HT. Greely. Crispr'd babies: Human germline genome editing in the 'he jiankui affair'. J Law Biosci. 2019;6(1):111-183.
40. M. Alonso and J. Savulescu. He jiankui´s gene-editing experiment and the non-identity problem. Bioethics. 2021;35(6):563-573.
41. L. Maeder and C. A. Gersbach. Genome-editing technologies for gene and cell therapy. Mol Ther. 2016;24(3):430-46.
42. M. Bibikova, D. Carroll, S. D. J, T. J. K, J. Smith, K. Y. G, and S. Chandrasegaran. Stimulation of homologous recombination through targeted cleavage by chimeric nucleases. Mol Cell Biol. 2001;21(1):289-97.
43. Bibikova, M. Golic, K. G. Golic, and D. Carroll. Targeted chromosomal cleavage and mutagenesis in drosophila using zinc-finger nucleases. Genetics. 2002;161(3):1169-75.
44. K. Szczesna. Crispr-cas9, talens and zfns-the battle in gene editing, 2023.
45. A. Eid and M. M. Mahfouz. Genome editing: the road of crispr/cas9 from bench to clinic. Exp Mol Med. 2016;48(10):e265.
46. R. Barrangou and J. A. Doudna. Applications of crispr technologies in research and beyond. Nat Biotechnol. 2016;34(9):933-941.
47. Y. Ishino, H. Shinagawa, K. Makino, M. Amemura, and A. Nakata. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in escherichia coli, and identification of the gene product. J Bacteriol. 1987;169(12):5429-33.
48. P. D. Explains. Crispr-cas9 genome editing technology, 2021.
49. S. Q. Tsai, Z. Zheng, N. T. Nguyen, M. Liebers, V. V. Topkar, V. Thapar, et al. Guide-seq enables genome-wide profiling of off-target cleavage by crispr-cas nucleases. Nat Biotechnol. 2015;33(2):187-197.
50. G. J. Kato, F. B. Piel, C. D. Reid, M. H. Gaston, K. Ohene-Frempong, L. Krishnamurti, et al. Sickle cell disease. Nat Rev Dis Primers. 2018:4:18010.
51. P. O. S, B. D. J, R. W. F, M. P. F, O. Castro, S. M. H, and K. P. P. Mortality in sickle cell disease. life ex- pectancy and

risk factors for early death. N Engl J Med. 1994;330(23):1639-44.
52. NHS. Symptoms - sickle cell disease, 2019.
53. L. Gragert, M. Eapen, E. Williams, J. Freeman, S. Spellman, R. Baitty, et al. Hla match likelihoods for hematopoietic stem-cell grafts in the u.s. registry. N Engl J Med. 2014;371(4):339-48.
54. O. from Elsevier. Beta-thalassemia - causes, symptoms, diagnosis, treatment, pathology, 2022.
55. J. Yin, X. Xie, Y. Ye, L. Wang, and F. Che. Bcl11a: a potential diagnostic biomarker and therapeutic target in human diseases. Biosci Rep. 2019;39(11):BSR20190604.
56. E. Mercuri and F. Muntoni. Muscular dystrophies. Lancet. 2019;394(10213):2025-2038.
57. C. Pasternak, S. Wong, and E. E. L. Mechanical function of dystrophin in muscle cells. J Cell Biol . 1995;128(3):355-61.
58. R. R. Bennett, J. den Dunnen, K. F. O'Brien, B. T. Darras, and L. M. Kunkel. Detection of mutations in the dys- trophin gene via automated dhplc screening and direct sequencing. BMC Genet. 2001:2:17.
59. Q. Q. Gao and E. M. McNally. The dystrophin complex: Structure, function, and implications for therapy. Compr Physiol. 2015;5(3):1223-39.
60. S. Sinha, K. Barbosa, K. Cheng, M. D. M. Leiserson, P. Jain, A. Deshpande, et al. A systematic genome-wide mapping of oncogenic mutation selection during crispr-cas9 genome editing. Nat Commun. 2021;12(1):6512.
61. M. K. White and K. Khalili. Crispr/cas9 and cancer targets: future possibilities and present challenges. Oncotarget. 2016;7(11):12305-17.
62. S. Mizuno, T. Huong, K. Kato, S. Mizuno-Iijima, Y. Tanimoto, Y. Daitoku, et al. Simple generation of albino c57bl/6j mice with g291t mutation in the tyrosinase gene by the crispr/cas9 system. Mamm Genome. 2014;25(7-8):327-34.
63. D. Oliver, S. Yuan, H. McSwiggin, and W. Yan. Pervasive genotypic mosaicism in founder mice derived from genome editing through pronuclear injection. PLoS One. 2015;10(6):e0129457.
64. M. Mehravar, A. Shirazi, M. Nazari, and M. Banan. Mosaicism in crispr/cas9-mediated genome editing. Dev Biol. 2019;445(2):156-162.
65. A. M. Jacobi, G. R. Rettig, R. Turk, M. A. Collingwood, S. A. Zeiner, R. M. Quadros, et al. Simplified crispr tools for efficient genome editing and streamlined protocols for their delivery into mammalian cells and mouse zygotes. Methods. 2017:121-122:16-28.
66. E. Zuo, Y.-J. Cai, K. Li, Y. Wei, B.-A. Wang, Y. Sun, et al. One-step generation of complete gene knockout mice and monkeys by crispr/cas9-mediated gene editing with multiple sgrnas. Cell Res . 2017;27(7):933-945.
67. F. Graham. Daily briefing: What happened to the 'crispr babies'? Nature. 2021.
68. F. Graham. Daily briefing: Crispr-baby scientist released from prison. Nature. 2022.
69. JM Boyle. Toward understanding the principle of double effect. Ethics. 1980;90:527–538.
70. H. Frangoul. The prospects of genome editing. 2023.

71. H. Ma, N. Marti-Gutierrez, S.-W. Park, J. Wu, Y. Lee, K. Suzuki, et al. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548(7668):413-419.
72. R. Baik, C. M. Kyle, S. E. Glenn, C. A. Vakulskas, K. O. Chmielewski, A. M. Dudek, et al. Transient inhibition of 53bp1 increases the frequency of targeted integration in human hematopoietic stem and progenitor cells. Nat Commun. 2024;15(1):111.

Citation of this Article

Yehia A. To What Extent Will Genome Editing Become an Available Cure for Genetic Disease? Mega J Case Rep. 2024;7(89):2001-2020.

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