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Mini Review

Assignment of responsibility for creating persons using germline genome-editing

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ABSTRACT

The 2018 announcement regarding safe childbirths via germline genome-editing (GGE) with parental consent shocked the world. This minireview examines the predictable risks, burdens, and potential harms of human GGE and explores the question of responsibility for using GGE in human reproduction. Although there is currently no international consensus on proving the absence of harmful off-target mutations in the genome, preclinical GGE study can demonstrate the non-existence under specific conditions. Initially, the clinical application will be limited to small studies without controls. In any case, individuals born via GGE should be followed up for long period. However, such persons can decline follow-up. Due to limited screening, an overlooked off-target mutation may harm the entire body. Some persons suffering such harm might claim damages on the ground that their life is less valuable. However, most jurisdictions will reject such claims. Practitioners are responsible for proving there are no harmful off-target mutations in each GGE case, although the appropriateness of proof is currently difficult to accept. Parents who consented to GGE, as well as practitioners, assume responsibility for the safety of genome-edited offspring; however, the fulfillment of responsibility ultimately depends on the offspring's autonomy. Meanwhile, practitioners and parents may be exempt from some damage claims by offspring harmed by unsafe GGE. The uncertainty of assigning responsibility may underpin GGE's prohibition in light of the unacceptable risks, burdens and potential harms for persons born via GGE; or it may oppositely underpin its permission if an acceptable risk-benefit balance is reached for parents and society.

1. Introduction

Since the 1960s, the potential of genetically modifying human germ cells (eggs and sperm cells) and one-cell stage embryos (termed germline), as well as somatic cells, has been suggested [1]. Older genetic modification techniques were often imprecise but feasible to some extent; however, those used in the germline can create persons whose cells are all genetically modified, and further the germline genetic modification becomes heritable via reproduction. Although human germline genetic modification could be acceptable for medical purposes, such as the prenatal prevention of genetic disease, it has been controversial surrounding the harm to and welfare for humans, changes to the nature of human reproduction and parent-child relationships. [2]. Subsequently, some countries legislated against human reproduction involving germline genetic modification [3]. On the other hand, in 2015, the UK permitted two types of germline mitochondrial DNA manipulation in order to prevent the maternal transmission of serious mitochondrial disease to offspring [4].

Genome-editing techniques facilitated far more precise and versatile gene modifications among them, clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 [5,6]. Microinjecting a designed Cas9 nuclease into the germline and then transferring genome-edited embryos into the uterus can produce animals with some beneficial traits [7]. In November 2018, a Chinese researcher announced the safe birth of twins via germline genome-editing (GGE) [8]. With parental consent, they applied CRISPR-Cas9 to the embryos for postnatal protection against HIV infection. However, their announcement shocked the world and drew stringent criticism for practicing unproven GGE for such a minor purpose, since HIV infection can be avoided by other means [9]. The current academic and policy-based positions regarding human GGE among various international organizations are divided: some statements do not permit human GGE 'currently', while others remain open to the clinical practice for other compelling reasons [10]. Notably, the latest report by the US National Academies/UK Royal Society in 2020 presented a clinical translational path for GGE under certain circumstances, assuming several criteria have been met [11]. Its first recommendation states the importance of achieving precise genome-editing without harmful off-target mutations in the embryos prior to clinical application. However, there is no risk or perfect safety in intervention in human subjects, which is the case with human GGE. If a more satisfactory and healthy childbirth in comparison with known and publicly available risks can be expected, some practitioners and prospective parents are likely to consider implementing human GGE [12–14]. In so doing, the

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ethical implications of the predictable risks and burdens of GGE upon genome- edited offspring should be carefully examined, as requested by the Declaration of Helsinki and others [14–21]. Moreover, the responsibility of parents, as well as practitioners, has to be explored, because the clinical implementation of GGE for human reproduction is consented to by prospective parents, not the genome-edited children [14–16,18,22].

The present minireview first examines the predictable risks and burdens of human GGE in the laboratory and clinic, while reviewing the risk assessment of the GGE in China. Then, we explore the sources of responsibility for to engage practitioners and participants in understanding the ethical and medical issues that surround this unproven technique. Those considerations underscore that the assignment of the entire responsibility for human GGE may be uncertain particularly in society. The uncertain assignment of responsibility is also discussed from a policy standpoint.

2. Risks, burdens, and potential harm of GGE

The GGE study in China intended to mutate the *CCR5* genes of resultant offspring to mimic a deletion mutation (Δ32) of the *CCR* gene that predisposes some people of European origin to resistance to HIV infection [8]. In December 2019, a court in Shenzhen found three researchers guilty of 'conducting illegal medical practices' [23]. Astonishingly, He Jiankui and two collaborators forged ethical review documents and misled practitioners into unknowingly implanting edited embryos into two women. The court also acknowledged that these two women who undergone the GGE gave birth to the twins and an infant, respectively. Although their research paper remains unpublished by any peer-reviewed journals, it is worth reexamining their risk assessment of GGE because 3 genome-edited children were born into the world.

2.1. GGE study in laboratory

According to the presentation in Hong Kong and the reported excerpts of their unpublished paper [8,24], He Jiankui and his collaborators first investigated the conditions for 'safely' mutate the *CCR5* genes in preclinical research. They then created 7 single-guide (sg) RNAs, optimized the microinjection conditions, and identified highly specific sgRNA that edited human embryos with a high efficiency and low mosaicism. Then, they investigated its potential off-target effect on each embryo at sites predicted by experiments and computational analyses of different genome resources from references and the parents of the embryo. They detected no off-target mutations in any of the embryos injected with Cas9 nuclease and a select sgRNA, except for one off-target deletion mutation found in only 1 of 19 tested embryos. The human embryonic stem cells established from those edited embryos had normal karyotypes and displayed pluripotency. *CCR5*- edited model mice were bred for 3 generations, and no obvious physiological or behavioral changes were observed.

As CRISPR-Cas9 employs the direct introduction of a designed nuclease into cells, the potential off-target effect is of paramount importance to clinical application [5,6]. However, the results of genome modification using CRISPR-Cas9 and its assessment of modification may be affected by human genetic variation and by the genomic instability frequently observed in early embryos [25–27]. To rule such factors out, the Chinese researchers used DNA samples of the parents of several embryos, in addition to a reference genome. They also employed several methods of genome sequencing for the 19 sets of an embryo and its parents. As a result, they identified one off-target deletion mutation in one of 19 embryos and concluded that the mutation site is unlikely to affect gene functions. Additionally, to assess the phenotypic impact of their *CCR5*-targeting CRISPR-Cas9 on individuals, they created edited mice and bred them, finding no observable changes. It appears that those researchers utilized multiple experimental systems to minimize and carefully assess the risks of GGE, as requested by the Declaration of Helsinki and others [17,20,28]. Yet, some would question the reliability and veracity of those researchers' demonstration of an absence of observable safety concerns in their GGE. In the first place, proving a negative outcome particularly in genetics is complicated and at best, problematic [29]. Indeed, other 3 groups recently showed that genome-editing may cause unwanted loss of targeted chromosome or heterozygosity in the human embryos [30–32]. While, there is currently no consensus on the assessment of off-target effect of genome-editing [33]. Under specific conditions, the Chinese researchers demonstrated that harmful off-target mutations were absent in the embryos. From a purely scientific standpoint, their conclusion may be acceptable [20,28]. However, it is necessary to examine the clinical and ethical implications of predictable risks and burdens of human GGE that may outweigh the importance of the scientific advancement.

2.2. GGE study in clinic

The Chinese researchers considered that the risks of their GGE were at an acceptable level and proceeded with a clinical GGE study to provide beneficial resistance to HIV infection for resultant children. They recruited 8 couples having wild-type *CCR5* genes, with each couple consisting of an HIV-positive husband and an HIV-negative wife [8,24]. After one couple canceled their participation, the remaining 7 couples underwent *CCR5*-mutating GGE. The researchers investigated the genomes of 3 to 5 cells biopsied from Cas9-injected embryos. After embryo transfer, 6 wives did not become pregnant and the remaining 2 delivered: one gave birth to twin girls and the other delivered one infant [23].

2.2.1. Study design

With regard to the design of clinical GGE research, other commentators have proposed phases I– III clinical trials to make GGE accessible to parents as soon as possible, while also conclusively demonstrating safety and efficacy [34]. In contrast, the clinical study in China was a small single arm GGE study without controls. The Chinese researchers indicated that they did not scope larger GGE studies, such as phase II-III trials [8]. Again, there is no medical intervention without risk. Given the potential risks to offspring, GGE studies must be restricted to small-scale study [14]. Moreover, controlled studies, such as those in which Cas9 without sgRNA is injected into embryos, are also unconceivable. Indeed, reproductive studies involving germline mitochondrial manipulation were all small single arm studies [35]. The power of such small studies without controls remains limited, and such studies cannot provide reliable data on the safety of the clinical practice of GGE.

2.2.2. Perinatal care for mother and (future) children

The Chinese researchers injected their CRISPR-Cas9 into embryos from the consenting couple. After analyzing several cells taken from edited embryos, of 4 injected embryos, 2 had one or more mutated *CCR5* gene: one embryo with *CCR5* genotypes of −14 bp/+1 bp and the other with that of WT/−15 bp. They also investigated all injected embryos for off-target mutations and large-scale deletions: one of the edited embryos had a possible 1 bp insertion in an intergenic off target region. They considered that the insertional mutation may not impact any biological function. The researchers informed the couple of the genetic information on all viable embryos. The couple consented to the transfer of those 2 edited embryos, one of which had the insertional mutation. During pregnancy, the researchers tested cell free DNA derived from the fetuses, performed ultrasound monitoring, and confirmed the 3 *CCR5* genotypes but did not detect the potential off- target 1 bp insertion. They also did not detect cancer-related mutations in the DNA

samples. After the 'healthy' birth of the twins, the researchers further investigated tissue samples from umbilical cord, umbilical cord blood and placenta. Consequently, they confirmed the *CCR5* genotypes were identical to those at the embryonic and fetal stages, and did not observe any off-target mutations, large deletions, or pathogenic cancer gene mutations.

Such information suggests that the Chinese researchers observed the Declaration of Helsinki [17]. Namely, they continuously monitored, assessed, and documented the risk of GGE from embryo transfer to delivery, while providing opportunities for the couple to choose embryos. One the other hand, the accuracy of genome sequencing depends on the DNA samples: only several cells taken from the embryos and cell free DNA derived from fetuses. If the wife found her pregnancy affected, she would have felt distressed about whether she should choose abortion or maintain pregnancy [19]. More importantly, they used only 3 available tissue samples to demonstrate the absence of off-target mutations in the twins. Of course, they sampled readily available tissues in order to avoid distressing the newborns [14]. Again, their demonstration in the embryos, fetuses and twins implies a 'probability' of no harmful off-target mutations in their GGE. While, their assertion of safe childbirths via GGE incurred criticism [21].

3. Societal standing of persons born via GGE

If an embryo in which an off-target mutation was overlooked was not screened out but transferred to the mother, it may later harm the newborn. Thus, the need for monitoring the safety of genome-edited offspring is often underscored. It is also imperative to explore a potential legal issue: damage claims by genome-edited offspring as well as their parents.

3.1. Follow-up of genome-edited offspring

Some proposed that genome-edited offspring should be followed up for decades, life, or generations [14,16,34,36]. First of all, long-term follow-up in intervention study is, unlike cohort study, uncommon and generally difficult to plan due to logistical and ethical issues, such as expenses and burdens of travel, examinations and testing [37]. The Chinese researchers planned an 18-year follow-up [8,24]. The parents might have taken the genome-edited twins to the hospital if the scandal had not been revealed. However, the physical, mental, and economic burdens might make such parents withdraw consent to follow-up [14], as illustrated by a reproductive study involving germline mitochondrial manipulation [38]. Concerning this conundrum, the need for 'mandatory' follow-up to confirm positive long-term outcomes was voiced [34]. However, even if practitioners pay the family's medical and travel expenses, mandatory long-term follow-up may impose on the family the unbearable physical and mental burdens of repeated hospital visits and DNA testing involving painful invasive sampling, particularly genome-edited children who never consented prior to GGE [14,36]. Thus, the autonomy of family members with human rights should be respected in the long-term follow-up, unless genome-edited offspring have not developed any health problems [14–16,36]. In the explanation of GGE prior to consent, practitioners and parents should discuss and understand the need for follow-up post-GGE sufficiently. Meanwhile, a survey of couples who had undergone a germline mitochondrial manipulation showed that only 1 out of 13 couples later informed their children that they had been bone via the germline genetic modification [39]. Practitioners and consenting parents should cooperate to inform genome-edited children and their progeny (In the case of intergenerational follow-up) of the circumstances of their birth via GGE and should obtain informed assent to follow-up [19,22]. Subsequently, practitioners should obtain informed consent, once they become legally competent [11,22]. Their autonomy should be respected and they should also understand that they may withdraw consent at any time [14,19,36]. Thus, it appears that such long-term or intergenerational follow-up post-GGE is extremely difficult to perform. In this regard, those Chinese researchers' follow-up plan, extending to the time that twins will reach their majority (18 years old in China) [8,24], seems appropriate from an ethical and practical standpoint. Ensuring the safety of genome-edited twins ultimately depends on their autonomy.

3.2. Damage claim by victims of unsafe GGE

As with animal GGE, human GGE could result in the birth of a person with a beneficial trait. But, the aforementioned limitations in screening edited embryos and fetuses risk reassessments suggest that some practices systemically harm resultant offspring by overlooking off-target mutations. Those offspring can suffer from cancer arising from a mutated tumor suppressor gene and infertility due to chromosomal translocations, as well as novel genetic disease [21,40,41].

As this is a tragic event, some parents might bring a wrongful birth lawsuit, arguing that the birth of a disabled child could have been avoided if the practitioners had performed prenatal testing more effectively and provided accurate information to the parents [42,43]. However, in human GGE, it seems difficult to demonstrate whether an offspring's disability was caused by the off-target effect or not. Chromosomal alternations and mutations that can occur naturally in gametogenesis and early embryonic development may hamper the demonstration, even if the genome of the disabled child is carefully compared with those of their parents. This could be the case with the concerned loss of targeted chromosome [30–32] and the trial may become protracted.

If someone informs genome-edited offspring and the progeny of their birth via GGE, they might sue for 'wrongful life' damages, asserting that they have been harmed by an off-target mutation so much that their life is less valuable and they should not have been born [42,43]. They would argue that a practitioner failed to discover off-target mutations or provide relevant information during or before embryo transfer or during pregnancy. If their mother or their grandmother had been provided with this information, they would also argue that she should have chosen abortion or should not have undergone harmful GGE at all. In the US, California, Washington and New Jersey allow wrongful life suits; however, claimable damages are restricted to economic damages that can be proven objectively, not monetary compensation for the entire experience of having a disabled life versus a healthy life [44]. In Europe, the Dutch Supreme Court fully upheld a wrongful life claim against medical personnel in 2005 [45]. However, such judgements are extremely rare around the world. Most jurisdictions have rejected wrongful life suits because allowing such a claim declares that the life of a disabled person is less valuable than that of a non-disabled person, and/or because allowing it will encourage other disabled persons, who were born irrespective of reproductive techniques, to claim damages against their parents and practitioners [43]. Therefore, it is difficult for the victims of unsafe GGE to claim their wrongfully-edited lives.

4. Responsibility for human GGE and policy

Again, there is currently no global consensus on proof of the absence of harmful off-target mutations in the edited genome. As discussed above, preclinical GGE research can demonstrate an absence under specific conditions. Practitioners are responsible for proving the non-existence of such mutations to each couple with a unique genome, although the appropriateness would be difficult to accept. Even if a clinical GGE study is approved based on a certain level of risk of in comparison with a foreseeable reproductive benefit, the irreversible intervention to future persons restricts clinical research to small single arm studies. However, this restriction on study design also limits the assessment of the risk of GGE. In the prior explanation, some prospective parents understand the risks, burdens, and potential harm to their future children and then give consent to GGE anyway because of their reproductive benefit. For this, both the consenting parents and practitioners assume responsibility for the follow-up of genome-edited offspring. Once

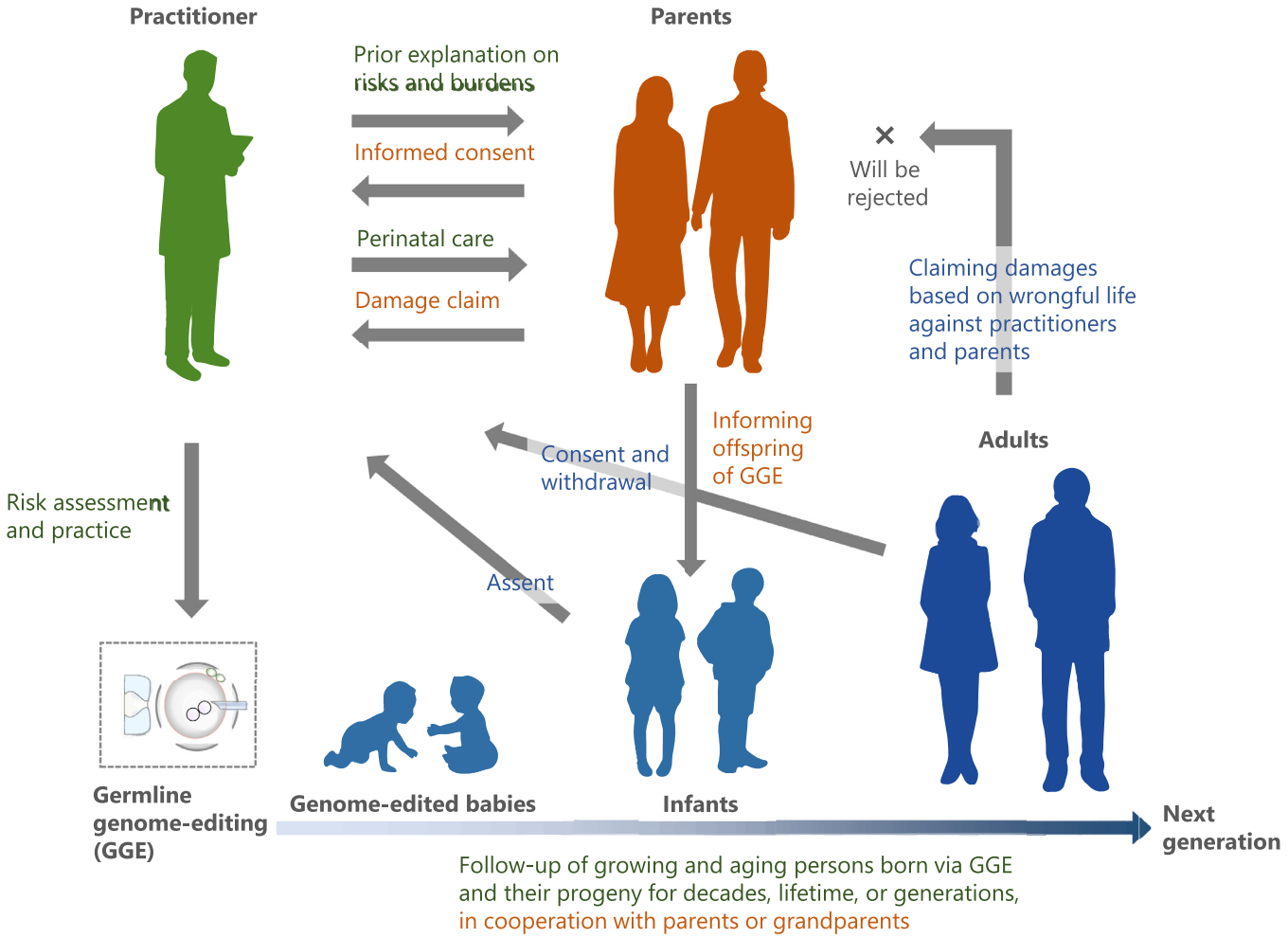

Fig. 1. Uncertainty of assignment of responsibility for creating persons using germline genome-editing (GGE).
The risks of human GGE can be assessed in preclinical research, despite the difficulty of accepting its appropriateness. However, it is difficult to assess and manage the risks of GGE in both clinic and society, primarily because the harmful genetic intervention is irreversible in genome-edited persons and because they will eventually become autonomous. In addition, damage claims by persons suffering from GGE are unlikely to be accepted or fully compensated for.

they reach the age of competence, the genome-edited offspring and their progeny can withdraw their previous assent or consent. Paradoxically, respecting the autonomy of genome-edited offspring can lead to a failure of fulfillment of responsibility for their lifetime or intergenerational safety. Due to limited screening and the uncertain safety of human GGE, an overlooked off-target mutation may actually harm all cells of a resultant human being. If the sufferers discover they were born via GGE, they might claim a wrongful life against the practitioners and/or parents. However, most jurisdictions will grant some exemption from such a claim on the ground that an edited life is no less valuable than others. Thus, the assignment of responsibility for the risks and burdens of human GGE may be uncertain in society (Fig. 1).

Consider the implications of uncertain assignment of responsibility for practicing human GGE from a policy standpoint. If countries acknowledge that the burdens and potential harms of GGE imposed upon resultant persons who can never give prior consent are untenable and unacceptable, they will adopt prohibitive policies toward relevant research or medicine, such as restricting public funding and enacting guidelines for practitioners or laws that are more enforceable for the whole nation [3,21].

On the other hand, some countries might find an acceptable balance between the predictable risk and the foreseeable benefit in creating persons using GGE and might thus further consider permitting its clinical practice as a benefit to prospective parents, as illustrated by the legalization of germline mitochondrial manipulation in the UK [4]. However, the UK regulations stipulate the permitted germline manipulations, applicable women and persons born of the woman, and licensing requirements of practitioners, including the planning of follow-up schemes. While, the follow-up is ultimately left up to the autonomy of parents and their offspring born from those techniques. Regarding adverse events after germline mitochondrial manipulations, the UK regulations request that practitioners report them immediately; however, the norm does not stipulate any items regarding compensation for damages [46]. Such a social consensus might be reached for human GGE if a foreseeable benefit of GGE use for prospective parents largely coincides with a social benefit. One of the potential uses is the prenatal prevention of the birth of persons with serious diseases because such a GGE use could reduce physical, mental, and economic burdens upon parents and also restrain social security costs in a country [11–13,47].

5. Conclusions

The present article examined the responsibility for using GGE in human reproduction and assessed the predictable risks and burdens of this practice. In preclinical GGE studies, the non-existence of off-target mutations can be assessed under specific conditions, despite the difficulty of accepting the appropriateness of off-target mutation assessment. In clinical studies, some applications of GGE will result in the birth of hu-

mans with a desired trait; however, it is difficult to assess the safety of GGE because such research involving genetic intervention in potential humans must be done in small studies without controls. It is also difficult to manage the risks of GGE, primarily because harmful off-target mutations can be overlooked, and those are irreversible in the body. However, long-term follow-up of genome-edited offspring can be difficult because some offspring will withdraw their assent or consent to follow-up. The victims of unsafe GGE might claim damages against the practitioners and/or parents on the grounds that wrongful GGE made their lives less valuable than others. However, such damage claims are unlikely to be accepted or fully compensated for in most legal systems, except in the Netherlands.

Parents who have consented to GGE, as well as practitioners, assume responsibility for the safety of the genome-edited offspring. Although the fulfillment of responsibility ultimately depends on the offspring's participation in follow-up post-GGE, parents and practitioners cannot coerce offspring into the follow-up. Meanwhile, practitioners and parents may be exempt from some damage claims made by offspring harmed by unsafe GGE. The uncertain assignment of social responsibility is likely to underpin the prohibition of GGE practice in light of the unacceptable risk to, burdens of, and potential harms for persons born via GGE. On the other hand, if an acceptable risk-and-benefit balance for prospective parents and society is reached, GGE might become an accepted practice in some countries; while, inevitably such a policy will leave some persons harmed by unsafe GGE unprotected.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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References

[1] Sinsheimer RL. The prospect for designed genetic change. Am Sci 1969;57(1):134–42.
[2] Frankel MS, CAR. Human Inheritable Genetic Modifications. Assessing Scientific, Ethical, Religious, and Policy Issues. American Association for the Advancement of Science.. Am Assoc Advanc Sci 2000.
[3] Baylis F, et al. Human Germline and Heritable Genome Editing: the Global Policy Landscape. The CRISPR Journal 2020;3(5):365–77.
[4] Dimond R, Stephens N. Legalising mitochondrial donation. enacting ethical futures in uk biomedical politics. Switzerland: Palgrave Pivot; 2018.
[5] Kim H, Kim JS. A guide to genome engineering with programmable nucleases. Nat Rev Genet 2014;15(5):321–34.
[6] Barrangou R, Doudna JA. Applications of CRISPR technologies in research and beyond. Nat Biotechnol 2016;34(9):933–41.
[7] Ishii T. Genome-edited livestock: ethics and social acceptance. Animal Frontiers 2017;7(2):24–32.
[8] He, J., *CCR5* gene editing in mouse, monkey and human embryos using CRISPR/Cas9, in Session 3 Human Embryo Editing at Second International Summit on Human Genome Editing. https://www.nationalacademies.org/event/11-27-2018/second-international-summit-on-human-gene-editing (accessed 3 March 2021). 2018.
[9] Cyranoski D, Ledford H. Genome-edited baby claim provokes international outcry. Nature 2018;563:607–8.
[10] Carolyn B. Do CRISPR Germline Ethics Statements Cut It? The CRISPR Journal 2018;1(2):115–25.
[11] National_Academy_of_Medicine and National_Academy_of_Sciences Heritable human genome editing. The National Academies Press; 2020.
[12] Gyngell C, et al. The Ethics of Germline Gene Editing. J. Appli. Philos. 2016. doi:10.1111/japp.12249.
[13] Koplin, J.J. et al. Germline gene editing and the precautionary principle. Bioethics 2020;34(1):49–59.
[14] Brokowski C, Adli M. Ethical Considerations in Therapeutic Clinical Trials Involving Novel Human Germline-Editing Technology. Crispr j 2020;3(1):18–26.
[15] Chan S. Playing it Safe? Precaution, Risk, and Responsibility in Human Genome Editing. Perspect Biol Med 2020;63(1):111–25.
[16] Schleidgen S, et al. Human germline editing in the era of CRISPR-Cas: risk and uncertainty, inter-generational responsibility, therapeutic legitimacy. BMC Med Ethics 2020;21(1):87 -87.
[17] World_Medical_Association_(WMA), Declaration of Helsinki - Ethical principles for medical research involving human subjects. https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/ (accessed 25 December 2020). Helsinki, 1964.
[18] Cwik B. Responsible Translational Pathways for Germline Gene Editing? Curr Stem Cell Rep 2020:1–8.
[19] Ishii T, Beriain IdM. Safety of Germline Genome Editing for Genetically Related "Future" Children as Perceived by Parents. The CRISPR journal 2019;2(6):370–5.
[21] de Wert G, et al. Responsible innovation in human germline gene editing. Background document to the recommendations of ESHG and ESHRE. Hum Reprod Open 2018 2018(1) hox024.
[20] Andorno R, et al. Geneva Statement on Heritable Human Genome Editing: the Need for Course Correction. Trends Biotechnol 2020;38(4):351–4.
[22] Adashi EY, Cohen IG. What would responsible remedial human germline editing look like? Nat. Biotechnol. 2020;38(4):398–400.
[23] Normile D. Chinese scientist who produced genetically altered babies sentenced to 3 years in jail. Science 2019.
[24] Regalado A. China's crispr babies: read exclusive excerpts from the unseen original research.. MIT Technology Review; 2019. https://www.technologyreview.com/2019/12/03/131752/chinas-crispr-babies-read-exclusive- excerpts-he-jiankui-paper/ accessed December 25 2020,..
[25] Canver MC, et al. Impact of Genetic Variation on CRISPR-Cas Targeting. Crispr j 2018;1(2):159–70.
[26] Hewes AM, et al. The Diversity of Genetic Outcomes from CRISPR/Cas Gene Editing is Regulated by the Length of the Symmetrical Donor DNA Template. Genes (Basel) 2020;11(10).
[27] Vanneste E, et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med 2009;15(5):577–83.
[28] Nordgren A. Designing Preclinical Studies in Germline Gene Editing: scientific and Ethical Aspects. J Bioeth Inq 2019;16(4):559–70.
[29] Hales SD. Thinking Tools: You can Prove a Negative Think 2005;4(10):109–12.
[30] Zuccaro MV, et al. Allele-Specific Chromosome Removal after Cas9 Cleavage in Human Embryos. Cell 2020;183(6):1650–1664.e15.
[31] Liang, D. et al. (2020) Frequent gene conversion in human embryos induced by double strand breaks. bioRxiv, 2020.06.19.162214.
[32] Alanis-Lobato, G. et al. (2020) Frequent loss-of-heterozygosity in CRISPR-Cas9-edited early human embryos. bioRxiv, 2020.06.05.135913.
[33] Joung JK. Unwanted mutations: standards needed for gene-editing errors. Nature 2015;523(7559):158.
[34] Evitt NH, et al. Human Germline CRISPR-Cas Modification: toward a Regulatory Framework. Am J Bioeth 2015;15(12):25–9.
[35] Ishii T. Reproductive medicine involving mitochondrial DNA modification: evolution, legality, and ethics. EMJ Repro Health 2018;4(1):88–99.
[36] Cwik B. Intergenerational monitoring in clinical trials of germline gene editing. J Med Ethics 2020;46(3):183–7.
[37] Hill KG, et al. Planning for Long-Term Follow-Up: strategies Learned from Longitudinal Studies. Prevention science: the official journal of the Society for Prevention Research 2016;17(7):806–18.
[38] Zhang J, et al. Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. Reprod Biomed Online 2017;34(4):361–8.
[39] Chen SH, et al. A limited survey-based uncontrolled follow-up study of children born after ooplasmic transplantation in a single centre. Reprod Biomed Online 2016;33(6):737–44.
[40] Hoffmann ER, Roig I. Cas9 in Human Embryos: on Target but No Repair. Cell 2020;183(6):1464–6.
[41] Ihry RJ, et al. p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med 2018;24(7):939–46.
[42] Krekora-Zając D. Civil liability for damages related to germline and embryo editing against the legal admissibility of gene editing. Palgrave Communications 2020;6(1):30.
[43] Frati P, et al. Preimplantation and prenatal diagnosis, wrongful birth and wrongful life: a global view of bioethical and legal controversies. Hum Reprod Update 2017;23(3):338–57.
[44] Fox D. Privatizing procreative liberty in the shadow of eugenics. J Law Biosci 2018;5(2):355–74.
[45] Sheldon T. Dutch Supreme Court backs damages for child for having been born. BMJ 2005;330(7494):747 -747.
[46] Human_Fertilisation_and_Embryology_Authority_(HFEA), HFEA Code of Practice Edition 9.0: 33. Mitochondrial donation. https://www.hfea.gov.uk/code-of-practice/33 (accessed 25 December 2020). 2019.
[47] Smith K. Time to start intervening in the human germline? A utilitarian perspective. Bioethics 2020;34(1):90–104.