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Treatment of heritable diseases using CRISPR: Hopes, fears, and reality

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ABSTRACT

CRISPR gene editing is poised to transform the therapeutic landscape for diseases of genetic origin. The ease and agility by which CRISPR can make specific changes to DNA holds great promise not only for the treatment of heritable diseases, but also their prevention through germline editing. CRISPR-based therapeutic strategies are currently under development for numerous monogenic diseases. These strategies range from proof of concept studies demonstrating pre-fertilization gamete editing to recently initiated clinical trials for postnatal *ex vivo* therapies. The promise of CRISPR's human genome editing potential has captivated the public's attention. It is of paramount importance that medical professionals who work with patients who may have or carry a monogenic heritable disease understand CRISPR technology in order to have informed and compassionate discussions with their patients. Understanding CRISPR means understanding its evolving therapeutic applications' nuances, limitations, and barriers to access as well as the regulatory landscape they inhabit. In this piece we provide a review of the promises and pitfalls of CRISPR germline gene editing and their implications for patient decision-making throughout various stages of the reproductive process.

There are an estimated 10,000 diseases that result from mutations in a single gene,[1] and heritable diseases collectively affect 5–7% of the human population.[2] The advent of CRISPR gene editing and the ease and agility by which it can make specific changes to DNA hold great potential for the treatment, prevention, or elimination of diseases with genetic origin.[3] CRISPR-based therapeutic strategies are currently being developed for a variety of heritable diseases such as sickle cell disease, hemophilia, muscular dystrophy, and cystic fibrosis. The promise of CRISPR's human genome editing potential has captivated the public's attention.[4] It is therefore important for medical professionals to understand the nuances and limitations of CRISPR technology so they can have informed and compassionate discussions with their patients.

The majority of CRISPR-based strategies currently in development intend to edit somatic (non-reproductive) cells in patients to correct or treat disease-causing mutations postnatally. For prospective parents affected by a heritable disease, CRISPR-editing of the germline (gametes, gamete precursors, or fertilized embryos[1]) could instead prevent transmission of

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[1]We adopt this expansive definition to include zygote and embryo editing consistent with the National Academy of Sciences Report discussed below – distinguishing germline editing based upon intent, not on the basis of type of cells edited.

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disease-linked genetic mutations altogether. Germline gene editing would result in an embryo in which every cell is free of the disease-causing mutation, including its gametes, to prevent the disease from being passed on to subsequent generations. Over multiple generations, germline editing could reduce disease prevalence in the human gene pool, and eventually even eradicate certain heritable diseases from the human population.

In contrast to editing somatic cells, gene editing of the human germline entails broader impacts for patients and society, and despite early calls for a temporary moratorium on making heritable changes to the human genome[5], research continues to proceed. In a recent report jointly commissioned by the National Academy of Sciences, the National Academy of Medicine, and the National Academies of Sciences, Engineering, and Medicine (NASEM) (hereafter referred to as the NASEM report) recommended that research on germline gene editing should continue, albeit cautiously, and only for therapeutic purposes to treat serious heritable diseases.[2] The clinical potential of human germline gene editing is therefore likely to soon become a technological reality, carrying with it considerable implications for assisted reproductive technologies.

Medical practitioners, particularly reproductive specialists and their embryologist and laboratory partners, may find themselves at the frontline of human germline gene editing, administering gene editing therapies along with their potential risks and benefits. Here we discuss the current options for prospective parents affected by heritable monogenic diseases who wish to avoid transmitting the genetic mutation to the next generation and how CRISPR gene editing may impact reproductive decisions in the future. We also review the current regulatory and legal structures that will shape the pace and extent of the clinical availability of these options for patients.

Current reproductive options for potential parents affected by monogenic heritable disease

In order to understand the potential paradigm-shifting significance of CRISPR germline editing, the current options for prospective parents affected by a monogenic heritable disease who seek to avoid passing their disease (or carrier gene for their disease) to their children must first be considered. While our focus here is on patients who wish to discuss and/or pursue options to prevent transmission of their disease, we note that there are some conditions in which parents may not wish to avoid passing on certain genetic traits, such as deafness.[6] It is equally important for providers to understand that *whether to* seek any intervention to avoid transmission is sometimes in itself a difficult and fraught decision.

The currently available options for prospective parents who wish to avoid transmission include: (a) avoiding producing a genetically-related embryo/child by abstaining from parenthood or abstaining from genetic parenthood through adoption, use of other gametes, or use of other embryos; (b) producing genetically-related embryos, only some of which contain the monogenic mutation and utilizing genetic screening to selectively implant embryos without the mutation; or (c) producing a genetically-related fetus, performing *in utero* genetic screening, and selectively terminating pregnancy of an affected fetus. Issues of identity and access arise in each potential mode to prevent transmission of a genetic mutation and many patients may have strong, core moral or religious convictions that are implicated in each instance. All of these factors are important for physicians to be aware of and empathetic to when counseling patients (Table 1).

Avoid genetic parentage

There are multiple options for prospective parents who are carriers of or affected by a monogenic heritable disease to avoid genetic parentage, and thus transmission of a disease-causing mutation. These patients can refrain from becoming a parent through clinically-effective family planning methods or pursue adopting a child. They can also substitute unaffected gametes, usually by procuring gametes from someone besides one of the individuals who intend to parent the child (so-called "third-party" gamete providers). Finally, prospective parents could procure a genetically unrelated embryo. As Table 1 illustrates, the accessibility of these options is not uniform across all patients. For instance, adoption may not be a viable option for an LGBTQ patient. Both adoption and procuring gametes (particularly oocytes) can be prohibitively expensive. For patients who highly value genetic parentage, these options may be distressing and/or perpetuate stigma, such as in the case of LGBTQ patients unable to access adoption. Some patients may also raise concerns regarding the ethics of paying for third-party gametes – whether doing so problematically commodifies human beings, places greater value on some traits than others (such as blonde hair) or exploits those providing the gametes. In addition, patients may raise concerns about destroying any excess embryos produced via *in vitro* fertilization (to the extent substituting third-party gametes necessitates IVF) and may wish to pursue options that avoid IVF.

Genetic parentage without genetic alteration

For some potential parents, maintaining a genetic connection is very important. For those patients who wish to maintain this connection while preventing transmission of their genetic disease, there are two currently-available reproductive options – preimplantation genetic diagnosis (PGD) and selective implantation, or *in utero* genetic screening and selective termination. However, the NASEM report highlights certain cases in which utilizing either of these methods to prevent disease transmission would be ineffective. If one prospective genetic parent is homozygous for a dominant disease-causing mutation (like Huntington's disease) or if both prospective genetic parents are affected by the same recessive disorder (for example, cystic fibrosis) it would be impossible to generate embryos or fetuses free of the disease-causing mutation without first correcting the underlying mutation in the intended parent(s)' gametes or resultant embryo.

Another circumstance to consider is when both parents are carriers of a recessive mutation. In such a case, only 25% of resultant embryos or fetuses would be free of the mutation. In other words, the likelihood of selective termination of the pregnancy in such a scenario would be 75%, or 75% of resulting embryos would not be implanted and thus discarded.

Table 1 – Current parentage options for prospective parents affected by a heritable disease that avoid or mitigate disease inheritance.

Patient Option	Genetic Parentage Maintained	Heritable Disease Transmission Prevented	Barrier(s) to access	Possible Patient Concerns/Objections
Refrain from becoming a parent	No	Yes	• Access to quality family planning health care and contraception methods vary	• Can cause emotional and psychological distress for people wishing to be parents • In the event of an unintended pregnancy, patients may object to termination
Adoption	No	Yes	• Cost prohibitive for some intended parents • Single, lesbian, gay, transgender, intersex, and queer people may face policy barriers • Racial and religious inequities in some adoption policies	• Can cause emotional and psychological distress for intended parents who wish to be genetic parents
Purchase embryos or gametes from outside parties	Yes, for one parent if one set of gametes purchased	Yes	• Cost of embryos and/or gametes and/or IVF prohibitive for some parents	• Purchase/sale of gametes and embryos is objectionable • If *in vitro* fertilization is required, "left over" embryos are likely to be discarded, which is ethically objectionable
In vitro fertilization (IVF) with preimplantation genetic diagnosis (PGD) and selective implantation	Yes	Not for every case: • Ineffective if a parent is homozygous for a dominant disease since all embryos would have the mutation • Ineffective if both parents are affected by the same monogenic disease	• Cost of IVF prohibitive for some intended parents • Some patients may have limited gamete reserves due to disease or medical treatments (i.e. chemotherapy)	• Ethical objections to discarding affected embryos
Prenatal genetic diagnosis with selective abortion of affected fetuses	Yes	Not for every case: • Ineffective if a parent is homozygous for a dominant disease since all embryos would have the mutation • Ineffective if both parents are affected by the same monogenic disease	• Abortion could be cost-prohibitive, although less so, as compared to IVF • Access to abortion may be limited for some parents.	• Could require abortion of a pregnancy if prenatal genetic diagnosis results are positive, and some patients find this ethically objectionable

Moreover, both of these "generate and screen" processes have limitations that germline editing methods discussed below address. Each could result in children that are not affected by the disease but remain carriers of a recessive genetic disorder. In contrast, the use of germline gene editing to correct recessive mutations could eventually eliminate disease burden for future generations. As noted above, any time *in vitro* fertilization is used (as is required for PGD), patients may raise concerns about the fate of the embryos not selected for implantation.

Possible future CRISPR gene editing options for potential parents affected by monogenic heritable disease

As is the case with existing options for potential parents affected by monogenic heritable diseases wishing to avoid transmission, there are multiple possible options that could become clinically available with the advent of CRISPR technology. They include: (a) using CRISPR to gene edit gametes

(or gamete precursor cells) and create a genetically-related embryo/child without the monogenic mutation; (b) producing genetically-related embryos with the monogenic mutation and utilizing CRISPR to gene edit the resulting embryo and remove the monogenic mutation before implantation; (c) producing a genetically-related embryo/child with the monogenic mutation and then utilizing somatic CRISPR-based gene therapy *in utero*; or (d) producing a genetically-related embryo/child with the monogenic mutation and then utilizing somatic CRISPR-based gene therapy postnatally. The gene editing of gametes or a pre-implantation embryo results in an embryo/child in which/whom every cell has been genetically altered; these two options are therefore often grouped together as "germline editing," as they were in the NASEM report, and we do here. Different proposed strategies for therapeutic interventions using CRISPR (some remain theoretical, while others are in clinical development) have unique technical considerations, as well as advantages and limitations for the patient (Fig. 1).

Gene editing of gametes and gamete precursors

One future option for potential parents may be to edit the gametes or gamete precursor cells of the affected intended genetic parent, resulting in an unaffected embryo. Human gamete gene editing remains largely theoretical, with only a handful of published proof-of-concept studies to date. However, based upon what we know about germline cells and the

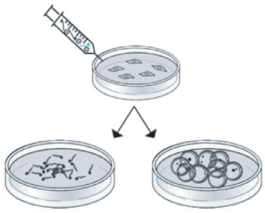
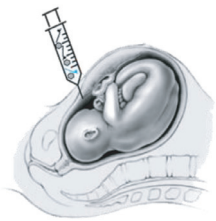
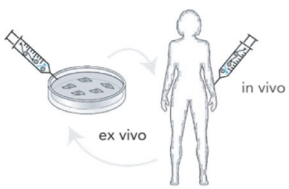
CRISPR Intervention	Clinical Status	Advantages	Risks and Limitations
Gamete or Gamete Precursors 	Theoretical with promising research	• May not necessarily require IVF • Gamete and gamete precursors may prove easier to edit, as compared to embryos • Eliminates risk of mosaicism • Avoids ethical issues about discarding embryos and/or abortion	• Gene editing procedures on gamete precursor cells could impair gamete differentiation, maturation, or replication • Effective *in vitro* methods for gamete differentiation are still under development • Gene edits to gametes or gamete precursors would be passed on to future generations and potentially impact the human gene pool
Embryo 	Proof of concept studies in human embryos	• Corrects the mutation in every cell of the embryo - important for diseases that impact multiple organs • Can avoid prenatal symptoms and prevent congenital defects • Could eventually eliminate a heritable disease from the human gene pool	• Require IVF • Potential for mosaicism • The gametes of a resultant embryo would be gene edited to potentially impact future generation and the human gene pool.
In utero 	Pre-clinical research using CRISPR in animal models	• Could treat disease before symptom onset • Small size of fetus can make for easier drug delivery • Immunological immaturity could reduce side-effects • Immune tolerance could be maintained for postnatal doses • Can target multiple organs • Increased number of progenitor cells available for gene editing	• Could disrupt normal organ development • Could result in edits to germline cells • There are likely to be associated risks to the mother or surrogate • Could increase risk of miscarriage
Postnatal 	Clinical trials have commenced	• Can specifically target certain tissues or cell types • Edits would not be passed on to future generations • *Ex vivo* editing allows for validation of corrected mutations and screening for off-target impacts prior to transfusion	• Limited in treating diseases that impact multiple organs • Effective *in vivo* drug delivery is a major therapeutic hurdle • Risk of off-target genetic and cellular effects • May induce immune response to viral delivery and/or CRISPR components

Fig. 1 – Potential CRISPR interventions to treat or prevent heritable diseases at various developmental stages.

CRISPR mechanism, when or if strategies to edit human gametes are developed they will likely involve CRISPR editing of gamete precursors (spermatogonia stem cells or germinal vesicle oocytes) or mature oocytes, since mature sperm will likely be difficult to target for gene editing.[7] To obtain corrected sperm, spermatogonia stem cells derived from testes biopsies could be edited using CRISPR and then differentiated in culture to mature sperm.[7,8] Mature oocytes could directly be gene edited using CRISPR or germinal vesicle oocytes could instead be collected, gene edited and then differentiated to maturity.[7] In theory, gametes differentiated from CRISPR-edited induced pluripotent stem cells (iPSC) obtained from a diseased patient could provide another alternative. Researchers have demonstrated successful differentiation of gametes from iPSCs isolated from mice[9,10] and CRISPR has been used to successfully correct cystic fibrosis mutations in human lung epithelial cells derived from patient iPSCs.[11] Once methods for *in vitro* culture and protocols for gamete differentiation improve, it may be scientifically possible for CRISPR-edited human gametes to be used to prevent disease transmission from affected parents to their offspring.

Patient concerns with any CRISPR germline editing may be both macro and micro: macro insofar as they implicate possible and unknown concerns for all future generations, and micro insofar as they, like many of the non-CRISPR options discussed, will likely require IVF and thus generate excess embryos. However, using CRISPR methods to edit gametes/gamete precursor cells do not implicate concerns raised regarding compensation for third-party gametes. In addition, should edited sperm cells eventually be viable as semen samples, methods like intrauterine insemination may be an option that circumvents IVF.

Gene editing in pre-implantation embryos

A second potential option for patients in the future could be to generate embryos and then use CRISPR gene editing to remove the genetic mutation from affected embryos. However, applications of CRISPR/Cas9 to human embryos are still in large part theoretical. An early proof-of-principle study recently published by Ma et al. at the Oregon Health and Science University demonstrated successful correction of a cardiac disease-causing mutation in viable human embryos using CRISPR/Cas9.[12] It should be noted that the human embryos used for these experiments were never intended for implantation and were not allowed to developed past 3 days post-fertilization. Three previous attempts to gene edit human embryos made by research groups in China had resulted in numerous off-target effects (editing of genes other than those related to the targeted gene) and high rates of mosaicism.[13–15] In contrast, the introduction of CRISPR components at the time of fertilization allowed Ma et al. to achieve a high percentage of uniformly gene-edited blastomeres (97% of corrected blastomeres). Ma et al. also reported low rates of off-target effects, a finding that has raised some debate in the scientific community over limitations of the sequencing methods employed.[16,17] To proceed towards clinical relevancy, technical procedures must be developed to eliminate risks of mosaicism and off-target effects in CRISPR-edited pre-implantation embryos.

Since embryos must be created, screened, and edited in pre-implantation methods, gene editing of embryos would not completely address patient concerns with preimplantation genetic diagnosis if *all* embryos are not ultimately implanted. However, in comparison to PGD and selective implantation, embryo gene editing could result in a smaller number of embryos being discarded; every embryo could be edited and used by the intended parent, or if embryo "adoption" were an option another intended parent could ultimately utilize the embryos, and thus address patient objections related to discarding embryos.

In utero *gene editing*

Editing the genes of an affected fetus *in utero* is another potential option for future parents. The delivery of CRISPR components to human fetuses *in utero* to edit disease-causing mutations remains purely theoretical. Studies of *in utero* gene therapy in animal models have however demonstrated successful treatment of a variety of diseases, including hemophilia and lysososomal storage disorders.[18] A recent report documented successful correction of the genetic mutation that causes the neurodegenerative disorder, neuronopathic Gaucher disease (nGD) by delivering the corrected gene to mouse fetuses while *in utero*.[19]

Studies have demonstrated successful *in utero* gene editing in animal models using either nanoparticles or electroporation to deliver CRISPR components to developing fetuses.[20,21] As the field of *in utero* gene editing expands, care must be taken to ensure safety for both the mother and developing fetus. *In utero* gene editing could be the best option for patients with concerns regarding discarding embryos or terminating pregnancy. Since no IVF is required for *in utero* corrections, no excess embryos are generated and *in utero* delivery may avoid some delivery and efficacy issues faced by postnatal gene editing therapies. *In utero* editing is also only likely to target fetal somatic cells, and thus avoid the ethical concerns that surround germline genetic alteration.

Postnatal gene editing

Finally, prospective parents may someday be able to rely upon CRISPR's somatic therapeutic intervention for an affected child after birth. Methods to achieve somatic gene editing using CRISPR in children or adults can be divided into two categories: *ex vivo* and *in vivo* gene editing. Of the CRISPR strategies discussed here, the scientific and medical evidence suggest that *ex vivo* therapies are the most likely to become a meaningful option for intended parents in the United States in the near future.

Ex vivo gene editing involves isolating a patient's cells, editing the cells in culture, and then transfusing corrected cells back into the patient. There are several clinical trials employing CRISPR-based strategies currently registered in the United States, most of which employ *ex vivo* gene editing strategies (www.clinicaltrials.gov). Pre-clinical *ex vivo* studies are nearing clinical application for various heritable blood disorders, such as sickle cell disease and β-thalassemia.[22–24]

To achieve *in vivo* gene editing, CRISPR components must be delivered either systemically to a patient or injected directly into easily targeted tissues like the eye or skeletal muscle. *In*

vivo gene editing is limited by the ability to deliver CRISPR to target organs. Viral delivery plasmids and lipid nanoparticles are the two most commonly used delivery platforms for CRISPR-based therapeutics, each with their own advantages and limitations, reviewed elsewhere.[25] The correction of inherited blindness, muscular dystrophy, and liver disorders, all of which impact easy to target tissues, have shown promising results in animal models and are likely be the first *in vivo* CRISPR therapeutics to enter clinical trials.[26–31]

Regulatory barriers (and bans) to realizing CRISPR's promises

While the biomedical community is understandably excited about the promise of CRISPR technologies to treat or prevent transmission of heritable diseases, multiple regulatory barriers stand between the captivating technology and its widespread clinical use. Like other drugs or biologics, the FDA regulates the approval and sale of CRISPR-related drugs and therapeutics. CRISPR-based therapeutic products are categorized as biologics by the FDA and as such are subject to oversight by the Center for Biologics Evaluation (CBER)[32] and require submission of a Biologics License Application.[33] The FDA has yet to approve the use of a single CRISPR gene therapy in the United States. This includes CRISPR-somatic *ex vivo* cell therapies, arguably the least controversial CRISPR therapeutic application that is also most-similar to existing gene therapy products FDA has approved/regulates. In fact, the FDA recently put one of the first CRISPR-related clinical trials in the United States on hold until the company organizing the trial could answer some unspecified questions.[34]

As with any other drug or biologic, CRISPR-based therapeutics must undergo three phases of clinical trials to receive approval from the FDA.[35] The first phase (Phase I) tests the product for safety only and is usually administered to a small cohort of healthy adults.[4] Phase II tests the product for efficacy, includes a much larger study population of participants who stand to benefit from the therapeutic agent, and typically compares effectiveness of the product against a placebo.[28] Phase III tests the efficacy and longer-term safety of the product (often including different doses of the therapeutic), involves a study population of several hundred to a few thousand participants who stand to benefit from the therapeutic agent.[35] While Phases I and II trails may take only a few months to complete, Phase III trials are the longest and most-rigorous and typically last between one to four years.[35] While it appears that the FDA is cautious in approving CRISPR-based somatic therapeutics, their path towards approval is clear insofar as methods of proving their safety and effectiveness fit into existing phased trials paradigm.

Under current U.S. law, there is no pathway to approval for clinical applications of CRISPR germline editing. The FDA has jurisdiction over CRISPR germline editing for the purpose of producing offspring without a heritable disease since it would require implanting altered cells into a human uterus for gestation.[36] Under federal law, the FDA is prohibited from "acknowledging applications for an exemption for investigational use of a drug or biological product in research in which a human embryo is intentionally created or modified to include a heritable genetic modification."[37] In other words, even if pre-clinical data showed robust, healthy embryos created from CRISPR-edited gametes or embryos, FDA approval would be required before such embryos could be implanted for gestation, and the FDA may not even acknowledge applications for such approval, effectively banning clinical applications of CRISPR germline editing in the United States. In addition, labs working on this research may face another layer of regulation as some states have restrictive laws governing human embryo and fetal tissue research.

The U.S. congress has similarly forbidden the National Institutes of Health (NIH) or any governmental organization from funding embryonic editing research, limiting researchers to private funding,[2] and NIH-supported guidance limits the length of time a human embryo may be allowed to develop *in vitro* to fourteen days.[38] The so-called "14 day rule" may limit development of clinical applications of CRISPR germline editing since it precludes assessments of efficacy, off-target effects, or other issues at later stages of embryonic development.

Conclusion – balancing hope and reality

It is important that doctors, nurses, reproductive specialists, and genetic counselors be prepared to help their patients with heritable genetic conditions navigate the difficult choices they face in deciding if and how to become a parent. In the future, CRISPR gene editing could provide a new option to prevent heritable disease transmission, while still enabling intended parents to have a genetic connection to their offspring. Someday CRISPR gene editing techniques could, in fact, provide alternatives that do not implicate common objections or barriers patients may struggle with using current technologies, particularly those related to IVF and procuring third-party gametes. Reproductive medical professionals will likely be confronted with inquiries about how CRISPR can benefit their patients. When faced with such inquiries, care must be taken to provide consultation that is informed by potential patient objections and that creates realistic expectations according to the technologies' potential benefits, risks, and limitations.

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