Genome Editing of the Germline: Broadening the Discussion

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We read with great interest two recent commentaries discussing the potential for genome editing of the human germ line. Lanphier *et al.*[1] propose an explicit moratorium on germline genome editing, whereas Baltimore *et al.*[2] propose a more nuanced approach that is reminiscent of the discussions of recombinant DNA technology in the early 1970s.[3] Here, we attempt to further the discussion by addressing the following points: (i) the practical limitations of zygote editing; (ii) the potential utility of editing spermatogonial stem cells; (iii) the need to distinguish between editing of true germ cells, embryos, and somatic cells, and the importance of preserving editing as a transformative research tool, echoing the recommendations by Baltimore *et al.*[2]; (iv) current practices for preventing transmission of disease-causing mutations; (v) the importance of taking patient perspectives into account; (vi) the need to define the principles and/or models that underlie ethical assessments; and (vii) the importance of involving a broad spectrum of interested parties, including patient-advocacy groups, in the discussion.

Scientists have been engineering genomes since the advent of molecular biology for research purposes and now have successfully engineered genomes to treat human disease.[4] Efficient modification of the genome of somatic human cells with spatial precision, down to the single nucleotide, was first described in 1998 by Russell and Hirata, who used recombinant AAV (rAAV) vectors, and then in 2003 by Porteus and Baltimore, who used engineered nucleases.[5,6] In the past decade, the field of genome engineering has exploded with the broader use of rAAV vectors and zinc-finger nucleases and the development of powerful new platforms: TAL effector nucleases (TALENs) and CRISPR/Cas9. Genome engineering can now be achieved with unprecedented efficiency. The possibility to correct disease-causing mutations or introduce precise sequence changes in *somatic* cells has become a therapeutic reality.[7]

The recent revolution in sequencing technology has greatly improved our ability to identify genetic associations for diseases. Nonetheless, the precise genetic underpinning of many complex diseases and traits such as eye color remains unknown. With this in mind, the advent of genome-editing technology alone is insufficient to lead to applications for nontherapeutic "designer" purposes. It is likely that only diseases with clearly associated genetic causes will be addressed, each in turn, with a particular set of genome-editing tools, and those tools would need to be evaluated by stringent efficacy, safety, and ethical standards before use.

The practical limitations of zygote injections

A powerful new application of engineered nucleases is the creation of genetically modified animals by injection into fertilized zygotes.[8,9] Injection of the nuclease without a donor DNA molecule can create insertions/deletions that knock out gene function. Injection of the nucleases with a donor molecule can create precise nucleotide changes in the genome. The success of these approaches in several mammalian species[10] implied that human genomes could be similarly modified; this has been accomplished recently by Liang *et al.* in human tripronuclear zygotes that are unable to develop into viable embryos.[11]

However, several important details preclude the adoption of zygote injection in humans, irrespective of any ethical concerns. First, only a fraction of injected zygotes give rise to viable offspring. Tens to hundreds of zygotes would need to be injected and implanted into several surrogate mothers to generate viable, genetically modified offspring. This strategy is simply not possible in humans. Furthermore, the resulting offspring would be *chimeric*, with only a fraction of somatic and germ cells carrying edited genomes. In subsequent generations, only a fraction of the offspring—possibly none—would be derived from a germ-line cell that had been edited. Finally, after editing reagents are injected into zygotes, the subfraction of cells that have undergone genome editing exhibit heterogeneity in the molecular nature of the resulting alleles (insertions/deletions, homologous recombination, or both). This variegation precludes any rational prediction of the resulting phenotype of an offspring. The mouse model of muscular dystrophy is an important example of the limitation of the zygote-editing strategy, as only a fraction of offspring exhibited sufficient chimerism in the muscle tissue to mitigate the clinical impact of mutations in the dystrophin gene.[12] Although the variegation can be dealt with in experimental systems, it limits the application to humans.

Editing of spermatogonial stem cells may eliminate concerns about off-target mutations

Germ-line genome editing to correct a disease-causing mutation must not create mutations at other sites. In contrast

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to the zygote-injection strategy, editing of stem cells that can be propagated *in vitro* enables characterization of the modified stem cells before use in therapy. Spermatogonial stem cells (SSCs)[13] ultimately give rise to haploid sperm. Recent developments in animal models have shown that SSCs can be grown as clones in culture and then transplanted back into the testis to generate sperm. Thus, a potential strategy is to isolate SSCs, use genome editing to precisely correct a disease-causing mutation, perform whole-genome sequencing of clones that have undergone gene correction, and use only the clones that are free from off-target mutations. A related strategy would be to directly generate sperm *in vitro* from edited SSCs to be used for *in vitro* fertilization.

Although unintended genomic changes during genome editing should be avoided, it is also useful to consider such potential changes in the context of the ongoing genetic diversity that is spontaneously created during spermatogenesis. Meiosis creates random genetic diversity by homologous recombination. SSCs also become genetically diversified *in vivo* through the effect of environmental and endogenous mutagens during proliferation. In fact, fathers appear to pass on two additional mutations each year that they age.[14] Thus, the potential off-target changes induced by genome editing should be put into the broader context of the tremendous continuous, natural genetic diversification that is occurring in the germ line.

Distinguishing different cell types and different uses of genome editing

It is important to distinguish between editing that results in germ-line transmission and editing of somatic cells or stem cells (such as SSCs or embryonic stem cells) that can create germ cells.[15] There is no controversy over the potential of curing patients by editing of somatic cells, and it is critical that the scientific community speak with a clear voice on this application. There is less consensus on the experimental use of editing to probe the biology of human germ cells. Nonetheless, we believe that it would be a serious setback to the biomedical research endeavor to preclude the experimental use of this transformative strategy to study fundamental germ cell biology. It is also important for the scientific community to clearly distinguish between discovery-based editing of germ cells and potential applications that would create humans.

Germ-line genome editing may be needed to cure certain human genetic diseases

A critical aspect of the discussion surrounding the potential of germ-line genome editing is that it may be the only way to cure certain genetic diseases. Genetic diseases that manifest themselves in a systemic way (e.g., cystic fibrosis or genetic mitochondrial disease), in a single but widespread tissue (e.g., muscular or myotonic dystrophy), or in a tissue not easily accessible (e.g., the basal ganglia in Huntington's disease) may all be resistant to somatic cell–based genome-editing approaches. Thus, a simple ban on the approach essentially precludes the potential for certain diseases to be cured using genome editing. We believe that before such a decision is codified, a wide community, including patient-advocacy groups, must be included in the conversation and decision-making process. Although editing of mitochondrial DNA might seem fundamentally different from editing of nuclear genes, we believe that, because it would also affect the genetic makeup and phenotype of future generations, it should also be part of the conversation.

Current approaches that create future generations without genetic disease

The issue of genome editing of the germ line must also be put in the ethical context of what is already being done. In societies with a high prevalence of certain genetic diseases, social mores and laws exist to prevent the marriage of individuals at risk of having affected children. People also utilize *in vitro* fertilization followed by diagnostic screening for healthy embryos. A consequence of this procedure is that the remaining embryos are destroyed. This practice is now performed routinely to avoid pregnancy with embryos carrying particular disease-causing alleles. Finally, certain mothers/couples make the extremely difficult decision to terminate a pregnancy when they discover their embryo is destined to suffer from a devastating genetic disease. Each of these approaches has its own ethical conundrums, and the issue of editing the germ line should be put into the ethical context of approaches that are already in use throughout the world. It is possible, for example, that the ethical concerns regarding the editing of the germ line may be fewer in comparison to the ethical concerns of an alternative approach or to no action at all. Finally, it is worth noting that the laudable goal of all of these approaches, including the potential of therapeutic editing of germ cells, is to *prevent* the occurrence of disease, rather than treat it after symptoms have developed.

Defining the ethical framework in which judgments are made

Many ethical frameworks have been developed through human history, ranging from those derived from sacred texts (e.g., the New and Old Testaments, the Quran, Buddhism, the teachings of the Pope or Confucius) and from different secular lines of thought (e.g., Utilitarianism, the Golden Rule of Kant, the "veil of ignorance" of Rawls, the theory of justice of Sandel). Hence, a clear set of ethical principles must be used and defined so as to have a constructive discussion of the societal implications of genome editing of the germ line. Although it is important to consider different ethical perspectives, we believe that Rawls's "original position," based on his concept of a "veil of ignorance," is a powerful approach—he asks us to imagine what world we would want if we did not know what traits, abilities, or social status we would have in that world. This framework would support the idea that exploration of genome editing of the germ line is ethically permissible as it would allow people who were born with

an expanded triplet repeat in the Huntingtin gene, for example, to reproduce without the 50% risk of passing along this incurable neurologic disease to their children. Countervailing this ethical argument is one that acknowledges the malignant uses of eugenics through human history and the fear that therapeutic editing of the human germ line might facilitate revival of this dark past.

Including the broadest community in the discussion

It is natural that societies rely on people whom they trust and consider knowledgeable to inform policy decisions. However, as a scientific community, we must be careful to avoid relying on self-appointed experts or small, invitation-only groups to make decisions that have far-reaching societal implications. We support the call of Baltimore and colleagues' commentary to convene a conference similar to the one in 1975 at Asilomar, which discussed the implications of recombinant DNA technology.[3] It is unlikely, however, that any single conference at a small venue such as Asilomar would accommodate all the relevant and interested parties. The issue of germ-line editing of the genome must be discussed in a series of events in a variety of forums. The American Society of Gene and Cell Therapy (ASGCT), the European Society of Gene and Cell Therapy (ESGCT) and the Japanese Society of Gene Therapy (JSGT) are important organizations to facilitate and lead these discussions. In this way, the broadest array of voices could be heard over time to allow the evolution of different thought processes and ethical considerations. Finally, it is important that the process not be controlled by the loudest or by the most politically powerful and well connected. Instead, it must include those most likely to be impacted by the discussion, such as children who might be born with devastating, multisystemic, inexorable diseases.

Summary

Genome editing that results in humans with precisely modified germ cells may never become practical. Nonetheless, the implications are great enough that we strongly support the idea of starting the conversation now, providing time for a broad consensus to be developed. We are confident that if diverse voices are heard, a consensus can be reached on a strategy in which societal mores are respected, the desires of parents are integrated, and the health of future generations is maximized.

DISCLAIMER

The Editors note that the views expressed in this unsolicited Commentary are those of the authors and do not necessarily represent the official position of the journal, its editors, or the ASGCT.

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Arresting the Colonial Destiny of Metastatic Seeds with DNA Aptamers

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In recent years, several studies have identified a class of carbohydrate-binding proteins known as (endothelial) E- and (platelet) P-selectins as potential therapeutic targets for inhibiting hematogenous tumor metastasis, owing to their critical role in the metastatic process. Selectins enable tethering and rolling of circulating tumor cells or cancer stem cells (CTC/CSCs) to the postcapillary venules, promoting their transmigration through the endothelium and subsequent homing to metastatic sites. In this issue of *Molecular Therapy,* Kang *et al.* describe the preclinical characterization of a nucleic acid DNA aptamer to E-selectin as a safe and effective therapeutic option for preventing this process and inhibiting breast cancer metastasis.[1]

One of the causes of cancer recurrence and therapy failure is the ability of cancer cells to leave their local surroundings and

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