A prudent path forward for genomic engineering and germline gene modification

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A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed

Genome engineering technology offers unparalleled potential for modifying human and nonhuman genomes. In humans, it holds the promise of curing genetic disease, while in other organisms it provides methods to reshape the biosphere for the benefit of the environment and human societies. However, with such enormous opportunities come unknown risks to human health and well-being. In January, a group of interested stakeholders met in Napa, California (*1*), to discuss the scientific, medical, legal, and ethical implications of these new prospects for genome biology. The goal was to initiate an informed discussion of the uses of genome engineering technology, and to identify those areas where action is essential to prepare for future developments. The meeting identified immediate steps to take toward ensuring that the application of genome engineering technology is performed safely and ethically.

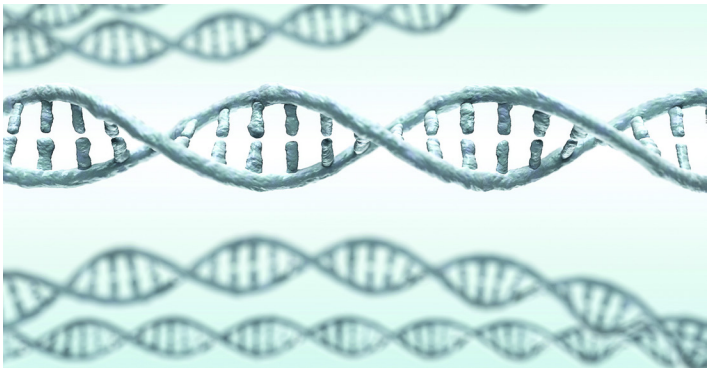
The promise of so-called "precision medicine" is propelled in part by synergies between two powerful technologies: DNA sequencing and genome engineering. Advances in DNA sequencing capabilities and genome-wide association studies have provided critical information about the genetic changes that influence the development of disease. In the past, without the means to make specific and efficient modifications to a genome, the ability to act on this information was limited. However, this limitation has been upended by the rapid development and widespread adoption of a simple, inexpensive, and remarkably effective genome engineering method known as clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 (*2*). Building on predecessor platforms, a rapidly expanding family of CRISPR-Cas9–derived technologies is revolutionizing the fields of genetics and molecular biology as researchers employ these methods to change DNA sequences—by introducing or correcting genetic mutations—in a wide variety of cells and organisms.

CURRENT APPLICATIONS. The simplicity of the CRISPR-Cas9 system allows any researcher with knowledge of molecular biology to modify genomes, making feasible experiments that were previously difficult or impossible to conduct. For example, the CRISPR-Cas9 system enables introduction of DNA sequence changes that correct genetic defects in whole animals, such as replacing a mutated gene underlying liver-based metabolic disease in a mouse model (*3*). The technique also allows DNA sequence changes in pluripotent embryonic stem cells (*4*) that can then be cultured to produce specific tissues, such as cardiomyocytes or neurons (*5*). Such studies are laying the groundwork for refined approaches that could eventually treat human disease. CRISPR-Cas9 technology can also be used to replicate precisely the genetic basis for human diseases in model organisms, leading to unprecedented insights into previously enigmatic disorders.

In addition to facilitating changes in differentiated somatic cells of animals and plants, CRISPR-Cas9 technology as well as other genome engineering methods can be used to change the DNA in the nuclei of reproductive cells that transmit information from one generation to the next (an organism's "germ line"). Thus, it is now possible to carry out

genome modification in fertilized animal eggs or embryos, thereby altering the genetic makeup of every differentiated cell in an organism and so ensuring that the changes will be passed on to the organism's progeny. Humans are no exception—changes to the human germ line could be made using this simple and widely available technology.

MOVING FORWARD. Given these rapid developments, it would be wise to begin a discussion that bridges the research community, relevant industries, medical centers, regulatory bodies, and the public to explore responsible uses of this technology. To initiate this conversation, developers and users of the CRISPR-Cas9 technology, and experts in genetics, law, and bioethics, discussed the implications and rapid expansion of the genome engineering field (*1*). This group, all from the United States, and which included some of the leaders in the original 1970s discussions about recombinant DNA research at Asilomar and elsewhere, focused on the issue of human germline engineering, as the methods have already been demonstrated in mice (*6*) and monkeys (*7*). The Napa discussion did not address mitochondrial transfer (*8*, *9*), a technique that does not use CRISPR-Cas9. Although characterized by some as another form of "germline" engineering, mitochondrial transfer raises different issues and has already been approved by the Human Fertilisation and Embryology Authority and by Parliament in the United Kingdom (*10*) and is being considered by the Institute of Medicine and the Food and Drug Administration in the United States (*11*). At the Napa meeting, "genome modification" and "germline engineering" referred to changes in the DNA of the nucleus of a germ cell.



The possibility of human germline engineering has long been a source of excitement and unease among the general public, especially in light of concerns about initiating a "slippery slope" from disease-curing applications toward uses with less compelling or even troubling implications. Assuming the safety and efficacy of the technology can be ensured, a key point of discussion is whether the treatment or cure of severe diseases in humans would be a responsible use of genome engineering, and if so, under what circumstances. For example, would it be appropriate to use the technology to change a disease-causing genetic mutation to a sequence more typical among healthy people? Even this seemingly straightforward scenario raises serious concerns, including the potential for unintended consequences of heritable germline modifications, because there are limits to our knowledge of human genetics, gene-environment interactions, and the pathways of disease (including the interplay between one disease and other conditions or diseases in the same patient). In the United States, such human research currently would require an Investigational New Drug exemption from the Food and Drug Administration, but value judgments about the balance between actions in the present and consequences in the future need deeper consideration of the ethical implications of human germline genome editing than the Investigational New Drug process provides.

RECOMMENDATIONS. To better inform future public conversations recommended by the Napa meeting, research is needed to understand and manage risks arising from the use of the CRISPR-Cas9 technology. Considerations include the possibility of off-target alterations, as well as on-target events that have unintended consequences. It is critical to implement appropriate and standardized benchmarking methods to determine the frequency of off-target effects and to assess the physiology of cells and tissues that have undergone genome editing. At present, the potential safety and efficacy issues arising from the use of this technology must be thoroughly investigated and understood before any attempts at human engineering are sanctioned, if ever, for clinical testing. As with any therapeutic strategy, higher risks can be tolerated when the reward of success is high, but such risks also demand higher confidence in their likely efficacy. And, for countries whose regulatory agencies focus on safety and efficacy but not on broader social and ethical concerns, another venue is needed to facilitate public conversation.

Given the speed with which the genome engineering field is evolving, the Napa meeting concluded that there is an urgent need for open discussion of the merits and risks of human genome modification by a broad cohort of scientists, clinicians, social scientists, the general public, and relevant public entities and interest groups.

In the near term, we recommend that steps be taken to:

1) Strongly discourage, even in those countries with lax jurisdictions where it might be permitted, any attempts at germline genome modification for clinical application in humans, while societal, environmental, and ethical implications of such activity are discussed among scientific and governmental organizations. (In countries with a highly developed bioscience capacity, germline genome modification in humans is currently illegal or tightly regulated.) This will enable pathways to responsible uses of this technology, if any, to be identified.

2) Create forums in which experts from the scientific and bioethics communities can provide information and education about this new era of human biology, the issues accom-

panying the risks and rewards of using such powerful technology for a wide variety of applications including the potential to treat or cure human genetic disease, and the attendant ethical, social, and legal implications of genome modification.

3) Encourage and support transparent research to evaluate the efficacy and specificity of CRISPR-Cas9 genome engineering technology in human and nonhuman model systems relevant to its potential applications for germline gene therapy. Such research is essential to inform deliberations about what clinical applications, if any, might in the future be deemed permissible.

4) Convene a globally representative group of developers and users of genome engineering technology and experts in genetics, law, and bioethics, as well as members of the scientific community, the public, and relevant government agencies and interest groups—to further consider these important issues, and where appropriate, recommend policies.

CONCLUSIONS. At the dawn of the recombinant DNA era, the most important lesson learned was that public trust in science ultimately begins with and requires ongoing transparency and open discussion. That lesson is amplified today with the emergence of CRISPR-Cas9 technology and the imminent prospects for genome engineering. Initiating these fascinating and challenging discussions now will optimize the decisions society will make at the advent of a new era in biology and genetics..

REFERENCES AND NOTES

1. IGI Forum on Bioethics, Napa, California; this meeting was sponsored by the Innovative Genomics Initiative at the University of California, Berkeley, and the University of California, San Francisco, on 24 January 2015; all the authors, except for GC and MJ, participated in the meeting.
2. J. A. Doudna, E. Charpentier, *Science* **346**, 1258096 (2014). Medline doi:10.1126/science.1258096
3. H. Yin *et al.*, *Nat. Biotechnol.* **32**, 551 (2014). Medline doi:10.1038/nbt.2884
4. P. Mali *et al.*, *Science* **339**, 823 (2013). Medline doi:10.1126/science.1232033
5. Z. Zhu, F. González, D. Huangfu, *Methods Enzymol.* **546**, 215 (2014). Medline doi:10.1016/B978-0-12-801185-0.00011-8
6. H. Wang *et al.*, *Cell* **153**, 910 (2013). Medline doi:10.1016/j.cell.2013.04.025
7. Y. Niu *et al.*, *Cell* **156**, 836 (2014). Medline doi:10.1016/j.cell.2014.01.027
8. http://www.hfea.gov.uk/8807.html
9. M. Tachibana *et al.*, *Nature* **493**, 627 (2013). Medline doi:10.1038/nature11647
10. The Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015; www.legislation.gov.uk/uksi/2015/572/contents/made (effective 29 October 2015).
11. U.S. Food and Drug Administration, Cellular, Tissue, and Gene Therapies Advisory Committee Meeting: Announcement, www.fda.gov/AdvisoryCommittees/Calendar/ucm380042.htm; www.iom.edu/activities/research/mitoethics.aspx.

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