Cornell University

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Responsible Conduct of Research (RCR) Symposium: Ethical Considerations about Genome Editing
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Principles of Responsible Conduct of Research (RCR) and format of symposium

All decision making in life involves ethical choices. In research, ethics can be categorized broadly into these nine areas: *research misconduct*, *authorship*, *peer review*, *collaborative research*, *human subjects research*, *use of live vertebrate animals*, *conflicts of interest, data management*, and *mentoring.* Federal agencies such as the NIH and NSF have specific requirements for RCR training that apply to students supported by government funds. At Cornell, the Office of Research Integrity and Assurance supports efforts to meet the government's requirements regarding RCR through various online trainings, symposia, workshops, and other events. To that end, this yearly January symposium has covered topics such as research misconduct, unauthorized use of blood samples, authorship, rigor and reproducibility in research, industry funding of academic research, and data sharing. The central features of these symposia have been an introductory talk, followed by small group discussions of hypothetical cases involving ethics in research, and a panel discussion to address questions that were raised in the groups.

Introduction to genome editing

In the past few years, a remarkably rapid and profound technological revolution has occurred, that makes it possible – easily and inexpensively – to create specific mutations at any desired site in genomes of diverse cells, including those of plants and animals (although the method is not perfect and can cause "off-target" mutations -- see summary in Appendix 2). In many cases it is straightforward, at least in principle, to make these mutations in the germline, and thus to create organisms, and from them, populations with such desired mutations. This new technology for genome editing is usually referred to as "CRISPR" or "CRISPR/Cas9," acronyms derived from the bacterial system from which this technology was developed. These technological advances raise ethical concerns, which are the topic of this symposium.

When considering organisms, it is important to distinguish mutations in somatic cells from mutations in germ cells. The former are used to study the function of genes in different tissues (for example in mice), and perhaps to ameliorate or even cure the symptoms of genetic defects. By contrast, the latter could become a permanent feature of a population of organisms.

As it applies to humans, consent for genome editing is fundamentally different between somatic and germline changes. For somatic editing, the individual whose DNA is to be changed presumably can decide if they are willing to accept the risks associated with unintended consequences, such as unanticipated harmful side effects or mutations in other places in the genome (so-called, "off target edits"). For germline editing, alterations (including off target edits) are passed on to future generations.

CASE STUDIES

The fundamental issues addressed in the examples below are:

1. What dangers might genome editing pose for animals or people?
2. How should we weigh possible dangers against the possible benefits?

Case 1: Genome editing in dogs

[Scenario 1: Somatic cell editing]

In humans, Duchenne muscular dystrophy (DMD) is a devastating X-linked human disease. DMD is caused by any of several different mutations in the dystrophin protein. A mutation similar to one of the known human DMD mutations, and with similar phenotype, is found in some beagles, making these dogs a model for the human disease. Recently a group reported [1] results from a preliminary attempt to correct the beagle dystrophin gene using CRISPR/Cas9, which in this case was introduced into one-month old dogs by injecting a non-pathogenic virus (AAV) engineered to carry the CRISPR/Cas9 module. The four dogs used in this study appeared to show major improvement of function.

Questions:

- Is this a justified use of dogs for research? Why or why not?
- What kind of regulatory approval would this lab need in order to carry out the experiments?
- What kind of evidence should be required to assure that risk of possible harmful effects of such editing is acceptable? And to whom does it need to be acceptable?

[Scenario 2: Germline editing (ex. 1)]

Suppose in the near future CRISPR/Cas9 can be used to edit the same canine gene in the beagle model as above, allowing creation of a population of dogs with the natural sequence of the canine DMD gene restored. Suppose further that these dogs are "cured" of canine DMD.

Questions:

- Other than demonstrating the technology, what would the results of this germline editing demonstrate beyond what could be shown in scenario 1, above?

[Scenario 3: Germline editing (ex. 2)]

(Example contributed by John Parker)

Originally from China, the Shar-Pei is a breed of dogs with small eyes, a large muzzle, and abundant folds of loose, overly thick skin about the head, neck, and shoulders (see picture). This phenotype appears to be due to over-expression of the Hyaluronic Acid Synthase 2 (HAS2) gene, which is involved in the synthesis of the extracellular polysaccharide hyaluronan, a major component of skin. The overexpression phenotype does not have a high penetrance, perhaps because the gene duplication is not very stable. The strange-looking bunches of skin folds is a characteristic probably continually selected by breeders, since this is a defining characteristic of the breed and thus is viewed as desirable. Another result of over-expression of HAS2 is distinctly negative, namely a recurring illness characterized by periodic fever syndrome.

Suppose a research group at a university decides to edit the germline of Shar-Pei dogs to lead to more permanent overexpression of HAS2, in effect enhancing the breed characteristics and allowing the easier breeding of the monetarily valuable Shar-Pei dogs.

Questions:

- Is this a justified use of dogs in research?
- Why should a university proposal to do this be approved or not approved by an IACUC?
- What if a commercial dog breeder were to undertake this germline editing? Could the breeder be prevented from doing this?
- Is germline editing to "improve" an animal species the same or different from simple breeding, as humans have done with animals and plants of all sorts for thousands of years?

Case 2: Genome editing in humans

[Scenario 1: Somatic cell editing]
A very small percentage of humans are naturally resistant to HIV infection, because they have a mutation in the gene encoding the HIV "co-receptor" that is found on the surface of some lymphocytes and macrophages in the immune system. The gene frequency of this mutation varies among populations. The mutation is not associated with any obvious deleterious phenotype.

At present it is not possible to "cure" a person who has been infected with HIV, but only to control spread of the virus in the body with a daily cocktail of antiviral drugs. HIV being a retrovirus, a copy of the viral genome resides in all of the infected cells, which in this case are mostly T cells of the immune system.

CRISPR/Cas9 in principle can be used to destroy or remove the viral DNA integrated into a chromosome, and also to re-create the natural mutation in the receptor that makes humans resistant to infection. In one recent report, both mutations were made simultaneously in immune cells taken from an infected patient, cultured and edited in cell culture [principles reviewed in 2]. These cells in principle could be infused back into the patient—analogous to what is done in treatment of some cancers like multiple myeloma, where stem cells from a patient are taken before patient irradiation or chemotherapy, and then re-introduced into the body where they can repopulate the bone marrow. In the hypothetical HIV case, after the DNA in the cells had been successfully edited, those cells would no longer have the capacity to give rise to live HIV virus and would be resistant to infection from external HIV virus. If sufficient numbers of these cells were growing in the patient, that patient would be "cured", i.e. spared the otherwise inevitable result of HIV-induced killing of all of the T4 cells.

Questions:

- How would one weigh the risks against the benefits of this genome editing? Making this judgment requires understanding of other possible treatments. Who would decide whether this treatment could proceed?
- In a technologically advanced country, given the expense of this treatment, how would it be decided who will receive the treatment? Who should bear the costs of such treatment?

[Scenario 2: Germline editing]
A recent note in Nature (see summary in [3]) describes the plans of a Russian scientist to use CRISPR to edit early human embryos obtained from an in vitro fertilization clinic, to correct a known genetic defect leading to deafness. A husband and wife, who are both deaf and carry the defective gene, were interviewed and are interested in proceeding with this genome editing, once it is approved by the appropriate ethics committee; they would like to have children who are not deaf.

Questions:

- If the deaf couple really wanted to proceed after being informed of the risks, who would have to approve this medical procedure?
- Is it within the parent's right to decide to change the germ line DNA of their offspring?
- Who could be held responsible if the editing failed to work as predicted? What would "responsibility' entail? Cost of the initial procedure? Cost of subsequent treatment for "pain and suffering" for the parents, or the child?

[Scenario 3: Futuristic germline editing]
Suppose that comparative genomic studies published in 2026 have led to the identification of genes that are major players in a measure of physical endurance physiologists call VO2max (basically the maximum rate of oxygen absorption by muscles). Individuals with unusually high VO2max have a clear advantage in many Olympic sports, especially events like bicycle racing. In a comprehensive genome analysis of 1000 individuals of exceptional VO2max, of the ~20,000 human genes, three stood out as possibly playing a role in the exceptional nature of this selected population, with most individuals carrying the same signature mutations in these genes that were not seen in the controls. As might be expected, the three genes all are involved in the energy metabolism of muscle cells.

A modern, high-tech clinic in the otherwise "underdeveloped" country of "Lilliput" does CRISPR-mediated human germline editing. Using embryos from their in vitro fertilization facility, they guarantee "correct" editing of any gene. For $1M for each gene, they will generate an implantable embryo with any desired edited sequence. A number of wealthy couples are standing in line to have their predicted super-athlete babies.

Questions:

- What are the essential differences between this scenario and scenario 2 above?
- Who should make decisions on this type of human genome editing?
- In many countries, women can elect to have an abortion quite late, for example if the fetus has a genetic defect. Similarly, one might argue that women should have the right to "improve" the genetic makeup of babies that they carry. Should cases of "cosmetic" edits be handled differently from those preventing genetic defects such as a disease leading to inevitable early death in infancy? If yes, who should make such decisions?

Appendix 1: References

General References

For recent human germ line editing scandal: https://www.nature.com/articles/d41586-019-00673-1

For recent review of human germ line editing including ethics: https://www.nature.com/articles/s41556-019-0424-0.pdf?draft=collection

For WHO reports on regulating human germ line editing:

1. https://www.who.int/ethics/topics/human-genome-editing/GenomeEditing-Report-2nd-Meeting-August_FINAL.pdf?ua=1
2. https://www.who.int/ethics/topics/human-genome-editing/en/

Citations in the text above:

1. DMD beagle somatic cell editing: https://science.sciencemag.org/content/362/6410/86.long
2. HIV receptor and provirus editing in humans, reviewed in Das et al, Curr Opin Virol 2019: https://doi.org/10.1016/j.coviro.2019.07.001
3. Deaf gene editing plans: https://www.nature.com/articles/d41586-019-03018-0

Appendix 2: Simplified Principles of Genome Editing

For eukaryotic genomes, ablating ("knocking out") or modifying (sometimes called "knocking in") genes, or introducing totally new genes ("transgenes"), are techniques first developed for yeast cells in the late 1970s (pioneered here at Cornell!). Some 20 years later it became possible to create mice with specific genome modifications (Nobel prize 2007), starting with cultured mouse embryonic stem cells whose genomes were modified. Embryonic stem cells can be inserted into early mouse embryos, which then can lead to mouse strains, for example with a particular gene knocked out in all tissues or only a single tissue. Depending on the gene, these mice may be totally normal, or may have different properties (for example have a higher incidence of cancer or be otherwise compromised), or be unviable (embryonic lethal). However, creating genetically modified mice is expensive and sometimes challenging, and similar experiments are much more difficult or impossible with other animal species.

In the past few years a technological revolution has occurred. It has become comparatively easy to accurately make specific modifications in genomes of diverse cells, including those of lower eukaryotic organisms, plants, and animals. All that is required is to introduce DNA into the target cells (often called "transfection"), which is usually not difficult to arrange. The tool for genome editing is based on a bacterial system that acts as a protection against invading bacterial viruses. In the slightly modified form used for purposes of genome editing, this system (typically called by the acronym "CRISPR/Cas9") comprises two parts. One is an endonuclease protein (Cas9) that cleaves DNA. The other is an RNA ("guide RNA") that is designed to be identical to a short stretch of the desired target sequence in the genome. When DNA coding for the endonuclease and for the guide RNA is introduced into a cell, the resulting nuclease will use the guide RNA to search and find the target sequence (which for a mammal would comprise less than 1/10,000,000 of the DNA!), and then to cleave it. The double strand break can lead to destruction of that gene ("non-homologous end joining", NHEJ; Figure 1A). Or if another DNA of identical or nearly identical sequence also is present in the cells (e.g. on an engineered DNA introduced into the target cells) the break can be repaired (called "gene conversion" or "template mediated repair"). Repair occurs by copying and insertion of the similar sequence (called the donor) at the break site (Figure 1B). Thus in summary, with CRISPR technology, any desired DNA sequence can be inserted at the desired site in a cell, and any sequence in a genome can be modified in any way that is desired.

Diagrams: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4022601/pdf/nihms-581755.pdf