Analyzing the Safety and Feasibility of Human Germline Editing Using CRISPR-Cas9 Systems

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The focus of our group for this project is genetic engineering in human embryos, or human germline editing. Our research question following this topic is "To what extent should CRISPR-Cas9 be limited when genetically editing the human germline?" CRISPR-Cas9, or clustered regularly interspaced short palindromic repeats and associated protein 9, is a system modified from a bacterial immune system that can be used to find and insert, delete, or edit any piece of DNA. This technology was discovered/developed in 2015 and is much more efficient, easy to use, and inexpensive than past technologies such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Because CRISPR-Cas9 is such an improvement over what already existed, it has opened many new possibilities that would have been much more difficult before. One of these possibilities, among others, is human germline editing.

Human germline editing is any process that changes the DNA of a germ (or reproductive) cell to be passed down to the next and following generations. This does not include genetic edits in somatic cells that would only affect some parts of an individual and would not be passed down to the next generation. We chose this topic because it is relatively specific, compared to gene editing in general, but still has a broad range of perspectives. Despite recent attempts to reach it, there is no broad consensus about human germline editing, within both the scientific community and the wider public. However, because CRISPR-Cas9 is so accessible, a consensus needs to be met.

The debates surrounding human germline editing have many different facets including ethical, social, economic, political, historical, and scientific. This paper will focus on the scientific side, because if human germline editing is not possible scientifically, the rest of the debates are pointless. As such, this paper will analyze the safety and feasibility of human germline editing, the main scientific issues.

Currently, there are three main safety concerns regarding our current practices for germline editing: mosaicism, off-target changes, and side effects of the new gene. Mosaicism is when the cells in an embryo are not uniformly edited, with a mix of "fixed" and unchanged cells. This usually occurs when CRISPR-Cas9 does not act quickly enough and the cells start to split before the DNA has been edited. Off-target changes are edits in the DNA at places other than the intended target. CRISPR-Cas9 is not perfect and makes mistakes when searching for the correct set of base pairs. Side effects of the new gene can occur when we don't know all of the functions

of a gene. Many genes have multiple functions and it is nearly impossible to know what will happen exactly when a gene is changed.

The first two, mosaicism and off-target changes, were evident in the three studies that attempted to edit genes in embryos. The first two, done in China in 2015 and 2016, used tripronuclear embryos (which are nonviable), to avoid the ethical debate surrounding the use of viable embryos. Besides being nonviable, tripronuclear embryos are suitable stand-ins for regular embryos. The results were only partially successful. Mosaicism was prevalent and off-target mutations were present. (Kang et al., 2016; Liang et al., 2015) These results indicate that while CRISPR-Cas9 is much better than previous technologies, the technology itself and the practices for its use both need to be much improved in order for it to be safe for human germline editing. The latest study, conducted in 2017, had better results because CRISPR-Cas9 was added at the same time as the sperm, instead of after fertilization, resulting in less prevalent mosaicism. In addition, no off-target changes were found. (Ma et al., 2017) This shows that the technology and practices are improving and that eventually mosaicism and off-target changes can be brought down to a minimum. These three studies were all published in peer-reviewed academic journals and seemed to follow good experiment procedures. They are all controversial, but this is largely due to the controversy that surrounds human germline editing and research with embryos in general, not the studies in particular.

A speech given at the International Summit on Human Gene Editing in 2015, elaborates on the third issue. Eric Lander, an expert in the human genome who even played an important role in the Human Genome Project, spoke about the complexity of disease and its relationship with human genetics. The genetic root behind a disease can be very difficult to find, requiring massive amounts of data, and even when a gene is known to be connected, exactly how the connection works is usually not known. Additionally, with polygenic diseases, most of the genes only have a small impact in risk of contracting the disease. Mendelian diseases, diseases that are caused by a single mutation, would be much easier to prevent, but even then many genes have multiple purposes. A gene that makes someone more vulnerable to one disease might protect them from another. (Lander, 2015) Obviously, there is a lot about genetics and disease that remains unknown and while this knowledge gap remains, we won’t be able to tell exactly what will happen when we change a gene.

According to one group of scientists, this unpredictability means that human germline editing is not worth it. They called for a voluntary moratorium against human germline editing due to its lack of safety. They also argued that any therapy offered by human germline editing could be achieved using other techniques, so it was not worth the risks. If anyone did move forward with it and something went wrong, somatic gene therapy might be associated with germline editing and be negatively affected by any negative public reaction. (Lanphier, Urnov, Haecker, Werner, & Smolenski, 2015) This would definitely harm the authors of the article, as all of them are involved with somatic gene therapy. Given their involvement in this field it should be kept in mind that they would also stand to benefit from a ban of human germline editing as it would most likely be competition to somatic gene therapy. So it stands to reason that they would be biased against human germline editing. Nevertheless, their position is valid.

The organizing committee of the International Summit on Human Gene Editing took a different position however. In their statement, they stated that basic and preclinical research is needed and that studies using human embryos were acceptable, as long as the embryos did not result in a pregnancy. Instead of warning off human germline editing entirely, they approved of moving with forward with proper precautions. The key safety issues mentioned before would need to be solved and the safety of the human germline editing would need to be demonstrated before it would be available for clinical use (including both clinical research and therapy). (Organizing Committee for the International Summit on Human Gene Editing, 2015) The organizing committee was made up of experts in the field and was obviously more open minded about human germline editing. They looked at the issue from multiple lenses and perspectives throughout the summit and based the statement off of the cases made at the summit. The summit was sponsored by the U.S. National Academy of Sciences, the U.S. National Academy of Medicine, the Royal Society, and the Chinese Academy of Sciences, all authorities on science and medicine.

Human germline editing using CRISPR-Cas9 is not yet safe enough to be feasible for clinical use, which is why further research is required. With more research and improvement on the technology and practices, the safety of the human germline editing could improve to be safe enough for clinical use, if ethical, social, and other debates are resolved.

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References

Kang, X., He, W., Huang, Y., Yu, Q., Chen, Y., Gao, X., … Fan, Y. (2016) Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. *Journal of Assisted Reproduction and Genetics, 33*(5), 581-588. https://doi.org/10.1007/s10815-016-0710-8

Lander, E. S. (2015) What we don't know. In *International Summit on Human Gene Editing: A Global Discussion Commissioned Papers*. Retrieved from http://nationalacademies.org/cs/groups/pgasite/documents/webpage/pga_170455.pdf

Lanphier, E., Urnov, F., Haecker, S. E., Werner, M., Smolenski, J. (2015). Don't edit the human germ line. *Nature, 519*(7544), 410-411. http://doi.org/10.1038/519410a

Liang, P., Xu, Y., Zhang, X., Chenhui, D., Huang, R., Zhang, Z., … Huang, J. (2015).CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes. *Protein & Cell, 6*(5), 363–372. https://doi.org/10.1007/s13238-015-0153-5

Ma, H., Marti-Gutierrez, N., Park, S., Wu, J., Lee, Y., Suzuki, K., … Mitalipov, S. (2017). Correction of a pathogenic gene mutation in human embryos. *Nature, 458,* 413–419. http://doi.org/10.1038/nature23305

Organizing Committee for the International Summit on Human Gene Editing. (2015, December 3). *On Human Gene Editing: International Summit Statement*. Retrieved from http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=12032015a