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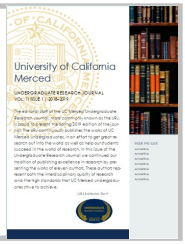

CRISPR V Culture

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New gene editing technologies give us the potential ability to bring back extinct species, help control the spread of invasive ones, and genetically modify those that spread diseases. They allow us to not only influence the evolutionary path of entire species, but entire ecosystems as well. In addition, gene editing has the potential to help us live healthier and longer lives. We have moved past rudimentary methods of DNA manipulation and can now remove individual genes from a strand of DNA. However, due to the complexity of this technology, and given that there are few who can use it to its full effect, people have largely failed to respond to its development, particularly regulators. It is not within the scope of this paper to explore the full implications of these various emerging technologies. Instead I will focus on CRISPR, a specific revolutionary gene editing technology first used in 2012, and the major developments that have taken place since then.

The first major development to consider is the use of CRISPR gene editing to alter the germline of mosquitos. The first instance for germline gene editing in a research study was undertaken in 2015 by a team at UC Irvine. The goal of this team was to use aggressive genetic engineering tactics to limit the ability of mosquitoes to act as a vector for malaria.[1] This research demonstrates that, when working in harmony with other more established gene editing techniques, CRISPR allows us to edit the genome of an entire population over the course of

just a few years. This is a scary fact. The trajectory of this line of research could lead to the genetic modification of entire species, possibly impacting our ecosystem in unknown ways. Nonetheless, this is most likely the first way germline gene editing will be used in practical application.

The methodology employed at UC Irvine was standard for most studies involving the raising and genetic modification of mosquitoes in the lab environment.[1,2] While the various genetic and biological systems at play are incredibly complex, the actual lab procedure itself is deceptively simple.[1] The researchers injected DNA containing their designed CRISPR-Cas9 complex and malaria antibody producing genes into the zygotes of a little more than 300 mosquitos. Embedded in this injected DNA sequence was an indicator that turned the progeny's eyes red if the desired genes were picked up and expressed. The mosquitos that had red eyes were then mated with normal non-genetically modified mosquitoes. It was shown that the resulting progeny overwhelmingly retained the malaria resistant genes from their parent—around 96% of them. The biologically correct term is to say that these gene drive traits, which have a greater than 50% chance of being passed on, exhibit greater than Mendelian or "super-Mendelian" inheritance. It is this super-Mendelian inheritance that makes these findings so impactful within the scientific and

1 See Vocabulary Appendix

medical community.

This is not the only example of a gene drive system being used to modify the genome of mosquitos for disease prevention purposes. A more recent study by researchers from Imperial College in the United Kingdom also considered a method for limiting the spread of malaria through the release of genetically modified mosquitoes into the wild.[2] There is a major difference, however, between the underlying approach of the UC Irvine research and the UK research. In the UC study the goal was to give mosquitoes a genetic resistance to the malaria infection, so they would be less likely to spread it to humans. In the UK study, genetic changes were made to simply increase the sterility rate among female mosquitoes, thus lowering the overall population of mosquitoes and lowering their disease vector capability. This approach, if implemented in the wild, would act like a hammer, as opposed to the approach taken in the UC study which would act more like a scalpel.

However, this hammer approach is not without merit. The central idea employed by the UK paper is simpler and more robust, making it easier to execute. The way in which mutagenesis was achieved in the UK research is also far more reliable, because it mimics the behavior of Homing Endonucleases, which are a type of protein expressed in the intronic regions of DNA famous for their prevalence throughout the genome of many organisms.[2]

Homing Endonucleases have the ability to copy themselves from one chromosome in a homologous pair to the other chromosome in the pair, meaning that they are guaranteed to be passed on to the progeny of a modified individual. In addition, the method employed in this study was quite simply trying to achieve less. The goal of the UC team was to implant three completely new genes into the genome of a mosquito, whereas the goal of the UK team was only to disrupt one gene that is already present in the mosquito's genome. This simplicity leads to fidelity, and they were consequently able to guarantee their mutations were passed on to the next generation 99.6% of the time.[2]

It should be noted that combating malaria by limiting a mosquito's ability to spread the disease, either through eradication or genetic modification, is not a new concept. The use of the pesticide DDT[2] is a famous, or rather infamous, example of an attempt to limit the spread of disease through the eradication of mosquitoes, which had disastrous results for both humans and wildlife (though it should be noted DDT is still used in disease prevention efforts). The release of genetically modified mosquitoes had proven to be a non-viable option in the past, because the methods for passing down the genetic modifications were either temperamental or took place over such a long time frame as to be ineffectual. With these two new CRISPR based, highly aggressive, gene-drive

2 DDT: an organochlorine used as an insecticide to combat the spread of malaria. It was promoted as an agricultural and household pesticide by the US government, but was ultimately found to have horrible effects on the environment and cause cancer.

systems genetic changes can be propagated throughout an entire species over the course of just a few years.[1,2]

As a scientist I am thrilled by the implications of these findings; but as a human they terrify me. Because while these aggressive gene-drive systems aren't going to be used on the human genome any time soon, scientists are already beginning to alter human DNA.

One of the most recent studies done concerning human gene editing was by Shoukhrat Mitalipov, a reproductive biologist at Oregon Health and Science University (OHSU). Mitalipov and his colleagues used CRISPR in human embryos to repair a mutation that causes heart disease.[3] Another recent study, done in September of 2017 by Kathy Niakan at the Francis Crick Institute in London, used CRISPR in human embryos to better understand human embryo-logical development.[4] While these two studies may seem similar in method they differ in purpose. While Niakan's research uses gene editing in a purely exploratory way to better understand embryonic development, the aim of the Oregon research is to use gene editing as a treatment for genetic diseases. It should be noted that the zygotes in these studies were not allowed to mature past 14 days, but both studies still raise questions for those who are concerned about human gene editing.

For many bioethicists, the main concern is how the genetic material is acquired—where and how the zygotes are made or harvested. Some bioethicists view the usage of eggs left over from IVF facilities as more ethical than harvesting eggs specifically for research purposes. In the case of the Niakan paper, leftover embryos from IVF clinics were utilized. In the case of the Oregon research however, eggs had to be specially harvested and fertilized because they were researching a genetic disease, and had to ensure the zygotes they were studying carried it.[5] Philosophers, researchers, and research organizations, on the other hand, are less concerned with where the genetic material comes from, and more concerned about what is done to it and why. Most within the regulatory and ethics community view the Niakan category of research, done purely to further the scientific body of knowledge, as harmless or low-stakes from a moral standpoint.[5] Research exploring gene modification as a treatment for specific diseases is more controversial. There are many researchers, philosophers, and even evolutionary biologists who think our gene modification abilities, and our knowledge of the human genome in general, are nowhere near ready to be used as treatments for diseases.

Just because there are those who think artificial gene editing shouldn't be used on humans doesn't mean that it hasn't been already. The first successful attempt to modify the DNA of a human embryo was completed in April of 2016

and resulted in the birth of a baby boy.[6] The boy's parents are a Jordanian American couple (referred to individually as Jane and John) who had been trying to have children for 20 years. Over that time, they had four miscarriages and only two children that survived past birth. Their first child, a daughter, died at age 6 from a rare genetic disease housed in her Mitochondrial DNA. Their second child to survive past birth died of the same disease after just 8 months. The couple then started to seek out a way to give birth to a child that didn't carry this genetic defect. They were eventually able to find a reproductive endocrinologist willing to help, who came up with a way for the couple to give birth to a healthy child.

First, a nucleus was taken from one of Jane's eggs and planted into a donor egg that had its nucleus removed. This egg was then fertilized with John's sperm. This means that if the child were to take a DNA test it would confirm that the Jordanian couple were his genetic parents. Yet, most of the biomass of the original zygote before it started to divide, including the child's now disease-free mitochondria, was from a third person.[6] While this procedure didn't make use of CRISPR or any other complex molecular gene editing technique, this still represents the first birth of an artificially genetically modified person. It should be noted that this entire process, including the actual birth of the child, took place in a clinic in Mexico because at the time, in the spring of 2016, a genetically

modified embryo was not allowed to mature past two weeks, nor could it be implanted into a womb in the United States.

That policy is seemingly no longer in place. In 2015 the International Summit on Human Gene Editing outlined a relatively clear way forward for germline human genome research. The International Summit released a statement saying:

> It would be irresponsible to proceed with any clinical use of germline editing unless and until: (i) the relevant safety and efficacy issues have been resolved, based on appropriate understanding and balancing of risks, potential benefits, and alternatives, and (ii) there is broad societal consensus about the appropriateness of the proposed application.[7]

Just 14 months after this statement was released, the US National Academy of Science (USAS) and the US National Academy of Medicine(USAM)—both of which had previously agreed with the international committee—released guidelines more accommodating to human germline editing. In their joint February 2017 report, Human Genome Editing: Science, Ethics, and Governance, the academies stated that, provided the research is only for compelling reasons, "clinical trials using heritable germline genome editing should be permitted." To their credit, the academies specified that human genetic editing trials should take place only under strict oversight and under

certain criteria, which includes "the absence of reasonable alternatives."

This change of stance did not go unnoticed by Francoise Baylis, Bioethics Research Chair at Dalhousie University in Canada, and arbiter of much of the Canadian government's legislation concerning scientific research. Baylis is a proponent of "slow science," a specific methodology which advocates for the very careful planning and oversight of projects that seek to modify the human genome. Baylis, who herself was actually on the planning committee for the International Summit on Human Gene Editing, helped draft their official views on germline editing. She later expanded on the international committee's statement by saying that the aforementioned "consensus" would be "inclusive among nations and engage a wide range of perspectives and expertise — including from biomedical scientists, social scientists, ethicists, health care providers, patients and their families, people with disabilities, policymakers, regulators, research funders, faith leaders, public interest advocates, industry representatives, and members of the general public."[7]

Baylis doesn't mince words identifying the USAS and USAM's possible motivations for changing their stance. In a May 2017 op-ed she said that "two likely motivational drivers are the pursuit of scientific prestige and capturing a highly lucrative commercial market." In an age when careers are being made with breakthroughs in gene editing technologies, and there are multiple patent wars

currently raging in the U.S. over these new technologies, it's hard to argue with her. Furthermore, it's hard to ignore the timing of this change of opinion, which came after the start of the patent war between UC Berkeley and The Broad Institute in Boston over technologies utilizing CRISPR, as well as the publishing of the two major papers exploring the cutting-edge gene drive systems discussed earlier. These gene drive systems are currently only used on mosquitos and other test animals, and no credible academic or private sector researcher would ever entertain the thought of using them on the human genome. However, without any scientific consensus on the limits of this new technology, and no serious regulations, it's hard to say for certain that we won't see gene drive systems used on human DNA in the future. And while a "broad social consensus" is nice in theory, the question of what exactly that looks like and how best to achieve it still has no good answers.

Baylis even admits, "[t]hese are good questions to which I don't have a complete answer," and this is perhaps why Baylis' voice has had little impact even in her own country, where genetic patents are being filed just as quickly as in the United States. As for how to achieve possible answers she is quick to fall back on her belief in "slow science" and says that:

> With the benefits of 'slow science' and 'slow ethics,' however, I am confident we could figure this out. We might, for example, begin with a critical review of strategies for decision-making by consensus that have been developed over time by various discrete communities."[7]

She then goes on to discuss the decision making techniques of the Navajo people and Quakers, and while some may hope that these traditional strategies will provide answers to these very modern and complex questions, personally I am cynical.

So where does this leave us? Truth be told, after conducting this research my views on gene editing are more complicated than ever, and it seems no one can illustrate a clear path forward. Private businesses, however, are going to start taking advantage of this technology, so the deadline for answers is fast approaching. Whether the technology or our culture are ready or not.

As for how Baylis' views fit into the larger narrative, her principles are sound, but her approach just isn't viable. The very term "slow science" doesn't sit well with many scientist in the fields of biochemistry and molecular biology, simply because they are anything but slow fields.[8] These are fields of science that in the past 20 years alone have seen more advancement than any other area of study over the same time frame. Biochemists and geneticists are synthesizing new unique DNA sequences and patenting new editing methods at

an unparalleled rate—5,000 new genetic patents a year—all to their individual benefit and profit. So simply asking them to slow down, I fear, is a proposition doomed to fail. Yet I feel that Baylis' principles of open communication , dialogue, and reaching a majority consensus on how to properly utilize this technology going forward are worthy goals.

As I see it, there are three forces at play in this debate. The first is creation, which includes our desire to create healthy offspring as well as the scientific and academic desire to discover something new. Second is competition—the desire to be first, or on the cutting edge of science. And finally commerce, the economic incentive to make a profit from science. The problem now is how best to reconcile these three forces with the best interests of our ecosystem and culture.

I think the answer is twofold. First, people attempting to appeal to scientists should pitch their appeal in the same way you would pitch to a potential investor. We need to make scientists realize that achieving a majority consensus on how we as a species should proceed with genome research is profitable, and in their best interest. Majority consensus has the best chance to yield strict and universal guidelines, which means scientists won't have to worry about their peers becoming their competitors for funding just because their research pushes the envelope (and bounds of morality) further.

This phenomenon, envelope pushing for the sake of increased profitability, is exemplified by the patent war that took place between UC Berkeley and The Broad Institute. UC Berkeley conducted research using CRISPR that underwent a year of planning, was overseen by multiple senior faculty, and was rigorously thought-out. However, they lost their chance to patent the resulting techniques because the Broad, whose research and patent filing took place after UC Berkeley's, was ultimately the one able to get the patent. This is because the Broad research was conducted using eukaryotic cells instead of bacterial cells, meaning they were able to work around the UC patent which applied to just prokaryotic cells. Essentially, just because the Broad research was more cutting edge, they stood to profit, even though their research had less oversight, less planning, and was far more ethically fraught.

This conflict illustrates why we need to outline a clear path forward for genetic research, to prevent scientists, both in academia and industry, from jumping the gun and undertaking controversial research just because it has the potential to be more profitable. A level playing field would mean equal opportunity and equal punishment for rule breakers, limiting the pressure of competition brought on by economic incentive.

In addition, regulators must re-strategize their appeals to scientists' humanity. Where I see most peoples' appeals for reform go wrong, particularly Baylis's, is in their argument for why reform is necessary. They should not simply say that achieving a majority decision on how to proceed with gene editing research is "the right thing to do." That rationale, no matter how true, is unspecific and unconvincing. Instead, they should show scientists why it is necessary, enumerating all the ways life on this planet could be disrupted by the unintended consequences stemming from the implementation of this technology. Even if scientists have considered all the outcomes, remind them anyway, because it is easy to sweep aside potential concern when you stand to profit. If we were to release mosquitos containing an aggressive gene-drive system into the wild right now, we simply don't know the effect it would have on the broader complex ecosystem.

Perhaps it would be fine, and have the desired targeted effect, or it could impact the environment in ways we can't even think of. The fact is, there is much we just don't know. If we started to allow human embryos containing genetically modified DNA to mature, come to term, and be born, we don't know what the full repercussions would be. We don't know how these genetic changes will affect development, longevity, or even the effectiveness of treatments using current technologies.[9,10] Again, perhaps it will all work out the

way we want it to, perhaps we will finally be able to eradicate genetic diseases from our genome and become true masters of our DNA. Or it could go wrong, and our mistakes will be felt by multiple genetic generations to come. We scientists need to realize the full ramifications our creations could have on our culture and ecosystem.

I don't mean to put all the burden onto the tactics used by regulators to galvanize scientists into action, nor are scientists the only stakeholders in this debate. This is a debate that affects countless people, and multiple facets of our culture. But this technology is outpacing our culture's ability to change, and this I feel is the underlying issue. Questions of ethics and morality, of how far we feel comfortable manipulating our own genome, or any genome, are questions that we as a culture need to come to terms with. That is why I feel it is so important for scientists to come to a consensus on the issues surrounding this new technology before it becomes available to the public. I feel it is the duty of scientists, innovators, and regulators to lead us along a clear and well-considered path into the future, to ensure our culture adapts and changes for the better. Right now, however, there is no clear path to be found. Scientists working on the human genome need to come to terms with the magnitude of the effect their research has on our lives. Even if scientists are modifying a genome other than our own they still can impact our ecosystem in multiple

untold and potentially irreversible ways. There may be those who feel a scientist's job is not to hold the hand of the public and make sure they are informed. That it is a scientist's job to be our reach, and that they need not concern themselves with our grasp. Yet, I believe that, if it is thoughtfully framed, consensus-building around the ethical and practical limits of gene-editing could be as liberating for researchers as it is vital for our culture. The fact is that the things being done with CRISPR are coming into conflict with the current boundaries our culture has established for scientific study, and we humans are rapidly becoming bystanders in this conflict.

Vocabulary Appendix

Gene drive system: A genetic system that insures that a given trait will be passed down on to the next generation and expressed regardless of whether it is dominant or recessive.

Mendelian Inheritance: The standard chance (usually 50%) that a given gene has to be passed on to the next generation under unaltered natural conditions.

Germline editing: The editing of not only the genetic material of a single organism but effecting a change that will continue to be present in future generations. Having the potential to change the entire genetic makeup of a species.

CRISPR–Cas9: Gene editing technique that allows for targeted removal of specific DNA sequences. First put to use in a groundbreaking 2012 study.

Gene: A distinct sequence of nucleotides forming part of a chromosome, the order of which determines the order of monomers in a polypeptide or nucleic acid molecule which a cell (or virus) may synthesize.

Gene (vernacular use): A unit of heredity that is transferred from a parent to off-spring and is held to determine some characteristic of the offspring. The genetic manifestation of physical traits or vice versa.

Vocabulary Appendix

In-Vitro: Testing done outside an organism. i.e in a test tube environment.

In-Vivo: Testing done within an organism and propagated throughout its body.

Zygote: A fertilized ovum, in other words the cell that will go on to form an embryo.

Mutagenesis: A process by which the genetic information of an organism is changed, resulting in a mutation. It may occur spontaneously in nature, or as a result of exposure to mutagens. It can also be achieved experimentally using laboratory procedures.

IVF: In Vitro Fertilization, which is the artificial insemination of an egg that was fertilized outside of the mother back into the uterus of the mother .

References

1.) Gantz, V. M., Jasinskiene, N., Tatarenkova, O., Fazekas, A., Macias, V. M., Bier, E., & James, A. A. (2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito anopheles stephensi. *Proceedings of the National Academy of Sciences, 112*(49), E6743.

2.) Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., . . . Nolan, T. (2016). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector anopheles gambiae. *Nature Biotechnology, 34*(1), 78-83. 10.1038/nbt.3439 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/26641531

3.) Baylis, F. (b). Human genome editing: We should all have a say. Retrieved from http://theconversation.com/human-genome-editing-we-should-all-have-a-say-81797

4.) Fogarty, N. M. E., McCarthy, A., Snijders, K. E., Powell, B. E., Kubikova, N., Blakeley, P., . . . Niakan, K. K. (2017). Genome editing reveals a role for OCT4 in human embryogenesis. *Nature, 550*(7674), 67. 10.1038/nature24033 Retrieved from https://www.nature.com/articles/nature24033

5.) Baylis, F. (a). Genome editing of human embryos broadens ethics discussions. Retrieved from http://theconversation.com/genome-editing-of-human-embryos-broadens-ethics-discussions-84888

6.) Hamzelou, J. Exclusive: World's first baby born with new "3 parent" technique. Retrieved from https://www.newscientist.com/article/2107219-exclusive-worlds-first-baby-born-with-new-3-parent-technique/

7.) Baylis, F. (2017). Human germline genome editing and broad societal consensus. Retrieved from https://www.nature.com/articles/s41562-017-0103

8.) Bosley, K. S., Botchan, M., Bredenoord, A. L., Carroll, D., Charo, R. A., Charpentier, E., . . . Zhou, Q. (2015). CRISPR germline engineering—the community speaks. Retrieved from https://www.nature.com/articles/nbt.3227

9.) Dance, A. (2017). Better beings? Retrieved from https://www.nature.com/articles/nbt.3998

10.) Hyun, I., & Osborn, C. (2017). Query the merits of embryo editing for reproductive research now. Retrieved from https://www.nature.com/articles/nbt.4000

Further References

1.) Flynn, R. L.Chemistry nobel DNA research lays foundation for new ways to fight cancer. Retrieved from http://theconversation.com/chemistry-nobel-dna-research-lays-foundation-for-new-ways-to-fight-cancer-48800

2.) Gyngell, C., & Savulescu, J.UK gene editing breakthrough could land an aussie in jail for 15 years: Here's why our laws need to catch up. Retrieved from http://theconversation.com/uk-gene-editing-breakthrough-could-land-an-aussie-in-jail-for-15-years-heres-why-our-laws-need-to-catch-up-84590

3.) Howard, Heidi C. Cornel, Martina. One small edit for humans, one giant edit for humankind? Points and questions to consider for a responsible way forward for gene editing in humans. On behalf of the European Society of Human Genetics and the European Society of Human Reproduction and Embryology: Retrieved from https://www.nature.com/articles/s41431-017-0024-z

4.) Howard, Heidi C. Cornel, Martina. One small edit for humans, one giant edit for humankind? Points and questions to consider for a responsible way forward for gene editing in humans. On behalf of the European Society of Human Genetics and the European Society of Human Reproduction and Embryology: Retrieved from https://www.nature.com/articles/s41431-017-0024-z

5.) Smalley, E. (2018). FDA warns public of dangers of DIY gene therapy. Retrieved from https://www.nature.com/articles/nbt0218-119

6.) Take stock of research ethics in human genome editing. (2017). Nature News, 549(7672), 307. 10.1038/549307a Retrieved from http://www.nature.com/news/take-stock-of-research-ethics-in-human-genome-editing-1.22632

7.) Wert, Guido De. Cornel, Martina. Responsible innovation in human germline gene editing: Background document to the recommendations of ESHG and ESHRE. On behalf of the European Society of Human Genetics and the European Society of Human Reproduction and Embryology: Retrieved from https://www.nature.com/articles/s41431-017-0077-z