COMMENTARY

The Lumbering Crawl Toward Human Germline Editing

Eli Y. Adashi and I. Glenn Cohen

It is in the nature of novelty that consensus is hard to come by. Such is clearly the lot of groundbreaking biomedical advances. History is no stranger to this phenomenon. The prospect of human germline editing is no exception. A fine illustration as any of this state of affairs is offered by Professor Drabiak in this issue of the *Journal of Law, Medicine, & Ethics*.[1] Squarely on the side of the skeptics, Professor Drabiak carefully lays out the relative shortcomings of the technology in question.[1] Our main focus is to complement Professor Drabiak's extensive account by highlighting the present state of the science of human germline editing. No judgement is being passed on the notion of human germline editing nor on the potential application thereof — we have discussed our own views in depth elsewhere.[2] Instead, we lay out the state of the science replete with the hindrances that would have to be overcome if human germline editing is to become a clinical reality. In this context, we highlight select areas wherein Professor Drabiak's arguments could be further elaborated:

- The mixing of Mitochondrial Replacement Therapy (MRT) and germline gene editing. Throughout the article, Professor Drabiak moves back and forth between policy treatment, concerns, and news events regarding MRT and those pertaining to gene editing.[3] While the two technologies have much in common and some would label "MRT" as "gene editing," the concerns pertaining to the latter often do not affect the former. This is particularly true if, as recommended by the National Academies, but not acknowledged by Professor Drabiak in her account, one were to limit MRT to male-only embryos thus avoiding transmission of heritable changes.[4]
- The effectiveness of the Congressional Rider. Professor Drabiak briefly mentions the appropriations rider, initially put in place in 2015 and renewed annually since, that prohibits FDA from considering applications "for an exemption for investigational use … in research in which a human embryo is intentionally created or modified to include a heritable genetic modification."[5] The FDA has interpreted the rider to bar the approval MRT, even for male-only embryos. Because of the rider, any attempt to use gene editing or MRT in the U.S. would violate the Food, Drug, and Cosmetics Act and subject the party to legal liability.[6] It is unclear what additional measures Professor Drabiak, who is clearly against these technologies, could seek, or why this means that the U.S., in her view, compares unfavorably to peer nations?[7]
- The legality of medical tourism from the U.S. for MRT or gene editing. In her discussion of the case of Dr. Zhang, Professor Drabiak writes that "despite the FDA asserting jurisdiction over MRT and genome editing, physicians may circumvent FDA regulations and unilaterally integrate experimental techniques modifying the human germline into clinical practice."[8] The answer, though, depends on the legality of the technique in the destination country, as well as on what elements of the process occur intra

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versus extra territorially and the physician's home country's own assertions of jurisdiction.[9] While Professor Drabiak acknowledges the FDA cease and desist letter issued to Dr. Zhang, it is not clear why she concludes it is not effective, as it appears to us to have been.[10]

On April 18, 2015, on the pages of *Protein & Cell*, Liang et al. reported on the first ever attempt to edit the genome of the human embryo.[11] Seeking to inactivate the wild-type β-Globin gene by applying the CRISPR/Cas9 endonuclease, the authors strove to generate a targeted Double-Strand Break (DSB) in the genome of tripronuclear (inviable) zygotes.[12]Amongst the outcomes noted, a low (14.5%) measure of editing efficiency stood out.[13] Modest editing specificity was similarly apparent due to on- and off-site insertions/deletions (indels).[14] Limited editing uniformity was also in evidence due to a significant measure of mosaicism.[15] The modest nature of the results notwithstanding, this proof-of-principle undertaking was nevertheless highly cited by dint of sheer novelty. Inclusion in "Nature's 10" listing for the year followed suit. In the aftermath, four additional communications explored the feasibility of DSB-dependent editing of the human embryo. The utility of DSB-independent editing was explored as well before too long.

It was not until August 24, 2017, however, on the pages of *Nature*, that a more substantive effort to assess the feasibility of human germline editing was undertaken by Ma et al. of the Oregon Health & Science University.[16] The disease target selected, Familial Hypertrophic Cardiomyopathy (FHC), an autosomal dominant malady, was attributable to a mutant Sarcomere Gene.[7] Incurable if amenable to symptomatic care, FHC all too often culminates in sudden cardiac death.[17] Two distinct sets of experiments were carried out.[18] In the first set of experiments, intact MII oocytes were fertilized by co-incubation with mutant sperm, edited 18h later, and the results analyzed in day-3 cleavage-stage embryos.[19] In the second set of experiments, intact MII oocytes were ICSI (Intracytoplasmic Sperm Injection)-fertilized as well as edited and the results analyzed in day-3 cleavage-stage embryos.[20] Editing of zygotes 18h post-fertilization increased the representation of non-mosaic wild-type homozygosity by 41%.[21] The mosaicism rate, however, stood at 24%.[22] Editing of zygotes at the time of fertilization increased the prevalence of non-mosaic wild-type homozygosity by 53% while reducing the mosaicism rate to 2.4%.[23] A modicum of on-target indels was noted for both experimental protocols.[24] Taken together, these observations suggest that zygotic editing at the time of fertilization enhances editing efficiency and uniformity if not specificity and that further optimization will be required if clinical application is to be entertained.

In 1963, Joshua Lederberg, PhD, one of the finest scientific minds of his time and a Nobel awardee for the discovery of bacterial conjugation, suggested that "we might anticipate the in vitro culture of germ cells and the direct control of nucleotide sequences in human chromosomes, coupled with recognition, selection and integration of the desired genes…" In hindsight, we can only assume that Professor Lederberg conjectured a functionality that we might refer to as editing the human germline. Fifty-five years later, that functionality is no longer in the realm of postulation. Actualization however, is a whole other matter.

These and previous observations bring one to an important fork in the proverbial road leading to mutation-free embryo transfers. One road will likely comprise selection via Preimplantation Genetic Diagnosis (PGD) in day-3 cleavage-stage embryos. The other road, however, might entail universal zygotic editing at the time of fertilization. These two technologies could not be more different. Selection via PGD is, of course, purely diagnostic in nature in that editing the multicellular embryo is infeasible at this time. Zygotic editing, on the other hand, is entirely restorative in nature. Neither approach is free of relative shortcomings. Selection via PGD has been found to display a significant falloff in the number of embryos available for transfer.[25] When screening for dominant disorders by PGD, 30% of egg retrievals failed to reach the embryo transfer stage.[26] The corresponding figure for recessive disorders was 20%.[27] This reality is further driven

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home in the PGD-dependent selection of savoir siblings wherein advanced maternal age may be associated with a retrieval to transfer falloff exceeding 90%. These observations suggest that PGD, while broadly applicable to the selection of mutation-free embryos, might be constrained by circumstances characterized by rate-limited pools of selectable embryos. PGD is also of limited utility in the context of rare parental constellations which give rise to universal embryonic affliction. Zygotic editing, on the other hand, may be uniquely suited for rate-limiting complements of embryo populations. Herein however, both mutant and intact zygotes are exposed to the editing mix, a reality the impact of which remains to be further evaluated. Technologies to validate editing rigor prior to embryo transfer have yet to materialize as well. Looking ahead, consideration might also be given to a third road to mutation-free embryo transfers, that is, the editing of gametes or else of stem cells destined to become gametes. Progress along these lines would facilitate the validation of editing rigor as well as eliminate the prospect of embryonic mosaicism.

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Note

The authors have no conflicts of interest to declare.

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