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Unintended Effects of Genetic Manipulation
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Posted: October, 2017

Researchers Agree Human Embryo Experiment Generated Unintended Effects, But Disagree on How to Interpret Surprise Results

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A set of controversial experiments that involved creating and genetically engineering more than a hundred human embryos *in vitro* for research purposes has sparked disagreement among prominent researchers about what the results actually show. Authors of the research claimed success, albeit through an unintended effect of their manipulations. But a group of critics say the data is insufficient to reach such a "stunning" conclusion – especially when other troubling possibilities are more likely.

The researchers conducting the experiments announced in August that they had succeeded in safely repairing a disease-related version of a gene by using CRISPR/Cas9, a powerful new biotechnology. But the international team of researchers from the U.S., Korea, and China reported that the correction they believed they detected in many of the embryos occurred in a surprising way – not the way they had tried to make it happen.

However, just weeks after the team reported their results in the prestigious journal *Nature*, to a blitz of media coverage, other prominent researchers posted online a major critique of the team's conclusions. The critique argued that the team, which was led by Oregon Health & Science University (OHSU)'s Shoukhrat Mitalipov, had wrongly interpreted their own results. The evidence, the critics added, is not sufficient to conclude that the abnormal gene was properly corrected in the embryos, and the team's suggestion about how such a correction could have happened was implausible.

The experiment at issue involved an attempt to prevent embryos from inheriting an abnormal gene associated with hypertrophic cardiomyopathy. That's a form of heart disease which can cause sudden cardiac death at any age, although according to a 2002 review study it more frequently results in "no or relatively mild disability and normal life expectancy." The mutant form of the gene is dominant, meaning offspring need inherit only one copy of it, from either the maternal or paternal side, to later develop the disease associated with it.

Fifty-eight embryos were created in the lab from the sperm of one male donor who carried one copy of the mutant gene and from eggs donated by women who carried two copies of the normal gene. The study's authors had first injected the eggs simultaneously with both the sperm and the CRISPR/Cas9 components, including synthetic DNA they had designed as a normal template to replace what they were trying to delete. Without these interventions, about 50 per cent of the embryos would be expected to be free of the mutant form of the gene. (The embryos could inherit from the paternal side either the normal form or the mutant form, but would always inherit a normal gene from the maternal side. So there was naturally a 50-per-cent chance of not inheriting any copy of the mutant gene.)

They concluded, though, that with their genetic manipulation, the actual rate of embryos with no mutant form of the gene was 72.4 per cent (or 42 out of 58), indicating that in many

cases they had successfully cut out the problematic material from the paternal gene and then repaired it. But they added that only one embryo showed evidence of having partially incorporated the synthetic DNA template, as was the study's intention. Instead, to their surprise the other embryos in which the paternal gene seemed to have been normalized had performed this self-repair by using material from the normal maternal copy of the gene, they reported. And even the one outlier, which included some cells with the synthetic template, also had other cells that were repaired by material from the maternal gene.

But the critique's authors argued that the actual published data from the experiment were not sufficient to draw such an unexpected and unlikely conclusion. Other scenarios are more likely, they suggested. In particular, they said the methods the researchers used to try to verify that the mutant gene was not present were not detailed enough to definitively make that call. It could be, they indicated, that the attempted repair unintentionally deleted enough genetic material that the presence of an abnormal paternal copy of the gene would not show up in the particular genetic analyses the researchers conducted. Future research should include a more detailed analysis to prove the presence of two normal copies of the gene, rather than relying on a lack of evidence of the mutant gene.

They also cautioned that if the embryos did have maternal genetic material in both copies of the gene, that might itself be linked to health risks down the line, for any children who would be born if the same procedure was tried in clinical trials with women who sought to carry such *in-vitro* embryos to term. It might, for example, signal some broader loss of inheritance of genetic material from the paternal side. One risk that would pose would be the possibility of a child inheriting a double copy of other genetic material from the mother that might be linked to recessive diseases. Such diseases can be passed down only when two copies of a mutant gene, not one, are inherited.

To more definitively conclude exactly what happened in the experiment, the critique emphasizes, the OHSU-led team would need to do more extensive genetic analyses than they described and presented in their paper. The critique's authors have submitted their own critical analysis of the research results to a peer-reviewed journal for consideration.

In addition to the disagreement among researchers about what happened in the experiment, this study is also highly controversial because it was attempting to engineer changes in the human germline (i.e., it involved human reproductive cells), the impacts of which – for good or ill – would be passed on to future generations. That raises a host of ethical, health, and safety issues. U.S. federal law, in fact, does not allow federal money to be used to conduct research on human embryos created only for research purposes. The study here was funded by OHSU's own institutional funds, three foundations, a Korean government-funded research organization, and a municipal government in China, where some of the researchers work. OHSU has already applied for a patent related to this study and has included the technology developed in the study on its list of technologies available for licensing that is posted on its website.

In early October, the journal *Nature* posted an Editor's Note to the original study, alerting readers that "some of the conclusions of this paper are subject to critiques that are being considered by editors . . . A further editorial response will follow the resolution of these issues."

Sources

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