



Unintended Effects of Genetic Manipulation

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The controversial study reported below, which concluded that a large number of unintended mutations in an experiment with mice were apparently caused by new CRISPR-Cas9 technology, has been officially retracted by the journal *Nature Methods*. The journal published a notice in late April, 2018 that it was retracting the paper “because the genomic variants observed by the authors in two CRISPR-treated mice cannot be conclusively attributed to CRISPR-Cas9 . . . without parental controls or more analysis of genetic background.” Two of the authors of the original paper agreed with the retraction, according to the journal, while the other four – including the two corresponding authors – did not. But all the authors agreed that “there is very little whole-genome sequencing data on the effects of CRISPR treatment *in vivo*,” and that “the question of whether CRISPR has effects on the *in vivo* genome will require further study.” The authors are continuing to conduct follow-up studies using whole-genome sequencing. The journal’s Retraction Notice is available [here](#). To read more about the original paper and the controversy it stirred, see our report below.

Posted: July, 2017

New CRISPR Genetic Engineering Technology Caused Large Number Of Unintended Mutations, Conclude Researchers in Controversial Mouse Study

Colleen Cordes

CRISPR-Cas9, a major new technique for trying to precisely design and insert changes in the genomes of organisms, apparently caused more than a thousand unintended mutations in a small new study involving mice, according to the study’s authors. The study, in turn, has sparked a controversy – fueled by the huge financial and academic interest in CRISPR technologies – about whether its evidence is strong enough to support the authors’ conclusion.

The researchers, in an earlier study, had used the CRISPR-based method to restore sight in blind mice by intentionally changing the particular mutated form of a gene in the mice that is linked to blindness. But the researchers were concerned that the typical computer-based algorithms for predicting possible unintended mutations that the CRISPR technique itself might cause would not be sufficient to help them identify all such surprise consequences. So they analyzed the whole genome of two of the CRISPR-engineered mice and compared it to the whole genome of a control mouse, down to the level of single nucleotides. (The control animal still had the mutated version of the gene linked to blindness.)

For one of the two experimental mice, they detected 1,736 unintended mutations in single nucleotides and 164 larger insertions and deletions of genetic material that were not intended, compared to the control animal’s genome. For the other CRISPR-treated mouse, in terms of unintended changes, they found 1,696 single-nucleotide mutations and 128 larger genetic insertions and deletions, compared to the control. Many of the unintended genetic changes did not seem to be randomly generated, since the two CRISPR-treated mice had both experienced 1,397 of the same single nucleotide changes and 117 of the same larger insertions and deletions of genetic material, the researchers noted. Their study was published in May 2017, in the peer-reviewed journal *Nature Methods*.

On the other hand, the researchers also found there were no mutations in any of the top 50 genetic sequences that computer modeling predicted would be most likely to show off-target effects. "Our results," the authors of the study wrote, "suggest current *in silico* modeling cannot predict bona fide off-target sites." They suggested that researchers working with CRISPR-based techniques in live organisms should use whole genome sequencing to doublecheck for unintended genetic changes, and to probe not only for unexpected larger insertions and deletions but also for single-nucleotide variants. That, they concluded, is "the most thorough method for identifying off-target mutations."

"The unpredictable generation of these variants is of concern," the researchers added. "The impact of the numerous mutations occurring in noncoding RNAs or other regulatory intragenic regions could be detrimental to key cellular processes." Their study, they noted, "shows a significantly higher number of potentially deleterious CRISPR–Cas9-induced mutations than have been previously reported."

They did not observe any obvious signs that the many unexpected mutations had affected the actual phenotypes (observable traits and behaviors) of the CRISPR-treated mice. But they suggested that such changes could still show up in the future, such as in response to stress or in later breeding with mice with a similar pattern of mutations. "More work may be needed to increase the fidelity of CRISPR–Cas9 with regard to off-target mutation generation before the CRISPR platform can be used without risk, especially in the clinical setting," they also concluded.

Stephen Tsang, one of the co-authors, in a press release issued by Columbia University Medical Center, reiterated the study's emphasis on the need for researchers to thoroughly evaluate the unintended effects of using CRISPR techniques.

"We feel it's critical that the scientific community consider the potential hazards of all off-target mutations caused by CRISPR, including single nucleotide mutations and mutations in non-coding regions of the genome," said Tsang, who is professor of ophthalmology and associate professor of pathology and cell biology in the Institute of Genomic Medicine and the Institute of Human Nutrition at Columbia University Medical Center.

He added: "Researchers who aren't using whole genome sequencing to find off-target effects may be missing potentially important mutations. Even a single nucleotide change can have a huge impact."

The study almost immediately elicited unusually strong, public criticism, especially from scientists who are financially involved with companies intent upon commercializing CRISPR technologies or who work at institutions claiming CRISPR patents. Critics have argued, for example, that what the researchers identified as unintended mutations due to CRISPR were most likely genetic differences between the mice that existed before the experiment and that the two experimental mice were more closely genetically related to each other than to the control mouse. The study authors, who reported no competing financial interests, have responded to one of the most prominent groups of critics, from the CRISPR-related company Editas Medicine. The Editas critique and two others — one associated with another major CRISPR-related company, Intellia Therapeutics, and the other by researchers at institutions claiming fundamental CRISPR patents — are included below in the sources below, as is the response from the authors to the Editas critique. (Unlike the study, neither the three critiques nor the authors' response were peer-reviewed before they were posted online at *bioRxiv*, which is a free online archive and distribution service for unpublished preprints in the life sciences. The site allows researchers to continue to update these preprints, as they receive comments on them.)

The journal that published the study at the end of May added this editorial note two weeks later: "readers are alerted that the conclusions of this paper are subject to criticisms that are

being considered by editors. A further editorial response will follow the resolution of these issues.”

Sources for the Study

Schaefer, Kellie A., Wen-Hsuan Wu, Diana F. Colgan et al. (2017). “Unexpected Mutations after CRISPR–Cas9 Editing *in vivo*,” *Nature Methods* vol. 14, pp. 547–48. [doi:10.1038/nmeth.4293](https://doi.org/10.1038/nmeth.4293)

Columbia University Medical Center (2017). “CRISPR Gene Editing Can Cause Hundreds of Unintended Mutations,” (News Release, May 30). <http://newsroom.cumc.columbia.edu/blog/2017/05/30/crispr-gene-editing-can-cause-hundreds-of-unintended-mutations/>

Sources for Follow-Up Critiques and Authors’ Response

Lareau, Caleb, Kendell Clement, Jonathan Y. Hsu et al. (2017) “‘Unexpected Mutations after CRISPR-Cas9 Editing *in vivo*’ Are Most Likely Pre-existing Sequence Variants and Not Nuclease-Induced Mutations,” *bioRxiv* 159707, posted July 5, 2017. doi.org/10.1101/159707. [The authors all list affiliations with The Broad Institute of MIT and Harvard, Harvard University units, or MIT. The Broad Institute, Harvard, and MIT [share fundamental CRISPR patents](#). Two of the authors also list, in a conflict of interest statement, their financial interests in Beacon Genomics, a company that sells technology to assess off-target effects in genome engineering.]

Lescarbeau, Reynald M., Bradley A. Murray, Thomas M. Barnes and Nesson Bermingham (2017). “A Reanalysis of Schaefer et al. Does Not Indicate Extensive CRISPR/Cas9 Mediated Off-Target Editing Events,” *bioRxiv* 159608, posted July 6, 2017. doi.org/10.1101/159608. [This is the critique associated with Intellia Therapeutics.]

Wilson, Christopher J., Tim Fennell, Anne Bothmer et al. (2017). “The Experimental Design and Data Interpretation in ‘Unexpected Mutations after CRISPR Cas9 Editing *in vivo*’ by Schaefer et al. Are Insufficient to Support the Conclusions Drawn by the Authors,” *bioRxiv* 153338, first posted June 21, 2017 and updated July 10, 2017. doi.org/10.1101/153338. [This is the critique associated with Editas Medicine.]

Schaefer, Kellie A., Wen-Hsuan Wu, Diana F. Colgan et al. (2017). “Response to Editas: [Unexpected Mutations after CRISPR–Cas9 Editing *in vivo*](#),” *bioRxiv* 154450 posted June 23, 2017. doi.org/10.1101/154450.