CRISPR joins battle of the bulge, fights obesity without edits to genome

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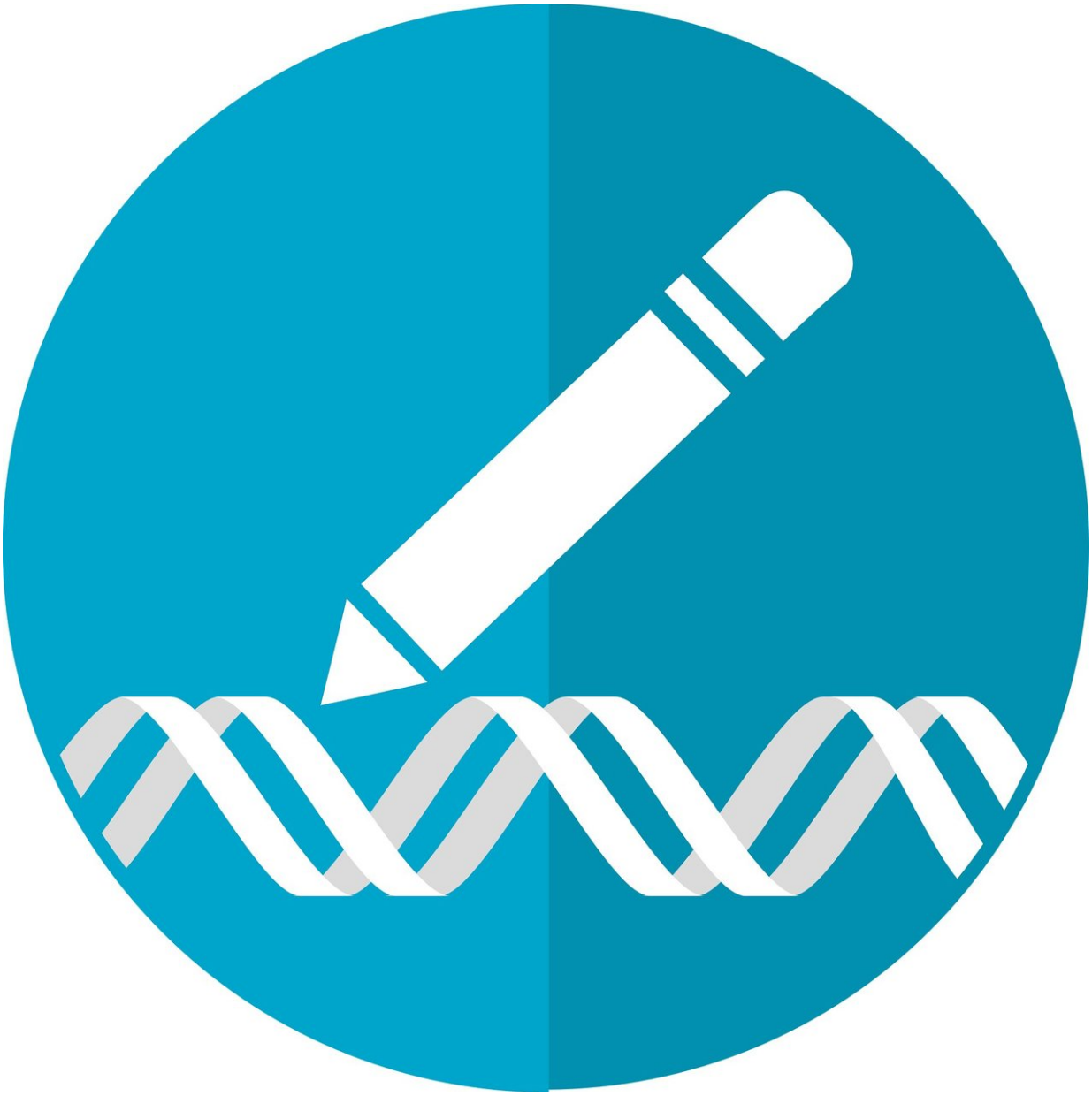

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A weighty new study shows that CRISPR therapies can cut fat without cutting DNA. In a paper published Dec. 13, 2018, in the journal *Science*, UC San Francisco researchers describe how a modified version of CRISPR was used to ramp up the activity of certain genes and prevent severe obesity in mice with genetic mutations that predispose them to extreme weight gain. Importantly, the researchers achieved long-lasting weight control without making a single edit to the genome.

Single-Copy Mutations Drive Many Human Diseases

Though the human genome contains two copies of every gene in an individual, one from each parent, scientists know of at least 660 genes where a mutation in just one copy can lead to diseases, some of which are devastating. One such condition is severe obesity, which the authors of the new study used as a model to develop a new therapeutic approach for treating these disorders.

Mutations in a single copy of SIM1 or MC4R—two genes critical for regulating hunger and satiety—are the most frequently observed mutations in severely obese individuals. When both copies of these genes are functioning, people are generally able to manage their food intake. But mutations can render one copy non-functional, forcing the body to rely exclusively on a single working copy, which on its own, doesn't sufficiently signal satiation, leaving afflicted individuals with an unrelenting appetite. As a result, they can't control their food intake and end up severely obese. But recent advances in CRISPR technology may offer a solution.

"We thought that if we could increase the dosage of the existing

functional copy of the gene, we could prevent many human diseases in individuals harboring these mutations," said Nadav Ahituv, Ph.D., professor of bioengineering and therapeutic sciences and senior author of the new study. "We were able to accomplish this by using a novel CRISPR-based technology developed right here at UCSF."

CRISPRa Activates Appetite-Suppressing Genes

The technology in question is CRISPRa (a for activation). Developed at UCSF in the lab of Jonathan Weissman, Ph.D., professor of cellular and molecular pharmacology, CRISPRa differs from conventional CRISPR in that it doesn't make cuts to the genome. It retains CRISPR's guidance system, which can be programmed to home in on a particular DNA sequence, but replaces the molecular scissors with a volume control knob. When CRISPRa finds its target, it amplifies the activity of that gene. No edits are made.

Recognizing its potential, the researchers created CRISPRa systems that targeted sequences that regulate the activity of SIM1 or MC4R. They used a viral-delivery system to introduce these CRISPRa constructs into the hunger-control regions of the brain in mice that were genetically engineered to have only one functional copy of either gene.

Mice that received the CRISPRa constructs produced more SIM1 or MC4R than those that didn't. Furthermore, the amounts were comparable to what mice with two working copies of these genes normally produce. Most importantly, the increased dose was enough to prevent the mice from becoming obese.

"The results were dramatic. Mice that were missing one copy of the SIM1 gene received the CRISPRa injections at four weeks of age and maintained a healthy body weight like normal mice. Mice that didn't receive CRISPRa injections couldn't stop eating. They started gaining

weight at six weeks of age, and by the time they were 10-weeks old, they were severely obese on a regular diet" said Navneet Matharu, Ph.D., a researcher in the Ahituv lab and lead author of the new study.

CRISPRa-treated mice were 30 to 40 percent lighter than their untreated counterparts. The effects were also long-lasting. The researchers monitored the mice for ten months—a significant fraction of a mouse's normal lifespan—and found that those that received a single CRISPRa treatment maintained a healthy weight for the duration of their monitoring.

"These results demonstrate that CRISPRa can be used to up the dosage of genes in diseases that result from a missing copy, providing a potential cure for certain forms of obesity as well as hundreds of other diseases," said Matharu.

CRISPRa Can Overcome the Limits of Gene Editing

The researchers believe they could have achieved similar results by using CRISPR to edit the genomes of these mice, but they argue that CRISPRa has a number of advantages over the standard version of the gene-editing technology.

"For therapeutic purposes, CRISPRa may be preferable to conventional CRISPR. It solves many of the problems associated with making permanent modifications to the genome, and it has the potential to treat a variety of genetic diseases for which gene editing isn't an option," said Christian Vaisse, MD, Ph.D., the Vera M. Long Endowed Chair in Diabetes Research at UCSF and co-author of the study.

Though CRISPR targets specific DNA sequences, off-target effects have been observed. With conventional CRISPR, this can lead to inadvertent but permanent changes to the genome with potentially

harmful outcomes. However, off-target effects associated with CRISPRa are less likely to be damaging because no permanent changes are made. In fact, the new study shows that using CRISPRa to target promoters and enhancers—noncoding DNA sequences that control when and where a gene is turned on—seems to prevent off-target effects while confining the desired effects to specific tissues of interest.

The researchers also note that CRISPRa could be used to treat other kinds of genetic disease. Diseases that arise from so-called "microdeletions"—a term that counterintuitively refers to the loss of large chromosome segments that span millions of nucleotides and multiple genes—are currently too large for conventional CRISPR to repair. In such cases, CRISPRa could be used to compensate for the deletion by increasing the activity of several genes on the unaffected copy of the chromosome. And in cases where a gene is completely lost, CRISPRa could activate another gene with a similar function to compensate for the missing gene, the researchers said.

"Though this particular study focused on obesity, we believe our system could be applied to any situation in which having only one functional copy of a gene leads to disease," Ahituv said. "Our method demonstrates tremendous therapeutic potential for numerous diseases, and we show that we can achieve these benefits without making any edits to the genome."

More information: "CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency" *Science* (2018). science.sciencemag.org/lookup/ … 1126/science.aau0629

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