Base editing of SMN2 gene restores production of SMN protein, curing spinal muscular atrophy in mice

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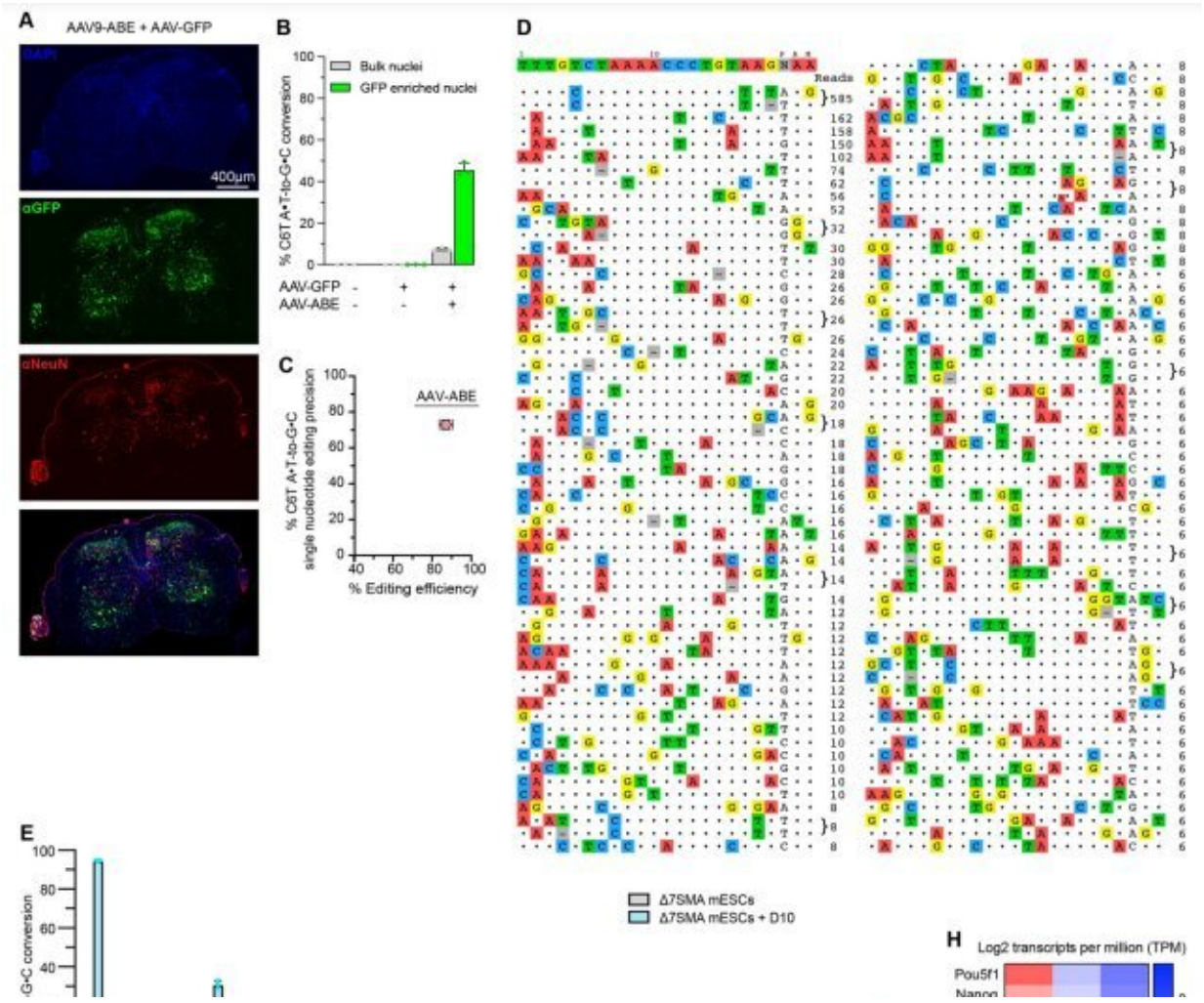

(A) Immunofluorescence images of spinal cord sections from wild-type Δ7SMA mice at 25 weeks that received AAV9-ABE + AAV9-GFP in a 10:1 ratio by

neonatal ICV injection, stained for GFP to indicate AAV transduction, NeuN as a marker of post-mitotic neurons, and DAPI to stain all nuclei. (B) In vivo base editing conversion of C6T in the spinal cord of Δ7SMA mice treated with AAV9-ABE + AAV9-GFP in bulk dissociated tissue, and GFP+ enriched nuclei. (C) CIRCLE-Seq nominations of candidate off-target sites in NIH3T3 cell genomic DNA treated in vitro with purified Spy-mac nuclease and P8 sgRNA. Mismatches at each off-target locus are shown relative to the sgRNA above. (D) On-target and off-target base editing of strategy D10 in Δ7SMA mESCs. Bars show editing of the highest edited nucleotide (P# shown in parenthesis) at each locus. (E) Fluorescence imaging of CND and MND differentiated Δ7SMAmESCs that harbor the Mnx1:GFP reporter of motor neurons and stably integrated with the D10 ABE strategy. (F) RT-qPCR for ABE8e expression in Δ7SMAmESCs (n=3) and differentiated MND (n=3) and CND (n=3) populations, previously transfected with the D10 strategy. (G) Gene expression analysis of Δ7SMAmESCs (n=3), and CND (n=3) and MND (n=3) differentiated cells showing expression levels of various motor neuron specific, neuron specific, spinal cord patterning, glia, and embryonic stem cell markers. Credit: *Science* (2023). DOI: 10.1126/science.adg6518

A team of medical researchers affiliated with a host of institutions in the U.S. has used base editing to restore the natural production of the SMN protein in mice, effectively curing spinal muscular atrophy (SMA) in the rodents. In their paper published in the journal *Science*, the group describes their base editing approach and its performance in restoring natural SMN production in mice afflicted with SMA.

SMA is one of the leading causes of infant mortality in humans. Babies born with the condition have a mutation in the SMN1 gene, resulting in production of insufficient amounts of the protein SMN, leading to neural deterioration and death. Many babies born with the condition who are diagnosed early enough are given drugs to increase production of SMN artificially, which slows progression of the disease, but cannot stop it completely. Thus, other therapies are needed.

In this new effort, the researchers used base editing, a kind of gene editing that is done chemically, to treat the disease in mice. Base editing is typically used to make single-nucleotide changes in a genome, as was done in this case.

In this particular instance, the change was made to the SMN2 gene, which normally partially encodes for production of SMN—the changes the team made fully activated the gene, allowing for more production of SMN. The SMN2 gene is related to the SMN1 gene, but there is an important difference: SMN2 has a C6>T mutation that makes it unable to regulate SMN protein production. Altering the mutation in a way that made it identical to the unmutated SMN1 gene removed this restriction, allowing the gene to encode for unlimited amounts of SMN.

Close monitoring of the altered mice showed the base editing restored production of SMN to normal levels, preventing neural degeneration. They also found that in cases where degeneration had already occurred, base editing led to regeneration and improved motor function. They also found that editing the mice's genes increased lifespan from an average of just 17 days (for a control group) to more than 100 days.

More information: Mandana Arbab et al, Base editing rescue of spinal muscular atrophy in cells and in mice, *Science* (2023). DOI: 10.1126/science.adg6518