

The article "*In Vivo* genome editing via CRISPR/Cas9 mediated homology-independent targeted integration" is about an experiment conducted in 2016 by multiple teams of scientists around the world. The purpose of the experiment was to investigate the viability of inserting gene-edited cells into living specimens. The authors point out that although there has been significant advancement in the field of gene-editing in recent years, current technology still does not allow for reliable *in vivo* gene integration, especially non-dividing cells in adults. In order to investigate possible avenues for further advancement of CRISPR technology, these researchers developed a plan to use a homology-independent targeted integration, or HITI. This method would be compared with another method of integration in order to evaluate its efficacy in successful expression of the edited genes. This method is called PITCH integration, and has an important difference from the HITI method in that it involves the use of bacterial sequences in integration, instead of involving only the replacement sequence as the proposed HITI method does. Both of these methods were tested on both embryonic and adult-aged rats by injection of cranial tissue with edited sequences of five different genes: DAPI, GFP mCherry, β -tubulin, and Merge. Specifically, the injections targets the visual cortex of the adult rats, and it was hypothesized that this would induce a degenerative eye condition in the rats known as retinitis pigmentosa. Affected areas of the brain were later dissected and stained in order to observe the changes in neuron connections as a result of genome editing. Other areas of non-dividing cells were also targeted with similar methods and reported that they produced similar increases in efficacy rates as those in the brain, around 5%.

The observers reported that methods that did not utilize bacterial sequences in their integration process were found to be more successful in the change of gene expression. While this difference was minor, it did result in an increase in both absolute and relative efficacy in all of the five observed genes, although the amount by which absolute efficacy improved was between 3-5% after 31 trials. The authors point out that these rats were collected and tested at multiple institutions and did not involve the randomization or specific usage of rats of different sexes, and that some of those used were pregnant, although they did not suspect that this would influence the results.

Ultimately, the researchers concluded that using the HITI method for *in vivo* transgene integration into adult neurons. They explain that further research using this method, especially in mammals, has the potential to lead to better and more viable methods of *in vivo* gene-replacement therapy.

Suzuki, K. et. al. *In vivo* genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. *Nature* **540**, 144-167 (2016).