European Cystic Fibrosis Society

Journal of Cystic Fibrosis
The Official Journal of the European Cystic Fibrosis Society

Cystic Fibrosis Research News

Title:

Comparison of Cas9 and Cas12a CRISPR editing methods to correct the W1282X-CFTR mutation

Lay Title:

Comparison of gene editing methods to correct the W1282X mutation

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What was your research question?

In this study, we wanted to understand if gene editing was effective in correcting the W1282X CFTR mutation. The first question was to compare the efficacy of two different editing methods, and then for the best one, to characterize the efficacy in rescuing CFTR expression and function.

Why is this important?

Even though remarkable breakthroughs have been done towards CF treatment with the approval of highly effective modulators, none of these are approved for patients with so-called nonsense or stop mutations. So here, we focused on W1282X, the second most frequent stop mutation accounting for 1.2% of CF mutations worldwide. In the absence of modulators, correction the underlying genetic defect by gene editing could provide a one-time cure.

What did you do?

Here, we compared two different gene editing methods for their ability to correct the W1282X mutation in human lung cells. We assessed the levels of precise correction and undesired editing. For the most effective editing method, we then performed a thorough characterization of the consequences of editing – assessing the levels of CFTR in terms of mRNA and protein and also the levels of protein function. The values were compared to those

observed in a population of cells which all show normal CFTR, to evaluate the possible therapeutic benefit achieved.

What did you find?

We found that one of the methods is better than the other - at least in the context of editing this specific mutation – with successful correction in 20% of the cells versus only around 10%. In the cell population, this 20% of correction led to an increase in the total levels of CFTR mRNA and protein, this last to around 10% of the levels observed in normal CFTR. More importantly, this level of correction could improve (rescue) CFTR function to around 20% of normal function. Characterization of a population where all cells are corrected showed that these are the same as cells with normal CFTR.

What does this mean and reasons for caution?

Altogether, this work demonstrates the potential of gene editing as a therapeutic strategy for CF directly correcting the root cause of the disease. As in all gene editing strategies, we need to be aware of potential side effects (off-target effects). In our case, we observed no undesired effects in candidate off-target sequences that when affected could have a negative effect.

What's next?

Our study shows that as a proof-of-principle gene editing can be used to correct W1282X-CFTR. The next relevant question is the selection of which cells are the most relevant to target in a person with CF, and the procedure to either deliver gene editing tools directly, or to edit their cells outside the body in the lab (ex vivo) and then deliver those corrected cells to the lung.

Original manuscript citation in PubMed

https://pubmed.ncbi.nlm.nih.gov/34103250/