Molecular Therapy
Nucleic Acids
Commentary

CRISPR-Cas9 in hiPSCs: A new era in personalized treatment for Stargardt disease

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https://doi.org/10.1016/j.omtn.2023.05.008

Inherited retinal dystrophies, including Stargardt disease, are a group of genetic eye diseases that currently lack effective treatment options. The CRISPR-Cas9 gene editing system and human induced pluripotent stem cells (hiPSCs) offer a promising avenue for treating Stargardt disease, a devastating genetic eye disorder. In a recent study published in *Molecular Therapy–Nucleic Acids*, Siles and colleagues demonstrated the accurate correction of two pathogenic variants in the *ABCA4* gene, which triggers Stargardt disease, in hiPSCs without any adverse effects.[1] This finding paves the way for personalized medicine and emerging gene and cell therapies for inherited retinal dystrophies.

Stargardt disease (STGD1) is a genetic disorder inherited in an autosomal recessive manner that affects the retina and causes vision loss. It is the second most prevalent pathology in this group and results in progressive retinal degeneration and vision loss in both children and adults.[2] STGD1 is caused by mutations in the ATP-binding cassette (ABC) transporter subfamily A4 gene (ABCA4), which encodes a protein involved in the transport of various molecules across cell membranes. These mutations lead to the accumulation of toxic substances in the retina, which leads to the death of photoreceptors. Stargardt disease is the most common form of inherited macular dystrophy, affecting 1 in 8,000 to 10,000 people worldwide. There are more than 1,500 known pathogenic variants of the *ABCA4* gene, most of which are missense or nonsense mutations. Mutations in noncoding regions are also being studied due to their effects on transcriptomic and proteomic complexity. The inherited retinal disease STGD1 leads to macular degeneration and visual loss, for which there is currently no cure. Developing therapeutic approaches to prevent disease progression is therefore crucial. Several therapeutic approaches have been developed to modulate Stargardt disease, including gene therapy, cell replacement therapy, and gene editing. Recent developments in gene editing technology, such as CRISPR-Cas9, have enabled the possibility of permanent gene correction.[3] Notably, CRISPR-Cas9 technology has made significant progress in the biotechnology and biomedicine sectors. CRISPR-Cas9-mediated gene editing has been used to study, model, and potentially treat inherited eye disorders, but there are concerns about the potential risks associated with this technology.

In this study, the authors aimed to correct two pathogenic variants from two STGD1 patients, who are unrelated, carrying compound heterozygous mutations using CRISPR-Cas9 technology. One of the variants related to STGD1 corresponds to a single-base substitution in an intronic region between exons 28 and 29 (c.4253+4C>T), which is predicted to cause a splicing defect. The other variant corresponds to an insertion of a GT in exon 22 of the *ABCA4* gene (c.3211_3212insGT), which is expected to result in a frameshift.[4,5] The authors effectively edited both pathogenic variants using single-stranded oligodeoxynucleotide and CRISPR-Cas9-mediated repair without causing genomic alterations in the predicted off-targets, as confirmed by whole-genome and Sanger sequencing.[6] Additionally, they found that gene editing did not compromise the expression of pluripotency markers in corrected clones compared with parental ones, indicating that the edited cells remained pluripotent. These findings encourage the investigation of CRISPR-Cas9 gene editing to revert pathogenic variants as a promising tool for STGD1 research and a potential therapeutic strategy for this inherited retinal dystrophy. Importantly, deep-intronic mutations account for only a small proportion of *ABCA4* described variants, and the approach used in this study shows precise single-nucleotide gene editing in the *ABCA4* sequence without detected off-target genomic alterations. As a result, the CRISPR-Cas9 investigation used in this work provides a promising approach for possible therapy of the STGD1 disease. Furthermore, this suggests that CRISPR-Cas9 gene editing can be a promising tool for future research and treatment of inherited retinal dystrophies.

The results of the study are promising, as the researchers were able to correct the mutation in the *ABCA4* gene with high efficiency in the patient-derived hiPSCs. The use of hiPSCs, which are generated from the patient's own cells, provides an exciting opportunity for personalized medicine as it eliminates the risk of immune rejection and allows for the development of patient-specific therapies. This research holds great potential for developing gene therapies for patients with Stargardt disease and other inherited retinal diseases. However, there are some important caveats and issues that warrant further analysis before this technology can be used in clinical settings. The efficiency of CRISPR-Cas9 gene editing may vary depending on the location and type of the mutation, and off-target affects can lead to unintended mutations and potentially harmful consequences.[7,8] Additionally, the study was conducted *in vitro*, and further testing in animal models and clinical trials is necessary to assess the safety and efficacy of the

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gene-edited cells. Moreover, the study only corrected the mutation in the hiPSCs, and it remains to be seen whether these corrected cells can be successfully differentiated into the desired cell types, such as retinal pigment epithelium cells or photoreceptor cells. Additionally, the long-term stability and safety of the corrected cells need to be evaluated, including the potential for off-target affects and immunogenicity. Ongoing clinical trials with human embryonic stem cell-derived retinal pigment epithelium cells for treating Stargardt disease are aimed at evaluating the safety of subretinal transplantation of these differentiated cells.[9] In conclusion, the use of CRISPR-Cas9 to correct mutations in hiPSCs derived from Stargardt disease patients is a significant advancement in gene editing and personalized medicine. However, further analysis and testing are necessary to fully evaluate the safety, efficacy, and long-term stability of this technology before it can be applied in clinical settings.

AUTHOR CONTRIBUTIONS

S.K. and P.R.C. conceived and wrote this commentary.

DECLARATION OF INTERESTS

The authors have no conflict of interest to declare.

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