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Commentary

Allele-specific CRISPR-Cas9 editing inactivates a single-nucleotide variant associated with collagen VI muscular dystrophy

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In the recent *Molecular Therapy Nucleic Acids* issue, Bolduc et al.[1] present a sophisticated exploration of the emerging potential of allele-specific CRISPR-spCas9 editing therapeutic approaches for collagen VI-related muscular dystrophy (COL6-RD), a dominant genetic disorder. The disease is triggered by mutations in the *COL6A1*, *COL6A2*, and *COL6A3* genes, encoding collagen α1, α2, and α3(VI) chains, respectively, each structured with a collagenous central triple helical domain composed of Gly-X-Y repeats.[2] Their research highlights the critical importance of precisely targeting disease-causing dominant variants. It underscores the significance of the findings in mitigating the impact of these debilitating disorders, thereby adding value to the field of genetics and molecular therapy.

Mutations in the genes encoding collagen VI, crucial for the structural integrity and function of skeletal muscle connective tissues, disrupt the collagen VI matrix. In particular, glycine substitutions, such as the G290R variant in the *COL6A1* gene (c.868G>A), act as a dominant negative and are associated with a severe form of COL6-RD. These conditions, often presenting early in life, are severely debilitating, underscoring the need for effective solutions and the significance of their research.

The study's findings hold immense potential. It specifically focuses on the pioneering application of CRISPR-Cas9 technology to target and inactivate the G290R allele in *COL6A* by incorporating an intentional mismatch in the guide RNA (gRNA) design. This approach aims to inactivate the mutant allele in particular and introduce frameshifting edits while selectively preserving the normal allele.

The authors designed two gRNAs to introduce frameshifting indels (insertions or deletions) at the site of the G290R variant, effectively inactivating the mutant allele. This approach, known as allelic-specific gene inactivation, does not require a repair template, simplifying the editing process and potentially reducing off-target effects. The study demonstrated that the CRISPR-Cas9 system could efficiently induce frameshifts at the G290R allele in patient-derived fibroblasts, leading to its inactivation. However, achieving allele specificity—ensuring that the editing selectively targeted the mutant allele without affecting the wild-type allele—was identified as a significant challenge.[3]

The authors introduced an intentional mismatch in the gRNA sequence to address this (Figure 1). This modification successfully increased the specificity of the gRNA for the mutant allele, although it also slightly reduced overall editing activity. This trade-off between specificity and efficiency is a recurrent theme in gene editing, highlighting the necessity for carefully optimizing gRNA design to balance these competing factors. The study's findings imply that by making further refinements, such as using truncated gRNAs or engineered Cas9 proteins with higher fidelity, we could enhance the precision of allele-specific editing, thus advancing the field closer to clinical applicability.[4] López-Márquez et al. also recently reported achieving allele-specific CRISPR-Cas9 editing of the G293R variant in *COL6A1* (c.877G>A). Their data suggest that the position of the protospacer may also contribute to high allele specificity.[5]

The study proves that inactivating the G290R allele corrects collagen VI assembly in patient-derived fibroblasts. Restoring the collagen VI matrix is a critical step toward ameliorating the disease phenotype, indicating that even partial inactivation of the mutant allele can have significant therapeutic benefits. However, the authors acknowledge that translating these findings into clinical applications will require further investigation, particularly in determining the threshold of corrected cells needed to achieve functional improvement *in vivo*.[6] This aspect is crucial for ensuring the efficacy of the treatment in a clinical setting.

One of this study's key strengths lies in its contribution to the broader field of gene therapy for dominant-negative disorders. The successful application of CRISPR-Cas9 to selectively target a pathogenic variant without needing a repair template expands the potential therapeutic toolkit for these challenging conditions. Moreover, the study's approach could be adapted to target other genes and mutations implicated in dominant-negative diseases, making it a versatile strategy for addressing a wide range of genetic disorders that have, thus far, been difficult to treat.[7]

However, the study also highlights several challenges before this approach can be translated into viable clinical therapies. Achieving high levels of allele specificity remains a significant hurdle, as even minimal off-target activity at the wild-type allele could have unintended and potentially deleterious consequences. Additionally, delivering CRISPR-Cas9 components to the relevant cells *in vivo*, particularly in muscle tissues, presents substantial logistical challenges that must be

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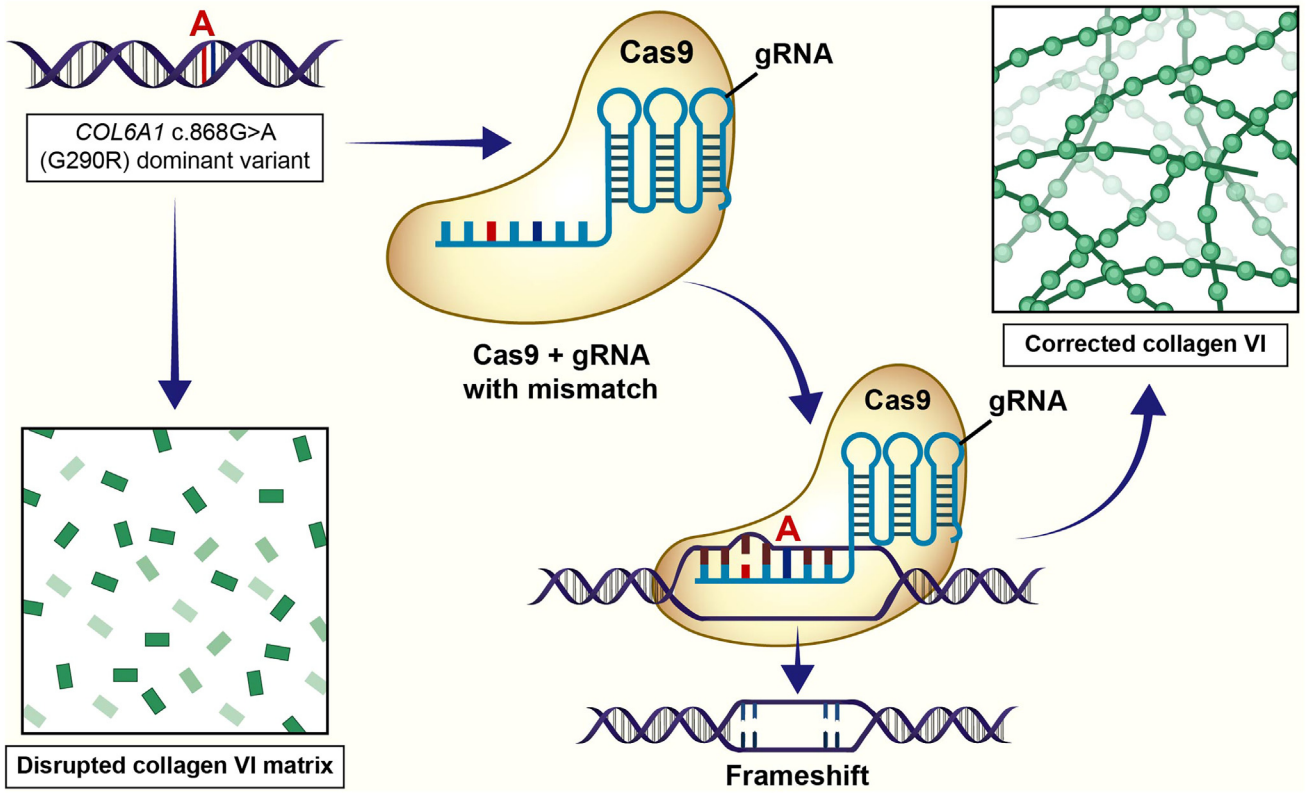

Figure 1. A schematic illustration of allele-specific CRISPR-Cas9 editing that inactivates a single-nucleotide variant associated with collagen VI muscular dystrophy

overcome to ensure the treatment's efficacy and safety. These challenges feature the complexity of translating gene editing technologies from the laboratory to the clinic.[8]

The researchers emphasize the need for further experimentation to refine the editing tools and develop effective delivery systems specifically targeting muscle interstitial fibroblasts, the primary producers of collagen VI in skeletal muscles. These steps are critical to advancing from proof-of-concept studies to practical clinical applications. Moreover, long-term studies will be necessary to assess gene edits' durability and monitor for any potential adverse effects, including immune responses to the CRISPR-Cas9 components or the edited cells. The safety and long-term efficacy of such gene editing interventions are paramount concerns that must be thoroughly addressed before clinical implementation.[9]

In conclusion, this study represents a significant advancement in gene editing for dominant-negative disorders, demonstrating the potential of CRISPR-Cas9 technology to inactivate pathogenic alleles associated with collagen VI muscular dystrophy selectively. The authors explain that CRISPR-Cas9 approaches can be tailored to target specific recurrent dominant disease-causing glycine variants in the *COL6A1* gene and likely other variants in the *COL6* genes. While promising, the study also emphasizes the need to carefully balance efficiency, specificity, and delivery to develop safe and effective gene editing therapies. The insights gained from their work will inform future strategies for treating collagen VI-related muscular dystrophies and a broader range of genetic conditions caused by dominant-negative mutations.

The path forward will require continued innovation and collaboration across the fields of molecular biology, genetics, and clinical medicine to bring these promising therapies from the laboratory to the clinic. The study's findings provide a foundation for further research and development, offering hope for treating these incurable disorders. The future of the CRISPR-Cas9 approach holds immense potential, and studies such as this are paving the way for transformative treatments that could redefine the management of genetic diseases.[10]

DECLARATION OF INTERESTS

The authors declare no competing interests.

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