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Restoration of the physiological levels of PMP22 in CMT1A patient cells via base editing of the Kozak sequence

A. Raoss (1), S. Sanniti (1), M.C. Diener (1), E. Destefanis (3), E. Rossi (1), G. Zappacosta (1), C. Ambrosini (2), V. Sidarovich (1), A. Quattrone (1)

(1) Dept CIBIO, University of Trento, Italy
(2) Fondazione Human Technopole, Milano, Italy
(3) Fralin Life Sciences Institute, Virginia Tech Cancer Research Center DC, Washington, D.C., United States

Hereditary peripheral neuropathies, including Charcot-Marie-Tooth (CMT) disease, are prevalent genetic disorders of the nervous system, affecting around 1 in 2500 people. CMT1A, the most common form of the disease, is caused by a duplication of the PMP22 gene on chromosome 17, which is sensitive to gene dosage. This leads to the overproduction of the PMP22 protein. Currently, there are no approved therapies that halt or reverse CMT1A at its root, so management remains symptomatic and supportive, focusing on preserving strength, mobility, and quality of life.

We applied advanced gene-editing technology — specifically, base editing — to control PMP22 protein levels precisely and moderately. Our approach was to modulate the translational efficiency of the PMP22 mRNA by introducing targeted point mutations into the Kozak sequence of the PMP22 gene to decrease its codon recognition ability. This original approach does not cause genomic damage and aims to fine-tune PMP22 protein levels while avoiding the adverse effects associated with the loss of an allele.

To identify the variants required to restore physiological protein levels, we screened PMP22 Kozak variants in a high-throughput manner to identify and validate those that reduce translational efficiency. We introduced the three best-performing variants into HEK293T cells overexpressing PMP22.

We achieved editing efficiencies above 30% for each variant. Western blot analysis revealed a 30% decrease in PMP22 protein levels in two of the edited variants. We will validate our approach further in patient-derived induced pluripotent stem cells (iPSCs) differentiated to Schwann cell precursors. This strategy has already been patented and, if successfully developed, could form the basis of an advanced clinical approach to CMT1A, overcoming the limitations of other proposed solutions and potentially paving the way for a 'one-shot' gene therapy treatment.