XIX CONVEGNO TRIENNALE
VERONA
15, 16, 17 e 18 OTTOBRE 2026
Camera di Commercio di Verona
AICE Associazione Italiana Centri Emofilia

XIX Convegno Triennale sugli Aspetti Clinici e Sociali dell'Emofilia ABSTRACT N. BEST-03

2.07 Novel therapeutic products and approaches (including gene therapy)

CRISPR-CAS9 CORRECTION IN SEVERE HEMOPHILIA A

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Background and Aims. Haemophilia A (HA) is a rare X-linked recessive bleeding disorder caused by variants in the *F8* gene, which encodes coagulation Factor VIII (FVIII). HA is characterized by FVIII deficiency, leading to impaired haemostasis and spontaneous bleeding episodes. In patients with HA, increasing FVIII activity above 2–5% can significantly improve quality of life. The aim of the study is to correct *F8* variant and to restore FVIII expression in a male patient with Severe Haemophilia A through CRISPR–Cas9 Base Editing in Liver Sinusoidal Endothelial Cells (LSECs).
Materials and Methods. Peripheral blood stem cells were isolated and reprogrammed into induced pluripotent stem cells (iPSCs) after transduction with a non-integrative Sendai virus system. The clones obtained were stabilized and their pluripotency was characterised by specific markers such as Oct4, Sox2, Klf4 and Nanog. Gene correction of HA-iPSCs was performed using CRISPR-Cas9 DNA base editing technology targeting the *F8* variant NM_000132.4: c.6967C>T. Following editing, cells were differentiated into LSECs using an optimised differentiation protocol and FVIII expression was assessed by immunofluorescence (IF) and real-time PCR (RT-qPCR).
Results. HA-iPSCs were positive for alkaline phosphatase staining, and IF validated the expression of pluripotency markers. The differentiated LSECs were positive for FCGR2B expression, as assessed by both IF and RT-qPCR. Furthermore, embryoid bodies formation were performed, demonstrating the expression of markers from all three germ layers. FVIII expression in HA-derived LSECs was evaluated before and after gene editing using IF, RT-qPCR, and western blot. The gene correction was confirmed, resulting in approximately 70% restoration of FVIII levels (*Figure 1*).
Conclusions. The iPSCs derived from the severe HA patient were successfully edited, highlighting the potential of CRISPR–Cas9 technology for future patient-specific correction of pathogenic variants. Furthermore, differentiation into LSECs proved to be a relevant and robust in vitro model for evaluating FVIII restoration in HA patients. In conclusion, given the limitations of current treatments, gene therapy may provide a promising and personalized therapeutic approach for these patients.

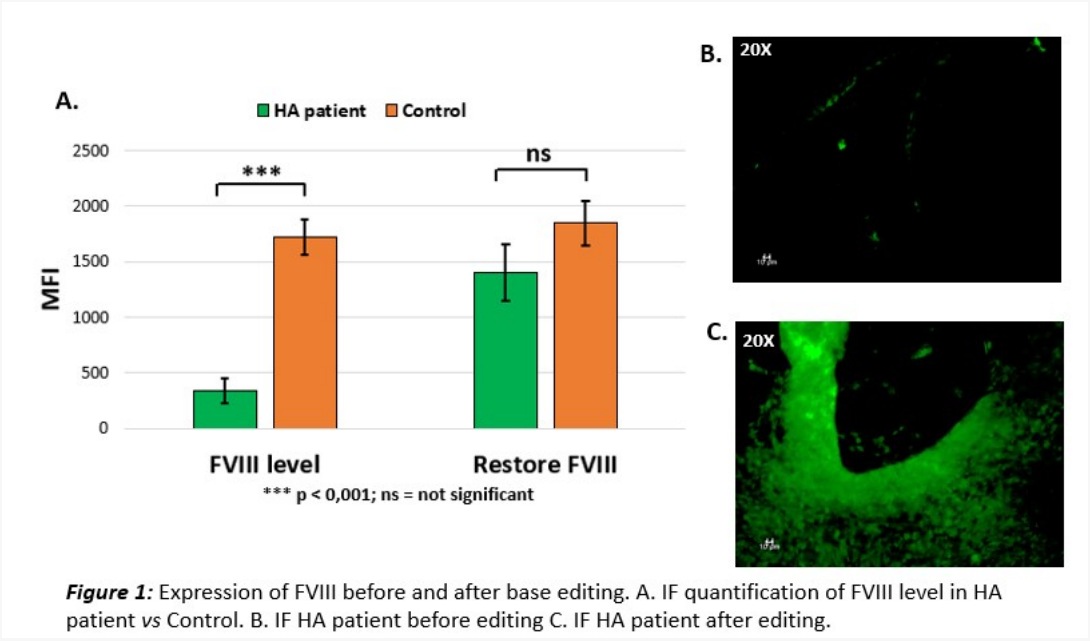


Figure 1: Expression of FVIII before and after base editing. A. IF quantification of FVIII level in HA patient *vs* Control. B. IF HA patient before editing C. IF HA patient after editing.