International Doctoral School
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THE USE OF CELL REPROGRAMMING AND GENE EDITING IN STUDIES OF THE PATHOGENESIS OF CONGENITAL NEUTROPENIA

FixNet
Fix Neutropenia

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The aim of the study is to use the CRISPR-Cas9 gene editing technology to repair ELANE gene mutations in induced pluripotent stem cells (iPSCs) derived from patients with congenital neutropenia.

INTRODUCTION

The *ELANE* gene, which encodes neutrophil elastase (NE), is the best-known gene responsible for over half of the Severe Congenital Neutropenia (SCN) cases, with mutations disrupting normal neutrophil maturation and function, ultimately leading to cell apoptosis through endoplasmic reticulum stress-mediated unfolded protein response (UPR) signaling (Figure 1).

The current treatment for SCN is limited to the administration of granulocyte colony-stimulating factor (G-CSF), which can increase neutrophil counts but is associated with a risk of secondary malignancy. Allogeneic hematopoietic stem cell transplantation is a potential curative therapy for G-CSF-resistant cases but has significant limitations, including graft-versus-host disease.

Current research efforts are focused on exploring the potential of CRISPR/Cas9-mediated gene therapy to restore neutrophil development and function in SCN patients using repaired induced pluripotent stem cells (iPSCs). This innovative approach holds significant promise for developing more effective and targeted therapeutic interventions for this rare and challenging genetic disorder.

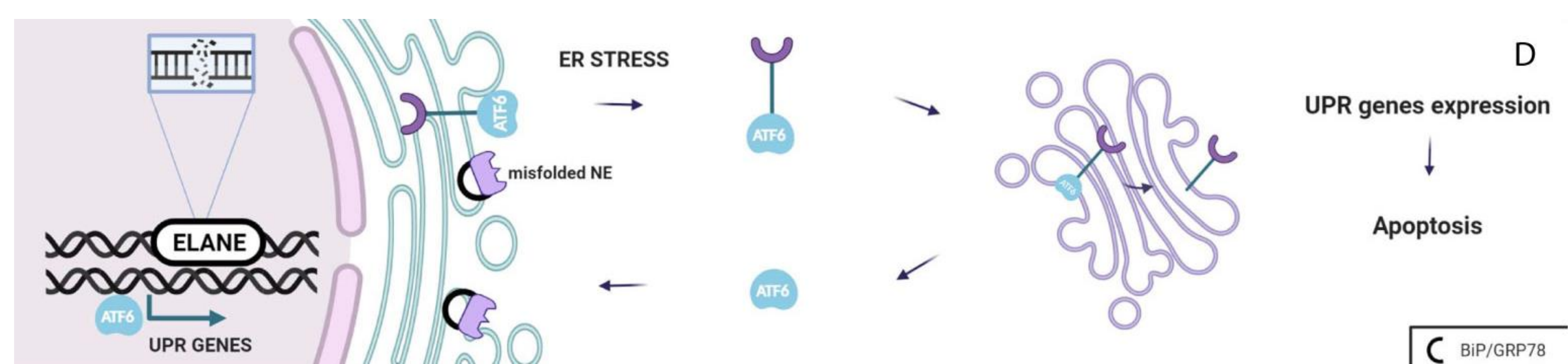


Figure 1. Mutations in the *ELANE* gene can cause abnormalities in the maturation and function of neutrophils, ultimately leading to endoplasmic reticulum stress-mediated unfolded protein response (UPR) signaling and apoptosis of the cells.

MATERIALS & METHODS

1. Patients and sample preparation: Three patients with *ELANE* gene mutations were recruited - two with significantly reduced neutrophil count and one with regular neutophils oscillations. Epithelial cells were isolated from their urine and reprogrammed into iPSCs.

2. Repair: The CRISPR/Cas9 system was used to attempt to repair the causative mutations. The eSpCas9 protein, a gRNA, and a DNA template were used and introduced to the cells with the nucleofection technique. Clonal cultures were set up and sequenced using Sanger sequencing to check the efficiency of the repair.

3. Neutrophil differentiation: Wild type iPSC and iPSCs from patients with *ELANE* gene mutations were differentiated into neutrophils, and surface markers were analyzed. Myeloid subtypes were separated using gating strategy (Figure 2). Control iPSCs were evaluated for morphology and neutrophil elastase location using staining and microscopy.

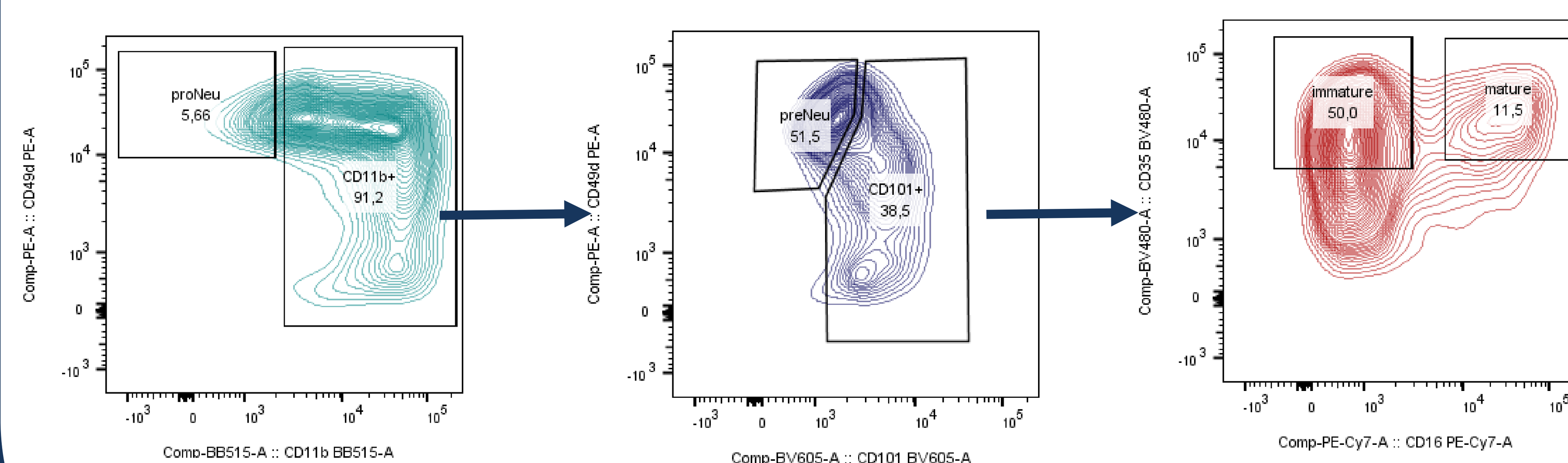


Figure 2. Gating strategy for various myeloid subtypes, such as proNeu, preNeu, immature-Neu, and mature Neu, representing myeloblasts, promyelocytes, myelocytes/metamyelocytes, and neutrophils, respectively.

RESULTS

Out of the three recruited patients, iPSCs were successfully generated from two patients with mutations in *ELANE* at positions c.163T>C p.Cys55Arg and c.597+1G>C V190_F199del. In cells from the patient with the Cys55Arg mutation, the genetic defect was repaired in 4 out of 30 tested cell colonies. Additionally, a homozygous p.Cys55Arg ELANE variant was generated for further functional studies, which can serve as a unique model for investigating neutrophil elastase activity (Figure 2A). In cells from the patient with the c.597+1G<C V190_F199del mutation, the genetic defect was repaired in 9 out of 29 sequenced colonies (Figure 2B). Control iPSCs (wild type) and iPSCs from patients were collected on day 23 and analyzed for the expression of characteristic surface markers of CD corresponding to individual myeloid precursors (Figure 3A). During differentiation into neutrophils, iPSCs obtained from patients with neutrophil elastase dysfunction showed inhibition of differentiation at the myeloblast stage compared to the control group (Figure 3B).

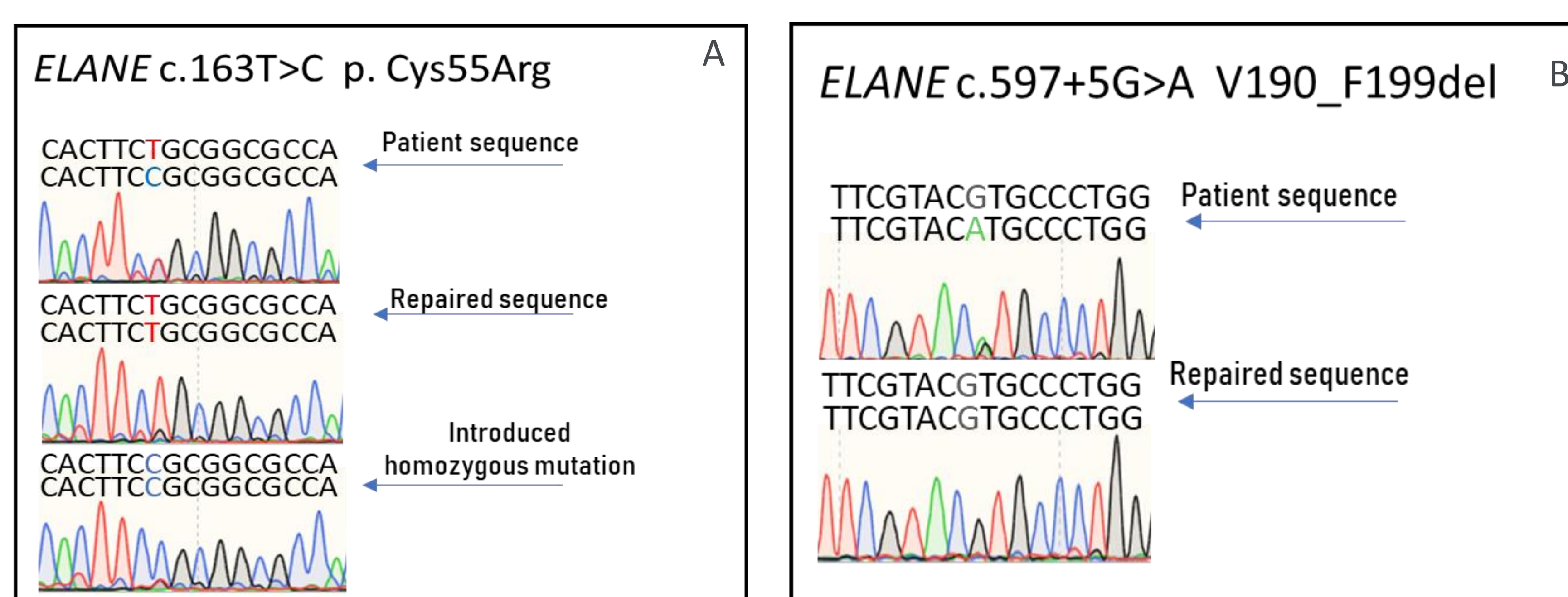


Figure 2. Seqencing results of homogenious colonies of a patient-derived iPSCs repaired using CRISPR/Cas9.
A) ELANE: NM_001972.3:c.[163T>C];[=], NP_001963:p.[(Cys55Arg)];[(=)]
B) ELANE:NM_001972.3:c.[597+5G>A];[=], NP_001963:p.[(Val190_Phe199del)];[(=)]

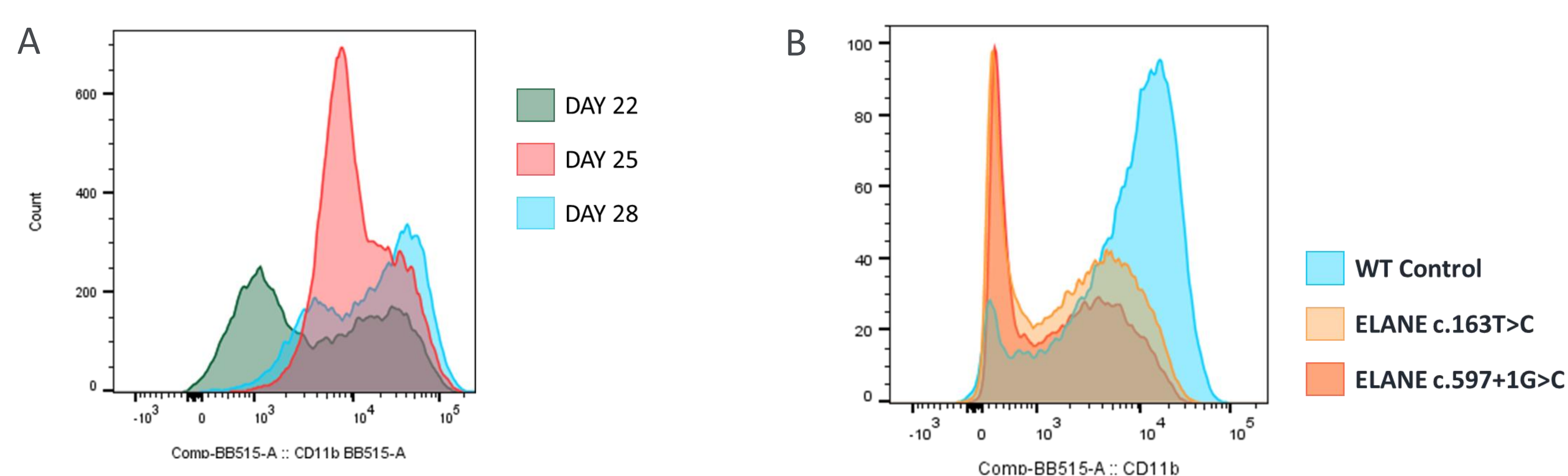
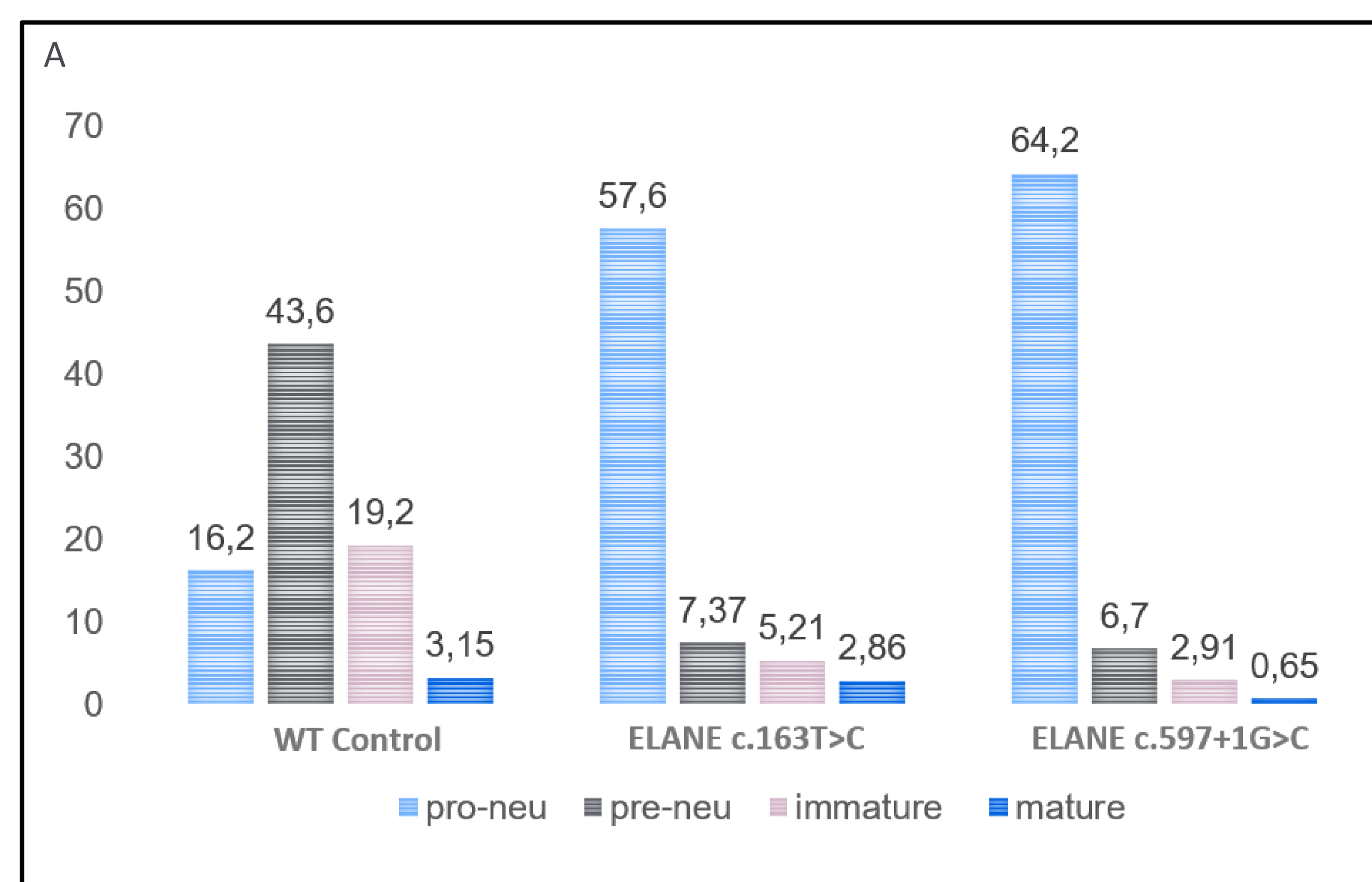


Figure 3. A. The expression of the neutrophil maturation marker CD11b assessed during the course of wild type iPSC differentiation on subsequent days. B. CD11b, a marker for neutrophil maturation, evaluated in iPSCs derived from patients with *ELANE* c.163T>C and c.597+1G>C substitutions, as well as in wild type iPSCs, on the 23rd day of differentiation,.



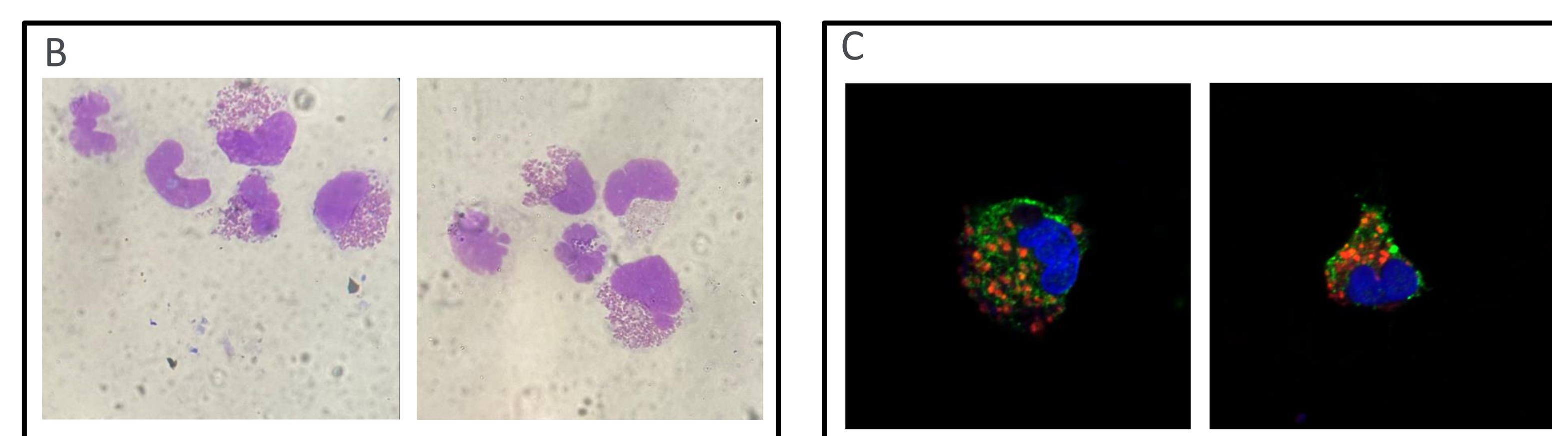


Figure 4. A. Control iPSCs (wild type) and iPSCs from patients with *ELANE* c.163T>C and c.597+1G>C substitutions analyzed for the expression of characteristic surface markers corresponding to individual myeloid precursors at day 23 of differentiation. B. Wild type iPSC differentiated towards neutrophils and neutrophilic-precursors. C. Localization of neutrophil elastase in iPSC-derived neutrophil granulocytes in wild type iPSC; anti-NE (red), anti-Bip (green), DAPI (blue).

CONCLUSIONS

iPSC cells obtained from patients with neutrophil elastase dysfunction are inhibited from differentiating into neutrophils at the myeloblast stage. To determine whether the ability to produce mature functional neutrophils is restored after correcting mutations in iPSC cells from our patient, the differentiation procedure should be conducted and the resulting neutrophils subjected to functional tests. The procedure utilizing corrected iPSC cells from patients with congenital neutropenia may initiate a modern individualized cell therapy for patients.