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ORAL ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Modeling TCIRG1 Neutropenia By Utilizing Patient Derived Induced Pluripotent Stem Cells

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Background: Severe congenital neutropenia (SCN) is a hereditary hematopoietic disorder characterized by recurrent infections and leukemic transformation. We previously identified a novel heterozygous missense mutation (R736S) in *TCIRG1* causing SCN in a large multigenerational family (PMID: 24753205). We also identified a statistically significant correlation in individuals with lower ANC with rare missense variants in *TCIRG1* in a large cohort of population-based genome screening (PMID: 27229898). Recently, we have discovered another *TCIRG1* heterozygousvariant: R736C in two unrelated families with congenital neutropenia. Independently, Shinwari et al identified a novel *TCIRG1* mutation, V52L, which was correlated with the neutropenic phenotype (PMID: 35573728). Homozygous mutations of *TCIRG1* are causing autosomal recessive osteopetrosis (ARO), which is characterized by osteopetrosis and secondary hematological and neurological abnormalities. The Genome Aggregation Database (gnomAD) has no data about *TCIRG1* R736S and R736C variants accruing in the healthy population. This is in contrast of numerous healthy individuals with a various heterozygous variants throughout the *TCIRG1* gene.
Aims: We aim to generate in-vitro model of *TCIRG1* neutropenia by utilizing patient derived induced pluripotent stem cells (iPSCs) and characterize the hematological abnormalities caused by mutant *TCIRG1* expression. We also aim to characterize the incidence of *TCIRG1*-associated neutropenia and the prevalence of *TCIRG1* variants by sharing this new information within the scientific community through identifying more cases/families with this disease.
Methods: Medical history, differential WBC counts and whole exome sequencing (WES) was used to identify the genetic basis of neutropenia in the family with R736S mutation. The R736C mutation in the new families also were identified by WES of the index cases. Medical history, blood counts and Sanger sequencing identified their affected relatives.
iPSC lines of 4 affected individuals from 2 families harboring *TCIRG1* R736S and R736C mutations and healthy volunteer were generated using standard methodology. S732F benign heterozygous *TCIRG1* variant was generated by utilizing CRISPR/Cas9 knock-In gene editing using a healthy volunteer iPSC line. iPSCs hematopoietic differentiation was achieved by using STEMdiff Hematopoietic Kit. Resultant CD34+ cells were differentiated towards neutrophils using a published protocol (PMID: 35795780). Cell proliferation, survival and differentiation characteristics were measured by automated cell counter and flow cytometry, cell viability staining and surface markers analysis for granulocytic differentiation.
Results: The discovery of these families came through the reputation and work of the Severe Congenital Neutropenia International Registry (SCNIR) and the expanding use of WES to identify the causes of hereditary disorders. With the identification of the index cases, we have identified several affected family members in each family. In all three families the severity of neutropenia is variable and correlates with the incidence of recurrent bacterial infections.
Myeloid differentiation of CD34+ cells derived from iPSCs of 4 patients from two families with R736S and R736C mutations revealed maturation, survival and proliferation abnormalities compared to volunteer cells and cell line harboring benign S732F heterozygous *TCIRG1* mutation. All patient cells had from 2 to 3-fold drop in cell proliferation and cell viability drop by over 70% for the patients with R736S and up to 90% with R736C mutation compared to the controls. The myeloid differentiation in the patients' cells, measured by flow cytometry and assessed by CD66b+/CD14+ and CD11b+/CD15+ markers was impaired by 38% and 23% respectively for the patients with R736S and 43% and 24% for the patients harboring R736C mutation.
Conclusions: The pathogenesis of *TCIRG1*-associated neutropenia is currently unknown. Carriers of pathogenic variants from these families appear to have neutropenia with variable severity. Our preliminary studies show that patient iPSC derived hematopoietic cells closely recapitulate the neutropenic phenotype. Population based genomic studies; careful family histories and WES will identify new patients with this disorder.

Disclosures No relevant conflicts of interest to declare.

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