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POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

SMC4 Loss of Function Is a New Cause of Inherited Bone Marrow Failure

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Inherited bone marrow failure syndromes (IBMFS) are a rare, heterogenous group of syndromes that are characterized by cytopenias, bone marrow hypocellularity, and in many cases an increased risk of transformation to myeloid malignancy. Presentation with severe pancytopenias at birth is reported but occurs very rarely, and more often neonatal pancytopenia is attributed to congenital and acquired infections or other acquired causes. We present the case of a preterm neonate with pancytopenia from birth and bone marrow failure of no known infectious, other acquired, or genetic etiology. Antenatal findings were notable for non-immune hydrops fetalis at 30 weeks of gestation prompting delivery. The initial laboratory evaluation revealed a normocytic, reticulocytopenic, severe anemia; leukopenia with an absolute neutrophil count of 500; and severe thrombocytopenia. Viral testing, including parvovirus, rubella, herpes simplex virus, cytomegalovirus, adenovirus, and enterovirus, was negative. Additional testing, including a chromosomal microarray, mitochondrial genome sequencing, chromosome stress testing, neonatal alloimmune thrombocytopenia panel, and telomere length analysis, was non-diagnostic. Bone marrow biopsy showed a remarkably hypoplastic marrow without evidence of dysplasia or cytogenetic abnormalities. The proband underwent matched unrelated donor hematopoietic stem cell transplant, and died approximately one month later from post-transplantation complications including hepatic veno-occlusive disease, thrombotic microangiopathy, CNS and pulmonary hemorrhages, pseudomonal sepsis, and multi-organ dysfunction. Autopsy was significant for microscopic pancreatic atrophy with lipomatous hypertrophy and osteopenia.

The proband and parents underwent clinical exome testing which was non-diagnostic and then consented to research reanalysis of the exome data. Research reanalysis identified the infant was compound heterozygous for predicted deleterious variants in the *Structural Maintenance of Chromosomes 4* gene *(SMC4)*, inherited in trans from each unaffected parent (c.2478_2479ins10 splice site variant predicted to alter splicing and c.T591G; p.Y197X). Germline variants in *SMC4* have not been reported as a cause of human disease; however, somatic variants are observed in several cancers including in acute myeloid leukemia (AML). SMC4 is a highly conserved protein that forms a heterodimer with Structural Maintenance of Chromosomes 2 (SMC2) in the condensin II protein complex. This complex is important for chromosome condensation, cell division, and DNA repair. Within hematopoietic tissue, SMC4 expression is higher in hematopoietic stem and progenitor cells when compared with mature lineage cells.

Given the predicted loss of function from both *SMC4* variants, we performed RNAseq on proband and control fibroblasts. This showed a roughly 10-fold decrease in total *SMC4* transcript levels (Figure 1A). A significant proportion of the transcript present was comprised of an aberrantly spliced transcript characterized by exon 15 skipping. We therefore hypothesized that loss of *SMC4* expression leads to a cell intrinsic defect in hematopoiesis. To test this hypothesis, we used CRISPR-Cas9 gene editing to generate knockout of *SMC4* in human cord blood CD34+ hematopoietic stem/progenitor cells (HSPCs) before culturing cells in an all-colony forming unit (CFU) assay. Using two independent small guide RNAs, we were able to achieve approximately 80% knock-down. After 14 days in culture, we observed a striking decrease in CFUs when compared with controls, though the composition of colony types was relatively unchanged (Figure 1B). Using next-generation sequencing, we observed a significant decrease in remaining *SMC4* genome edits at the end of the culture period, suggesting a relative disadvantage of *SMC4*-edited cells for survival and/or proliferation.

In summary, we describe a preterm infant with congenital bone marrow failure without an identifiable cause and link this phenotype to biallelic loss-of-function variants in *SMC4*. This is the first study identifying *SMC4* as a novel gene that causes inherited bone marrow failure, and further experiments are underway to validate this finding.

Disclosures Warren: *X4 Pharmaceuticals:* Consultancy.

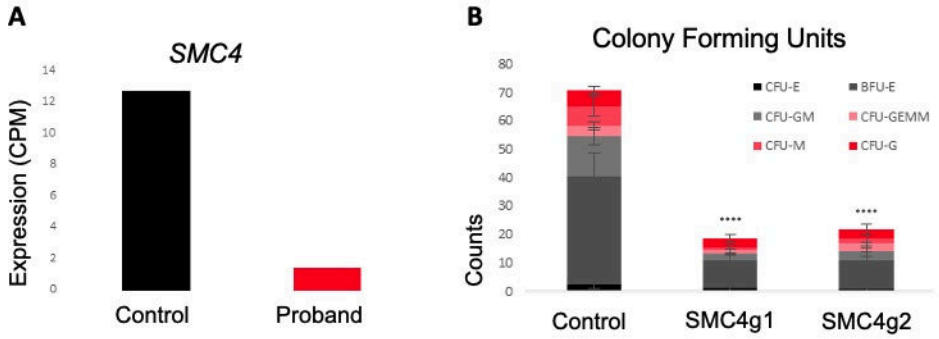

Figure 1 A) Graph comparing expression of *SMC4* RNA in control vs. patient fibroblasts using RNAseq. B) Colony forming unit counts 14-16 days after edited HSPCs plated in complete MethoCult media. Control is AAVS1 safe harbor locus edited cells; SMC4g1 and SMC4g2 are two independent small guide RNAs targeting the *SMC4* gene. **** p<0.05 using one-way ANOVA.

Figure 1

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