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Functional evaluation of a novel RPL30 mutation and its role in Diamond Blackfan anemia (DBA)

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Background

- DBA is a bone marrow failure syndrome with red cell aplasia
- Hallmark ribosomopathy, disease with defects in ribosome biogenesis
- *RPS19* has been most commonly reported and studied, 19 others have been recognized, *RPL30* not previously reported
- Teenage patient diagnosed with DBA with novel, heterozygous noncoding mutation in *RPL30* (Figure 2), identified in mother and suspected in maternal grandmother (Figure 1)

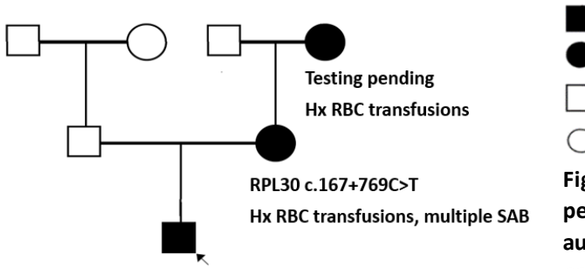


Figure 1. Patient's pedigree demonstrating autosomal dominant pattern. SAB- spontaneous abortion.

Project aims

- → **Generate and validate cell culture model of *RPL30* mutant**
- → Evaluate ribosome production, protein translation, and signal pathways*
- → Visualize nucleolar morphology as biomarker of stress and disease*
- → Assess hematopoietic differentiation, specifically erythroid *

Experimental model

- **Retinal pigment epithelial cell line (RPE-1)-** Wild type (WT) and four *RPL30* mutant homozygous clones achieved with novel CRISPR three guide approach
- **Human induced pluripotent cell line (hiPSC)***

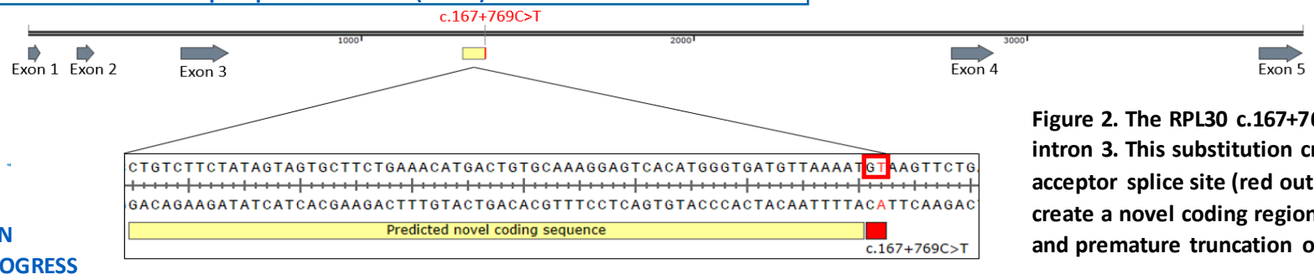


Figure 2. The RPL30 c.167+769C>T variant located in intron 3. This substitution creates an alternative GT acceptor splice site (red outline) that is predicted to create a novel coding region, resulting in frameshift and premature truncation of the *RPL30* transcript.

* IN PROGRESS

Alternative splice site in *RPL30*

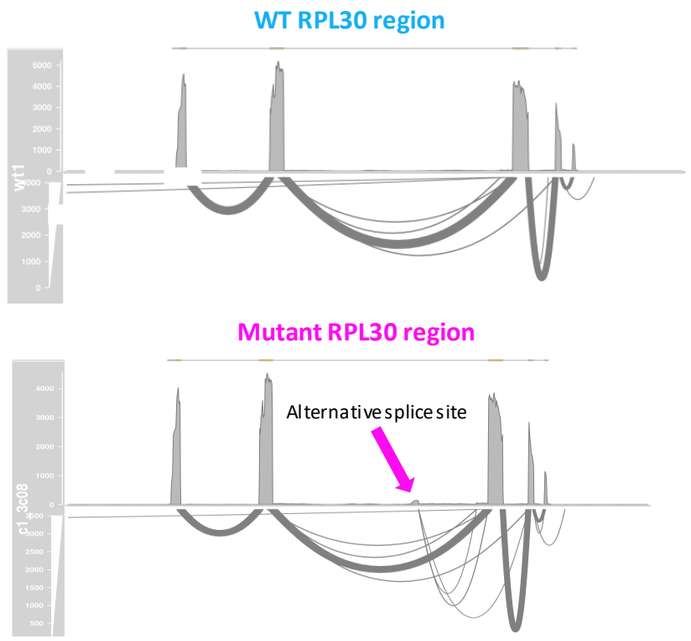


Figure 3. Sashimi plots from RNAseq data comparing RPE-1 WT (blue) and a representative RPL30 mutant clone (pink). Alternative splice site is depicted with pink arrow and was seen in all four clones.

RNASeq altered in *RPL30*

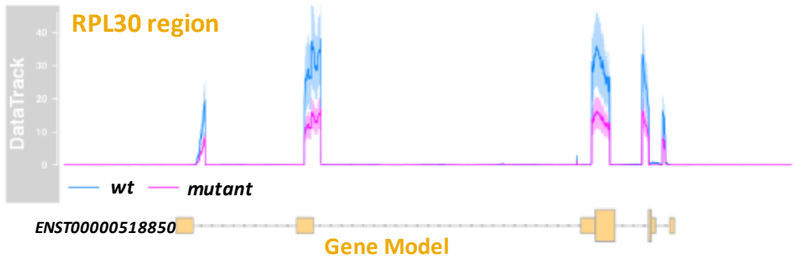
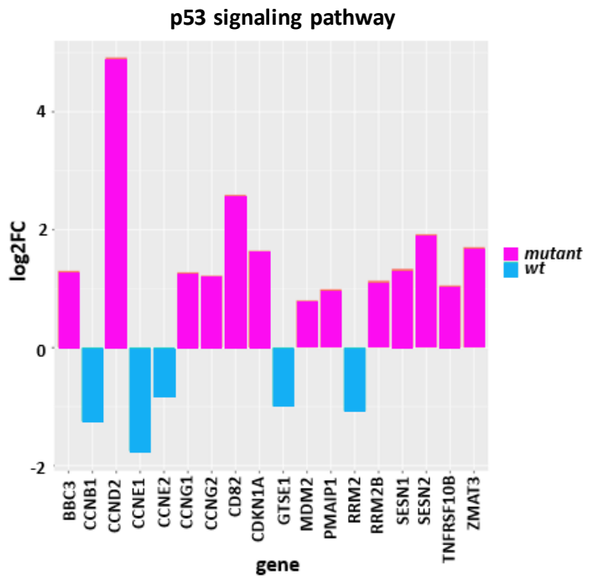


Figure 4. RNA sequencing (RNASeq) coverage for *RPL30* shows decreased RPL30 in RPE-1 mutant clones (pink) compared to RPE-1 WT (blue).

p53 pathway upregulated in *RPL30*

Figure 5. With RNASeq data, performed KEGG pathway enrichment analysis showing significant impact on multiple genes involved in the p53 signaling pathway as seen here.



p53 protein increased in *RPL30*

p53 upregulated in ribosomal protein mutations and proposed to lead to apoptosis in DBA, seen in all 4 clones (Figure 6)

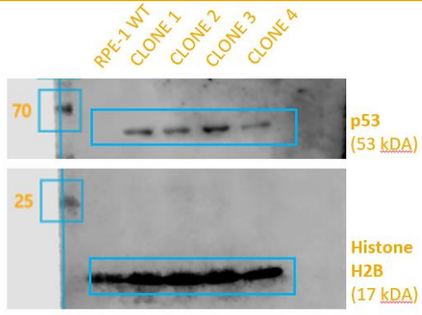


Figure 6. Western blot with LI-COR reagents. Anti-H2B utilized for protein loading control. Chemiluminescence with horseradish peroxidase (HRP) for p53.

Next steps...

- → Explore differential gene expression of RPL30 mutant identified in RNASeq, specifically signal pathways
- → Estimate global protein translation with OPP Click-IT assay
- → Assess ribosome assembly with polysome profiling
- → Develop hiPSC for hematopoietic differentiation and colony forming units

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