Identifying Gene Expression Differences in Blood and Fibroblasts of Patients with Bainbridge-Ropers Syndrome, a Rare Neurodevelopmental Disorder

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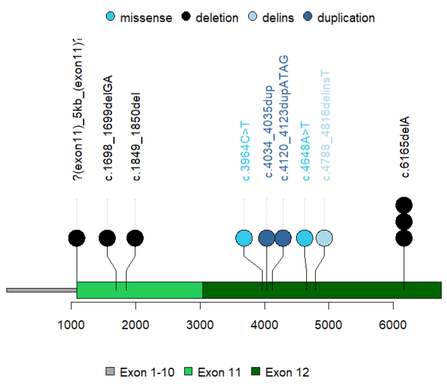
Abstract

Additional Sex Combs-Like 3 (*ASXL3*) encodes a chromatin modifier protein that plays an important role in epigenetic regulation and transcriptional activation. *De novo* truncating mutations in *ASXL3* cause Bainbridge-Ropers Syndrome (BRS), a rare neurodevelopmental disorder that affects various biological processes in addition to neurological development. However, the mechanism by which the mutation causes BRS is poorly understood. Previous research has established that *de novo* mutations alter the transcriptome in patient fibroblasts. We performed RNA sequencing of BRS blood and fibroblasts to identify differentially expressed genes across tissues. Results showed that BRS blood and fibroblasts had 351 and 161 differentially expressed genes (DEGs), respectively. Gene ontology revealed immune and neurological genes dysregulated in BRS blood, while fibroblasts showed additional dysregulation in organ development, craniofacial development, and gene regulation factors. Pathogenic variants in *ASXL3* affect the transcriptome of genes related to development and cell differentiation across tissues.

Background

- BRS is a neurodevelopmental disorder characterized by intellectual disability, cranio-skeletal abnormalities, feeding issues and is associated with autism spectrum disorder (ASD).
- Caused by *de novo* mutations in exon 11 and 12 of ASXL3 (Figure 1).
- ~300 people diagnosed worldwide.
- ASXL3 interacts with the PR-DUB complex to deubiquitinate H2A and repress gene transcription.



- **How do ASXL3 mutations alter a cell's transcriptome, and does it cause similar changes across tissue types?**
- **Truncating mutations in ASXL3 are predicted to alter the protein's structure and function as a chromatin modifier and likely contribute to the development of BRS.**

Methods

- Samples were collected (35 individuals) with a total of 28 blood samples, and 15 skin biopsies
  - 10 BRS blood samples and 5 BRS fibroblast samples

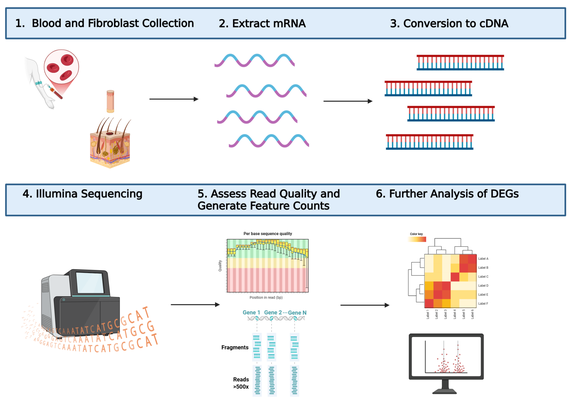


Figure 2: Workflow of RNA Sequencing from Collection to Analysis. RNA library prep was performed using TruSeq. rRNA depletion was performed and the mRNA was later sequenced using Illumina. Read quality was assessed using FastQC, and reads were mapped onto hg38 genome using STAR. FeatureCounts was used to extract gene counts table and analysis was performed using DESeq2 package in R and a p-adjusted value of 0.05 was used in all analyses.

Results

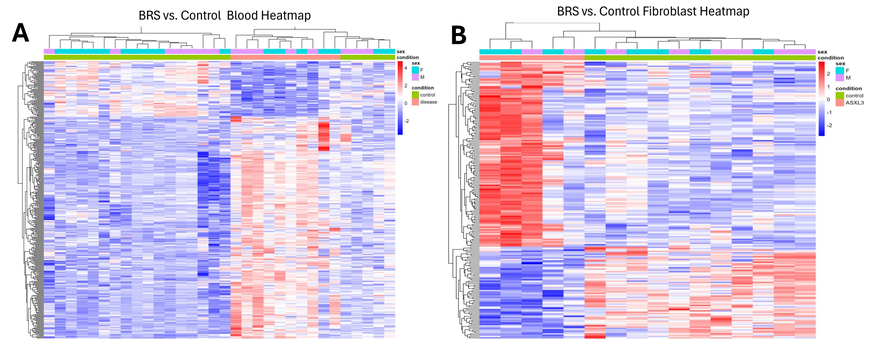


Figure 3: Gene Expression Profiles are Distinct in BRS Blood and Fibroblasts from Control Samples. Relative gene expression levels were normalized using Z-score standardization. The log2 fold change of all upregulated and downregulated DEGs in (A) blood and (B) fibroblasts (351 and 205, respectively) are visualized as a heatmap. The control and patient samples mostly cluster together in both tissue types.

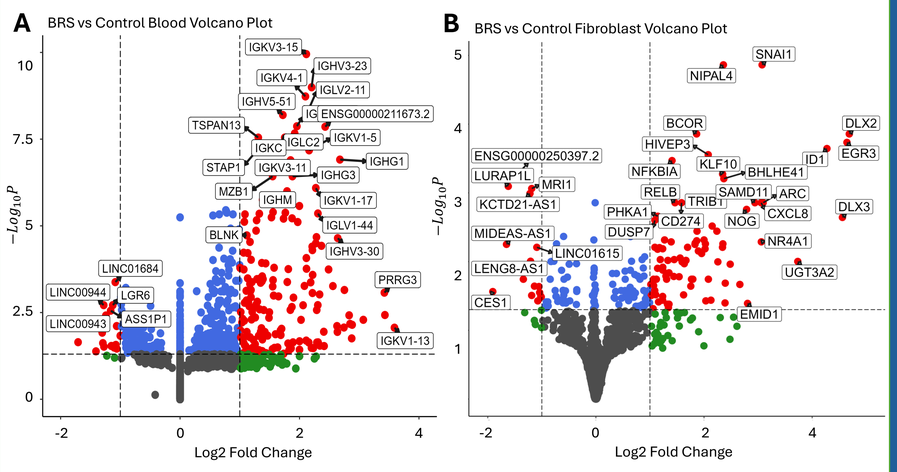


Figure 4: Upregulation of Immune and Developmental Genes in Distinct Tissue Types. DESeq2 results ordered and labeled using ENSEMBL. EnhancedVolcano package was used to visualize the data with a p-adjusted <0.05 and absolute L2FC >0.58. Significant genes shown in red with some relevant genes labeled. (A) 351 blood DEGs and (B) 161 fibroblast DEGs are shown.

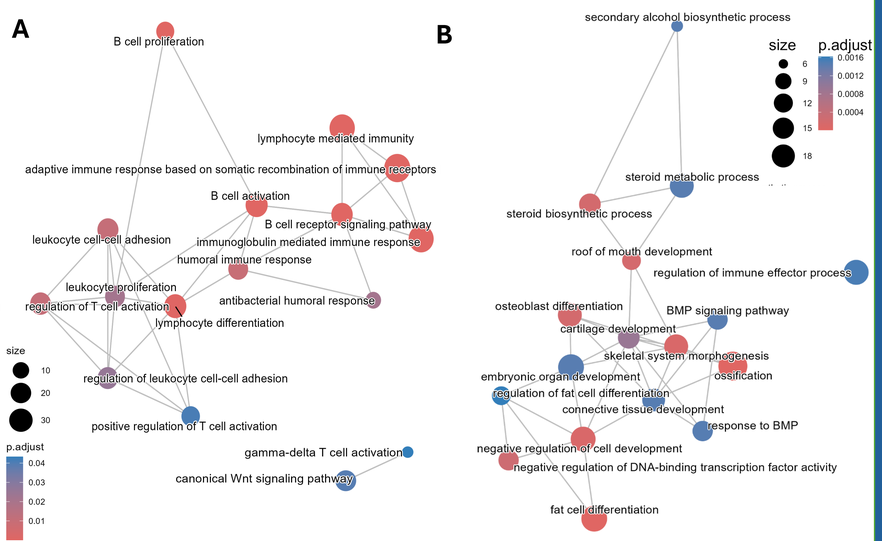


Figure 5: GO Analysis Shows Distinct Systems Dysregulated in Blood and Fibroblasts. GO analysis was used to analyze the biological processes associated with transcript differences. Genes were filtered with an absolute L2FC >0.58 and a p-adjusted value <0.05, then simplified using clusterProfiler. (A) Blood revealed immune genes were the most highly dysregulated, along with canonical Wnt signaling in blood, and (B) various developmental systems were dysregulated in fibroblasts including BMP signaling, and skeletal and organ development.

Blood vs Fibroblast DEGs

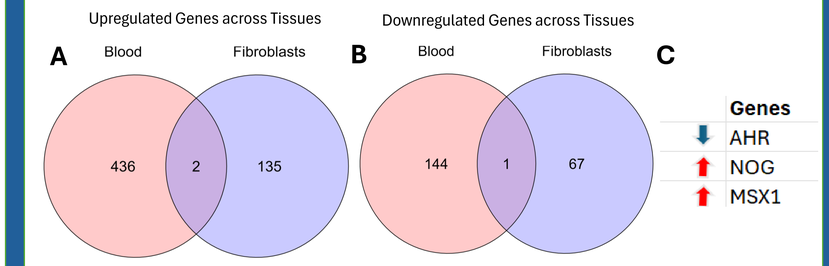


Figure 6: Three Genes Differentially Expressed Across Tissue Types. Only genes mapped to ENSEMBL and filtered using p-adjusted <0.05 included; genes were separated into upregulated (L2FC >0) and downregulated (L2FC < 0). (A) Noggin (NOG) and Msh Homeobox 1 (MSX1) upregulated while (B) Aryl Hydrocarbon Receptor (AHR) was downregulated across tissue types. (C) Complete list of genes dysregulated across tissues shown.

Conclusions and Future Directions

Conclusions

- BRS patients and control samples cluster together with distinct gene expression profiles, showing clear transcriptomic differences between wild type and disease tissues.
- Blood DEGs primarily related to immune response and the canonical Wnt signaling pathway → ASXL3 is lowly expressed in blood samples → possible that mutations in ASXL3 don't impact this tissue significantly.
- ASXL3 mutations implicated in melanoma → fibroblasts are more relevant cell type → GO analysis showed dysregulation in key bone development pathways, BMP signaling, and cell differentiation.
- Comparison reveals three genes similarly dysregulated → all related to bone and CNS development
  - NOG → inhibit BMP pathway
    - Upregulation could cause irregular skeletal development, confounded by other dysregulated genes
  - MSX1 → craniofacial development and limb development
  - AHR → immune response and impact the central nervous system and contribute to diseases across many biological systems

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Acknowledgements

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