ASXL1 mutations that cause Bohring Opitz Syndrome (BOS) or acute myeloid leukemia share epigenomic and transcriptomic signatures

Isabella Lin[1,2,3*], Zain Awamleh[4*], Angela Wei[1,2,3,5], Bianca Russell[6], Rosanna Weksberg[4,7,8], Valerie A. Arboleda[1,2,3,5,9,10]

*These authors contributed equally

[1] Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA
[2] Department of Computational Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA
[3] Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA
[4] Department of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON M5G 0A4, Canada
[5] Interdepartmental Bioinformatics Program, University of California, Los Angeles, CA
[6] Department of Pediatrics, Division of Genetics, University of California, Los Angeles, UCLA
[7] Department of Pediatrics, Division of Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, ON M5G 1X8, Canada
[8] Institute of Medical Sciences and Department of Molecular Genetics, University of Toronto, Toronto, Canada
[9] Molecular Biology institute, University of California, Los Angeles, CA
[10] Jonsson Comprehensive Cancer Center, University of California, Los Angeles CA

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Abstract

ASXL1 (*Additional sex combs-like 1*) plays key roles in epigenetic regulation of early developmental gene expression. *De novo* and truncating mutations of *ASXL1* cause Bohring-Opitz Syndrome (BOS, OMIM #605039), a rare genetic disorder characterized by multiple congenital anomalies including: profound intellectual disability, seizures, heart defects, long and thick hair, and severe skeletal defects giving rise to 'BOS posture'. These same BOS-causing *ASXL1* mutations are also high-prevalence somatic driver mutations in acute myeloid leukemia (AML). We utilize primary cells from BOS patients (n = 18) and controls (n = 27) to dissect gene regulatory changes caused by *ASXL1* mutations. We asked whether DNA methylation epigenetic and transcriptional networks were shared between BOS and AML with *ASXL1* mutations. AML is a heterogeneous disorder and often harbors multiple genetic mutations and chromosomal abnormalities that drive cancer evolution. Using The Cancer Genome Atlas (TCGA), we identified DNAm profiles of AML cases with and without ASXL1 mutations. We used the publicly available methylation data to cluster these samples using CpG sites used in our previously validated BOS DNA methylation episignature. When clustering all individuals with AML and individuals with BOS, individuals with AML associated-ASXL1 mutations cluster more closely with individuals with BOS, whereas individuals with AML and no *ASXL1* mutations cluster separately. To assess transcriptomic signatures, we compared RNA-seq data from 27 AML cases with *ASXL1* mutations from the Beat AML cohort with non-AML blood controls with the differential gene expression analysis of our BOS cases compared with controls. After correcting for batch effects, we identified 821 genes with an absolute fold change greater than 1.5 that were shared between both data sets. A majority of the changes occur in the same direction (615/821, 74.9%) with 53.1% (327/615) of DEGs showing increased gene expression associated with presence of *ASXL1* mutations. Overall, we have demonstrated clear links in the gene regulatory pathway dysregulation driven by ASXL1 across distinct disease entities: Bohring Opitz Syndrome (BOS) and AML.

Despite the significant overlap between genes causing developmental delay and established cancer driver genes (1), few studies have established the clear presence of common downstream effects of these high-impact mutations across disease-types in patient-derived samples. The initial conceptual links between development and cancer were hypothesized over 50 years ago when cancer was defined as an "error of development" by Dr. Beatrice Mintz (2, 3); more specifically, that genetic aberrations in stem cells gave rise to cancer through reversion to a more undifferentiated state (3, 4). During human development, genes that regulate the epigenome, called epigenes (5), play pivotal roles in driving cell-specific RNA expression and complex biological signaling programs. Mutations in epigenes, such as *ASXL1*, are hypothesized to be primary drivers in cancer and act to activate aberrant developmental programs that revert to an malignant stem-cell state(6). While the dual essentiality of epigenes in human development and cancer has been established, exploration of the similarity of molecular mechanisms and epigenetic biomarkers across seemingly distinct disease types has not been fully elucidated.

In this letter, we consider the gene, *Additional sex combs-like 1* (*ASXL1*), where germline, *de novo*, truncating mutations in *ASXL1* have been identified in 75% of cases of a severe pediatric syndrome, Bohring-Opitz Syndrome (BOS, MIM#605309), which is characterized by intrauterine growth restriction, microcephaly, intellectual disability, dysmorphic features and a characteristic posture. The same pathogenic *ASXL1* mutations in BOS are identified as somatic driver mutations in 30% of acute myeloid leukemias (AML)(7). BOS is not currently associated with cancer-predisposition in affected children and to date, no BOS patients have been reported to develop AML or myeloid dysplastic syndromes. The extent to which the same molecular mechanisms that drive developmental anomalies *in utero* and drive leukemia remain unclear. To explore whether *ASXL1* mutations dysregulate common epigenetic and transcriptomic targets in development and in AML, we compared the transcriptomic and DNA methylation dysregulation in BOS patients and in patients with AML harboring *ASXL1* somatic mutations (**Figure 1A**). Our results highlight common dysregulated epigenomic and transcriptomic states driven by protein truncating

mutations in *ASXL1*. These point to direct effects of the truncated *ASXL1* protein that are common between germline and somatic pathophysiology.

Informed consent was obtained from all research participants according to the protocol approved by the Hospital for Sick Children (REB# 1000038847) and UCLA (IRB #11-001087). For the methylation assay, DNA was extracted from whole blood, bisulfite converted, and processed as previously described(8). Peripheral blood was collected in PAXgene Blood RNA Tubes (Qiagen) and extracted using the MagMAX™ RNA Isolation Kit (Ambion). RNA libraries were prepared using Truseq Stranded Total RNA Library Prep Gold (Illumina) with QiaSelect rRNA and globin Depletion (Qiagen). Indexed libraries were pooled were sequenced to 40 million reads per sample at the UCLA Technology Center for Genomics and Bioinformatics. We compared transcriptomic data from 27 AML cases with *ASXL1* mutations from the Beat AML cohort (9) with non-AML blood controls. All RNA-seq data was processed through our RNA-seq pipeline described in (10), corrected for batch effects, sex, age and disease state (Supplemental Tables).

Transcriptomic analysis comparing BOS patient-derived blood to controls (**Figure 1B,** vertical) identified 2,118 significant differentially expressed genes (DEGs) ($p_{adj} < 0.05$), with 1097 DEGs with an absolute fold change greater than 1.5. Parallel analysis comparing AML-ASXL1 patient samples compared with control blood (**Figure 1B**, horizontal) identified 23,976 DEGs (11,394 protein coding DEGs), likely due to the significant cell-type heterogeneity in AML specimens. Our previous work identified commonly dysregulated genes and pathways across different BOS patient tissues, suggesting that ASXL1 has common genetic mechanisms that act across many cell types. To identify DEGs that are across different disease-types driven by *ASXL1*-mutations, we plotted fold change for the transcriptomic analyses across their respective axes (**Figure 1B, center**) which highlighted 821 significant genes with an absolute fold change greater than 1.5. Most of the changes occur in the same direction (615/821, 74.9%) with 53.1%

(327/615) of DEGs showing increased gene expression associated with presence of ASXL1 mutations. The intersection of these gene lists was shown to be significant by fisher exact test (p-value= 2.059301e-90). Our data here highlight the same genes identified in our global transcriptomic studies in dermal fibroblasts(11), including genes such as *VANGL2*, a member of the planer cell polarity pathway(12) and *GRIK5*, a pre- and post-synaptic receptor for glutamate, a crucial excitatory neurotransmitter in many regions of the central nervous system(13).

To investigate methylation differences, Illumina 450K DNA methylation (DNAm) data for six individuals with AML was obtained from The Cancer Genome Atlas (TCGA)(14) data on the Genomic Data Commons (GDC) repository(15). Of these, three individuals harbor a somatic variant in *ASXL1,* while the remaining three harbor somatic mutations in other genes. We compared the DNA methylation profiles of individuals with AML to the DNA methylation profiles of our cohort of individuals with BOS, using principal component analysis (PCA) and hierarchical clustering (dendrogram) at the established BOS DNA methylation signature sites (763 CpG sites)(8). The analysis included DNA methylation profiles of typically developing controls (grey), individuals with BOS (orange), the three AML samples with *ASXL1* mutations (green) and three without *ASXL1* mutations (purple). The PCA plot shows the three individuals with AML-ASXL1 variants (green) that are clearly distinguishable from controls on the first principal component (35%) and individuals with BOS (orange) on the third principal component (10%) (Figure 1C). The three individuals with AML not carrying *ASXL1* mutations (purple) are also distinguished from both controls and individuals with BOS on the second principal component (21%). When clustering all individuals with AML and individuals with BOS, individuals with AML associated-ASXL1 mutations cluster more closely with individuals with BOS, whereas individuals with AML and no *ASXL1* mutations cluster separately. This clustering pattern is recapitulated in the dendrogram and heat map (Figure 1D), which shows greater overlap in methylation patterns between individuals with BOS and AML caused by *ASXL1* mutations (green-orange) compared to AML caused by somatic mutations in other genes (purple). This demonstrates that the DNA methylation signal associated

with *ASXL1* mutations supersedes the DNA methylation signals associated with disease status (BOS vs AML), age, and sex.

In conclusion, these findings clearly demonstrate that truncating mutations in *ASXL1* drive common molecular dysregulation, regardless of disease-types or whether the mutations originate as germline or somatic mutations. The cell-type and developmental contexts remain critical to determining the full effects of *ASXL1*-mutation, as *ASXL1* is known to act as part of larger protein complexes which require co-expression of protein factors(16). Our data shows that *ASXL1*-truncating mutations dysregulate common gene pathways, regardless of whether the mutation originates as germline or somatic. Therefore, we believe that targeted treatments for *ASXL1*-mutated leukemias may be repurposed for patients with BOS to restore some molecular functions. Conversely, identification of molecular mechanisms in rare disease populations can define how driver mutations lead to cancer and thereby lead to a much larger impact beyond rare disease populations. Targeting the epigenome is an emerging approach to treating cancer(17) yet there are no precision-targeted epigenomic treatments for rare disease patients(18). These data highlight the potential of for epigenetic therapies designed for cancer to be repurposed in rare pediatric syndromes driven by shared mutations and mechanisms.

Public Data Sets.
https://portal.gdc.cancer.gov/projects/TCGA-LAML.

Conflict of interest statement.
The authors declare that no conflict of interest exists.

Author Contributions.
VA, ZA and IL designed and conceptualized the study and wrote the paper. IL, SL and AW performed data generation and transcriptomic analysis for patient-derived samples. BR coordinated sample collection for patient samples. ZA and RW performed data generation and analysis of DNA methylation data. All authors contributed to the writing of the manuscript.

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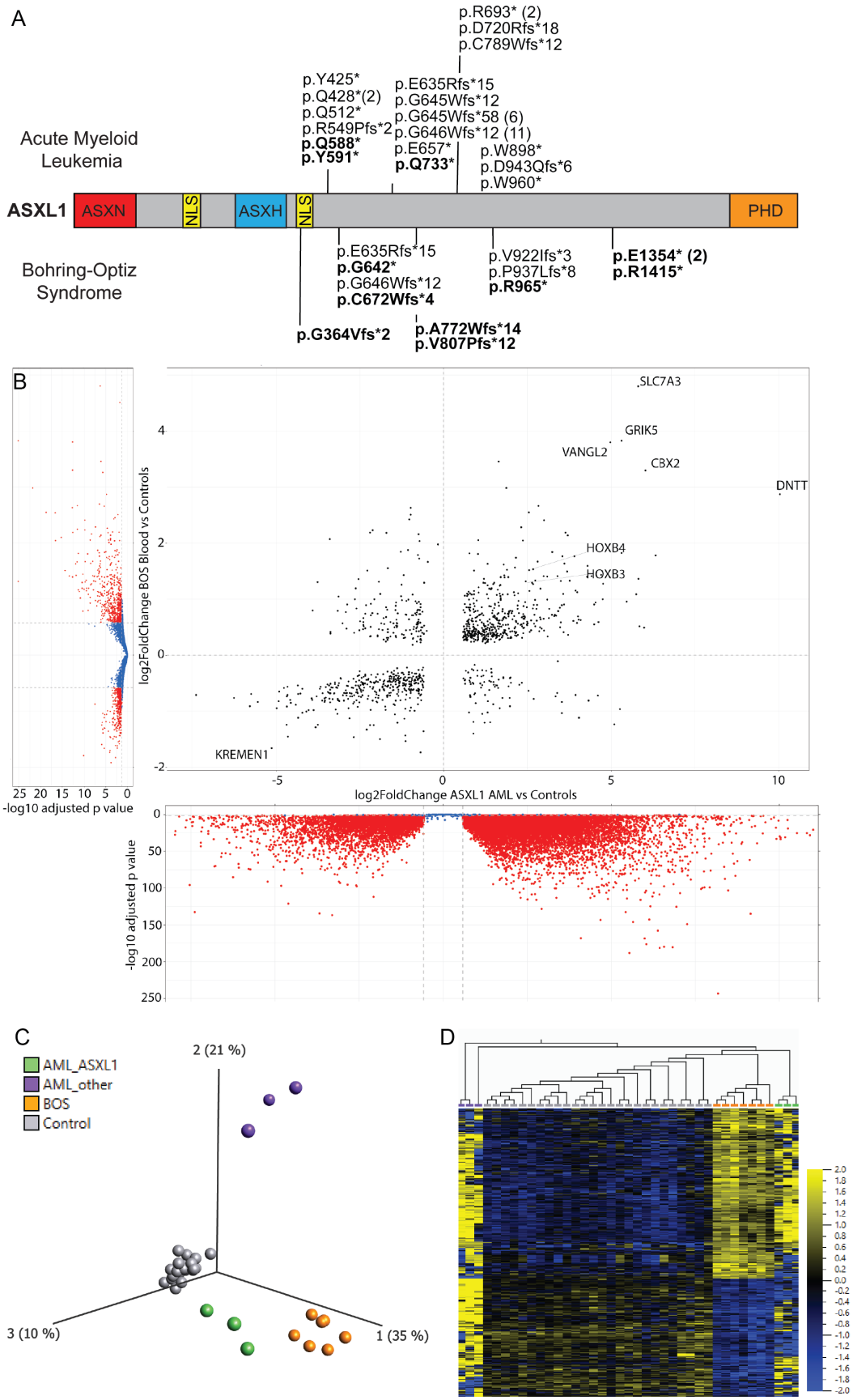
A
Acute Myeloid Leukemia
Bohring-Optiz Syndrome
ASXL1
ASXN
NLS
ASXH
NLS
PHD
p.R693* (2)
p.D720Rfs*18
p.C789Wfs*12
p.Y425*
p.Q428*(2)
p.Q512*
p.R549Pfs*2
p.Q588*
p.Y591*
p.E635Rfs*15
p.G645Wfs*12
p.G645Wfs*58 (6)
p.G646Wfs*12 (11)
p.E657*
p.Q733*
p.W898*
p.D943Qfs*6
p.W960*
p.E635Rfs*15
p.G642*
p.G646Wfs*12
p.C672Wfs*4
p.V922Ifs*3
p.P937Lfs*8
p.R965*
p.E1354* (2)
p.R1415*
p.G364Vfs*2
p.A772Wfs*14
p.V807Pfs*12
B
SLC7A3
GRIK5
VANGL2
CBX2
DNTT
HOXB4
HOXB3
KREMEN1
log2FoldChange BOS Blood vs Controls
log2FoldChange ASXL1 AML vs Controls
-log10 adjusted p value
-log10 adjusted p value
C
AML_ASXL1
AML_other
BOS
Control
2 (21 %)
3 (10 %)
1 (35 %)
D

Figure 1. Pathogenic variants in *ASXL1* drive common epigenomic and transcriptional dysregulation in germline disorders and Acute Myeloid Leukemia (AML). A) Schematic representation of the ASXL1 protein (GenBank: ASXL1; NM_015338.6; GRCh37), its functional domains, and mutations causing BOS and AML. Red, HB1, ASXL, restriction endonuclease HTH domain (HARE-HTH or ASXN, 11–83); blue, Asx homology domain (ASXH, 236–359); orange, C-terminal plant homeodomain (PHD, 1506–1539). Mutations in bold have both RNA-seq and DNA methylation data. B) Global transcriptomic analysis of BOS-patient whole blood compared to controls identified 2118 p-adj significant genes (blue and red) and 1097 differentially expressed genes (DEGs, red) with absolute log fold change great than 1.5 (vertical axis). Transcriptomic analysis comparing *ASXL1*-mutated AML with samples without *ASXL1* mutations identified 23,943 DEGs (horizontal axis). DEGs are Bonferroni-corrected p-value, $p < 0.05$). DEGs with a log2 fold change > 0.58, corresponding to an absolute fold change greater than 1.5 are shown in red). Our study identified 821 genes that were differentially expressed across all ASXL1 mutated samples, regardless of sample or disease types. **C)** principal component analysis (PCA) plot and **D)** heatmap showing clustering of individuals with BOS (n=8; orange), typically developing controls (n=26; grey), individuals with AML caused by a somatic mutation in *ASXL1* (n=3; green), and individuals with AML caused by somatic mutations in other genes (n=3; purple). Clustering is based on DNA methylation values for each individual at the 763 CpG sites identified in the BOS DNAm signature. The heatmap color gradient indicates the normalized DNAm value ranging from -2.0 (blue) to 2.0 (yellow). Euclidean distance metric is used in the heatmap clustering dendrograms.

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