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Germline Variants Associated with Cancer Predisposition and Bone Marrow Failure Are Common in KMT2A-r Infant Acute Lymphoblastic Leukemia Patients

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Germline Variants Associated with Cancer Predisposition and Bone Marrow Failure Are Common in KMT2A-r Infant Acute Lymphoblastic Leukemia Patients

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Background

- Leukemias in infants (<12 months) are extremely rare, ~40 cases/million infants diagnosed in the US each year.
  - Of these, infant acute lymphoblastic leukemia is the most common
- Prognosis remains poor with an overall survival of less than 50%.
- Approximately 70% of cases are characterized by a chromosomal rearrangement of *KMT2A* (*KMT2A-r*) on chromosome 11q23.
  - >100 partner genes, many of which are known transcriptional elongation factors
  - Strongest independent predictor of a poor prognosis

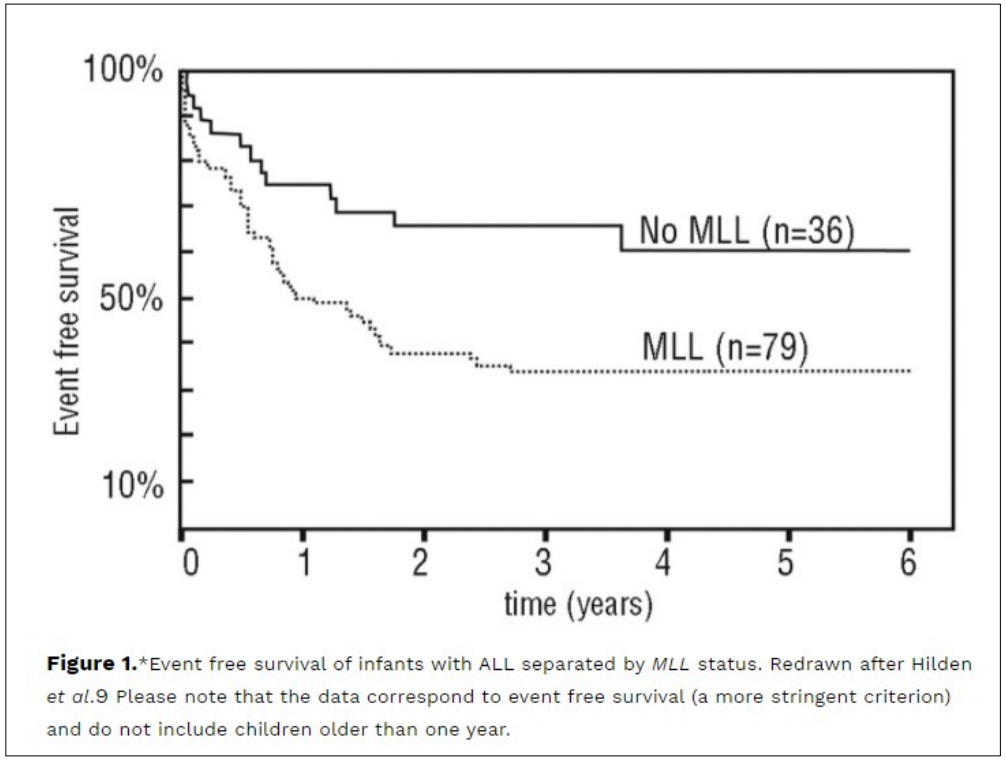
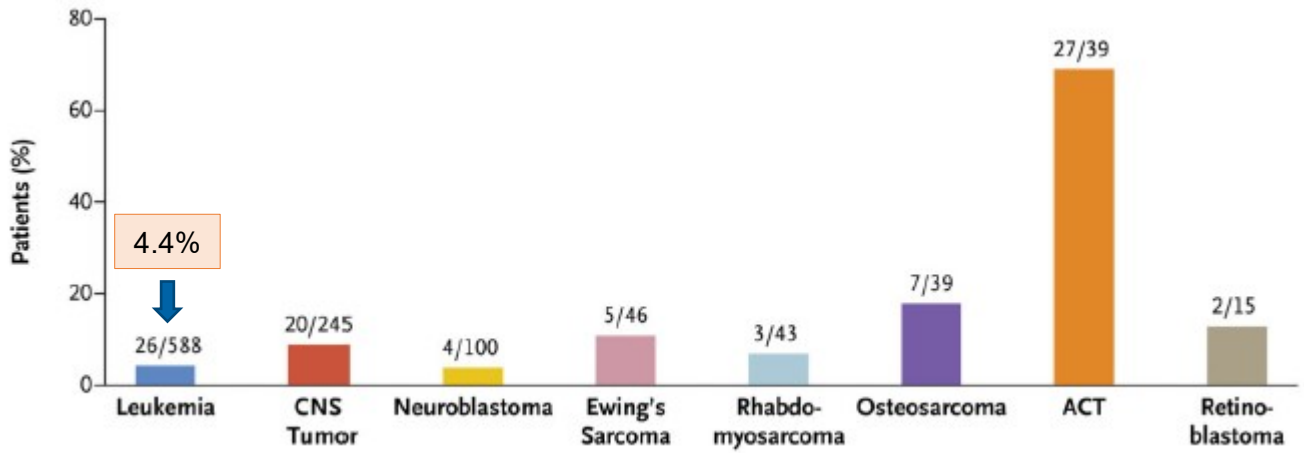


Figure 1.*Event free survival of infants with ALL separated by *MLL* status. Redrawn after Hilden *et al.*9 Please note that the data correspond to event free survival (a more stringent criterion) and do not include children older than one year.

Slany R. 2009. *Haematologica*

Background

- Aside from *KMT2A*-r itself, studies examining somatic variation have demonstrated a low mutational frequency.
- Germline mutations in cancer predisposition genes are found in 8.6% of pediatric malignancies (Zhang J et al., *N Engl J Med* 2015).



- We hypothesize that **germline variants may play a role in the development of *KMT2A*-r ALL in infants.**

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Benign	Likely Benign	Variants of Uncertain Significant	Likely Pathogenic	Pathogenic

Predicting Pathogenicity of a Gene Variant

- Type of gene variant
  - Missense, nonsense, frameshift, splice site → how will it effect function?
- Population data
  - prevalence in control populations
- Computational and predictive data
- Functional data
  - In vitro or in vivo studies supporting damaging effect on gene/gene product
- Segregation/familial data
- Allelic data
- Phenotypic data

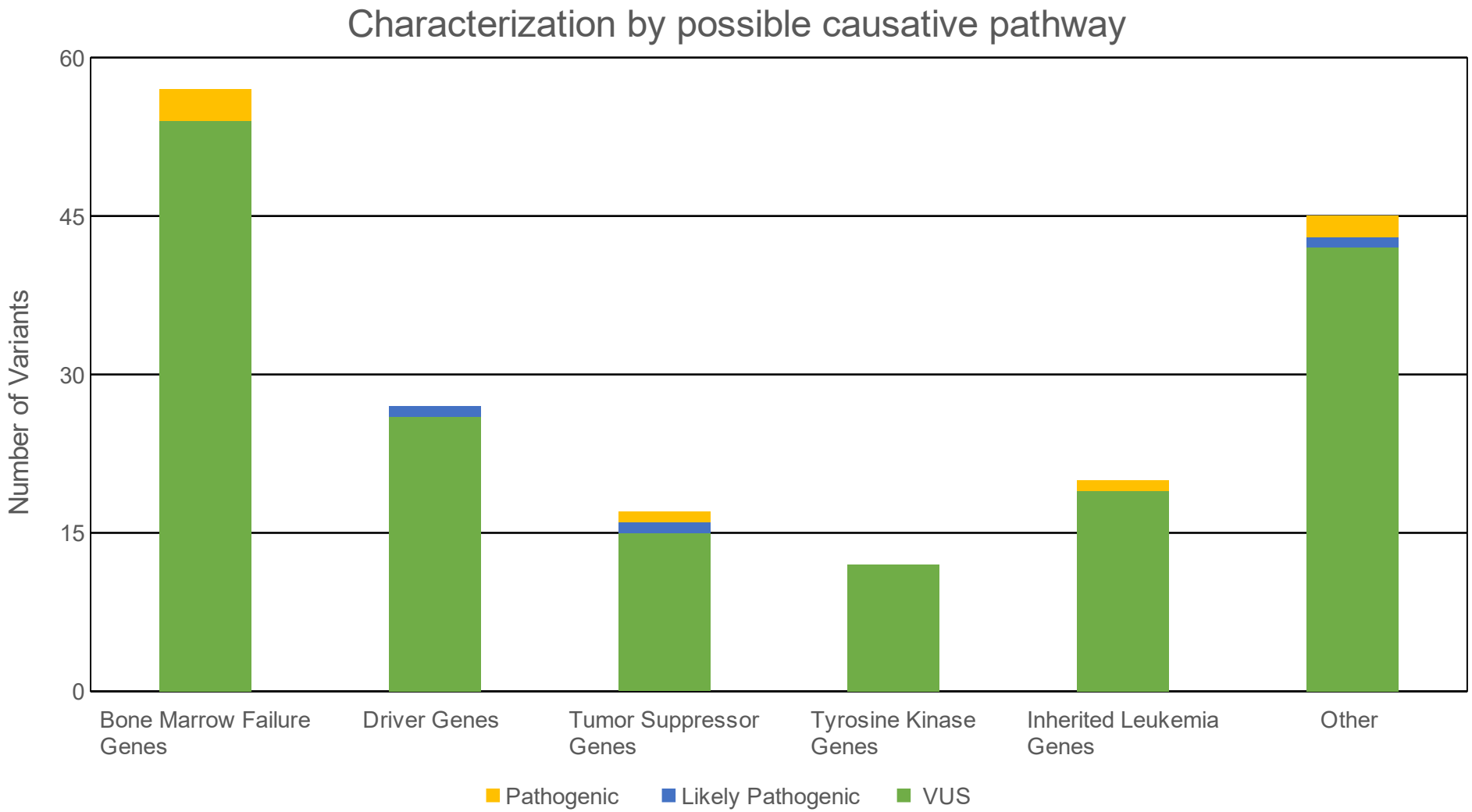
Methods

- We performed whole genome sequencing (WGS) and whole exome sequencing (WES) on DNA isolated from peripheral blood at time of remission from 36 *KMT2A*-r infant ALL cases enrolled on COG trial AALL15P1.
- Sequencing was performed using Illumina Hiseq 4000 or 2500 to a minimum depth of 90Gb (WGS) and 15Gb (WES). Alignment and variant calling were performed using the Dragon Bio-IT platform (v 3.2.8, Illumina)
- We compiled a list of 346 genes associated with cancer predisposition, bone marrow failure, and cancer driver genes (as identified in somatic variant analysis by Ma X et al., *Nature* 2018)
- Variants meeting the following criteria were interpreted using ACMG/AMP 2015 guidelines for sequence variants:
  - Variant allele frequency ~50%
  - Minor allele frequency <1% in our Children's Mercy internal variant warehouse database

Results

- 351 germline variants were identified (mean 9.75 variants per patient sample)
  - Benign/Likely Benign (B/LB) = 199
  - Variants of Uncertain Significance (VUS) = 143
  - Likely Pathogenic (LP) = 3
  - Pathogenic (P) = 6
- 19.4% of unique patient samples displayed at least one LP/P variant

Results


Characterization by possible causative pathway
Number of Variants
60
45
30
15
0
Bone Marrow Failure Genes
Driver Genes
Tumor Suppressor Genes
Tyrosine Kinase Genes
Inherited Leukemia Genes
Other
Pathogenic
Likely Pathogenic
VUS

Results

Likely Pathogenic/Pathogenic Germline Variant Characterization in *KMT2A-r* infant ALL							
Case Number	Gene	Nucleotide Change	Amino Acid Change	Transcript	Clinical Disorder	Inheritance	ACMG Classification
1	*ERCC2*	c. 466C>T	p. R156*	NM_000400.3	Xeroderma Pigmentosum (early onset skin cancer)	AR	Pathogenic
1	*FANCI*	c.3853C>T	p. R1285*	NM_001113378.1	Fanconi anemia (BMF)	AR	Pathogenic
3	*CTC1*	c.724_727 del	p. KA242*	NM_025099.5	Coats plus syndrome, Dyskeratosis congenita (BMF)	AR	Pathogenic
12	*ATM*	c.8988-1G>A	p. ?	NM_000051.3	Ataxia-telangiectasia syndrome (lymphoma and leukemia)	AR	Pathogenic
18	*MITF*	c.952G>A	p. E318K	NM_000248.3	Increased risk of melanoma and renal cell carcinoma	AD	Pathogenic
18	*HOXB13*	c.251G>A	p. G84E	NM_006361.5	Hereditary prostate Cancer	Unknown	Pathogenic
21	*CHEK2*	c.707T>C	p. L236P	NM_007194.3	Increased risk of breast cancer and colorectal cancer	AD	Likely Pathogenic
30	*RAG1*	c.994C>T	p. R332*	NM_000448.2	Severe Combined Immunodeficiency, Alpha/Beta T-cell lymphopenia	AR	Likely Pathogenic
33	*ERCC2*	c.2150C>G	p. A717G	NM_000400.3	Xeroderma Pigmentosum (early onset skin cancer)	AR	Likely Pathogenic

Conclusion

- Our *KMT2A*-r infant ALL cohort presents a higher mutation rate of 19.4% in germline variants in cancer predisposition and bone marrow failure genes than has been previously reported, however the significance of this finding is unclear.
- Variants in *ERCC2*, which encodes a protein involved in DNA repair mechanisms, were found to be recurrent within our cohort.
- We have continued this investigation, exploring germline analysis of other infant and non-infant ALL samples to determine the frequency of this recurrent *ERCC2* variant in relation to other pediatric leukemias.

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- Patrick A. Brown

Children's Mercy
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Thank you for your time.

Please contact me at semcdermott@cmh.edu with any questions.