THE GENOMICS OF EARLY AGE ACUTE PROMYELOCYTIC LEUKEMIA

Francianne G. Andrade[1,2], Ph.D.; Ingrid Sardou-Cezar[2]; Ivo S. Muskens[1], M.D.; Patrícia C. de Brito, M.D.[3]; Gustavo Zamperlini, M.D.[4]; Eugênia Terra-Granado, Ph.D.[2], Joseph L. Wiemels[1], Ph.D.; Maria S. Pombo-de-Oliveira[2], M.D., Ph.D.

[1] Center for Genetic Epidemiology, Department of Preventive Medicine, University of Southern California, California, United States
[2] Pediatric Hematology-Oncology Program, National Cancer Institute, Rio de Janeiro, Brazil
[3] Department of Pediatric Oncology, Araújo Jorge Hospital, Goiás, Brazil
[4] Department of Pediatric Oncology, Hospital Infantil Darcy Vargas, São Paulo, Brazil

BACKGROUND

- Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia characterized by the master drive t(15;17)(q24;q21)/*PML-RARα* and distinctive cytopathology.
- APL seldom occurs in early childhood, suggesting a long latency for the accumulation of genomic and/or epigenomic alterations that leads to overt disease.
- The genomic events of leukemia in early-age are mostly initiated *in utero* however, they have not been explored in APL.

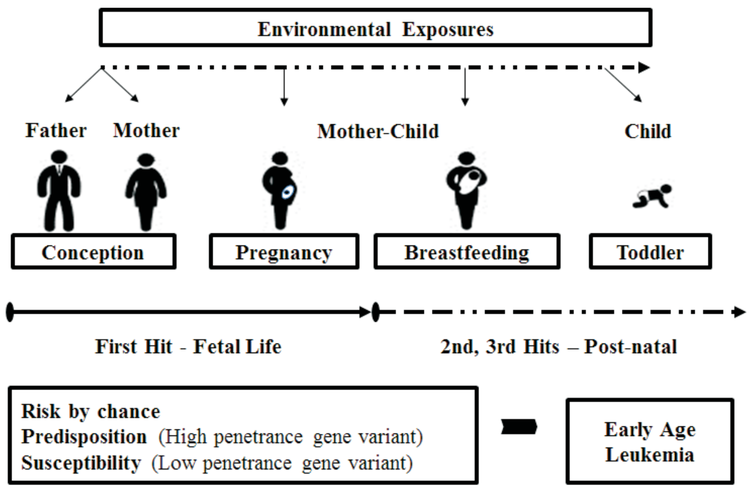

Figure 1. Model of the hypothesis for the modulated risk of developing leukemia in early age by the complex interactions between inherited predispositions at genetic and epigenetic levels, environment exposures to damaging agents and chance of events. *Pombo-de-Oliveira MS & Andrade FG, Arch Med Res, 47(8):593-606, 2016.*

AIM

- To perform a comprehensive variant analysis in order to describe the genomics of APL in young children (up 2 years of age).
- To identify the somatic and germline variants of APL to provide insight into molecular events associated with early onset of the disease.

MATERIAL AND METHODS

Patients and Samples. Two children (17 and 25 months of age at diagnosis) with typical APL with *PML-RARα* were assessed from a dataset of the hospital-based registry from a central laboratory (Pediatric-Hematology-Oncology Program, National Cancer Institute, Brazil) that is a reference for leukemia diagnostic assistance. Samples included the paired DNA from archived neonatal blood spot and diagnosis of the two patients.

Whole exome sequencing and variant calling. DNA was extracted from 85 mm^2 sections of dried blood spots (collected at birth; Figura 2) and from bone marrow (collected at diagnosis) using QIAamp DNA Micro/Mini kit (Qiagen). Whole exome sequencing was performed in 50 ng of DNA using the SureSelect QTX Target Enrichment for Illumina Multiplexed Sequencing (Agilent Technologies).

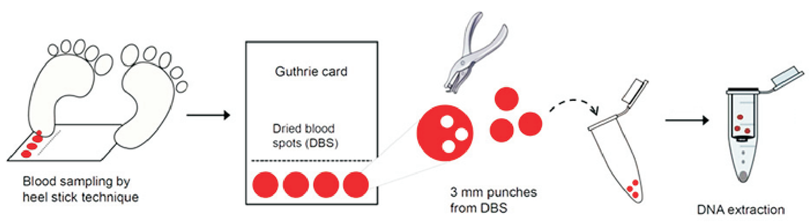

Figure 2. Schematic illustration for newborn DNA isolation.

Somatic and germline variants were called using Genome Analysis Tool Kit 4.0 (GATK) best practices guidelines. Variants with a total read depth <5 supporting reads and genotype quality (GQ) <20 were removed.

Annotation was performed using ANNOVAR, for which incorporated information for Gencode v2625, Genome Aggregation Database (gnomAD) 2.0.2 exome allelic frequencies, and ClinVar. BCFtools 1.9 was used to annotate for Trans-Omics for Precision Medicine (TOPMed) program Freeze 5 allelic frequencies, and CADD scores (version 1.4), and mean gnomAD exonic coverage. Germline variants were removed if gnomAD exonic or TOPMed allelic frequency was > 0.0001. The remaining candidate variants were compared between tumor (diagnostic) and normal (pre-diagnosis, at birth) samples in Ingenuity Variant Analysis (Qiagen).

RESULTS

The bioinformatic analyses focused on two different approaches: identification of variants present in both samples as candidate function-impacting (germline calling) and variants present in the leukemic samples that were wild type in the paired germline samples (somatic calling). The pipeline identified an average of 129 germline variants, including stop gains (3.9%), indels (7%), and single nucleotide variants (64.7%) in exonic regions, with 27.7% predicted deleterious.

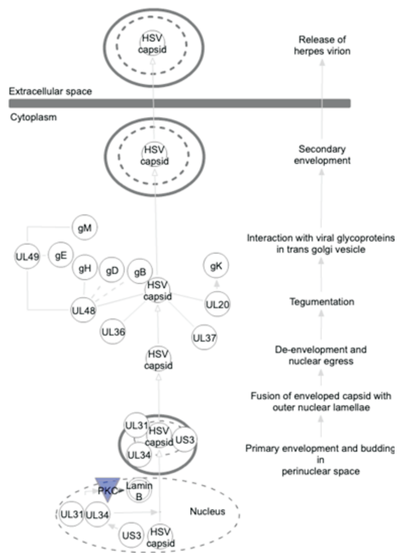

Figure 3. Mechanisms of Viral Exit from Host Cells. Cannonical pathway resulted from germline variant calling in Patient #1.

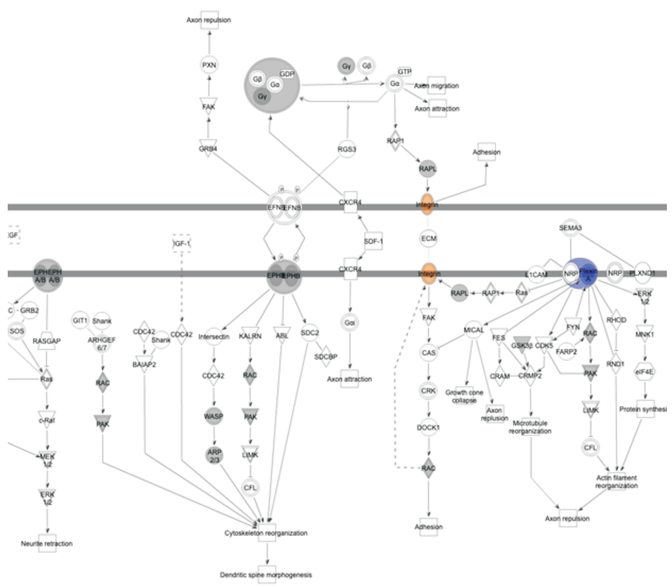

Figure 4. Axonal Guidance Signaling. Cannonical pathway resulted from germline variant calling in Patient #2.

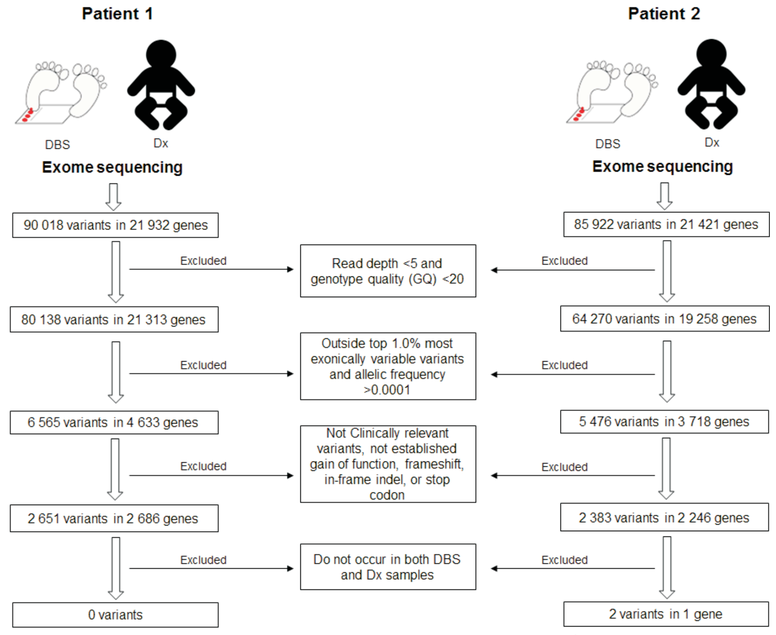

Figure 5. Somatic variant filtering. Variants were included if annotated as Pathogenic, Possibly/Probably Pathogenic, Unknown Significance OR Disease-associated according to HGMD OR clinically relevant variants from CentoMD OR established gain of function in the literature OR gene fusions OR inferred activating mutations by IVA OR predicted gain of function by BSIFT OR Frameshift, in-frame indel, or stop codon change OR Missense unless predicted to be innocuous by SIFT or Polyphen-2 OR predicted deleterious by having CADD score > 20.0 OR predicted to disrupt splicing by MaxEntScan OR deleterious to a microRNA OR in copy number loss genes OR in promoter binding site OR in enhancer OR in evolutionary-conserved region with a phyloP p-value of ≤ 0.01 OR in untranslated region OR within 2 bases into intron kept which are hemizygous OR heterozygous alterations that are known or predicted to directly affect: Acute Myeloid leukaemia (AML) (Acute myeloid leukemia) (affected genes and had an allele frequency ≤ 0.0001 in TOPMED and gnomAD.

The filtering for selection of variants did not identify significant alteration in the patient with 17 months of age at diagnosis (patient #1). Patient #2 presented rare somatic variants in *MSH6* gene; the in silico analysis identified the mismatch repair pathway the top category associated with the variants (p=0.006; Figure 5).

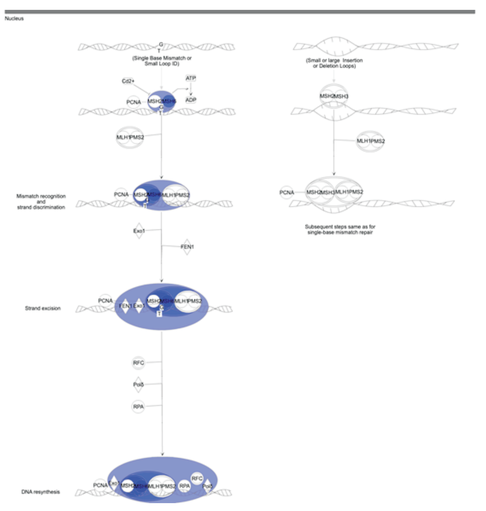

Figure 5. Mismatch repair pathway. Cannonical pathway resulted from somatic variant calling in Patient #2 .

CONCLUSION

We analyzed for the first time the paired neonatal blood spot derived DNA and diagnostic samples of patients with APL in early age. The rare germline variants identified are patient-specific and may play important roles in APL predisposition. The absence/low number of somatic variants identified suggest that the short time frame is not enough for the accumulation of genomic events. APL in early age might present mediating mechanisms other than variants in exonic regions involved in leukemia transformation. The lack of exome-wide significant association indicates the particularity of etiology underlying early age APL.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA