Birth Defects and Childhood Cancer: Harnessing the Power of the Gabriella Miller Kids First Pediatric Research Program

Philip Lupo, PhD, MPH
Department of Pediatrics
Section of Hematology-Oncology
Baylor College of Medicine
Birth defects

• **Definition:** structural or functional anomalies present at the time of birth

• **Nomenclature**
  - Anomalies
  - Malformations
  - Birth defects

• >100s of birth defects

https://www.cdc.gov/ncbddd/birthdefects/types.html
Childhood cancer

Data from the Surveillance, Epidemiology, and End Results (SEER) Database
Birth defects and cancer risk

- **Chromosomal anomalies**
  - Example: Trisomy 21 and acute lymphoblastic leukemia
  - 6%

- **Single gene defects**
  - Example: Costello syndrome and rhabdomyosarcoma
  - 7.5%

- **Non-syndromic birth defects**
  - Multifactorial; cancer risk largely unknown
  - 86.5%

March of Dimes; Taub JW, *J Pediatr Hematol Oncol*. 2001; Genetics Home Reference
(Some) research questions

1. Which birth defects are associated with which cancers?

2. Do specific birth defect-cancer associations represent undiscovered Mendelian syndromes?

3. Why do some children with birth defects develop cancer while others do not?
GOBACK (Genetic Overlap Between Anomalies and Cancer in Kids) Study

GOBACK

<table>
<thead>
<tr>
<th>Texas</th>
<th>Michigan</th>
<th>Arkansas</th>
<th>North Carolina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Defect Registries</td>
<td>Linking</td>
<td>Cancer Registries</td>
<td></td>
</tr>
</tbody>
</table>

>10 million births
Non-syndromic birth defects and childhood cancer

<table>
<thead>
<tr>
<th>Birth Defect</th>
<th>Cancer</th>
<th>HR (95% CI)1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular septal defect</td>
<td>Hepatoblastoma</td>
<td>10.6 (5.8-19.2)</td>
</tr>
<tr>
<td>Pulmonary valve atresia</td>
<td>Hepatoblastoma</td>
<td>22.6 (9.1-55.7)</td>
</tr>
<tr>
<td>Pulmonary valve atresia</td>
<td>Neuroblastoma</td>
<td>7.6 (3.8-15.3)</td>
</tr>
<tr>
<td>Left ventricular outflow tract defects</td>
<td>Neuroblastoma</td>
<td>7.8 (3.5-17.3)</td>
</tr>
<tr>
<td>Biliary atresia</td>
<td>Non-Hodgkin lymphoma</td>
<td>164.2 (77.8-346.8)</td>
</tr>
<tr>
<td>Craniosynostosis</td>
<td>Hepatoblastoma</td>
<td>9.7 (4.3-22.2)</td>
</tr>
<tr>
<td><strong>Choanal atresia</strong></td>
<td><strong>Acute leukemia</strong></td>
<td><strong>9.2 (3.8-22.1)</strong></td>
</tr>
</tbody>
</table>

1. Adjusted for maternal age, child’s sex and state of birth. Models including hepatoblastoma are adjusted for birthweight. Models including ventricular septal defect are adjusted for birthweight and gestational age.

*JAMA Oncol*. 2019 Jun 20;5(8):1150-8
Cancer risk increased for children with multiple non-syndromic birth defects

Adjusted HR (95% CI) | Number of cancer cases
--- | ---
No defect | 1.00 | 13,111
1 defect | 1.32 (1.20-1.45) | 477
2 defects | 3.51 (3.19-3.56) | 446
3 defects | 4.62 (4.08-5.22) | 261
≥4 defects | 5.85 (5.31-6.44) | 432
GOBACK family cohort

- Recruit cohort of families
- Whole genome sequencing
  - Variant analysis
    - SNVs/indels
    - *SVs

GOBACK

- Texas
- Michigan
- North Carolina
- Arkansas

Birth Defect Registries

Cancer Registries

>10 million births

Identify novel associations

*SVs

Sharon Plon, MD, PhD
Aniko Sabo, PhD
De novo heterozygous 5kb deletion in USP9X

Genomic location: chrX:41066285-41071603

PCR validation of the heterozygous deletion

Saumya Sisoudiya
Female proband: multiple birth defects and leukemia

• Birth defects
  ▪ Coloboma
  ▪ Heart defects
  ▪ Choanal atresia
  ▪ Growth Retardation
  ▪ Genital anomalies
  ▪ Ear anomalies

• Cancer: Precursor B-lymphoblastic leukemia

<table>
<thead>
<tr>
<th>Birth defect</th>
<th>Cancer</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choanal atresia</td>
<td>Acute leukemia</td>
<td>9.2 (3.8-22.1)</td>
</tr>
</tbody>
</table>
USP9X involved in several developmental and cancer pathways

Adapted from Murtaza et. al., *Cell and Mol Life Sci.*, 2015
Created with BioRender.com
Germline LoF/missense variants in *USP9X* are associated with a female-specific syndrome with developmental delay and multiple birth defects: 42 cases

Jolly et al., 2016, Au et al., 2017; Tsurusaki et al., 2019; Sinthuwiwat et al., 2019; Lenberg et al, 2019; Vianna et al., 2020; Jolly et al., 2020; Meira et al., 2021; Li et al., 2022
Females with LoF variants present with the core phenotypic features of the syndrome.
USP9X somatically mutated in childhood cancers

Ma et al, Nature, 2018

USP9X is a novel ALL susceptibility gene associated with a CHARGE-like syndrome
Next steps: Kids First GOBACK X01

Leverage Children’s Oncology Group Project: EveryChild (PEC)

1. Determine the frequency of known cancer predisposition variants among children with congenital anomalies and cancer

2. Identify variants that underlie novel anomaly-cancer predisposition syndromes and describe the landscape of somatic alterations in these children

Sharon Plon
Logan Spector
7. Does the patient have any structural birth defects known at this time?

- Cleft lip: __________
- Cleft palate: __________
- Clubfoot: __________
- Gastroschisis: __________
- Heart defect: __________
- Other specific: __________

8. Does the patient have any known genetic disorder?

- Down Syndrome: __________
- Ullrich-Turner Syndrome: __________
- Neurofibromatosis Type 1: __________
- Other specific: __________

9. Does the patient have any known autoimmune disease?

- Juvenile Idiopathic Arthritis: __________
- Celias disease: __________
- Diabetes mellitus Type 1: __________
- Inflammatory bowel diseases (Crohn’s or ulcerative colitis): __________
- Other specific: __________

10. Which of these describe the patient? Check all that apply.

- White: __________
- African American: __________
- American Indian, Alaskan, Eskimo: __________
- Asian: __________
- Other specific: __________
- Unknown: __________
- Other Spanish/Hispanic origin includes European: __________

Please return to hospital or clinic staff. Phone: __________ FAX: __________ Thank you for your information!
Kids First GOBACK Sequencing

• 700 blood and 500 tumor DNA samples for whole genome sequencing (WGS) at 30X coverage

• 500 tumor DNA samples for exome sequencing at 100X coverage

• 120 tumor RNA samples for transcriptome sequencing
### Patient Contact Information

**Patient’s Address:**

- Street Address:
- City: 
- State/Province: 
- Zip/Postal Code: 
- Country: 

**Phone Number:**

- City: 
- Country: 
- Phone number: 
- Email: 

**Driver’s license number:**

- Driver’s license issued state: (If applicable)

### Parent/Guardian Future Contact Information

**Parent/Guardian:**

- First Name: 
- Middle Name: 
- Last Name: 

**Address:**

- Street Address: 
- City: 
- State/Province: 
- Zip/Postal Code: 
- Country: 

**Phone number:**

- City: 
- Country: 
- Phone number: 
- Email: 

Please indicate the language spoken in the home, circle all that apply: English French Spanish Other, specify.

### Other Key Contact Information

**Key Contact:**

- First Name: 
- Middle Name: 
- Last Name: 

**Address:**

- Street Address: 
- City: 
- State/Province: 
- Zip/Postal Code: 
- Country: 

**Phone number:**

- City: 
- Country: 
- Phone number: 
- Email: 

Please indicate the language spoken in the home, circle all that apply: English French Spanish Other, specify.

---

### APEC14B1, Project EveryChild Registry

**1. Where was the patient born?**

- City: 
- State/Province: 
- Zip/Postal Code: 
- Country: 

**2. Was this patient a single or multiple birth?**

- Single [ ]
- Twins [ ]
- Triplets or more [ ]

2a. If twin, specify: [ ] Identical [ ] Fraternal [ ] Unknown [ ]

2b. If twin, specify sex: [ ] Both female [ ] Both male [ ] Male/female [ ]

Please indicate the language spoken in the home, circle all that apply: English French Spanish Other, specify.

**3. Was patient conceived through use of In vitro fertilization?**

- Yes [ ]
- No [ ]

**4. Was cord blood banked at birth?**

- Yes [ ]
- No [ ]

**5. Has anyone in the patient’s immediate family (Biological mother, father, brothers, sisters) ever had cancer? If yes, please record information below:**

My child’s: 

<table>
<thead>
<tr>
<th>Types of cancer</th>
<th>Not sure</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ] Mother</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ ] Father</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ ] Full brother</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ ] Full sister</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ ] Son</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[ ] Daughter</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please indicate the language spoken in the home, circle all that apply: English French Spanish Other, specify.

**6. Please indicate the name and relationship of at least one person/guardian:**

<table>
<thead>
<tr>
<th>Name (First Name)</th>
<th>Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please return to hospital or clinic staff. Phone: ____________________ FAX: ____________________ Thank you for your information!
Kids First GOBACK R03

1. Collect extensive phenotypic and clinical data from children with congenital anomalies and cancer enrolled in Project:EveryChild

2. Integrate phenotypic and clinical data from Project:EveryChild into the Gabriella Miller Kids First Pediatric Data Resource Center

Allison Heath
Adam Resnick
GOBACK conclusions

• Birth defects are associated with an increased risk for childhood cancer, especially embryonal tumors and germ cell tumors

• Birth defects account for ~10% of childhood cancers

• WGS of informative families
  ▪ Yield new genetic insights about birth defects and childhood cancer
  ▪ *USP9X*: a novel leukemia susceptibility gene
Down syndrome-associated leukemia
Down syndrome (DS) and leukemia

- First reported 1930
- First systematic study in 1957
- ~20-fold increased risk
- Cumulative risk of 2% by age 5
- Comprises ~2% of childhood acute lymphoblastic leukemia (ALL) and 10% of childhood acute myeloid leukemia (AML)

Hasle et al, Lancet 2000
DS-ALL questions

• Why does ALL arise more often in children with DS?

• Does ALL differ between children with and without DS?

• Are there germline genetic variants associated with the ALL susceptibility in children with DS?
**Abstract:** DESCRIPTION (provided by applicant): Down syndrome (DS), which occurs due to trisomy 21, is one of the strongest risk factors for both congenital disease (CHD) and acute leukemia. For instance, children with DS have a 2000-fold increased risk of atrioventricular septal defects (AVSD) and a 20-fold increased risk of acute lymphoblastic leukemia (ALL). An important and innovative aspect of the Kids First program is the use of genomic analysis to understand the genetic basis of these diseases. The project aims to identify novel genetic markers and pathways that may contribute to the development of these conditions in DS patients. The sequencing of this project is partially supported by the Investigation of Co-occurring conditions across the Lifespan to Understand Down syndrome (INCLUDEd) Project.
Aims

1. Identify genetic variants underlying AVSD in children with DS

2. Identify genetic variants underlying ALL in children with DS
   - Particular attention to rare, structural, and chromosome 21 variants
   - Evaluation of relationship between germline and somatic features (WGS of paired leukemia-germline samples)
Regular Article

Genomic landscape of Down syndrome-associated acute lymphoblastic leukemia

DS-ALL WGS study
ALL subtype classification by DS status
• Age at diagnosis was younger for those with DS-ALL compared to those with non-DS-ALL
• This was especially true for \textit{CRLF2-r}
Preliminary DS-ALL germline findings
DS-ALL GWAS identifies novel (blue/purple) and known (gray) loci
### Top variants associated with DS-ALL

<table>
<thead>
<tr>
<th>Locus</th>
<th>RSID</th>
<th>CHR</th>
<th>POS, b38</th>
<th>Risk allele</th>
<th>DS-ALL freq</th>
<th>DS freq</th>
<th>OR (95% CI)</th>
<th>P-value</th>
<th>SNPs in Locus P&lt;5e-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD1 (intron 1)</td>
<td></td>
<td>1</td>
<td>201,394,520</td>
<td>C</td>
<td>0.0113</td>
<td>0.0003</td>
<td>2.04 (1.58-2.63)</td>
<td>4.6E-08</td>
<td>1</td>
</tr>
<tr>
<td>4q13.1</td>
<td>rs17290452</td>
<td>4</td>
<td>58,639,220</td>
<td>T</td>
<td>0.0992</td>
<td>0.0472</td>
<td>1.17 (1.11-1.23)</td>
<td>2.4E-08</td>
<td>1</td>
</tr>
<tr>
<td>IKZF1 (downstream)</td>
<td>rs28462675</td>
<td>7</td>
<td>50,406,172</td>
<td>G</td>
<td>0.4051</td>
<td>0.2618</td>
<td>1.11 (1.08-1.14)</td>
<td>1.1E-13</td>
<td>37</td>
</tr>
<tr>
<td>CDKN2A (exon)</td>
<td>rs3731249</td>
<td>9</td>
<td>21,970,917</td>
<td>T</td>
<td>0.0751</td>
<td>0.0280</td>
<td>1.23 (1.15-1.32)</td>
<td>1.5E-09</td>
<td>4</td>
</tr>
<tr>
<td>PTCSC2 (intron)</td>
<td></td>
<td>9</td>
<td>97,746,216</td>
<td>A</td>
<td>0.0127</td>
<td>0.0003</td>
<td>2.01 (1.58-2.56)</td>
<td>1.8E-08</td>
<td>1</td>
</tr>
<tr>
<td>PCBP2 (intron 1)</td>
<td></td>
<td>12</td>
<td>53,452,389</td>
<td>T</td>
<td><strong>0.0227</strong></td>
<td><strong>0.0000</strong></td>
<td><strong>2.24 (1.85-2.72)</strong></td>
<td><strong>2.1E-16</strong></td>
<td>22</td>
</tr>
<tr>
<td>GOLGA8B (upstream)</td>
<td></td>
<td>15</td>
<td>34,603,388</td>
<td>A</td>
<td>0.0142</td>
<td>0.0010</td>
<td>1.83 (1.48-2.27)</td>
<td>2.3E-08</td>
<td>1</td>
</tr>
<tr>
<td>CHST6 (upstream)</td>
<td></td>
<td>16</td>
<td>75,497,610</td>
<td>T</td>
<td>0.0142</td>
<td>0.0010</td>
<td>1.82 (1.48-2.26)</td>
<td>3.0E-08</td>
<td>2</td>
</tr>
<tr>
<td>KRT222-KRT24</td>
<td></td>
<td>17</td>
<td>40,688,678</td>
<td>T</td>
<td>0.0142</td>
<td>0.0000</td>
<td>2.03 (1.58-2.59)</td>
<td>1.8E-08</td>
<td>1</td>
</tr>
</tbody>
</table>
DS-ALL conclusions

• Distinct spectrum of subtypes in DS-ALL
  ▪ CRLF2-r >50%
  ▪ Other subtypes in non-DS ALL are under-represented

• DS-ALL patients are younger than non-DS ALL patients: onset of CRLF2-2 ALL is almost 10 years earlier in children with DS

• Novel and known germline variants play a role in DS-ALL susceptibility
DS-ALL future directions

• Germline analyses of DS-ALL
  ▪ Further evaluate novel loci
  ▪ Assess rare and structural variants
  ▪ Evaluate relationship between the inherited germline and somatic features

• Conduct deep phenotyping of children with DS-ALL
  ▪ Identify the role of co-occurring birth defects on DS-ALL features and outcomes
  ▪ Linkages with COG and other data sources (e.g., National Death Index)
Birth defects, childhood cancer, and Kids First: Overall conclusions

• Evaluating the overlap between birth defects and cancer provides novel insights into develop and carcinogenesis

• Insights into factors influencing cancer among children with birth defects may guide improved genetic counseling, surveillance, and treatment interventions

• Kids First has...
  ▪ Accelerated the timeline of birth defects-childhood cancer discoveries
  ▪ Provided a springboard for new funding opportunities
  ▪ Fostered collaborative research
Acknowledgements

Baylor/TXCH
- Sharon Plon
- Karen Rabin
- Jeremy Schraw
- Austin Brown
- Olga Taylor
- Dani Mitchell
- Lauren Sanclemente
- Michael Scheurer
- Jacob Junco
- Saumya Sisoudiya
- Tiffany Chambers

COG
- Logan Spector
- Mignon Loh
- Stephen Hunger
- Meenakshi Devidas
- Yunfeng Dai
- Michael Borowitz
- Brent Wood
- Nyla Heerema
- Andrew Carroll

Crnic Institute
- Joaquin Espinosa

Kids First
- Valerie Cotton
- James Coulombe
- Marcia Fournier

The patients and families who participated in this research
**USP9X** codes for a highly conserved deubiquitinase

Adapted from Murtaza et. al., *Cell and Mol Life Sci.*, 2015

Created with BioRender.com
USP9X is expressed from active X (Xₐ) as well as the inactive X (Xᵢ) chromosome in humans.

Roman et. al., 2021