Using metabolomics with neonatal blood spots to discover causes of childhood leukemia

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Known or suspected causes of childhood leukemia (mostly ALL)

- Genes (< 10% of risk)
- Exposures
  - Associations with radiation, paternal smoking and some environmental chemicals
- Early life exposures important
  - Identical twins diagnosed as infants have very high concordance rates
  - Most ALLs diagnosed before age 5
Age at diagnosis of CL

- Approximately 2,500 new cases per year among children <15 years in the US
- Highest rates in Whites, Hispanics, and males

Figure 4. Age-specific Incidence Rates of Acute Lymphocytic Leukemia (ALL) by Race/ethnicity and Acute Myeloid Leukemia (AML) All Races Combined, 2001-2010

Source: Surveillance, Epidemiology, and End Results (SEER) Program, 18 SEER Registries, National Cancer Institute.
The blood exposome

EXPOSURES ARE CHEMICALS …
and the blood exposome includes all chemicals in the body.

Archived neonatal dried blood spots (ANBS) contain information about the fetal exposome

- Collected from 98% of newborns in the United States, 24-48 hours after birth
- Used to test for congenital disorders
- If stored, can be used in epidemiological studies
- Unique and important biospecimen for understanding causes of pediatric disease
Scheme for performing exposomics with ANBS

ANBS (4.7-mm punch ~ 8 µL blood)
Solvent extraction
Centrifugal filtration
Small molecules
Add Int. Std.
LC-HRMS
Add Int. Std.
Proteins (mostly HSA)
Trypsin
Digest

Untargeted metabolomics and adductomics

Lauren Petrick
Will Edmands
Hasmik Grigoryan
Katie Hall
Yukiko Yano
LC-High resolution mass spectrometry

Sample

UPLC analysis

Retention Time (min)

Abundance

MASS SPECTRUM (MS/MS)

Fragmentation

Will Edmands
Lauren Petrick
Small molecules in ANBS (100 CL cases & matched controls)

<table>
<thead>
<tr>
<th>Small-molecule features</th>
<th>ESI (-) mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td>66,096</td>
</tr>
<tr>
<td>After filtering (fold change above background &gt; 5)</td>
<td>8,852</td>
</tr>
<tr>
<td>Features with CV &lt; 20%</td>
<td>3,919</td>
</tr>
<tr>
<td>Testable clusters</td>
<td>665</td>
</tr>
</tbody>
</table>
A glimpse of the fetal exposome

- Fatty acids
- Monosaccharides
- Steroids & hormones
- Glutathione
- Lipids
- Microbial metabolites
- Nucleotide
- Phosphate
- Sulphate
- MS2-matched feature

Will Edmands
Tests of association with childhood leukemia ($n = 665$ Features after filtering)

Machine learning algorithms (lasso & random forests) selected the same 7 discriminating molecules

Courtney Schiffman
Lauren Petrick
Sandrine Dudoit
Findings

• Seven small molecules in ANBS predict childhood leukemia status in a learning sample
  ○ Molecular identities are being confirmed

• Currently repeating analysis with a validation sample of ANBS (100 cases/100 controls)
Thanks

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