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

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and the Environment

CEB
Center For
Exposure Biology

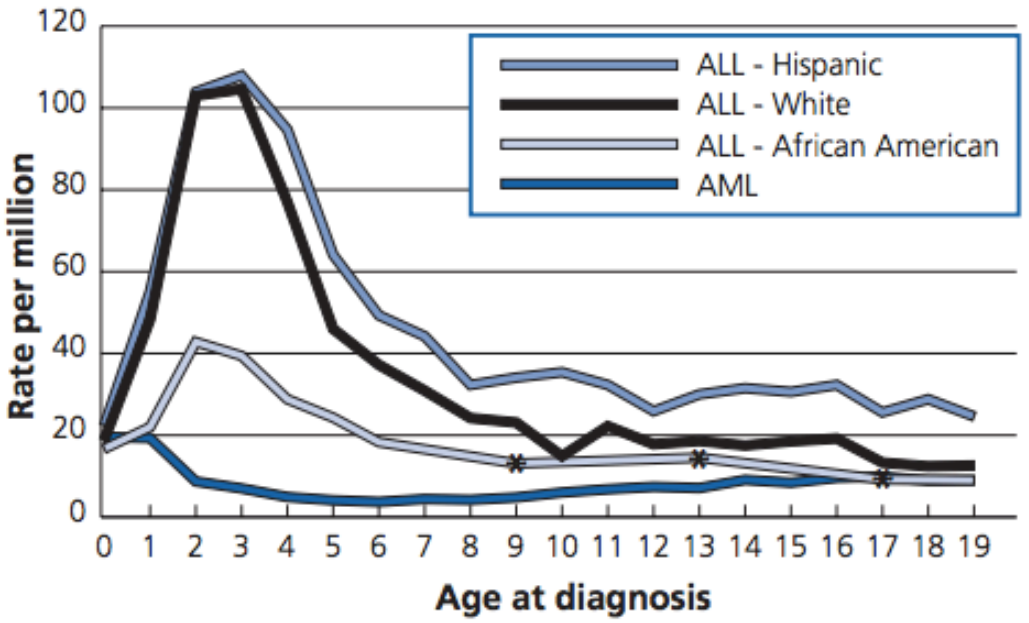
Known or suspected causes of childhood leukemia (mostly ALL)

- 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.

Cancer Facts & Figures 2014: Special Addition, ACS

The *blood exposome*

EPIDEMIOLOGY

Environment and Disease Risks

Stephen M. Rappaport and Martyn T. Smith

A new paradigm is needed to assess how a lifetime of exposure to environmental factors affects the risk of developing chronic diseases.

Although the risks of developing chronic diseases are attributed to both genetic and environmental factors, 70 to 90% of disease risks are probably due to differences in environments (*1*–*3*). Yet, epidemiologists increasingly use genome-wide association studies (GWAS) to investigate diseases, while relying on questionnaires to characterize "environmental exposures." This is because GWAS represent the only approach for exploring the totality of any risk factor (genes, in this case) associated with disease prevalence. Moreover, the value of costly genetic information is diminished when inaccurate and imprecise environmental data lead to biased inferences regarding gene-environment interactions (*4*). A more comprehensive and quantitative view of environmental exposure is needed if epidemiologists are to discover the major causes of chronic diseases.

An obstacle to identifying the most important environmental exposures is the fragmentation of epidemiological research along lines defined by different factors. When epidemiologists investigate environmental risks, they tend to concentrate on a particular category of exposures involving air and water pollution, occupation, diet and obesity, stress and behavior, or types of infection. This slicing of the disease pie along parochial lines leads to scientific separation and confuses the definition of "environmental exposures." In fact, all of these exposure categories can contribute to chronic diseases and should be investigated collectively rather than separately.

To develop a more cohesive view of environmental exposure, it is important to recognize that toxic effects are mediated through chemicals that alter critical molecules, cells, and physiological processes inside the body. Thus, it would be reasonable to consider the "environment" as the body's internal chemical environment and "exposures" as the amounts of biologically active chemicals in this internal environment. Under this view, exposures are not restricted to chemicals (toxicants) entering the body from air, water, or food, for example, but also include chemicals produced by inflammation, oxidative stress, lipid peroxidation, infections, gut flora, and other natural processes (*5*, *6*) (see the figure). This internal chemical environment continually fluctuates during life due to changes in external and internal sources, aging, infections, life-style, stress, psychosocial factors, and preexisting diseases.

The term "exposome" refers to the totality of environmental exposures from conception onwards, and has been proposed to be a

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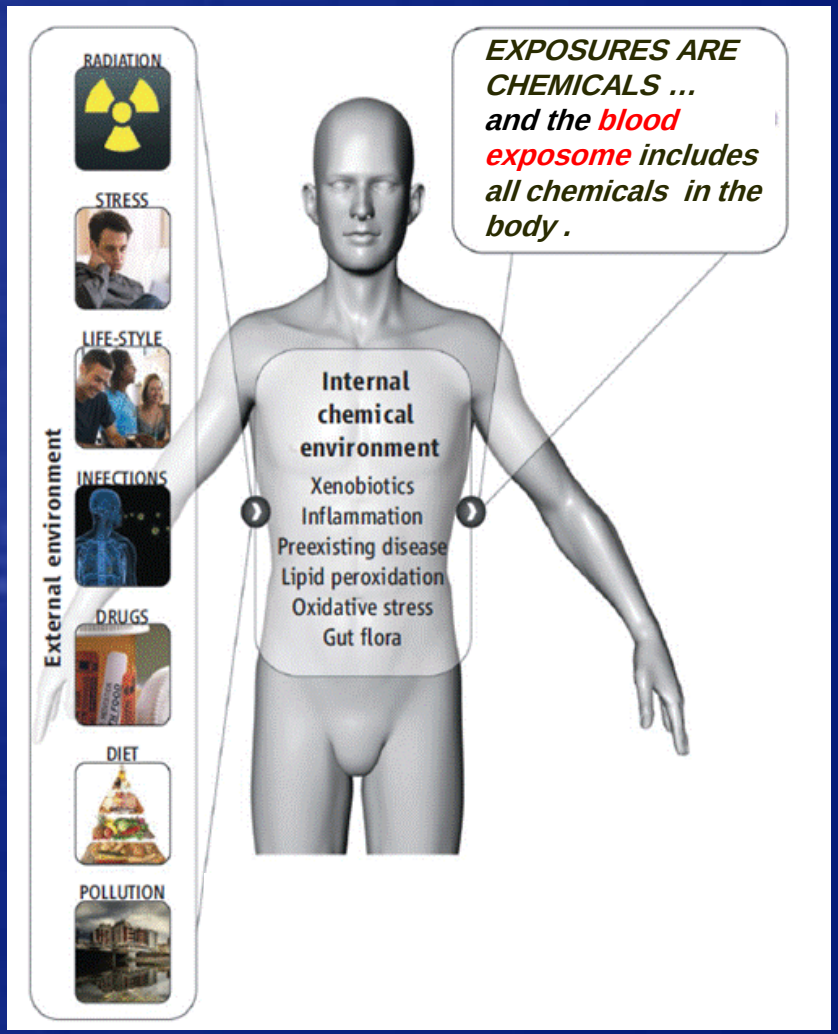
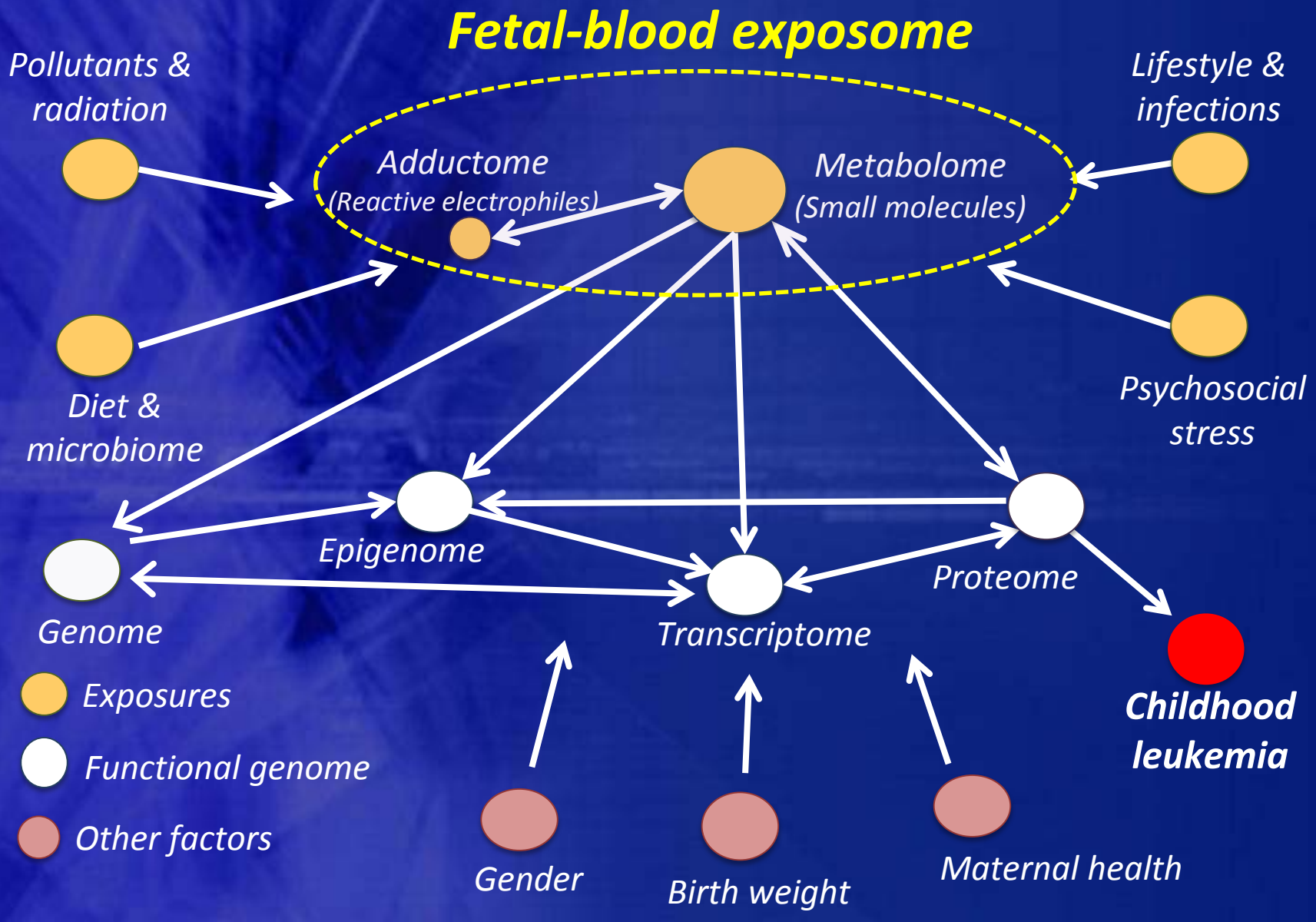


Chemical communication



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

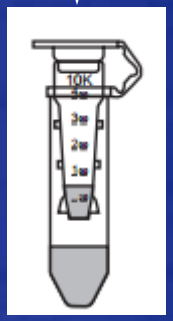
- Collected from 98% of newborns in the united states, 24-48 hours after birth
- Used to test for congenital disorders
- If stored, can be used in epidemiological studies
- Unique and important biospecimen for understanding causes of pediatric disease

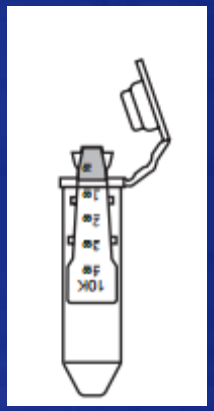
Scheme for performing exposomics with ANBS

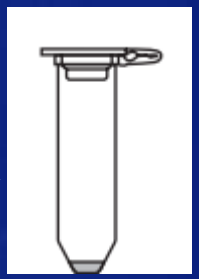
ANBS (4.7-mm punch ~ **8 µL blood**)

Solvent extraction

Centrifugal filtration

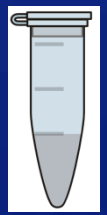






Proteins (mostly HSA)

Trypsin



Digest

Small molecules

Add Int. Std.

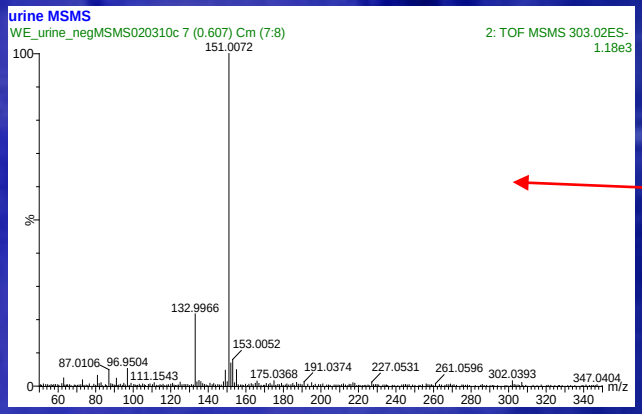
LC-HRMS

Add Int. Std.

Untargeted *metabolomics and adductomics*

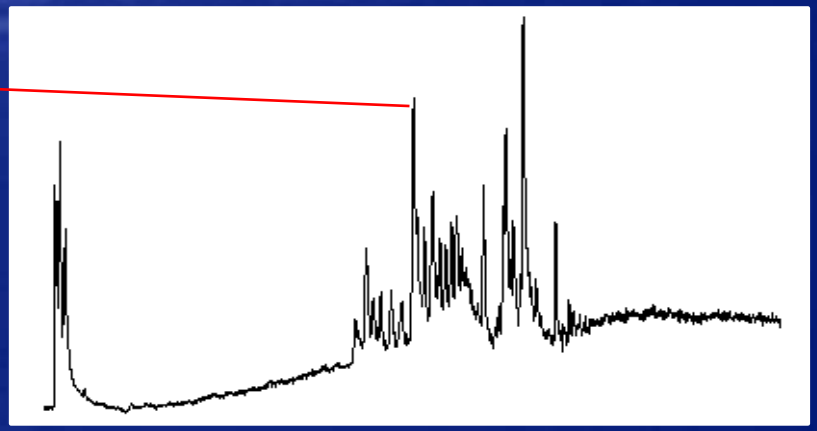
Lauren Petrick
Will Edmands
Hasmik Grigoryan
Katie Hall
Yukiko Yano

LC-High resolution mass spectrometry



MASS SPECTRUM (MS/MS)

Fragmentation

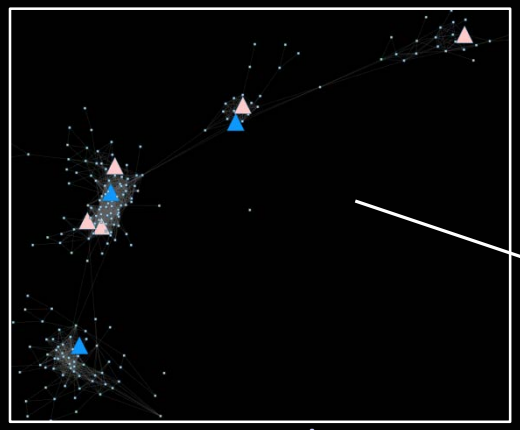


Will Edmands
Lauren Petrick

Small molecules in ANBS (100 CL cases & matched controls)

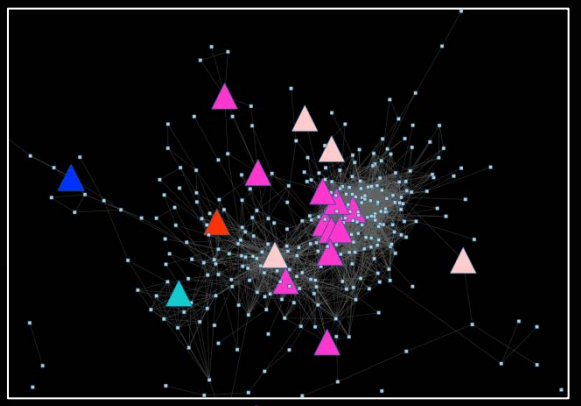
Small-molecule features	ESI (-) mode
Detected	66,096
After filtering (fold change above background > 5)	8,852
Features with CV < 20%	3,919
Testable clusters	665

A glimpse of the fetal exposome

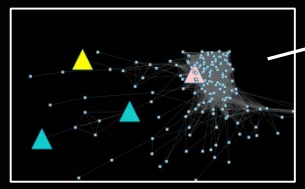


Fatty acids

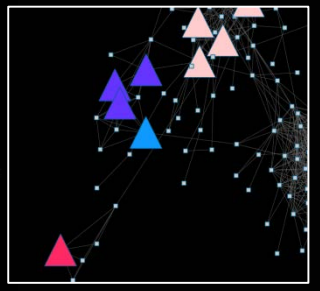
Glutathione



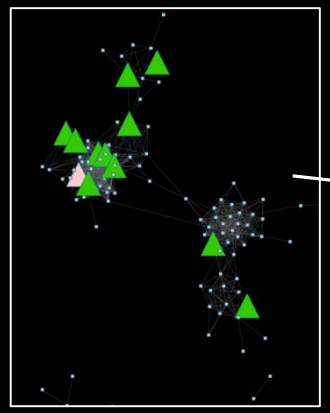
Lipids



Monosaccharides



Microbial metabolites

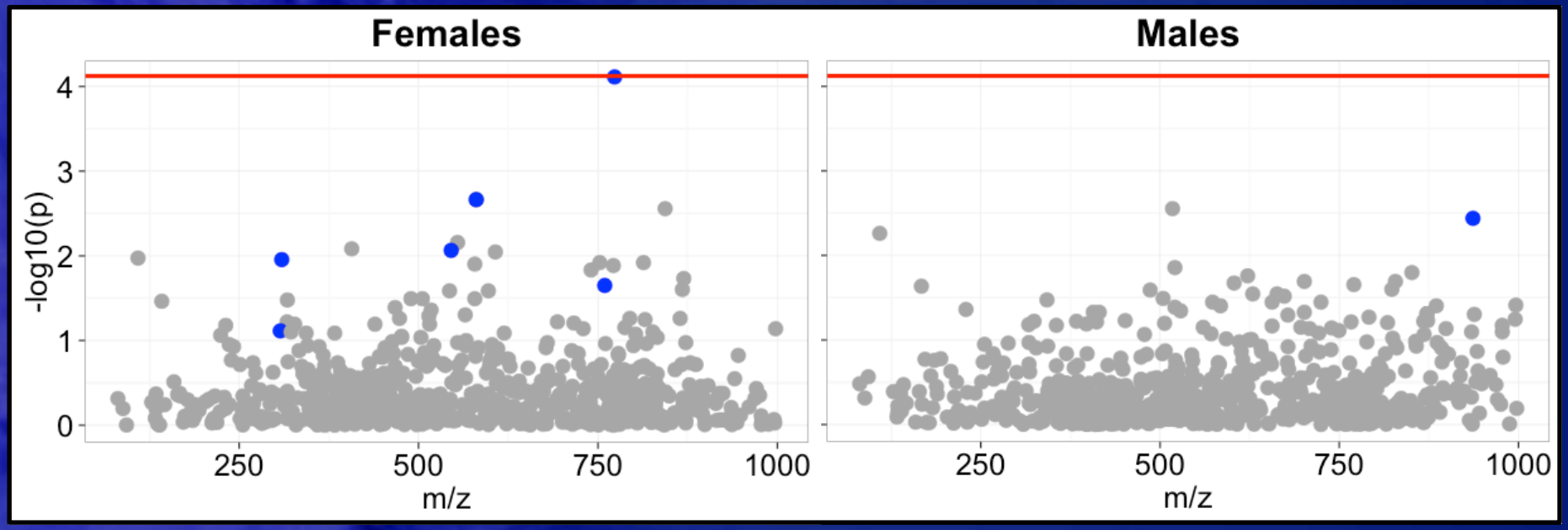


Steroids & hormones

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)

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