The Importance of Understanding Interindividual Variability in Response to Chemical Exposures

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NAS ESEH Committee Meeting
Washington, DC
30 September 2015
Types of Variability

- Binary vs. continuous variation

- Among Subjects (Interindividual) vs. Within Subject (Intraindividual)

Dolinoy et al. 2007
Sources of Interindividual Variability

- Genetics
- Environment
  - Diet
  - Exposures/Exposome
  - Stress
  - Socioeconomic
  - Behavioral
  - Microbiome
  - Infectious agents

Intraindividual Variability
- Tissues
- Cells
- Life stage
- Sensitivity of apical endpoints
Interindividual Variability Leads to Health Disparities

10% of asthma patients suffer severe symptoms despite regular use of corticosteroids.

Identified 2 SNPs in glucocorticoid signaling pathway gene associated with decreased clinical response to inhaled corticosteroids in asthmatic children and young adults.

Suggests genetic variation contributes to interindividual variability in clinical response.

Proteomics identified 111 altered proteins in cord blood of infants prenatally exposed to arsenic.

Interindividual differences: as maternal arsenic levels increased, 60% of infants had above average protein expression (activators); 40% of infants had lower expression (repressors).

Activator males had decreased head circumference; no significant difference between activator/repressor females.

African Americans disproportionately affected by prostate cancer aggressiveness, and lower plasma 25(OH)D3.

Those with highest percentage of African American ancestry had lowest mean plasma 25(OH)D3.

Higher plasma levels associated with increased odds of aggressive prostate cancer among African Americans, but not European Americans.
Genes, Environment and Health Initiative: The Vision

**EXPOSURE BIOLOGY PROGRAM**
- Develop technology and biomarkers
  - Diet
  - Physical Activity
  - Environmental Exposures
  - Psychosocial Stress and Addictive Substances

**GENETICS PROGRAM**
- Identify genetic variants
  - GWA Studies
  - Data Analysis
  - Replication
  - Sequencing
  - Database
  - Function
  - Translation

GxE (Genes x Environment)
Example of genetic leading to Interindividual variability in humans
Our Environment is Complex and Multifaceted
The Exposome

Define the exposome
- An untargeted (hypothesis free) assessment of the totality of environmental exposures
- External, internal, or both?

How to measure it
- New tools necessary to monitor exposure
- Can use biomonitoring or untargeted metabolomics to begin to do ‘top-down’ approach

Mixtures
- Chemical
- Non-chemical (infectious agents, diet, psycho-social)

Microbiome

Rappaport and Smith (2010), Science, 330:460-461
Mixtures

Demographics

Nutrients

Smoking

Pesticides

PCBs

Hydrocarbons

• Map how exposures associated with disease identified by EWAS correlate with other exposures

• “Exposome globe”
  ---positive correlation,
  ---negative correlation

• Understanding how exposures are correlated may help define mixtures relevant to human health

(Modified from Patel et al. 2015)
Epigenetic changes lead to heritable variability
Epigenetic Changes Have Been Implicated in a Wide Variety of Human Diseases

**Normal processes**
- Development
- Cell differentiation
- Aging

**External influences**
- Environmental exposures
- Nutrition
- Chemical toxins
- Metals
- Mediators of stress
- Drugs of abuse
- Infection (including HIV)

**Adverse health outcomes**
- Cancer
- Cardiopulmonary disease
- Autoimmune disease
- Obesity
- Diabetes
- Neurodevelopmental disorders
- Schizophrenia
- Addiction
- Depression

**GENOME**

**EPIGENOME**

**DISEASE**
The Roadmap Epigenomics Program: a public resource of reference epigenomic maps of normal human cells

Adult cells/tissues, fetal cells/tissues, pluripotent (ESC and iPS) cells
Diet

Susceptibility

1. Sugar-sweetened beverage consumption in early childhood
2. Soy consumption
3. Developmental phthalate exposure and high-fat diet
4. Methylmercury contaminated seafood maternal diet

Disease

1. Obesity in pre-pubertal and pubertal life stage
2. Obesity and early pubertal onset in offspring
3. Glucose intolerance
4. Cognitive defects
The Microbiome: A New Paradigm

- New genomic technologies have enabled comprehensive identification of the microbial community inhabiting our bodies: the “microbiome”

- Studies reveal associations between the composition of the microbiome and health and disease

- Commensal microbes have a role in metabolism of environmental toxicants

- Environmental exposures to chemicals, metals, antibiotics, etc., influence the composition of the microbiome
What about preconceptional exposures to mom and dad?
Windows of Susceptibility

• Maternal Smoking & Children’s Obesity
  – NTP Review of 23 Studies
  – Studies range from 2001 – 2010
  – Pooled data show:
    • Odds Ratio=1.5 for obesity (95%CI=1.35-1.65)
    • Odds Ratio=1.6 for overweight (95%CI=1.42-1.90)
How do we integrate *interindividual variability* into basic research?
Using Mice as a “Model” for Human Exposures

Genetically Diverse Human Population

Inbred Strain (C57BL/6J) (B6C3F1)
Diversity Among Inbred Strains: Acetaminophen Toxicity
Differential Host Response to Toxic Exposure: Benzene Clearance

French et al., EHP, 2014
“Modeling” Reference Human Populations with Reference Populations of Mice

Genetically Diverse Mouse Population

Genetically Diverse Human Population
Diversity Outcross (DO): Complementary Resource Produced from Collaborative Cross Lines

• 8 way advanced intercross
• Continuously breeding “outbred” colony—each mouse different
• “Controlled” genetic complexity
• Enhanced mapping resolution

Churchill, Threadgill, Pardo-Manuel deVillena, and colleagues
Proof of concept of DO: Benzene 28 Day Inhalation Study

- Diversity outbred (J:DO) male mice: 7 & 8\textsuperscript{th} randomly outbred generations; selected from 175 breeding pairs

- Due to broad phenotypic diversity, mice were randomly assigned to exposure group by weight

- **Dose levels:** 0, 1, 10, 100 ppm benzene, 28 days, 6 hr TWA

- 75 male mice per exposure group, 300 mice/study

- 600 mice total: 2 separate cohorts to assess reproducibility

- Endpoint for genetic damage assessment
  - Micronuclei in bone marrow and blood reticulocytes and erythrocytes
  - Mouse Universal Genotyping Array (9K SNPs; MUGA)
  - Mapping & Linkage analysis (QTLRel)

French, Churchill and colleagues
Change in %MN-RET before and after exposure

"We propose a shift from primarily *in vivo* animal studies to *in vitro* assays…"

"…virtually all routine toxicity testing would be conducted *in vitro* …using high throughput robotic assisted methodologies…"
Types of High Throughput In Vitro Phenotyping Assays

- Phenotypic readouts
  - Cytotoxicity
  - Apoptosis
  - Membrane integrity
  - Mitochondrial toxicity
  - Cardiomyocyte toxicity

- Genetox
- Cell Signaling
  - Stress response
  - Immune response
  - Others...

- Epigenetics
  - Locus DeRepression (LDR)

- Drug metabolism

- Target specific assays
  - Nuclear receptors
  - hERG channel
  - Isolated molecular targets

- Genetic variation: > 1,000 human lymphoblastoid cell lines

Pie chart showing the distribution of different types of assays.
Incorporating “In Vitro Genetic Studies” into Tox21

Reference population of Mouse Lines (Human in the future)

ES Derivation from Blastocysts

iPS reprogramming

Reference population of ES/iPS Cell Lines
“In Vitro Genetic” Studies

Reference population of ES/iPS Cell Lines from the CC/DO

Transcriptomics

Proteomics

Metabolomics

High Content Cell-based Screening
Challenges faced with *in vitro* screens

How do we develop *in vitro* screens that capture interindividual variability?
Precision Medicine

- Multidisciplinary research
- Multifaceted environment
- Interindividual variability
- Intraindividual variability

Precision Prevention
Thank You!

NIEHS Strategic Plan Website
http://www.niehs.nih.gov/strategicplan
Mixtures

- Low dose
- Chronic exposure
- Lifetime (womb to adulthood)
- Non-monotonic dose-response relationships
- Synergistic effects
- Combinatorial complexities
- Adverse Outcome Pathways
- Cumulative effects
- Subpopulation vulnerabilities
“ENVIRONMENT” Includes:

- Industrial chemicals
- Agricultural chemicals
- Physical agents (heat, radiation)
- By-products of combustion and industrial processes (dioxin)
- Infectious agents
- Microbiome (gut flora)
- Foods and nutrients
- Prescription drugs
- Lifestyle choices and substance abuse
- Social and economic factors
Benzene, 100 ppm – Bone Marrow MN-RET/1000 Chr 10 QTL (LOD = 14.59)

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>MN-RET/1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4 ± 0.2</td>
</tr>
<tr>
<td>1</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>10*</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>100**</td>
<td>19.5 ± 1.2</td>
</tr>
</tbody>
</table>

*\(p = 0.0131\)

**\(p < 0.0001\)

French, Churchill and colleagues
Benzene, 100 ppm – Bone Marrow %MN-RET Chr 10 QTL

Sult3a1 – sulfotransferase family 3A, member 1 is a cytosolic phase 2 amine/phenol sulfotransferase

Nkain2 (Tcba1) - Na+/K+ transporting ATPase interacting 2 is a transmembrane protein that interacts with Na,K-ATPase (ATP1B1)

French, Churchill and colleagues
Epigenetic changes lead to heritable variability
Epigenetics

• The study of changes in DNA expression that are independent of the DNA sequence.

• A person’s DNA base sequence doesn’t change, but expression of DNA is affected by changes in DNA “packaging.”

• Environment is critical factor in DNA expression; we’re born with genes, but environment affects epigenetic changes.
Epigenetics in Action: $A^{vy}$ mutation of agouti
Influence of Diet in $A^{yy}$ mutants

Waterland and Jirtle, MCB 2003
Precision Medicine

Multidisciplinary research
Grant Support
NIGMS
NCRR
NIEHS
NIDDK
NHLBI

Multifaceted environment

*Inter*individual variability

Subpopulation vulnerability = stroke

Precision Prevention

Warfarin

VKORC1

Subpopulation vulnerability = stroke
Mouse Diversity Array

- 623,124 phylogenetically informative SNPs
- Known ascertainment
- 916,269 copy number genomic probes
- Mouse Universal Genotyping Array (MUGA)

Churchill, Threadgill, Pardo Manuel deVillena, Williams and colleagues