Using the Diversity Outbred mice to identify gene by environment interactions

Alison Harrill, PhD
Uncovering environmental cancer causes @ National Toxicology Program

Identify environmental causes of cancer subtypes (ex. early onset colorectal cancer, breast cancer)

Protect public health by informing regulators of potential chemical threats and their potencies
Preserve patient quality of life via Precision Medicine

Toxicology - Study how drugs cause injury to tumor and healthy tissue

Pharmacogenetics - Determine which genetic sequences affect drug response in individual patients

Choose drug based on patient DNA

Preserve health of patient

PRECISION TOXICOLOGY - MINIMIZE COLLATERAL ADVERSE EVENTS
Pharmacogenomics (PGx)

Seeks to understand how a patient's gene sequence affect how she responds to a medication (or chemical)

Drug causes **adverse event**, but is **efficacious**

Drug is not toxic and not efficacious

Drug causes **adverse event** and not efficacious

Drug not toxic, but is **efficacious**

Same diagnosis, Same prescription
**Genetic Terms & Definitions**

**GENE**
A direct sequence of nucleotides forming part of a chromosome

**ALLELE / GENOTYPE**
One of 2 or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome

**SINGLE NUCLEOTIDE POLYMORPHISM**
An allele that encompasses a single nucleotide in the sequence

Person 1: ATGCTTGGCGTA
Person 2: ATGCATGGCGTA

**MAJOR ALLELE**
The common allele / variation / nucleotide

**MINOR ALLELE**
The less common allele / variation / nucleotide

95% People: ATGCTTGGCGTA
5% People: ATGCATGGCGTA

**PHENOTYPE**
A set of observable characteristics arising from the individual's genotype and its interactions with the external environment

Allele A
Allele B
Genetic Sequence Variability Can Affect Response to Drugs in Several Ways

**PHARMACOKINETICS**
plasma clearance, distribution of drug to target organs/cells, metabolite production

Example: Warfarin dose setting is guided by PGx test for CYP2C9 and VKORC1

**PHARMACODYNAMICS**
Genes down or upstream of metabolism play a role in modulating drug effect

Example: Direct binding of abacavir to HLA-B*5701 causes allergic skin rash
GUIDE TREATMENT
Your cancer drug has been found to cause an adverse reaction in a subset of patients. A pharmacogenomic test can allow the drug to reach patients who would benefit, while minimizing risks for those who would be harmed.

PREVENT ENVIRONMENTAL CAUSES OF CANCER
You want to determine the mode of action of sensitivity to the drug/chemical. Alternatively, you want to know if there are shifts in dose response across the population that can affect safe human exposure thresholds of environmental chemicals.
A Problem

Most toxicology studies performed before drugs move to clinical trials are done in genetically limited animals.

We typically do not test drugs on genetically diverse populations of animals -> that means we will miss individual differences in response due to different alleles (versions of gene sequences).
Utility of Animal Models for Toxicology

Controlled Experiments
Can set variables: timing and dose of drug/chemical administration, diet, environment/stress, known genetics

Experiments Can Be Replicated
We standardize reagents (ex 99.9% pure compound) - animals can also be thought of as a reagent and should be well-defined

Ethics
We don't want to dose humans with a chemical of unknown toxicity
Utility of Animal Models (GxE) for Pharmacogenomics

Humans and mice share 92% genetic sequence homology
Like humans, mice have migrated across globe with gene flow across continents

Subspecies of mice/rats have a varied geographic origin
■ GENETICALLY IDENTICAL

■ HOMOZYGOUS AT MOST LOCI

No difference in the allele that comes from mom vs the one that comes from dad

■ LOW PHENOTYPIC VARIABILITY

Assuming all conditions and environment are the same/well-controlled

■ LOW N NEEDED TO POWER STATISTICS

■ EASY TO MAINTAIN GENETIC FIDELITY

Inbred Strains Characteristics

Inbreeding can lead to genetic disease and poor health status, which is one reason these are favored as disease models.
GENETICALLY UNIQUE INDIVIDUALS

HETEROZYGOUS AT MANY LOCI
Better mimic human populations because this is common for us too

HIGH PHENOTYPIC VARIABILITY
Different shapes and sizes, just like people

HIGHER N NEEDED TO POWER ANALYSIS
Tradeoff for having greater phenotypic diversity

MUST DILIGENTLY MAINTAIN GENETIC FIDELITY

Outbred populations mimic human diversity better than single inbred strains
Models with utility for assessing gene by environment interactions to assess AEs associated with chemotherapy

USE A PANEL OF COMMON INBRED STRAINS

Presentations are tools that can be used as lectures.

USE RECOMBINANT INBRED STRAIN PANEL

Presentations are tools that can be used as lectures.

USE A HIGHLY DIVERSE OUTBRED STOCK

Presentations are tools that can be used as lectures.
GENETICALLY DIVERSE OUTBRED MOUSE STOCK
Different mice > different genetics

GENETICALLY DIVERSE HUMAN POPULATION
What we're seeking to mimic
Different people > Different genetics

Use a mouse population to inform human susceptibility to chemicals
COLLABORATIVE CROSS AND DIVERSITY OUTBRED

All founder strains have been fully sequenced

Includes representatives of 3 mouse subspecies
  M.m. domesticus
  M.m. musculus
  M.m. castaneous

3 strains were recently derived from the wild

Collaborative Cross
  >100 Recombinant Inbred lines

Diversity Outbred Stock
  Each individual is genetically unique
  Sequence mice using MUGA SNP array

An advantage is increased genetic mapping resolution
Translational pharmacogenomics

Use mouse populations to predict human population responses
TESTING
Expose diverse mice to chemical and collect samples for quantitative analysis

GWAS
Use statistics to determine regions of genome (loci) that contain variants influencing the trait

SINGLE GENE ANALYSIS
Narrow down candidate gene list, confirm via sequence analysis in mice

TRANSLATION
Sequence candidate genes in humans and confirm association with trait

Genome Wide Association Study (GWAS)
AN OVERVIEW
ASSESS GENETIC SUSCEPTIBILITY

Basics of GWAS

GWAS is a statistical approach that assesses at each location whether there is a significant difference in the mapped phenotype depending on the locus genotype.

It's on Chr 3 that we'll expect to find a pharmacogenetic risk factor for the drug toxicity.
An example of GWAS using Diversity Outbred (DO) Mice

INVESTIGATING GENETIC CAUSE OF RARE, BUT SERIOUS LIVER INJURY THAT OCCURRED DUE TO HERBAL SUPPLEMENTS CONTAINING GREEN TEA EXTRACT
Green Tea Extract

Epigallocatechin gallate

Gene x Environment = Liver Injury

A small fraction of the animals (16%; 43/272) exhibited severe hepatotoxicity (10-86.8% liver necrosis) that is analogous to the clinical cases.
For green tea extract, there was a significant peak on the distal arm of Chr 4

We can expand the significant gene region

Because we have the complete sequence and haplotype structure of each DO mouse, can determine what is the effect on the phenotype of having inherited that DNA "chunk" from one of the 8 founders
We can use the founder effects to narrow down the list of quantitative trait genes in the QTL support interval.

Focus targeted sequencing on just those genes that contain a unique NOD founder allele.
DO GWAS - Green Tea Extract

Sequencing candidate genes in 15 clinical cases of green tea extract induced liver injury

<table>
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<tr>
<th>Gene Symbol</th>
<th>SNP ID (Array)</th>
<th>Gene Name</th>
<th>Chromosome</th>
<th>Position</th>
<th>P value for clinical association</th>
<th>Risk/Protective allele</th>
<th>Effect</th>
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<td>exm1 6762</td>
<td>period circadian clock</td>
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<td>Missense (K/W)</td>
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<td>mitofusin 2</td>
<td>2</td>
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<td>Missense (I/V)</td>
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<td>VPS13D</td>
<td>exm1 6480</td>
<td>vacuolar protein sorting 13 homolog D (S. cerevisiae)</td>
<td>3</td>
<td>1234349</td>
<td>0.043064</td>
<td>A/T</td>
<td>Missense (R/S)</td>
</tr>
</tbody>
</table>

Mitofusin 2, involved in mitochondrial regulation and maintenance, may contribute to susceptibility to EGCG-induced liver injury by herbal supplement use.
Investigating gene by environment for chemotherapeutic toxicity

Once you've identified susceptible and resistant mice - mechanistic and biomarker analysis becomes tractable
Differential kidney toxicity responses in DO mice due to chemotherapeutic cisplatin

MARKERS OF KIDNEY INJURY ELEVATED
5 mg/kg cisplatin i.p.; 3 days after exposure

Urinary biomarkers of proximal tubule injury elevated only in susceptible DO mice

Harrill et al. Exp Biol Medicine 2018
Myelosuppression caused by chemotherapy

GENETIC SUSCEPTIBILITY

Investigation of genetic variants in DO mice that increase susceptibility to decreased neutrophil counts in response to:

doxorubicin
cyclophosphamide
docetaxel

Gatti et al. Pharmacogenomics J 2018
Effect of drugs and chemicals on brain development of offspring

Emphasis on developmental brain injury in 200 male and female Diversity Outbred neural progenitor cell lines
Including genetic diversity in toxicology and in disease modeling can improve understanding and prediction of human outcomes, enabling precision medicine.
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THANK YOU FOR YOUR ATTENTION!