Genetic Studies in the Drug-Induced Liver Injury Network (DILIN)

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The Drug-Induced Liver Injury Network (DILIN)

https://dilin.dcriduke.edu/
Proposed Mechanisms for DILI

- cholestasis
- mitochondrial dysfunction
- chemical stress
- drug bioactivation
- covalent binding
- apoptosis
- hepatocyte hypertrophy
- hepatocyte hyperplasia
- hepatocyte necrosis
- fibrosis
- activation of the innate immune system
- activation of the adaptive immune system

Multicellular and multifunctional organ; Multiple and variable forms of disease
DILIN: Enrollment for GWAS

- 579 cases (all drugs)
- 565 with available genotypes
- Genotyped on Illumina 1Mduo array
- Controls: 2,314 individuals from the 1958 Birth Cohort genotyped on the 1Mduo (WTCCC)
- Top n drugs:
  - Amoxicillin/clavulanic acid (Augmentin): 59 EU
  - Nitrofurantoin: 24 EU
  - Valproic Acid: 16 EU
  - Isoniazid: 14 EU, 9 AA
Principal Components Analysis (EU)
Isoniazid-induced Liver Injury

- Idiosyncratic, suspected genetic predisposition
- (Weak) evidence for familial clustering
- Essential component of treatment for tuberculosis
- Liver injury due to isoniazid (relatively) common and severe
Isoniazid (INH) GWAS Results

African American \( (n = 9) \)  
European American \( (n = 14) \)
## Drug-specific Analyses: Isoniazid

### African American (n = 9)

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Can we identify genetic variants predisposing to DILI generally?
EU Non-Amox/Clav Cases (n=339)
Synthetic Associations

Dickson SP et al., PLoS Biology 2010
Example of a Synthetic Association: GWAS of Sickle Cell Disease
Example of a Synthetic Association: GWAS of Ribavirin-induced Anemia

Fellay J et al., Nature 2010
Example of a Synthetic Association:
GWAS of Ribavirin-induced Anemia
Conclusions from GWAS

- Top-associated SNPs in MHC region for amoxicillin-clavulanate and other drugs reveal both shared and drug-specific genetic risk factors

- For most drugs, no genome-wide significant associations have been detected via GWAS

- Association signals attributed to common SNPs may represent synthetic associations of multiple rare variants with a variety of precipitant drugs

- Follow-up studies should be performed with this possibility in mind
GWAS: Future Directions

• Misclassification errors – can we refine the phenotype given such small sample sizes?
  – Stratification by Injury Type (hepatocellular, cholestatic, mixed)
  – Stratification by Severity, Age, Time to Onset
  – Structural similarity (drug or metabolite)

• Investigation of other ancestral populations
  – Asian: 21 Cases, 219 Controls
  – Hispanic: 41 Cases, 33 Controls
  – Identify novel risk alleles, refine LD span(s)
Searching for Rare DILI Risk Variants by Whole Exome Sequencing

- Genome-wide association studies have revealed no common genetic risk factors for INH-DILI
- Common variation cannot be highly predictive of DILI, by definition
- Whole-exome sequencing allows essentially complete survey of both common and rare functional variation across the genome
- Goal: Identify genetic risk factors causal for INH-DILI, with the aim of developing a diagnostic test with clinically useful predictive power
Study Design Overview

Sequence genomes of SAE cases

Identify and validate all variant sites

Exclude common variants (those previously evaluated in GWAS)

Prioritize variants for next step:

a. Variants with predicted function:
   - Missense and nonsense variants
   - Splicing and ncRNA regions
   - CNVs

b. Variants enriched in SAE cases
   - dbSNP entries
   - HapMap frequencies (when available)
   - 1000 genomes project

Genotype selected variants on expanded sample set to confirm associations
Sequencing Costs

- Targeted exomic sequencing
  - $3-4K/exome
- 21X coverage or higher
  - >99% probability of discovering unique variant at any particular site (<1% false negative rate)
- Roughly 1 week per exome
- Significant cost reduction vs whole-genome sequencing (roughly $10-20K/genome currently)
Data Analysis and Interpretation: Sequence Variant Analyzer (SVA)
Data Analysis and Interpretation

• SVA: annotate and prioritize variants based on:
  – 1) enrichment in SAE cases
  – 2) likelihood of functional consequence

• Test immediately for association by comparison to public databases (dbSNP, HapMap, 1000genomes) or available control genomes (Duke CHGV) within SVA

• Test for association by targeted genotyping in larger cohorts of case and control subjects
Conclusions

• GWAS have revealed several common variants of moderate effect on DILI risk
  – Tendency for drug-specific effects

• Additional common risk factors may yet be identified via GWAS
  – Power limitations: sample size, genetic architecture

• Comprehensive evaluation of rare functional variation may soon allow for identification of high-impact genetic determinants of DILI
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