An Examination of the Genetic Architecture of Alcohol Withdrawal

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BACKGROUND:

- **Alcohol is the most abused drug in the United States, but alcohol dependence (AD) remains poorly understood because of its heterogeneous and polygenic features**.
- **The emergence of a negative emotional state when a person is unable to access alcohol (i.e., withdrawal) is a key motivational factor that may provide insight into the effects of chronic use**.
  - Approximately 27% of alcohol dependence individuals meet criteria of alcohol withdrawal (AW).
  - Studies suggest AW is under genetic influence (Levey et al. 2014, Palmer et al., 2015, Wang et al. 2012), but no specific loci have been found that survive correction (Bonferroni p-value threshold $p < 5x10^{-8}$) for multiple testing.
  - Integrative whole genome-methods (see Figure 1 (right side) are anticipated to aid in overcoming this limitation (Palmer et al., 2012).

OBJECTIVES:

- The goal of this project is to identify genetic loci, genes, and an optimal gene set involved in AW. We intend to:
  - Confirm candidate genes derived from previous literature and experimental studies.
  - Identify novel loci through whole-genome analysis.

SAMPLE DESCRIPTION

- Mean age: 37.37 (range 7-88); 50% female.
- Withdrawal symptoms assessed using the Semi-structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994).

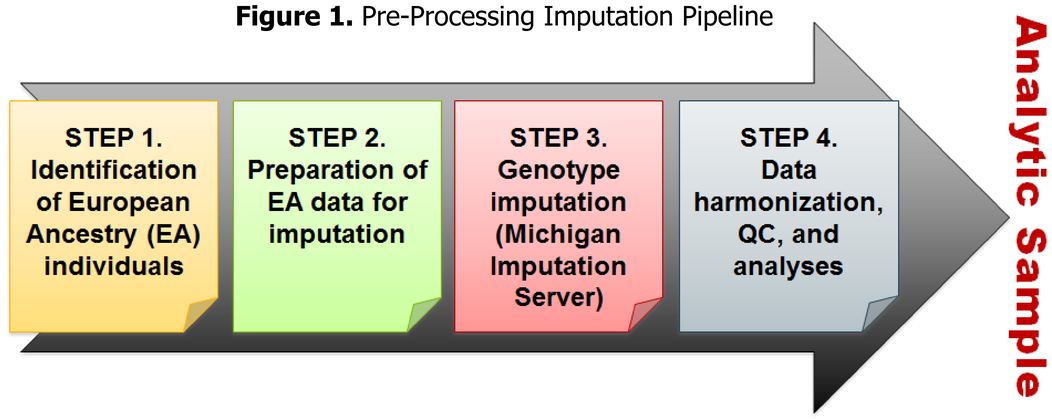
Table 1. Summary Statistics for Withdrawal Symptoms in combined sample

Symptoms						
Symptoms	No	Yes	Total N			
Shakes	2617	749	3366			
No Sleep	2488	875	3363			
Anxious / Depressed	2171	1192	3363			
Sweats	2610	754	3364			
Heart Beating Quickly	2679	683	3362			
Nausea	2884	479	3363			
Physically Weak	2453	910	3363			
Headache	2725	639	3364			
Hallucinations	3110	255	3365			
Other Alcohol Measures						
Variables	No	Yes	Total N			
Ever Drink	238	4141	4379			
Drink to Avoid Symptoms	347	931	1278			
2+ Symptoms at a time	3110	255	3365			
	Min	Median	Mean	Max	NA's	Total N
Age Regularly Drinking	5	18	19.16	65	298	4142
Age First Drunk	2	16	17.45	68	347	4075
Max Drinks in 24 hrs	0	15	21.34	274	299	4123

PROCEDURAL STEPS:

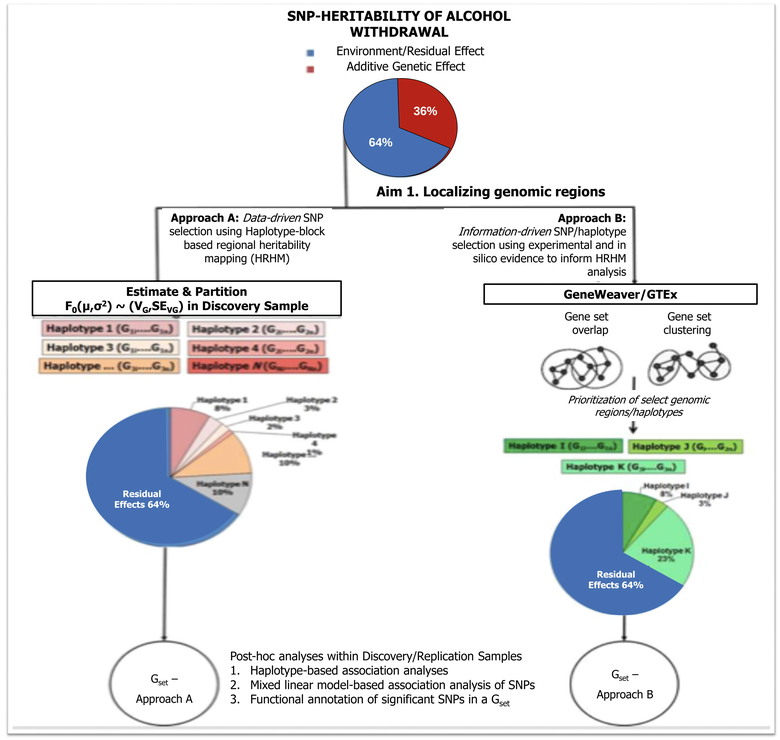
1. Integrate several assessments from the Collaborative Study on the Genetics of Alcoholism (COGA) to form a single dataset (discovery sample).
2. Impute genomic data using the 1000 Genomes Project as a reference (see Figure 1).

Figure 1. Pre-Processing Imputation Pipeline



3. Identify 'a priori' genomic loci and genes using curated databases (GeneWeaver and PubMed literature Review); excluding association-type studies.
4. Apply two main computational methods to observe pattern trait-locus patterns and to test the identified gene sets.
   - Family-based genomewide association analysis and genomic-relatedness maximum likelihood analysis. All analyses will account for variation in ancestral background, age, and gender.
   - Jaccard Similarity will be used to compare "top" SNPs from the GWA analysis to the information-driven gene set.
5. Epistatic interactions will also be explored.

Figure 2. Data Analysis Pipeline



EXAMPLE GENE LIST:

- These six genes have been associated with delirium tremens (DT) (Baker 2012).
- It will be contrasted with many other gene-sets.
- In this example, only 1/6 genes overlap with 17 other gene-sets identified.

Table 2. Example Gene-set

Gene Name	System
ALDH2	Glutamate Decarboxylase
DBH	GABA receptors
DRD2	Neurotransmitter Agents
NPY	cortical excitability and stress response
SLC6A3	DA transporter
SLC6A4	SER transporter

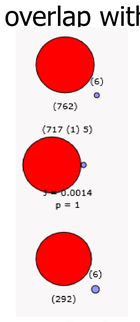


Figure 3. Gene-set Intersection graph

LIMITATIONS

- Power for the current analyses are limited by sample size, however we seek to minimize this by controlling for confounders, in particular ancestry, by using ancestral principal components based on the six major super populations.
- A priori gene set analyses are primarily based on study results reported to the GeneWeaver ontological engine, which we hope to supplement using a PUBMED search with the following parameters:
  - Date of Publication since 1970.
  - Text contains:
    - "alcohol withdrawal" AND "genetic"
    - "alcohol withdrawal" AND "GWAS"
    - "alcohol withdrawal" AND "candidate gene"

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