MARC Project 5:

MOLECULAR EPIDEMIOLOGY OF ALCOHOLISM AND COMORBID DISORDERS

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ABSTRACT

- **The MARC research project 5 builds upon gene-discovery projects such as COGA (Collaborative Study on the Genetics of Alcoholism: PIs Begleiter and Reich) and similar projects (e.g. PIs Hill, Kendler) which are studying treatment-ascertained alcoholics and their relatives, and the MARC-affiliated Alcohol-QTL IRPG consortium (PIs Heath, Martin, Madden, Todd), which is studying community-ascertained alcoholics and heavy smokers and their adult relatives, by incorporating a molecular genetic component into 4 mature, prospective longitudinal studies (PIs Chassin, Cooper, Heath, Sher) spanning the age-range from early adolescence into young adulthood, with 3-7 waves of prospective assessment.**

ABSTRACT (continued)

- In addition to collecting DNA from the target samples, this research project combines secondary data-analysis and genotyping, proceeding in 4 stages:
  - (i) longitudinal and other phenotypic analyses to establish consistent phenotype definition across informative data-sets (not all data-sets will be informative for all phenotypes of interest);
  - (ii) behavioral genetic analyses on existing twin data sets (MOAFTS, and other U.S. and Australian data-sets to which we have access through the MARC) to confirm heritability of phenotypes defined at stage (i), and where possible determine whether that phenotypic operationalization is optimal for understanding genetic effects (which may not be the case if the structures of genetic and environmental influences are very different);
  - (iii) genotyping for a limited number of candidate genes; and
  - (iv) genetic association analysis.

ABSTRACT (continued)

- This carefully staged approach is necessary to minimize the dangers of multiple testing when combining candidate gene data and rich longitudinal data sets. For the same reason, we focus on a limited number of candidate phenotypes where prospective data are expected to be informative for understanding the etiology of alcoholism.
- Selection of candidate phenotypes and candidate genes is guided by the MARC focus on the roles of overlapping mechanisms of behavioral under control, negative affect regulation and pharmacologic vulnerability in the etiology of alcohol use disorders (AUDs), emphasizing AUD phenotypes associated with (a) externalizing symptoms, (b) tolerance and quantitative consumption indices, (c) cognitive aspects of alcohol use (expectancies), (d) co-occurrence with tobacco dependence, and (e) negative affect (depression, suicidality).

Specific Aims

- **Project Goals**
  - **1.1. To obtain blood samples for DNA extraction from 1,000 additional participants in the Heath longitudinal study of female twins, adding to those collected from 4 prospective longitudinal studies (Chassin, Cooper, Heath, Sher).**
  - **1.2. To Combine data from 5 longitudinal studies (Anokhin, Bucholz, Chassin, Heath, Sher) to derive phenotypes of alcohol involvement (use & problems), co-occurring features based on longitudinal course, operationalized across two or more data-sets and focused on 4 domains: (i) externalizing symptoms; (ii) consumption, as well as cognitive aspects of alcohol use (expectancies); and co-occurrence with (iii) tobacco dependence and (iv) early trauma and depression. Two approaches will be emphasized– developmental (e.g. using mixture modeling to identify trajectories through time); and state/trait modeling (e.g. modeling chronicity of effects).**

Specific Aims

- **Project Goals (continued)**
  - **1.3 To test for candidate gene effects in all 5 samples on these phenotypes (choice of candidate gene will be revised in the light of emerging findings from ongoing gene-discovery efforts, including MARC-affiliated projects, by the time the next genotyping phase of the project begins):**
    - **(i) AUDs and externalizing symptoms (DRD4, DRD5, SLC6A3 (DAT1 in old notation), GABRA2, GABRG2, CHRM2);**
    - **(ii) AUDs and alcohol consumption (ALDH2 promoter polymorphism, ADH1B (ADH2 in old notation), ADH1C (ADH3 in old notation), ADH4, ADH7, NTSR1);**
    - **(iii) AUDs and tobacco consumption (CHRNA4, CHRNA5/CHRNA3/CHRNB4, CHRNA7, CHRNB2, CHRNB3);**
    - **(iv) AUDs and negative affect and stress-regulation, CRF, NPY, SLC6A4 (5HTT in old notation), OPRM1). Many of these polymorphisms are not adaptable to DNA array analysis and require individual genotyping.**

Specific Aims

- Project Goals (continued)
  - 1.4. For samples with adequate DNA yields, to expand candidate gene analyses to a custom DNA array using the top 1,000 associated single nucleotide polymorphisms (SNPs) and flanking markers (about 20,000 SNPs total) which replicate across ongoing genome wide association studies of alcoholism in the Collaborative study on the Genetics of Alcoholism (COGA) and Interactive Research Project Grant (IRPG) studies.
  - 1.5. To test if the most significant markers from GWA studies of AUD are also associated with the longitudinal phenotypes in 1.2 .

Preliminary Results

- Efforts have been devoted to the organization of d data sets and analytic approaches for the determination of phenotypes of interests for genetic analysis and the completion of an initial screening project to determine interest of subjects participating in this project. Current progress for this study has occurred on two fronts.
- First, regular meetings of key investigators and biostatisticians have been held over the last year to discuss analytic approaches to the development of phenotypes across data sets and to begin joining data sets. The consensus is that phenotypes will be initially developed in the two University of Missouri data sets then tested for heritability in the MOAFTS (Missouri Adolescent Female Twin Study) data set. Common items and constructs are being developed.

Preliminary Results (continued)

- **Second, a test of how to approach subjects for blood collection was conducted in the Sher study: 257 individuals (~½ of the target sample) was contacted to assess interest in the study; 249 agreed to participate (a 96.9% initial cooperation rate). Materials for blood collection were mailed to potential participants. The participants are asked to go to local clinics or laboratories for blood drawing and samples are then express shipped to the laboratory at Washington University. The next wave of interviews with the MOAFTS sample has begun, and blood collection efforts with cooperative twins is being conducted with these.**

Preliminary Results (continued)

- **Progress to Date on Sample Collection and Genotyping**
  - Blood and buccal swab samples are now being collected for DNA isolation from all four prospective studies populations.
  - To date ~3,300 specimens have been received in the lab: Chassin (N = 583), Heath (N=1,504), Sher (N = 255), Bucholz (N=416), Anokhin (N = 552).
  - DNA has been prepared and under gone quality control checks for 2,300 individuals. Functional repeat polymorphism genotypes have been produced for DRD_4, and $SLC6A_4$ (HHT) loci on 1318 samples, and for DRD_5, and $SLC6A_3$ (DAT1) on 1,594 samples

Preliminary Results (continued)

- **Immediate Plans**
  - During the next year we anticipate completing sample collection, repeat polymorphism genotyping and most single nucleotide polymorphism (snp) genotyping as well as developing phenotypes for joint samples.