2004

Progress toward identification of alcoholism susceptibility genes on chromosome 7 in the COGA dataset

Alison M. Goate
Washington University School of Medicine in St. Louis

Progress Toward Identification of Alcoholism Susceptibility Genes on Chromosome 7 in the COGA dataset

Alison M. Goate, Ph.D.

This research is supported by NIH Grant (U10AA08403) from the National Institute on Alcohol Abuse and Alcoholism (NIAAA).

There are no interests to disclose.

Genetics of alcoholism

1) To what extent do genetic influences impact the trait of interest?
2) Can we identify these genetic influences?
3) How do these genes act and interact with other genetic/environmental influences?

The heritability of alcoholism

- Twin & adoption studies
  - → Denmark, Sweden, Finland, Australia, USA
- Across birth cohorts
  - → late 1800s to early 1970s
- Across methods of assessment
  - → In-patient hospitalizations, gov't records, diagnostics interview
- Across diagnostic criteria
  - → Feighner, DSMIII-R, DSMIV, ICD
- Heritability estimates
  - →51-65% in females
  - →48-73% in males

COGA

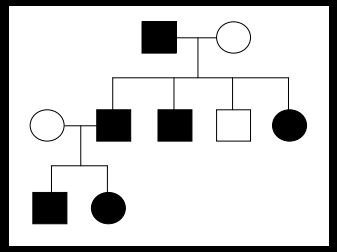
Collaborative Study of the Genetics of Alcoholism

"The long-term objective of this multi-dimensional interdisciplinary research project is to *characterize the genetic factors* involved in the determination of predisposition to alcoholism. This substantial undertaking involves the expertise of biochemists, clinicians, geneticists, neuropsychologists, neurophysiologists, and statisticians."

--original COGA grant application, 1989

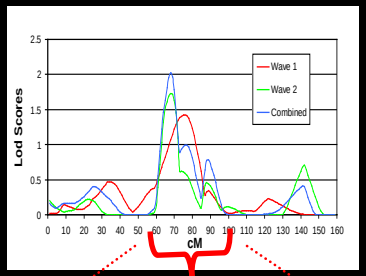
COGA strategy

1. Ascertain alcoholic families



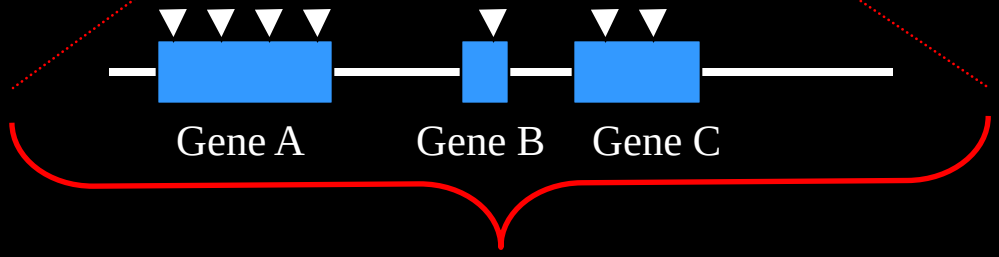
Polydiagnostic interview
Electrophysiological data

2. Linkage analyses to identify chromosomal regions



↑ allele-sharing among affecteds within a family

3. Association analyses to identify specific genes



COGA

Collaborative Study of the Genetics of Alcoholism

- Patients identified through inpatient/outpatient treatment programs at 6 sites
  - General sample: 1,227 families (n=9265)
    - Semi-structured Assessment for the Genetics of Alcoholism Interview (SSAGA)
  - Genetic sample: 262 families (n=2282)
    - Blood draw, EEG/ERP, neuropsychological assessments
    - 2 waves of data collection

Phenotypes used in the genetic analyses

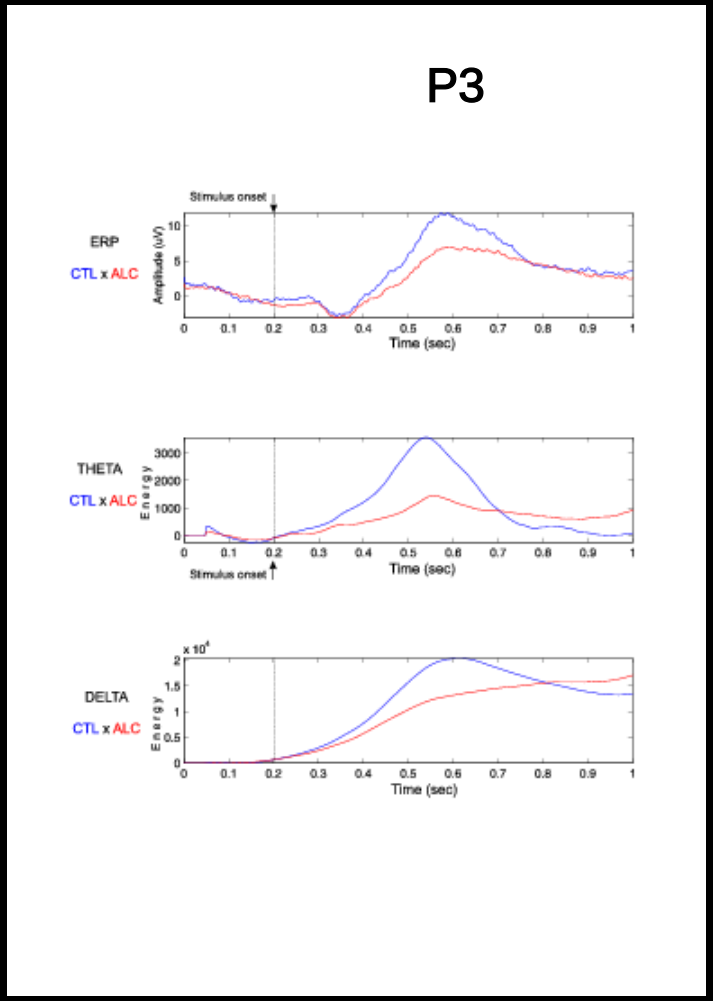
- Alcohol dependence
  - → COGA= DSMIIIR plus Feighner criteria
  - → DSMIV
  - → ICD10
- Other substance abuse
  - → Cocaine dependence
  - → Marijuana dependence
  - → Habitual smoking (2 packs/day for at least 6 months)
- Comorbid disorders
  - → depression
- Endophenotypes
  - → Electroencephalogram (EEG)
  - → Event related potential (ERP)

Neurophysiological Endophenotypes

- **S-transform of evoked activity to target in VP3**
  - → **yields time-frequency characteristics of signal**
- **Endophenotypes: Theta band (3-7 Hz) + delta band (1-2 Hz) between 300-700 ms (when P3 component is maximum) in brain regions (frontal, central, parietal)**
  - → **Time-frequency distribution mean value**
- **SOLAR linkage analysis**
  - → **Using 1340 individuals in 253 families**

Jones et al.

Theta + Delta Oscillations Underlying GO NO-GO P3 Are Reduced In Alcoholics



Control

Alcoholic

Kamarajan et al., 2003

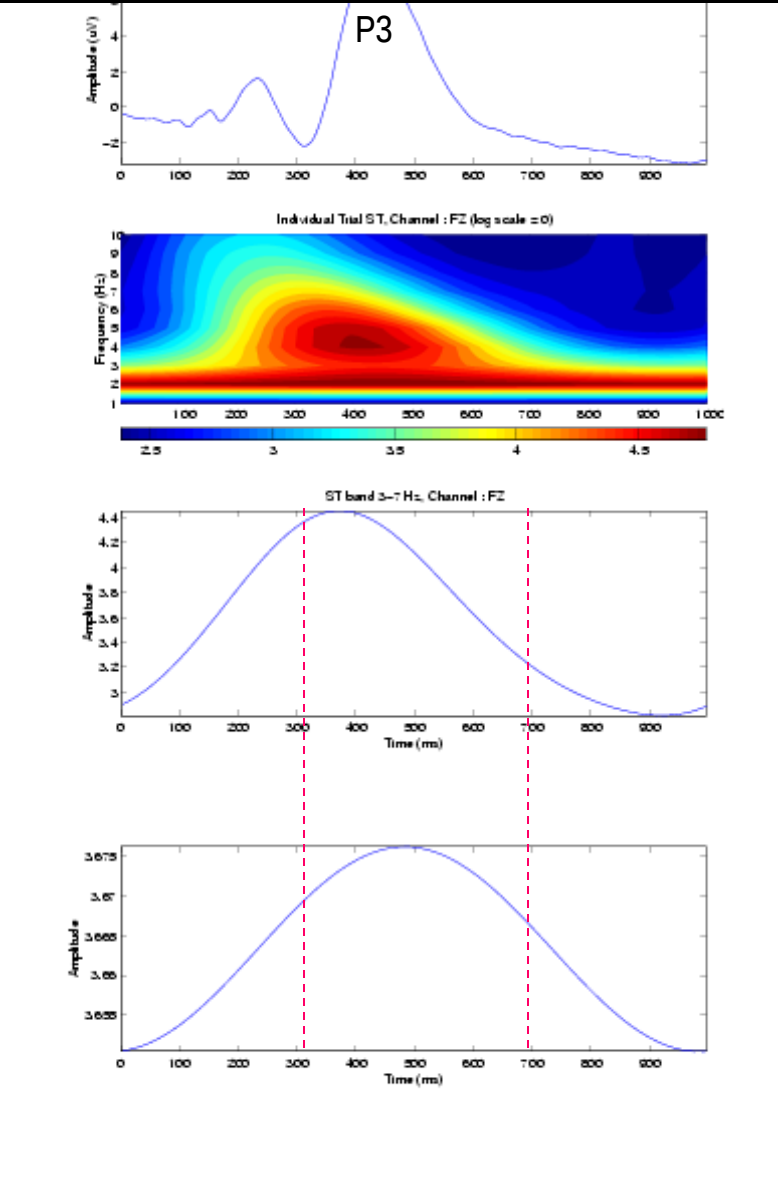
SOLAR Linkage Analysis:Theta + Delta Oscillations VP3 Target (S-Transform)

ERP TARGET

TIME by FREQ

THETA

DELTA



N=1340/253

Difficulties with Complex Disorders

- Many genes of small effect
- Genetic heterogeneity
- Gene-gene interaction
- Gene-environment interaction
- Phenotype definition

EEG Summary

- Imbalance in excitation/inhibition (CNS disinhibition) in alcoholics and individuals at risk
- Hypothesis: CNS disinhibition involved in genetic predisposition for development of alcohol dependence
- EEG as an endophenotype for alcohol dependence

EEG Heritabilities

Frequency band	Mean h2
Delta (1.5-3.5 Hz)	76%
Theta (4-7.5 Hz)	89%
Alpha (8-12.5 Hz)	89%
Beta (13-25 Hz)	86%

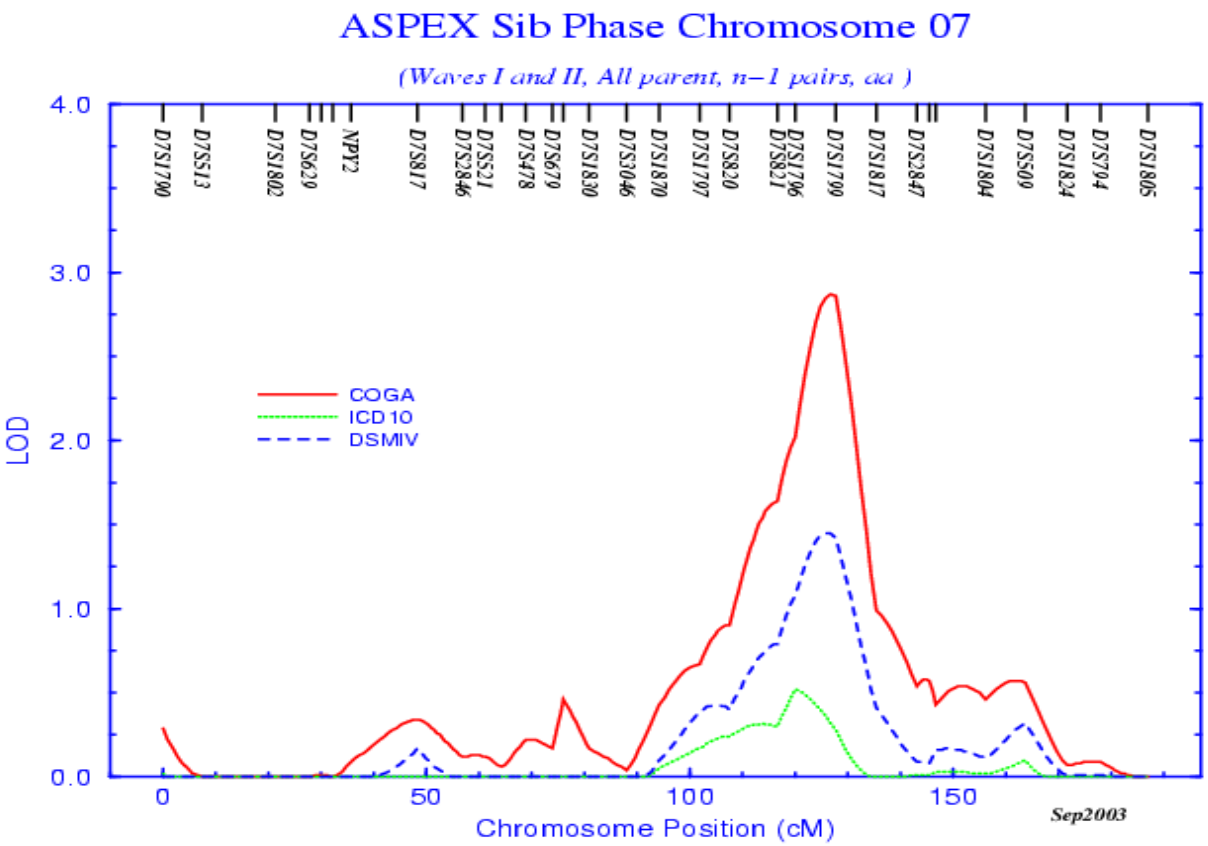
Van Beijsterveldt et al., 1996

Linkage Results in COGA

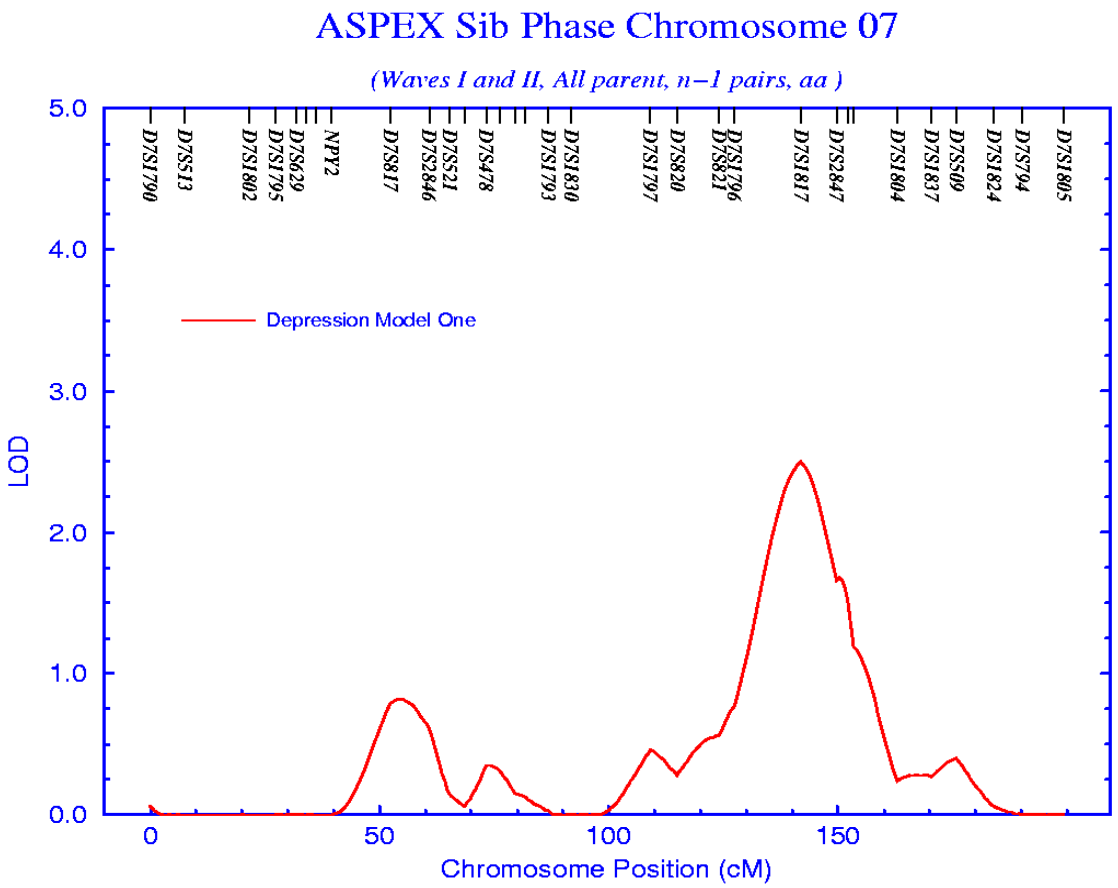
Results of the initial genome screen for COGA alcohol dependence

- 3 chromosomal regions showed evidence for a susceptibility locus
  - Chromosome 1 near D1S1588
  - Chromosome 2 near D2S1790
  - Chromosome 7 near D7S1793
- 1 chromosomal region showed evidence for a protective locus
  - Chromosome 4 near ADH3

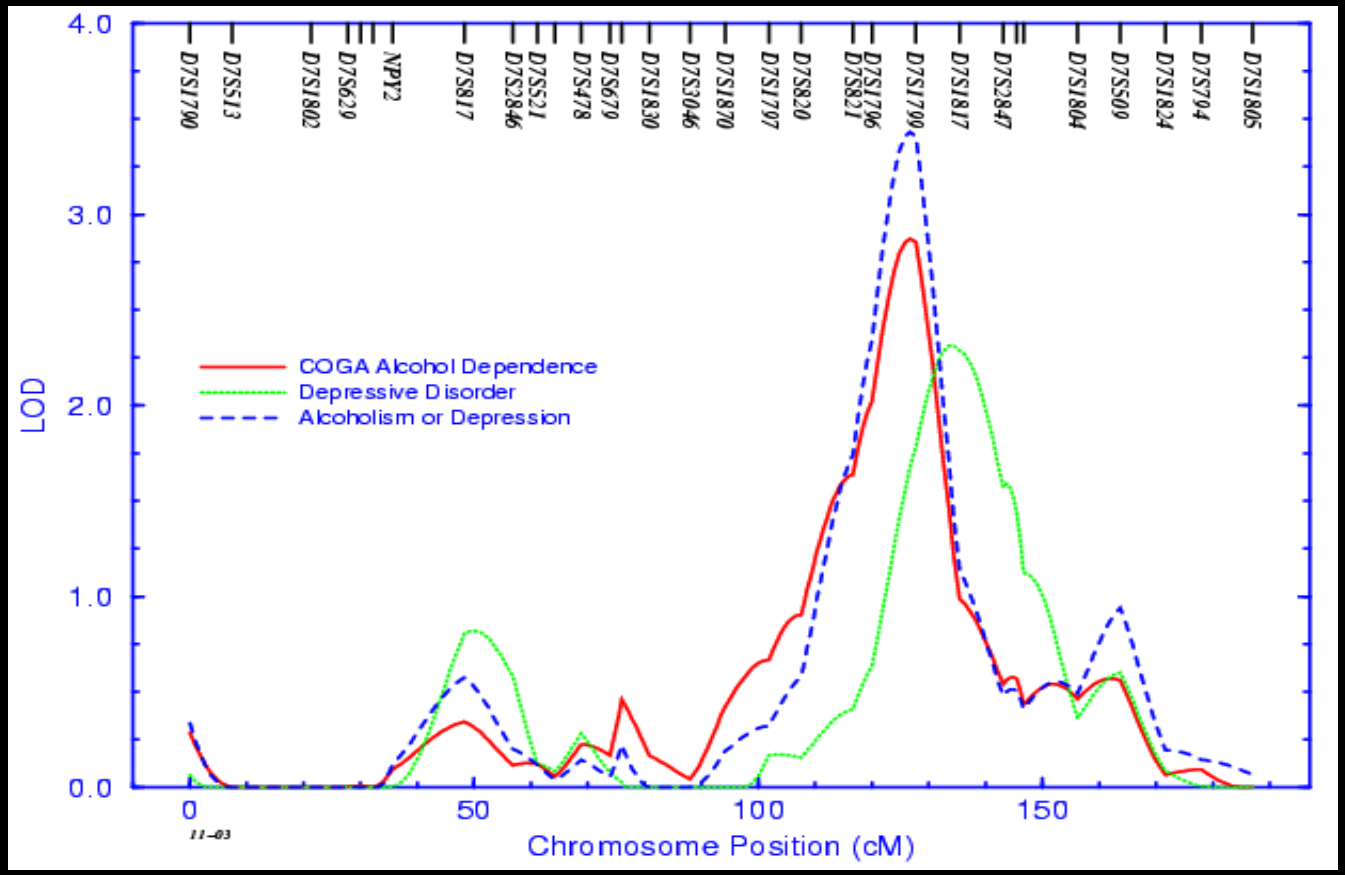
Evidence for a susceptibility gene for alcohol dependence on chromosome 7



Evidence for a susceptibility gene for depression on chromosome 7



Linkage to chromosome 7



Linkage Analysis Of Theta ERO: Chromosome 7

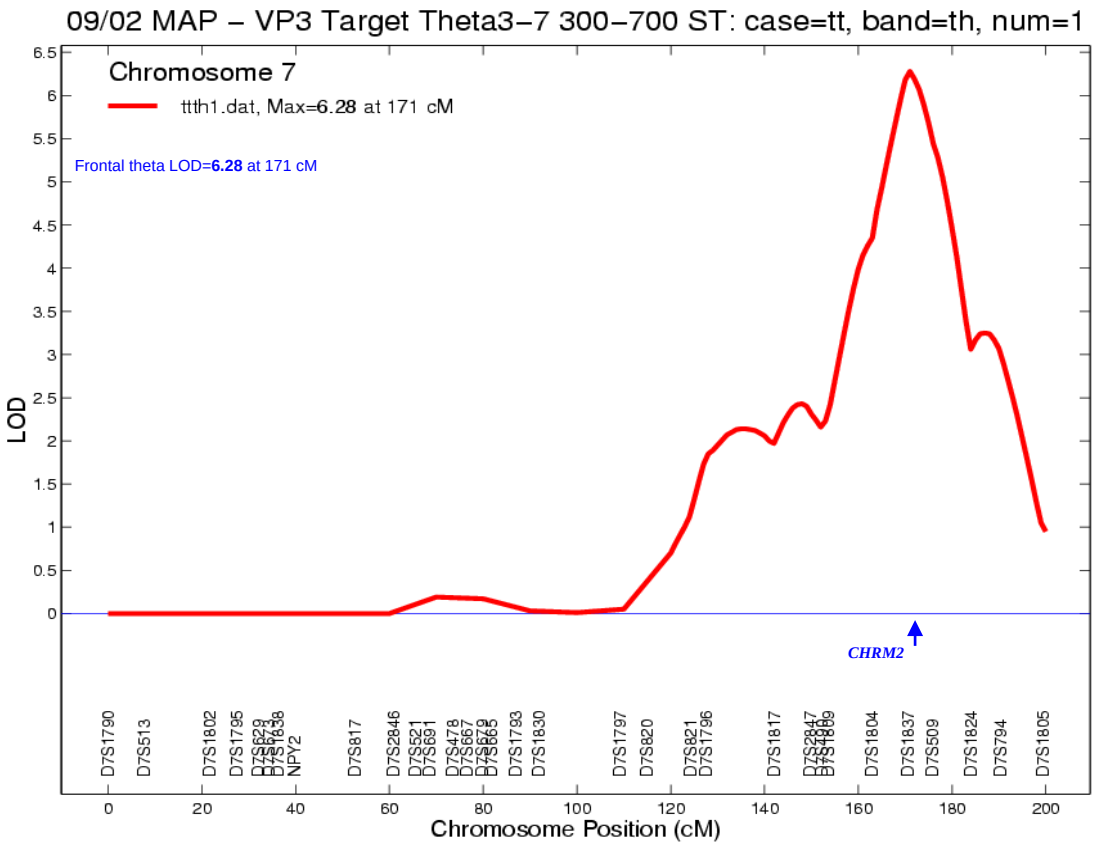
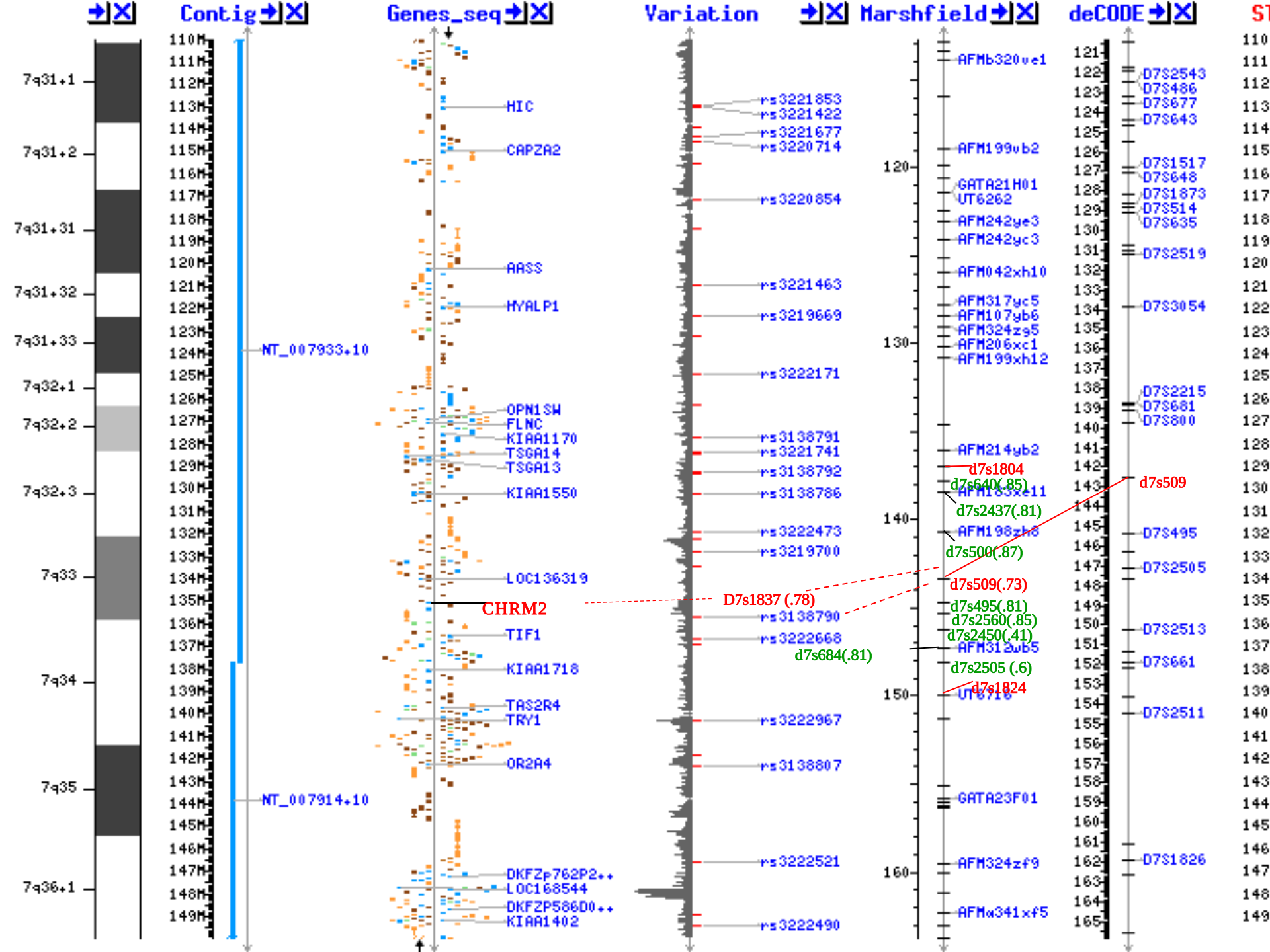


Figure 1. Linkage analysis of evoked theta on Chromosome 7

Jones et al.

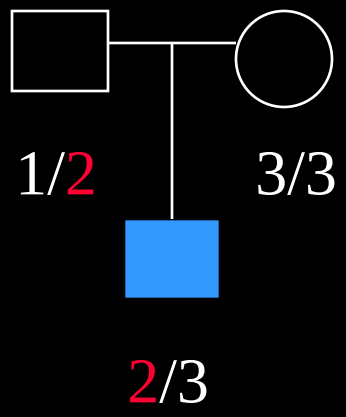

Contig
Genes_seq
Variation
Marshfield
deCODE
7q31.1
7q31.2
7q31.31
7q31.32
7q31.33
7q32.1
7q32.2
7q32.3
7q33
7q34
7q35
7q36.1
NT_007933.10
NT_007914.10
HIC
CAPZA2
AASS
HYALP1
OPN1SW
FLNC
KIAA1170
TSGA14
TSGA13
KIAA1550
LOC136319
CHRM2
TIF1
KIAA1718
TAS2R4
TRY1
OR2A4
DKFZp762P2++
LOC168544
DKFZP586D0++
KIAA1402
rs3221853
rs3221422
rs3221677
rs3220714
rs3220854
rs3221463
rs3219669
rs3222171
rs3138791
rs3221741
rs3138792
rs3138786
rs3222473
rs3219700
rs3138790
rs3222668
rs3222967
rs3138807
rs3222521
rs3222490
D7s1837 (.78)
d7s684(.81)
AFMb320ve1
AFM199vb2
GATA21H01
UT6262
AFM242ye3
AFM242yc3
AFM042xh10
AFM317yc5
AFM107yb6
AFM324zg5
AFM206xc1
AFM199xh12
AFM214yb2
d7s1804
d7s640(.85)
d7s2437(.81)
d7s500(.87)
d7s509(.73)
d7s495(.81)
d7s2560(.85)
d7s2450(.41)
AFM312wb5
d7s2505 (.6)
d7s1824
GATA23F01
AFM324zf9
AFMa341xf5
D7S2543
D7S486
D7S677
D7S643
D7S1517
D7S648
D7S1873
D7S514
D7S635
D7S2519
D7S3054
D7S2215
D7S681
D7S800
d7s509
D7S495
D7S2505
D7S2513
D7S661
D7S2511
D7S1826

COGA association strategy

- Multiple analytic methods: family-based
  - → Extended families (PDT; trios & discordant sibs)
  - → Classic TDT trios (TRANSMIT; SAGE)
- Multiple SNPs in each gene
- LD across the region
- Consistency!

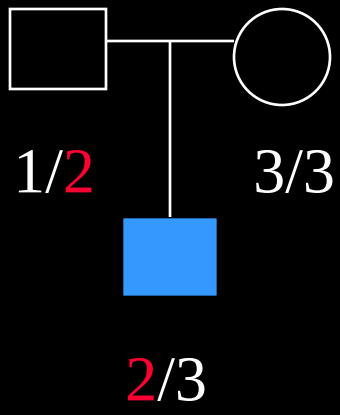
Family-based association methods

TDT – Transmission Disequilibrium Test

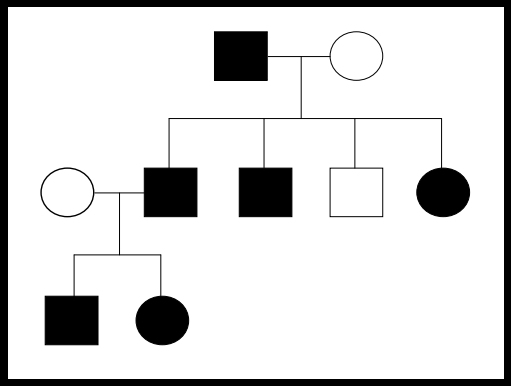


Family-based association methods

TDT – Transmission Disequilibrium Test



PDT – Pedigree Disequilibrium Test



All possible trios
Affected vs unaffected siblings

Location of SNPs within and flanking the *CHRM2* gene on chromosome 7

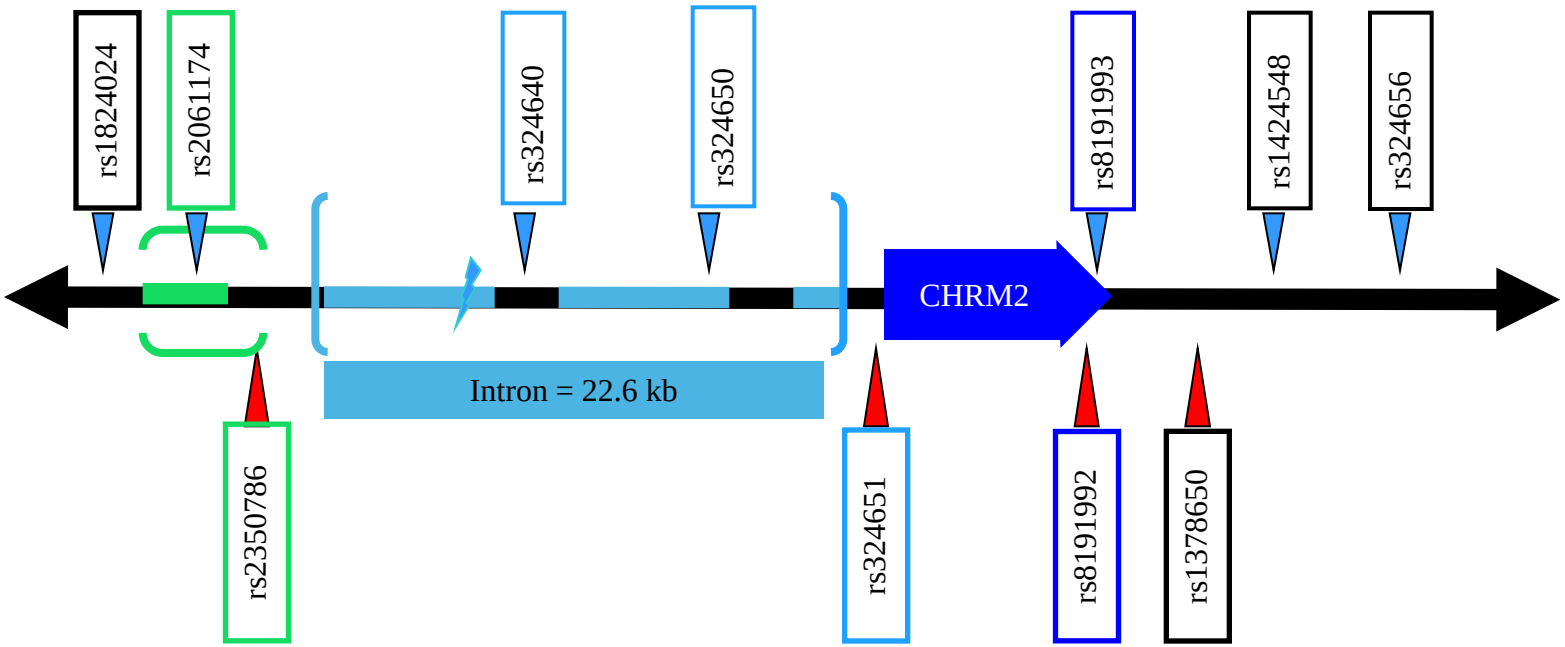


Table 2. Pedigree disequilibrium test (PDT) of 9 SNPs within and flanking the CHRM2 gene and Major Depressive Disorder.

SNP	p-value
rs2350786	0.034
rs324640	0.064
rs324650	0.047
rs324651	0.649
M164040.078	
M256	0.056
rs1378650	0.122
rs1424548	0.710
rs324656	0.486

Transmit disequilibrium test (TDT) of 9 SNPs within and flanking the CHRM2 gene and alcohol dependence.

Diagnosis SNP	COGA_M1 (N=888)		DSM4_M1 (N=756)		ICD10_M1 (N=575)	
	Chi	p-val	Chi	p-val	Chi	p-val
rs2350786	0.02	0.888	0.00	0.995	0.01	0.905
rs324640	1.01	0.315	0.53	0.468	0.01	0.934
rs324650	0.98	0.321	0.73	0.394	0.07	0.796
rs324651	1.44	0.231	2.13	0.145	0.49	0.485
M16404	0.27	0.603	0.17	0.679	0.03	0.862
M256	0.01	0.928	0.01	0.918	0.03	0.865
rs1378650	0.20	0.653	0.29	0.591	0.09	0.766
rs1424548	0.65	0.421	0.00	0.954	0.01	0.912
rs324656	0.02	0.896	0.05	0.828	0.46	0.496

Model 1: Unaffected subjects are defined as individuals who drink but do not endorse any symptoms of alcohol dependence.

Pair-wise disequilibrium between SNPs in the CHRM2 gene

SNP D' r^2 r^2	rs2350786	rs324651	M16404	rs1378650
rs2350786 (A:0.29/G:0.81)		0.67	0.44	0.60
rs324651 (G:087/T:0.13)	0.03		0.97	0.83
M16404 (A:0.41/T:0.59)	0.13	0.09		0.79
rs1378650 (C:0.58/T:0.42)	0.23	0.07	0.60	

Pedigree disequilibrium test (PDT) of 4 SNPs within and flanking the CHRM2 gene and alcohol dependence

	SNP (P-values)			
Disease Model	rs2350786	rs324651	M16404	rs1378650
COGA M1	0.733	0.195	0.036	0.032
COGA M2	0.682	0.264	0.050	0.051
DSM4 M1	0.811	0.025	0.008	0.022
DSM4 M2	0.707	0.107	0.023	0.043
ICD10 M1	0.704	0.061	0.079	0.196
ICD10 M2	0.677	0.289	0.167	0.203

Model 1: Unaffected subjects are defined as individuals who drink but do not endorse any symptoms of alcohol dependence.
Model 2: Unaffected subjects are defined as individuals who drink but do not meet diagnostic criteria for alcohol dependence.

Haplotype analysis three SNPs within the CHRM2 gene using SIMWALK with DSM IV diagnosis Model 1.

rs324651	M16404	rs1378650	Haplotype frequencies in founders (N)		Parental Contribution Discordant Sibs			
					Trans	Not trans	Affected	Unaffected	F	
G	A	C	0.072	(87)	44	33	19	14	0.654	
G	A	T	0.393	(479)	298	308	148	105	-1.431	
G	T	C	0.326	(396)	274	251	140	102	2.642	
G	T	T	0.081	(98)	34	31	32	20	0.208	
T	A	C	0.008	(10)	1	2	0	0	-1.000	
T	A	T	0.101	(123)	70	93	39	31	-1.956	
T	T	C	0.010	(12)	2	2	0	0	0.000	
T	T	T	0.009	(11)	3	6	0	0	-1.069	

Global PDT test of SNP haplotypes (excluding the 3 rare haplotypes) with DSM IV_m1 = χ^2=10.43 p=0.034, 4 d.f.

CHRM2 SNPs LD SOLAR MEASURED GENOTYPE DELTA TARGET

rs2350786	Frontal	0.66	rs8191992	Frontal	0.21
	Central	0.09		Central	0.0046
	Parietal	0.08		Parietal	0.0014
rs324640	Frontal	0.99	rs8191993	Frontal	0.69
	Central	0.02		Central	0.39
	Parietal	0.08		Parietal	0.96
rs324650	Frontal	0.82	rs1378650	Frontal	0.21
	Central	0.048		Central	0.008
	Parietal	0.01		Parietal	0.004
rs324651	Frontal	0.36	rs1424548	Frontal	0.95
	Central	0.12		Central	0.66
	Parietal	0.2		Parietal	0.61
			rs324656	Frontal	0.24
				Central	0.033
				Parietal	0.065

Conclusions I – Association Studies

- COGA strategy for testing candidate genes
  - → Within family tests
  - → Multiple SNPs in each gene
  - → Consistency between association results and patterns of LD
- Evidence for association
  - → GABRA2 on chromosome 4
  - → GABRG3 on chromosome 15

$GABA_A$ receptor genes & alcoholism

- Why association with some GABA-A receptors and not others?
  - → Chromosome 4:
    - GABRA2 (not GABRG1, GABRA4, GABRB1)
  - → Chromosome 15:
    - GABRG3 (not GABRB3, GABRA5)
- What about these genetic variants alters risk?
  - → Sequencing, no amino acid substitutions
  - → Regulatory differences

What next?

- Demonstrated genetic influence on alcoholism
- Identified specific genes
- Characterizing the risk associated with these genes

Conclusions

→From identifying genetic influence

→To identifying specific genes

→To characterizing the risk associated with those genes

- Gene-gene & gene-environment interaction
- Developmental trajectories associated with genetic risk factors

Acknowledgments - COGA

H. Begleiter, SUNY HSC @ Brooklyn, Principal Investigator
H. Edenberg, Indiana University, Co-Principal Investigator

This collaborative study includes nine different centers where data collection, analysis, and storage take place. The nine sites and Principal Investigators and Co-Investigators are: Howard University (R. Taylor), Indiana University (H. Edenberg, J. Nurnberger, Jr., P.M. Conneally, T. Foroud), Rutgers University (J. Tischfield), Southwest Foundation (L. Almasy), SUNY HSC @ Brooklyn (B. Porjesz, H. Begleiter), University of California, San Diego (M. Schuckit), University of Connecticut (V. Hesselbrock), University of Iowa (R. Crowe, S. Kuperman), Washington University (L. Bierut, C.R. Cloninger, J. Rice, A. Goate)

Lisa Neuhold, NIAAA Staff Collaborator

This national collaborative study is supported by the NIH Grant U10AA08403 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA).