Genome-Wide Association Study of Opioid Dependence: Multiple Associations Mapped to Calcium and Potassium Pathways

Joel Gelernter, Henry R. Kranzler, Richard Sherva, Ryan Koesterer, Laura Almasy, Hongyu Zhao, and Lindsay A. Farrer

Background: We report a genome-wide association study (GWAS) of two populations, African-American and European-American (AA, EA) for opioid dependence (OD) in three sets of subjects, to identify pathways, genes, and alleles important in OD risk.

Methods: The design employed three phases (on the basis of separate sample collections). Phase 1 included our discovery GWAS dataset consisting of 5697 subjects (58% AA) diagnosed with opioid and/or other substance dependence and control subjects. Subjects were genotyped with the Illumina OmniQuad microarray, yielding 890,000 single nucleotide polymorphisms (SNPs) suitable for analysis. Additional genotypes were imputed with the 1000 Genomes reference panel. Top-ranked findings were further evaluated in Phase 2 by incorporating information from the publicly available Study of Addiction: Genetics and Environment dataset, with GWAS data from 4063 subjects (32% AA). In Phase 3, the most significant SNPs from Phase 2 were genotyped in 2549 independent subjects (32% AA). Analyses were performed with case-control and ordinal trait designs.

Results: Most significant results emerged from the AA subgroup. Genome-wide-significant associations ($p < 5.0 \times 10^{-8}$) were observed with SNPs from multiple loci—*KCNG2**rs62103177 was most significant after combining results from datasets in every phase of the study. The most compelling results were obtained with genes involved in potassium signaling pathways (e.g., *KCNC1* and *KCNG2*). Pathway analysis also implicated genes involved in calcium signaling and long-term potentiation.

Conclusions: This is the first study to identify risk variants for OD with GWAS. Our results strongly implicate risk pathways and provide insights into novel therapeutic and prevention strategies and might biologically bridge OD and other non–substance dependence psychiatric traits where similar pathways have been implicated.

Key Words: Calcium signaling, complex traits, convergence, genome-wide association, opioid dependence, potassium

Opioid dependence (OD) is associated with serious medical, legal, and social problems and co-occurring psychiatric disorders. The cost of OD to society in 2002 was approximately $181 billion (1). Although genome-wide association study (GWAS) is a method of choice to identify risk genes for complex traits, none has been published for OD, despite an estimated heritability of >.60 (2). The strongest GWAS-derived noteworthy and replicable genome-wide significant (GWS) results so far for drug dependence (DD) traits identified a set of loci mapping to a chromosome 15 nicotinic receptor gene cluster [e.g., Thorgeirsson *et al.* (3)] for nicotine dependence (ND) and related traits; we also reported a GWS association of the *FAM53B* locus to cocaine dependence (CD) (4).

Few other DD GWAS studies have been attempted, and those that have been published are underpowered by modern standards, partly because they used dichotomous traits (i.e., DD diagnoses). Here, we used a relatively large sample and augmented power with an ordinal trait analytic design that allowed us to take into account both the presence or absence of OD and the severity of affection (including the ability to distinguish between subjects with zero and those with one or two symptoms). This increased power by enabling us to use more of the available phenotypic information than standard diagnosis-based analyses. Some of these strategies have been used previously in successful efforts to map ND risk alleles, most notably the use of large clinical samples (3). We further increased our analytic power by including, for some analyses, data from the Study of Addiction: Genetics and Environment (SAGE) sample (5,6), which includes SD trait information. This dataset is available to the scientific community through an application process and will henceforth be referred to as "public domain."

Our GWAS discovery sample consisted of 2379 European-Americans (EA), including 1383 subjects with OD; and 3318 African-Americans (AA), including 683 subjects with OD. A second phase sample of 4603 EAs and AAs from the SAGE study and a third phase sample including 2549 EAs and AAs ascertained in a manner identical to that of the discovery sample were used to replicate and extend our findings.

Thus, our study took place in three "phases" that differed with respect to samples and genotyping. Phase 1 designates our own GWAS sample. Phase 2 designates the addition of SNP data from

From the Department of Psychiatry (JG), Division of Human Genetics, Departments of Genetics and Neurobiology, Yale University School of Medicine; VA Connecticut Healthcare Center (JG), West Haven; Departments Biostatistics and Genetics (HZ), Yale School of Public Health, Yale University, New Haven, CT; University of Pennsylvania Perelman School of Medicine (HRK); Philadelphia VA Medical Center (HRK), Philadelphia, Pennsylvania; Department of Medicine (Biomedical Genetics) (RK, RS, LAF), Boston University School of Medicine; Departments of Neurology, Ophthalmology, Genetics and Genomics (LAF), Epidemiology and Biostatistics, Boston University Schools of Medicine and Public Health, Boston, Massachusetts; and the Department of Genetics (LA), Texas Biomedical Research Institute, San Antonio, Texas.

Address correspondence to Joel Gelernter, M.D., Yale University School of Medicine, Department of Psychiatry, Division of Human Genetics; and VA CT Healthcare Center and Departments of Genetics and Neurobiology, VA CT 116A2, 950 Campbell Avenue, West Haven, CT 06516; E-mail: joel.gelernter@yale.edu.

Received May 31, 2013; revised Jul 29, 2013; accepted Aug 27, 2013.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2013.08.034

Table 1. Demographic and Diagnostic Information and Subject Characteristics

	Phase 1 (SNFs)				Phase 1 (Unrelateds)				Phase 3				Site Total		
	AA		EA		AA		EA		AA		EA		AA	EA	Total
Recruiting Sites	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female			
Yale (APT Foundation)	199	257	141	108	453	370	485	290	223	198	474	477	1700	1975	3675
UConn	174	227	155	161	455	355	451	296	127	93	315	299	1431	1677	3108
MUSC	42	84	52	47	53	109	33	29	21	24	47	47	333	255	588
McLean Hospital	44	36	42	30	10	6	18	11	0	2	2	3	98	106	204
UPenn	9	11	0	0	288	136	20	10	51	64	43	39	559	112	671
									Phase 2						
SAGE (Phase 2)									643	668	1222	1530	1311	2752	4063
Total Phases 1+2+3													5432	6877	12,309

Subject Characteristics

	AA				EA			
Phase/OD Status	*n*	Male (%)	Mean Age	Mean Symp. Count	*n*	Male (%)	Mean Age	Mean Symp. Count
Phase 1/OD Cases	683	62	44.2	6.1	1383	60	37.2	6.5
Phase 1/OD Abusers	49	76	44.8	1.5	57	72	36.9	1.6
Phase 1/Control Subjects	2550	49	40.6	.04	854	55	39.6	.1
Phase 1/Exposed Control Subjects	639	61	42.2	.14	366	64	37.1	.21
Phase 2/OD Cases	105	61	39.9	6	193	60	35.6	5.9
Phase 2/OD Abusers	2	100	40.5	0	5	100	41	0
Phase 2/Control Subjects	1204	48	40	.03	2554	43	38.8	.03
Phase 2/Exposed Control Subjects	158	67	40.8	.2	383	69	35.9	.2
Phase 3/OD Cases	142	67	45.2	5.9	407	60	35.6	6.4
Phase 3/OD Abusers	10	90	44.8	2.2	22	55	36.9	1.1
Phase 3/Control Subjects	601	47	39.9	.02	1255	46	42.3	.02
Phase 3/Exposed Control Subjects	85	78	42.3	.13	137	61	36.7	.16

Recruiting sites: Yale University School of Medicine (APT Foundation), New Haven, CT; University of Connecticut Health Center (UConn), Farmington, CT; the University of Pennsylvania School of Medicine (UPenn), Philadelphia, PA; the Medical University of South Carolina (MUSC), Charleston, SC; and McLean Hospital (Harvard Medical School; Belmont, MA).

AA, African-Americans; EA, European-Americans; OD, opioid dependence; SAGE, Study of Addiction: Genetics and Environment; SNFs, small nuclear families; Symp, symptom.