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NKAIN1–SERINC2 is a functional, replicable and genome-wide significant risk gene region specific for alcohol dependence in subjects of European descent☆

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ABSTRACT

Objective: We aimed to identify novel, functional, replicable and genome-wide significant risk regions specific for alcohol dependence using genome-wide association studies (GWASs).
Methods: A discovery sample (1409 European-American cases with alcohol dependence and 1518 European-American controls) and a replication sample (6438 European-Australian family subjects with 1645 alcohol dependent probands) underwent association analysis. Nineteen other cohorts with 11 different neuropsychiatric disorders served as contrast groups. Additional eight samples underwent expression quantitative locus (eQTL) analysis.
Results: A genome-wide significant risk gene region (*NKAIN1–SERINC2*) was identified in a meta-analysis of the discovery and replication samples. This region was enriched with 74 risk SNPs (unimputed); half of them had significant *cis*-acting regulatory effects. The distributions of $-\log(p)$ values for the SNP-disease associations or SNP-expression associations in this region were consistent throughout eight independent samples. Furthermore, imputing across the *NKAIN1–SERINC2* region, we found that among all 795 SNPs in the discovery sample, 471 SNPs were nominally associated with alcohol dependence ($1.7 \times 10^{-7} \leq p \leq 0.047$); 53 survived region- and cohort-wide correction for multiple testing; 92 SNPs were replicated in the replication sample ($0.002 \leq p \leq 0.050$). This region was neither significantly associated with alcohol dependence in African-Americans, nor with other non-alcoholism diseases. Finally, transcript expression of genes in *NKAIN1–SERINC2* was significantly ($p < 3.4 \times 10^{-7}$) associated with expression of numerous genes in the neurotransmitter systems or metabolic pathways previously associated with alcohol dependence.
Conclusion: NKAIN1–SERINC2 may harbor a causal variant(s) for alcohol dependence. It may contribute to the disease risk by way of neurotransmitter systems or metabolic pathways.

1. Introduction

Several lines of evidence demonstrate a substantial genetic component in the risk to developing alcohol dependence. Siblings of alcoholic probands have a 3- to 8-fold increase in the risk of also developing alcohol dependence (Reich et al., 1998). The heritability of risk for alcohol dependence is estimated to be ~39% by studies of the adopted-away offspring of affected and unaffected parents (Cloninger et al., 1981) and as high as 60% by twin studies (Heath et al., 1997). These studies provided evidence that genetic factors constitute a significant cause of alcohol dependence.

Numerous risk loci have been reported for alcohol dependence by the candidate gene approach. Most of these risk genes implicated have been from (1) classical neurotransmitter signaling systems, including the dopaminergic (e.g., *MAOA*, *COMT*, and *NCAM1-TTC12-ANKK1-DRD2*; Bau et al., 2001; Hutchison et al., 2002; Dick and Foroud, 2003; Olsson et al., 2004; Dahmen et al.,

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2005; Kohnke et al., 2005; Gelernter et al., 2006; Stapleton et al., 2007; Huang et al., 2008; Yang et al., 2008), serotoninergic (e.g., *SLC6A4* and *HTR2B*), GABAergic (e.g., *GABRA2* and *GABRG1*), and cholinergic systems (e.g., *CHRM2* and *CHRNA5-CHRNA3-CHRNB4*); (2) non-classical neurotransmitter signaling systems (e.g., *CRHR1*); (3) the ethanol metabolic pathway (e.g., *ADH1B*, *ADH1C*, *ADH4* and *ALDH2*; Luo et al., 2006b; Uhl et al., 2008); and (4) the opioidergic signaling pathway (e.g., *OPRM1*, *OPRD1* and *OPRK1*; Gelernter and Kranzler, 2009; Ray et al., 2006; Zhang et al., 2006). In recent years, genome-wide association studies (GWASs; Treutlein et al., 2009; Bierut et al., 2010; Edenberg et al., 2010; Heath et al., 2011) also reported risk loci for alcohol dependence (summarized previously in Zuo et al., 2012). In particular, using the datasets of the SAGE (the Study of Addiction: Genetics and Environment), COGA (the Collaborative Study on the Genetics of Alcoholism) and OZ-ALC (the Australian twin-family study of alcohol use disorder) separately, several GWASs have detected some risk loci for alcohol dependence and alcohol consumption in subjects of European and African descents (Bierut et al., 2010; Edenberg et al., 2010; Chen et al., 2011; Heath et al., 2011; Wang et al., 2011) (summarized previously in Zuo et al., 2012). Different from these previous studies that reported the top-ranked risk SNPs for alcohol dependence, we reanalyzed the SAGE, COGA and OZ-ALC data, using some new analytic strategies with the goal of identifying replicable risk genes for alcohol dependence. Using the European-Americans as the discovery sample and the African-Americans as the validation sample, we identified *KIAA0040* as risk gene for alcohol dependence (Zuo et al., 2012). Using the African-Americans as the discovery sample and the European-Americans as the validation sample, we identified *PTP4A1-PHF3* as risk gene region for alcohol dependence (Zuo et al., 2011). In the present study, we used the European-Americans as the discovery sample and the European-Australians as the validation sample, with the goal of identifying novel (i.e., previously unimplicated) risk loci specific for alcohol dependence. Using these relatively homogenous samples in one association study might reduce the false negative rates due to sample heterogeneity, increasing the chance to detect novel risk loci.

In the present study, we combined SAGE and COGA datasets, hoping to increase the sample sizes and, in turn, the study's statistical power, thereby enhancing our ability to detect novel risk loci that were missed previously. Furthermore, we examined the specificity of these risk loci for alcohol dependence, by testing their associations with ten non-alcoholism neuropsychiatric disorders including attention deficit hyperactivity disorder (ADHD), autism, major depression, bipolar disorder, schizophrenia, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), early onset stroke, ischemic stroke, and Parkinson's disease. The data of these disorders were all of those with neuropsychiatric and neurological disorders available for our analysis from the dbGaP database. Both these non-alcoholism disorders and alcohol dependence have been related to dopaminergic, serotoninergic, cholinergic, GABAergic, glutamatergic, and/or adrenergic neurotransmission systems. Additionally, it has been reported that alcoholism has high rates of comorbidity with numerous psychiatric disorders including anxiety disorders, major depression, bipolar disorders, schizophrenia, PTSD, etc. (Regier et al., 1990; Kessler et al., 1996; Grant et al., 2004). Thus, it is important to test any risk gene (or genes) identified in the present study, especially the novel gene (or genes) related to these systems, in these non-alcoholism neuropsychiatric disorders, to see if they are specific for alcohol dependence. We note that not all neuropsychiatric and neurological disorders were exhaustively examined in the present study, which may be a limitation of this specificity test.

2. Materials and methods

2.1. Subjects

The discovery sample included 1409 European-American cases with alcohol dependence (38.3 ± 10.2 years) and 1518 European-American controls (38.4 ± 10.4 years) and the replication sample included 6438 European-Australian family subjects with 1645 alcohol dependent probands. A total of 39,903 subjects of European or African descent from 19 other cohorts with 11 different neuropsychiatric disorders served as contrast groups (Table S1[1]).

The discovery sample came from the European-American subjects in the merged SAGE and COGA datasets (Bierut et al., 2010; Edenberg et al., 2010; Zuo et al., 2012) (1477 subjects in COGA that overlapped with SAGE were excluded), and the European-Australian replication sample came from the OZ-ALC dataset (Heath et al., 2011). Affected subjects met lifetime DSM-IV criteria for alcohol dependence (American Psychiatric Association, 1994). The control subjects were defined as individuals who had been exposed to alcohol (and possibly to other drugs), but had never become addicted to alcohol or other illicit substances (lifetime diagnoses). Detailed demographic information for these samples is shown in Table S1[2] or available elsewhere (Bierut et al., 2010; Edenberg et al., 2010; Heath et al., 2011; Zuo et al., 2013). The discovery sample was genotyped on the Illumina Human 1 M (for SAGE and COGA) and the replication sample was genotyped on Illumina Human CNV370v1 with the same genotype clustering algorithms.

The diagnoses, dataset names, ethnicities, designs, sex and age structures, average ages and sample sizes of a total of 21 cohorts are shown in Table S1.[3] More detailed demographic information including dbGaP accession numbers and genotyping platforms for those non-alcoholism diseases is also available elsewhere (Zuo et al., 2013). These subjects were genotyped on ILLUMINA, AFFYMETRIX or PERLEGEN microarray beadchip platforms (Zuo et al., 2013). All subjects gave written informed consent to participating in protocols approved by the relevant institutional review boards (IRBs). All subjects were de-identified in this study that was approved by Yale IRB.

2.2. Association analysis

Whole genome data in the discovery sample and the region-wide imputed genotype data in other 20 cohorts were analyzed. Before association analysis was conducted, we stringently cleaned the phenotype data and then the genotype data within each ethnicity. Detailed procedures of data cleaning were described previously (Zuo et al., 2012). After cleaning, our subjects had high levels of ancestral homogeneity within each phenotype group (QQ plots were presented for discovery and replication samples previously (Zuo et al., 2011, 2012); $\lambda = 1.07$ and 1.01 in EA discovery sample and Australian replication sample, respectively). This selection process yielded 805,814 SNPs in the discovery sample (1409 cases and 1518 controls) and 300,839 SNPs in 6438 Australian replication samples. Cleaned SNP numbers in other datasets are shown in Table S2.[4]

2.2.1. Genome-wide association tests in the European-American discovery sample. The allele frequencies were compared between cases and controls in the European-American discovery sample using genome-wide logistic regression analysis as implemented in the program PLINK (Purcell et al., 2007). Diagnosis served as the dependent variable, alleles served as the independent variables, and ancestry proportions, study sites (COGA, COGEND and FSCD), sex, and age served as the covariates. The ancestry proportions for each individual were estimated by integrating the ancestry information content of 3172 independent ancestry-informative SNPs (Zuo et al., 2012) using the program STRUCTURE. The Manhattan plot and the QQ plot were shown previously (Zuo et al., 2011, 2012). The most significant gene region (i.e., Na^+/K^+ transporting ATPase interacting 1 gene (*NKAIN1*)–serine incorporator 2 gene (*SERINC2*) region in the present study) was tested in the replication sample and other non-alcoholism samples, to examine its replicability and specificity (see below).

2.2.2. Family-based association tests in the Australian family replication sample. We imputed the genotype data 1 Mb surrounding the most significant gene region that covers the entire *NKAIN1–SERINC2* region, to fill in the missing markers (see imputation below). Associations between all available markers within this 1 Mb region and alcohol dependence were tested in Australians using a family-based association test program (FBAT; Horvath et al., 2001), adjusting for covariates including sex, age and admixture rates (ancestry proportions; see above), and assuming an additive genetic

[1] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

[2] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

[3] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

[4] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

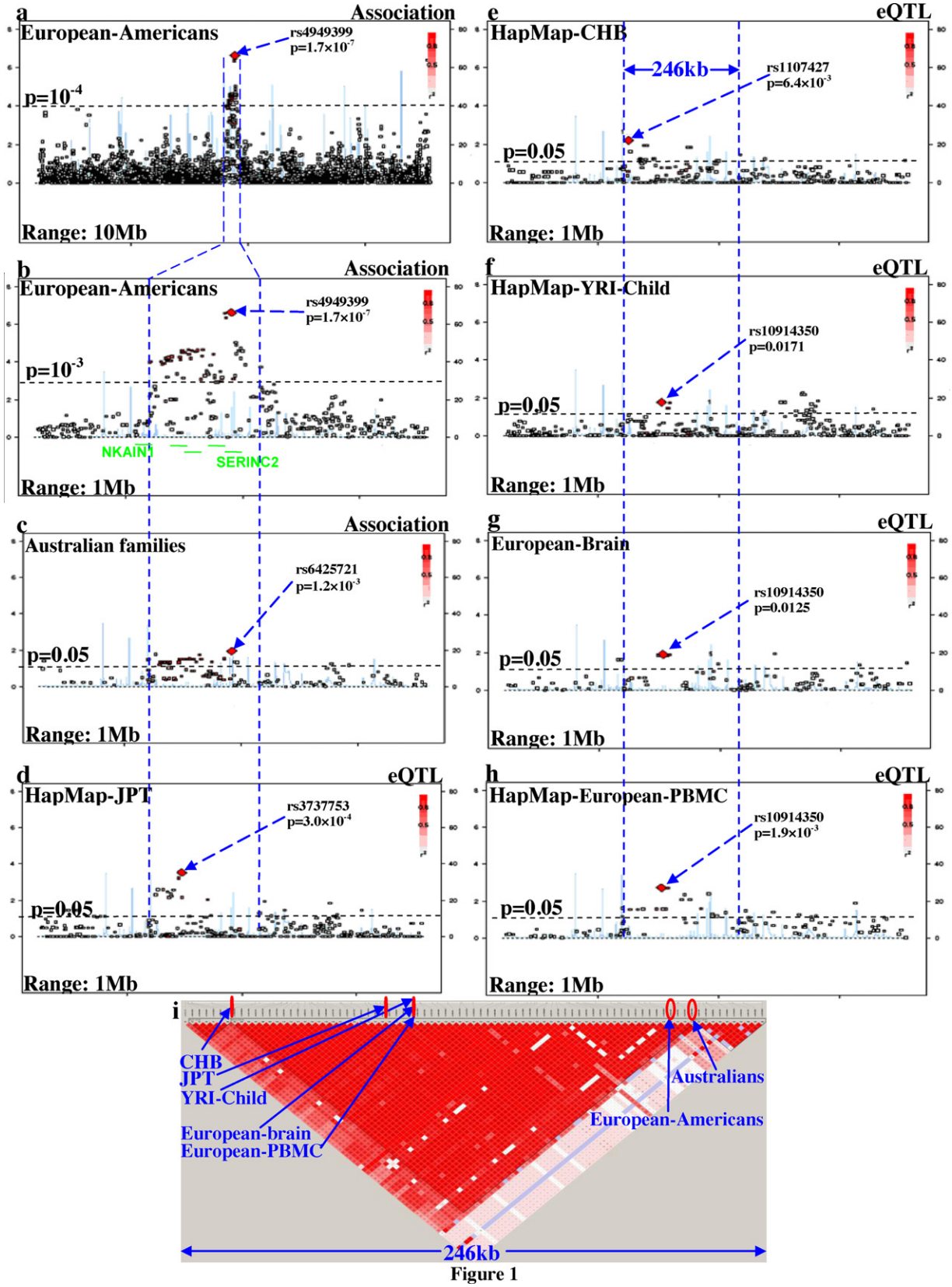

Fig. 1. Regional association and *cis*-eQTL plots around *NKAIN1–SERINC2* region. [Left *Y*-axis corresponds to −log(*p*) value; right *Y*-axis corresponds to recombination rates; quantitative color gradient corresponds to r^2; red squares represent peak SNPs. (A) regional association plot in European-American discovery samples for a 10 Mb region around the peak association SNP (rs4949399) in *NKAIN1–SERINC2*; (B and C) regional association plots in European-American discovery samples and Australians for a 1 Mb region around the peak association SNP (rs4949399) in *NKAIN1–SERINC2*; (D–H) regional eQTL plots in HapMap populations and European samples for a 1 Mb region around rs4949399; only the effects on local gene expression are illustrated (the effects on flanking gene expression are presented in Table S4); (I) LD map for *NKAIN1–SERINC2* region; red bars on the top represent the peak SNPs in this region in each population. All markers in this figure are non-imputed.]

model under the null hypothesis of no linkage and no association, biallelic mode, minimum number of informative families of 10 for each analysis and offset of zero (results same as those using the program DFAM implemented in PLINK; data not shown). The associations that were replicated across European-American discovery samples and Australians are shown in Table 2 and Fig. 1. This replication design helped to control for the false positive findings. Meta-analysis was performed to derive the combined p values between European-American discovery sample and Australians using the program METAL. An overall *z*-statistic and *p*-value for each SNP were calculated from a weighted sum of the individual statistics. Weights were proportional to the square-root of the number of individuals examined in each sample and selected such that the squared weights sum to 1.0. For these meta-analysis results, the genome-wide Bonferroni-corrected α was set at 5×10^{-8} (by one million markers) and the region- and cohort-wide corrected α was set at 1.7×10^{-5} (by 139 effective markers; see Table S2[5]).

2.2.3. Specificity of associations. To test the specificity of this most significant locus to alcohol dependence, the associations between this locus and 11 alcoholism or non-alcoholism disorders were tested in other 20 cohorts. These samples included case-control and family-based samples of European or African descent. We imputed this locus (Chr1: 31,425,179-31,732,987) using the same reference panel (i.e., 1000 genome project and HapMap 3 panel) across all disease groups with the program IMPUTE2 (Howie et al., 2009), reference CEU panel for the samples of European descent and YRI panel for the samples of African descent, respectively, to make the genetic marker sets highly consistent across different samples. This program uses a Markov Chain Monte Carlo (MCMC) algorithm to derive full posterior probabilities, not "best-guess", of genotypes of each SNP (burnin = 10,000, iteration = 10,000, k = 100 and Ne = 11,500). If the probability of one of the three genotypes of a SNP was over the threshold of 0.99, the genotypes of this SNP were expressed as a corresponding allele pair for the following association analysis; otherwise, they were treated as missing genotypes. For SNPs that were directly genotyped, we used the direct genotypes rather than the imputed data.

For unrelated case-control samples, we used the logistic regression analysis implemented in PLINK to test associations between genotypes and phenotypes, with ancestry proportions (Zuo et al., 2012), sex, age and alcohol drinking behavior (i.e., at least 12 alcoholic drinks in the past 12 months) as covariates; for family samples, we used FBAT to test associations. The cleaned marker numbers, the minor allele frequencies (MAFs) of the most significant risk SNPs and the minimal p values, are shown in Table S2.[6] The significance levels (α) were corrected for the number of effective markers [calculated by a Bonferroni-type program SNPSpD that takes LD structure into account (Li and Ji, 2005)] and the number of cohorts (i.e., 21). The number of risk SNPs that were nominally ($p < 0.05$) or significantly ($p < \alpha$) associated with phenotypes are shown in Table S2.[7] Finally, an overall π_0 value for associations with multiple comparisons was estimated from p values within each cohort using the R package QVALUE (Storey and Tibshirani, 2003). A π_0 value is the overall proportion of true null hypotheses, reflecting the overall behavior of the estimated *q*-values (false discovery rates). For example, in our European-American discovery sample, 795 SNPs were tested for associations to derive 795 *p* values. From these 795 *p* values, we estimated an overall π_0 value to be 0.037 (see Table S2[8]); that is, only 3.7% of these association tests were false positive. In other words, associations between the entire marker set across *NKAIN1–SERINC2* region and alcohol dependence in this phenotype group were highly likely (96.3%) to be true positive.

2.2.4. cis- and trans-acting genetic regulation of expression analysis. There is a substantial gap between SNP-alcohol dependence associations and understanding how these SNPs contribute to alcohol dependence. The functional effects of SNPs on alcohol dependence can be mediated through several mechanisms. Variation in gene expression is an important mechanism underlying susceptibility to alcohol dependence. The abundance of a gene transcript is directly modified by polymorphism in regulatory elements. Consequently, transcript abundance might be considered as a quantitative trait that can be mapped with considerable power, which has have been named expression QTLs (eQTLs). Using the same analytic strategies as previously published (Zuo et al., 2011, 2012), we performed *cis*-acting expression of quantitative locus (*cis*-eQTL) analysis on the risk SNPs in lymphoblastoid cell lines (Stranger et al., 2005), brain tissues and peripheral blood mononuclear cell (PBMC) samples (Heinzen et al., 2008). We also performed transcriptome-wide *trans*-eQTL analysis on the risk SNPs ($\alpha = 2.4 \times 10^{-8}$), genome-wide *trans*-eQTL analysis of *NKAIN1–SERINC2* transcript expression ($\alpha = 4.7 \times 10^{-8}$), and transcriptome-wide expression correlation analysis ($\alpha = 3.4 \times 10^{-7}$) in brain tissues and PBMC samples (Heinzen et al., 2008; Tables 3 and S3–S5,[9] and Fig. 1).

3. Results

In the European-American discovery sample, the three top-ranked genes were *SERINC2*, *KIAA0040*, and *IPO11*, which contained eight top-ranked SNPs ($p < 5 \times 10^{-7}$ as well as FDR < 0.05), including 5 SNPs in *SERINC2* ($1.7 \times 10^{-7} \leq p \leq 4.4 \times 10^{-7}$), 2 SNPs in *KIAA0040* ($2.8 \times 10^{-7} \leq p \leq 3.9 \times 10^{-7}$; Zuo et al., 2012), and 1 SNP in *IPO11* ($p = 2.8 \times 10^{-7}$; Zuo et al., 2012). *SERINC2* was the most significant risk gene for alcohol dependence and thus was the focus of the present study. *KIAA0040* and *IPO11* were reported previously (Zuo et al., 2012).

Testing all markers 10 Mb surrounding *SERINC2* in the European-American discovery sample, we found that all association signals with $p < 10^{-4}$ were concentrated in a narrow region around *SERINC2* (Fig. 1A), within which all association signals with $p < 10^{-3}$ were concentrated in a 246Kb *NKAIN1–SERINC2* region (Fig. 1B). This region contained five genes including *NKAIN1, SNRNP40, ZCCHC17, FABP3* and *SERINC2*; full names and biological functions of these genes and their associations with human diseases are summarized in Table S6.[10] Among all of the 97 unimputed SNPs in this region, 74 SNPs were nominally associated with alcohol dependence ($1.7 \times 10^{-7} \leq p \leq 0.026$) in the European-American discovery sample (data not shown). These 74 risk markers were located in three linkage disequilibrium (LD) blocks that were defined by Gabriel et al. (2002) using the program Haploview (Fig. 1I). Imputing across the *NKAIN1–SERINC2* region, we found that among all 795 SNPs, 471 SNPs were nominally associated with alcohol dependence ($1.7 \times 10^{-7} \leq p \leq 0.047$), 53 of which survived region-wide and cohort-wide correction for multiple testing (corrected $\alpha = 1.7 \times 10^{-5}$). The risk alleles of all 471 markers were minor alleles ($f < 0.5$). The top-ranked 27 SNPs ($p < 10^{-6}$; Table 1) were all located in *SERINC2* in LD Block 2. Ninety-two SNPs of these 471 risk markers were also nominally associated with alcohol dependence in the Australian replication sample ($0.001 \leq p \leq 0.049$; Table 2), all of which had a same effect direction (OR > 1) and similar effect sizes to those in the European-American discovery sample, and most of which were located in LD Block 2.

Meta-analysis showed that a total of 523 SNPs were nominally associated with alcohol dependence ($3.1 \times 10^{-8} \leq p \leq 0.049$), including 3 SNPs that survived genome-wide Bonferroni correction ($p = 3.1 \times 10^{-8}$ for rs4478858, $p = 3.8 \times 10^{-8}$ for rs2275436 and $p = 4.7 \times 10^{-8}$ for rs1039630; $\alpha = 5 \times 10^{-8}$), 34 SNPs with $p < 10^{-6}$, 158 SNPs with $p < 10^{-5}$, 244 SNPs with $p < 10^{-4}$, and 183 SNPs that survived region-wide and cohort-wide correction ($\alpha = 1.7 \times 10^{-5}$). Furthermore, all 92 replicable SNPs were nominally associated with alcohol dependence in meta-analysis ($3.1 \times 10^{-8} \leq p \leq 0.020$), including the aforementioned 3 SNPs that survived genome-wide Bonferroni correction ($\alpha = 5 \times 10^{-8}$), 7 SNPs with $p < 10^{-6}$, 69 SNPs with $p < 10^{-5}$, 83 SNPs with $p < 10^{-4}$, and 77 SNPs that survived region-wide and cohort-wide correction ($\alpha = 1.7 \times 10^{-5}$).

This risk region was specific to alcohol dependence in subjects of European descent. It was neither significantly associated with any other non-alcoholism diseases nor alcohol dependence in African-Americans. π_0 was less than 0.05 only in the European-American discovery sample ($\pi_0 = 0.037$, Table S2[11]).

[5] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

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[7] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

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[10] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

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Table 1
Top-ranked risk SNPs for alcohol dependence in *NKAIN1–SERINC2* ($p < 10^{-6}$).

SNP	Position	Gene	Location	Risk allele	European-Americans		Meta-analysis	
					OR	*p*-Values	*Z*	*p*
rs1039630◄	31654059	5′ regulatory region	940 bp to 5′ of SERINC2	C	1.32	$\mathbf{2.6 \times 10^{-7}}$	5.46	$\mathbf{4.7 \times 10^{-8}}$
rs2839939◄	31654224	5′ regulatory region	775 bp to 5′ of SERINC2	C	1.31	3.9×10^{-7}	5.23	1.7×10^{-7}
rs6701521◄	31655882	*SERINC2*	Intron 2	A	1.31	5.1×10^{-7}	5.21	1.9×10^{-7}
rs12033669◄	31656352	*SERINC2*	Intron 2	A	1.30	8.0×10^{-7}	5.09	3.6×10^{-7}
rs4478858◄	31656512	*SERINC2*	Intron 2	C	1.31	$\mathbf{4.4 \times 10^{-7}}$	5.53	$\mathbf{3.1 \times 10^{-8}}$
rs1934290◄	31656767	*SERINC2*	Intron 2	C	1.31	6.9×10^{-7}	5.15	2.6×10^{-7}
rs7545902◄	31657523	*SERINC2*	Intron 2	A	1.30	7.8×10^{-7}	5.09	3.5×10^{-7}
rs10914375◄	31658446	*SERINC2*	Intron 2	G	1.31	6.9×10^{-7}	5.15	2.6×10^{-7}
rs10914376◄	31659151	*SERINC2*	Intron 3	C	1.30	7.8×10^{-7}	5.09	3.5×10^{-7}
rs10914377◄	31659420	*SERINC2*	Exon 4	A	1.30	8.0×10^{-7}	5.09	3.6×10^{-7}
rs12729234	31660092	*SERINC2*	Exon 4	T	1.30	8.0×10^{-7}	5.09	3.6×10^{-7}
rs10737357	31661423	*SERINC2*	Intron 5	C	1.30	8.0×10^{-7}	5.09	3.6×10^{-7}
rs3862890	31661807	*SERINC2*	Intron 5	A	1.30	8.0×10^{-7}	5.09	3.6×10^{-7}
rs10798850	31664735	*SERINC2*	Intron 5	T	1.30	8.2×10^{-7}	5.09	3.7×10^{-7}
rs4949398	31665434	*SERINC2*	Intron 5	G	1.33	1.7×10^{-7}	5.23	1.7×10^{-7}
rs4949399	31665498	*SERINC2*	Intron 5	G	1.33	1.7×10^{-7}	5.23	1.7×10^{-7}
rs12132936	31668518	*SERINC2*	Intron 5	G	1.31	7.3×10^{-7}	5.11	3.3×10^{-7}
rs1320584	31669650	*SERINC2*	Intron 6	A	1.31	3.7×10^{-7}	5.23	1.7×10^{-7}
rs1317251	31669880	*SERINC2*	Intron 6	T	1.30	8.3×10^{-7}	5.07	4.1×10^{-7}
rs4949209	31670550	*SERINC2*	Intron 7	T	1.31	3.2×10^{-7}	5.26	1.5×10^{-7}
rs4949400	31670719	*SERINC2*	Intron 7	T	1.32	2.3×10^{-7}	5.33	9.7×10^{-8}
rs4949401	31670749	*SERINC2*	Intron 7	T	1.31	3.4×10^{-7}	5.25	1.5×10^{-7}
rs4949402•	31670821	*SERINC2*	Exon 8	C	1.32	2.6×10^{-7}	5.39	7.3×10^{-8}
rs4949403	31670866	*SERINC2*	Intron 8	T	1.32	2.4×10^{-7}	5.30	1.2×10^{-7}
rs2275436	31671050	*SERINC2*	Intron 8	G	1.32	$\mathbf{2.4 \times 10^{-7}}$	5.50	$\mathbf{3.8 \times 10^{-8}}$
rs2275435	31671123	*SERINC2*	Intron 8	G	1.31	3.0×10^{-7}	5.27	1.4×10^{-7}
rs2297885	31674046	*SERINC2*	Intron 10	C	1.30	8.5×10^{-7}	5.18	2.2×10^{-7}

All of these SNPs are located in LD Block 2. ◄, these markers are located in transcription factor binding sites; •, this marker is located in an exonic splicing silencer or enhancer. The non-imputed SNPs are underlined.

Thirty-two SNPs of the 74 unimputed risk markers, including 16 replicable SNPs, had functional *cis*-eQTL signals in at least one of the three HapMap populations (CHB, JPT and YRI-Parent) and the two European samples ($0.003 \le p \le 0.044$ for gene-level expression and $1.4 \times 10^{-4} \le p \le 0.006$ for exon-level expression; Table 3). There were other non-risk markers in this region that had significant *cis*-eQTL signals too ($3.0 \times 10^{-4} \le p \le 0.049$ for gene-level expression and $4.2 \times 10^{-7} \le p \le 0.001$ for exon-level expression; Table S3[12]). Additionally, many of the risk markers also had nominal *trans*-acting regulatory effects, which, however, were not significant after Bonferroni correction (data not shown).

Within a 1 Mb range, the most significant risk SNPs in the European-American discovery sample, the Australians and their combined sample (meta-analysis), and the most significant functional SNPs in the JPT, CHB and European PBMC tissue (with effects on local gene expression), and in the European brain tissue (with effects on flanking gene expression) were all located in the 246Kb *NKAIN1–SERINC2* region (Fig. 1, Tables 1 and S2[13]). The peak SNPs within this 246 kb region in the YRI-Child, European brain tissue and PBMC tissue were the same one (i.e., rs10914350). All peak SNPs in this region across all populations were in high LD; most were in the LD Block 2. Additionally, the distributions of $-\log(p)$ values for associations in all markers throughout the entire *NKAIN1–SERINC2* region were consistent across the European-American discovery sample, the Australians, the HapMap CEU-Child, CEU-Parent, JPT, YRI-Child, European Brain sample and European PBMC sample ($0.264 \le r \le 0.704$; $2.9 \times 10^{-5} \le p \le 0.031$; Table 4).

The transcript expression of *NKAIN1* was significantly regulated by *TMEM178* ($p = 4.8 \times 10^{-9}$), *CRY1* ($p = 1.1 \times 10^{-8}$), *PSKH2* ($p = 3.1 \times 10^{-8}$) and *KDM4B* ($p = 4.7 \times 10^{-8}$) and the transcript expression of *SERINC2* was significantly regulated by *SORBS2* ($p = 3.1 \times 10^{-8}$; Table S5[14]). Additionally, expression of *NKAIN1–SERINC2* transcripts was significantly correlated with expression of numerous alcoholism-related genes (Chang et al., 2002; Luo et al., 2003; Oroszi et al., 2005; Luo et al., 2006a; Ehringer et al., 2007; Zuo et al., 2007; Wetherill et al., 2008; Lind et al., 2009; Edenberg et al., 2010; McHugh et al., 2010), although some associations between these genes and alcoholism have not yet been well replicated. These genes were mostly from the dopaminergic, serotoninergic, cholinergic, GABAergic, glutamatergic, histaminergic, endocannabinoid, metabolic, neuropeptide and opoidergic systems ($\alpha = 3.4 \times 10^{-7}$; Table S4[15]).

Ten top-ranked markers located within *SERINC2* or within the 5′ regulatory region of *SERINC2* (Table 1), and nine replicable risk markers (Table 2) were located in transcription factor binding sites. One marker (rs4949402 in *SERINC2*) was located in an exonic splicing silencer or enhancer. Additionally, *SERINC2* harbors numerous structural variants (see NCBI dbSNPs) that are close to alcoholism-risk SNPs identified in the present study. For example, the genome-wide significant risk SNP rs1039630 was 37 bp far from a frameshift SNP rs138193519 (indel -/C) and 344–347 bp far from a cluster of frameshift SNPs including rs143900963, rs3050485, rs68042003, rs56365042 and rs68140019.

4. Discussion

In the present study, after merging 480 COGA subjects into the SAGE sample, most results were consistent with a previous study that used the SAGE sample alone (Bierut et al., 2010). The

[12] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.
[13] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.
[14] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.
[15] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

Table 2
Replicable risk SNPs for alcohol dependence between discovery and replication samples.

SNP	Position	Gene	Location	Risk allele	European-Americans		Australians		Meta-analysis	
					OR	*p*	OR	*p*	*Z*	*p*
rs10798835	31481642	*NKAIN1*	Intron 1	T	1.24	1.5×10^{-4}	1.32	0.021	4.31	1.6×10^{-5}
rs10798836	31481801	*NKAIN1*	Intron 1	C	1.23	2.6×10^{-4}	1.36	0.011	4.28	1.9×10^{-5}
rs6425720	31482142	*NKAIN1*	Intron 1	A	1.23	1.3×10^{-4}	1.36	0.010	4.46	8.3×10^{-6}
rs1007012	31482655	*NKAIN1*	Intron 1	G	1.23	1.6×10^{-4}	1.45	0.002	4.56	5.1×10^{-6}
rs1007013	31482857	*NKAIN1*	Intron 1	C	1.23	1.4×10^{-4}	1.36	0.010	4.45	8.7×10^{-6}
rs12752761	31483475	*NKAIN1*	Intron 1	T	1.23	1.4×10^{-4}	1.36	0.010	4.45	8.7×10^{-6}
rs6425723	31487791	*intergenic*	2.5 kb to 5′ of *NKAIN1*	T	1.23	4.4×10^{-4}	1.42	0.010	4.11	4.0×10^{-5}
rs11577679	31498772	*intergenic*	6.2 kb to 3′ of *SNRNP40*	T	1.23	2.0×10^{-4}	1.31	0.013	4.32	1.6×10^{-5}
rs7524117	31511003	*SNRNP40*	Intron 8	A	1.24	1.5×10^{-4}	1.31	0.018	4.35	1.3×10^{-5}
rs4949372	31511444	*SNRNP40*	Intron 8	G	1.24	8.5×10^{-5}	1.25	0.050	4.35	1.4×10^{-5}
rs3766289	31532715	*SNRNP40*	Intron 4	G	1.24	9.4×10^{-5}	1.26	0.044	4.35	1.4×10^{-5}
rs7521463	31534523	*SNRNP40*	Intron 3	T	1.24	9.4×10^{-5}	1.26	0.044	4.35	1.4×10^{-5}
rs2062951	31535568	*SNRNP40*	Intron 3	T	1.24	9.4×10^{-5}	1.26	0.044	4.35	1.4×10^{-5}
rs4949380	31536747	*SNRNP40*	Intron 3	A	1.25	5.9×10^{-5}	1.26	0.042	4.46	8.3×10^{-6}
rs1532861[a]	31539078	*SNRNP40*	Intron 1	T	1.24	9.4×10^{-5}	1.26	0.044	4.35	1.4×10^{-5}
rs6679393[a]	31543531	*ZCCHC17*	Intron 1	T	1.26	2.7×10^{-5}	1.27	0.037	4.66	3.2×10^{-6}
rs1566963[a]	31544793	*ZCCHC17*	Intron 1	G	1.25	4.3×10^{-5}	1.29	0.026	4.59	4.4×10^{-6}
rs1566961[a]	31545037	*ZCCHC17*	Intron 1	C	1.25	3.1×10^{-5}	1.27	0.037	4.62	3.8×10^{-6}
rs4622114[a]	31545158	*ZCCHC17*	Intron 1	A	1.25	4.4×10^{-5}	1.29	0.026	4.59	4.4×10^{-6}
rs11576603[a]	31546618	*ZCCHC17*	Intron 1	C	1.25	3.8×10^{-5}	1.25	0.047	4.54	5.6×10^{-6}
rs4949382	31549318	*ZCCHC17*	Intron 1	A	1.25	3.3×10^{-5}	1.25	0.047	4.57	4.8×10^{-6}
rs10798842	31551235	*ZCCHC17*	Intron 1	A	1.25	3.1×10^{-5}	1.26	0.040	4.61	4.0×10^{-6}
rs6425735	31556532	*ZCCHC17*	Intron 2	T	1.25	6.2×10^{-5}	1.44	0.003	4.72	2.4×10^{-6}
rs6425736	31556963	*ZCCHC17*	Intron 2	T	1.25	3.1×10^{-5}	1.25	0.047	4.59	4.5×10^{-6}
rs6679365	31560453	*ZCCHC17*	Intron 2	G	1.26	2.3×10^{-5}	1.25	0.047	4.65	3.3×10^{-6}
rs7523438	31560900	*ZCCHC17*	Intron 2	A	1.25	4.0×10^{-5}	1.27	0.036	4.57	4.9×10^{-6}
rs7523509	31560916	*ZCCHC17*	Intron 2	A	1.25	3.1×10^{-5}	1.25	0.047	4.59	4.5×10^{-6}
rs7556340	31561040	*ZCCHC17*	Intron 2	T	1.25	3.2×10^{-5}	1.25	0.047	4.58	4.7×10^{-6}
rs6425737	31562875	*ZCCHC17*	Intron 2	T	1.25	3.1×10^{-5}	1.25	0.047	4.59	4.5×10^{-6}
rs3806255	31564165	*ZCCHC17*	Intron 2	A	1.26	3.0×10^{-5}	1.25	0.047	4.59	4.4×10^{-6}
rs4949384	31564728	*ZCCHC17*	Intron 3	A	1.26	3.0×10^{-5}	1.25	0.047	4.59	4.4×10^{-6}
rs11590120	31564911	*ZCCHC17*	Intron 3	A	1.26	3.0×10^{-5}	1.25	0.047	4.59	4.4×10^{-6}
rs1566964	31565381	*ZCCHC17*	Intron 3	C	1.26	3.0×10^{-5}	1.25	0.047	4.59	4.4×10^{-6}
rs4949385	31565843	*ZCCHC17*	Intron 3	C	1.24	6.5×10^{-5}	1.28	0.034	4.47	8.0×10^{-6}
rs11576621	31568047	*ZCCHC17*	Intron 3	A	1.26	2.5×10^{-5}	1.25	0.047	4.64	3.6×10^{-6}
rs10914355	31569572	*ZCCHC17*	Intron 3	G	1.26	2.9×10^{-5}	1.25	0.047	4.60	4.2×10^{-6}
rs10798843	31569681	*ZCCHC17*	Intron 3	A	1.26	2.9×10^{-5}	1.25	0.047	4.60	4.2×10^{-6}
rs7526095	31570168	*ZCCHC17*	Intron 3	C	1.26	3.0×10^{-5}	1.25	0.047	4.59	4.4×10^{-6}
rs7547897	31570180	*ZCCHC17*	Intron 3	G	1.26	3.0×10^{-5}	1.25	0.047	4.59	4.4×10^{-6}
rs6681286	31571971	*ZCCHC17*	Intron 3	G	1.26	3.0×10^{-5}	1.25	0.047	4.59	4.4×10^{-6}
rs4949205	31573166	*ZCCHC17*	Intron 3	A	1.25	3.1×10^{-5}	1.25	0.047	4.59	4.5×10^{-6}
rs6676354	31573333	*ZCCHC17*	Intron 3	A	1.25	3.1×10^{-5}	1.25	0.047	4.59	4.5×10^{-6}
rs6687819	31573699	*ZCCHC17*	Intron 3	G	1.25	3.1×10^{-5}	1.25	0.047	4.59	4.5×10^{-6}
rs6685728	31575137	*ZCCHC17*	Intron 3	T	1.25	3.5×10^{-5}	1.25	0.047	4.56	5.2×10^{-6}
rs4949387	31576027	*ZCCHC17*	Intron 3	T	1.22	6.8×10^{-4}	1.40	0.020	3.90	9.8×10^{-5}
rs7543348	31577392	*ZCCHC17*	Intron 3	A	1.25	3.6×10^{-5}	1.32	0.019	4.67	3.1×10^{-6}
rs6655946	31578315	*ZCCHC17*	Intron 3	G	1.25	3.5×10^{-5}	1.32	0.019	4.67	3.0×10^{-6}
rs12126725	31578440	*ZCCHC17*	Intron 3	G	1.26	2.8×10^{-5}	1.25	0.047	4.61	4.1×10^{-6}
rs68008576	31578492	*ZCCHC17*	Intron 3	T	1.25	3.3×10^{-5}	1.25	0.047	4.57	4.9×10^{-6}
rs6676682	31582403	*ZCCHC17*	Intron 3	C	1.26	2.9×10^{-5}	1.25	0.047	4.60	4.2×10^{-6}
rs11582342	31583268	*ZCCHC17*	Intron 4	A	1.26	2.4×10^{-5}	1.25	0.047	4.64	3.4×10^{-6}
rs34960310	31588784	*ZCCHC17*	Intron 5	A	1.26	2.6×10^{-5}	1.25	0.047	4.62	3.8×10^{-6}
rs10914360	31590063	*ZCCHC17*	Intron 5	G	1.26	2.5×10^{-5}	1.25	0.047	4.63	3.7×10^{-6}
rs10914361	31590129	*ZCCHC17*	Intron 5	T	1.26	2.5×10^{-5}	1.25	0.047	4.63	3.7×10^{-6}
rs4531329	31590397	*ZCCHC17*	Intron 5	A	1.26	2.5×10^{-5}	1.25	0.047	4.63	3.7×10^{-6}
rs6679360	31590523	*ZCCHC17*	Intron 5	G	1.26	2.5×10^{-5}	1.25	0.047	4.63	3.7×10^{-6}
rs10798845	31595223	*ZCCHC17*	Intron 7	G	1.25	4.9×10^{-5}	1.25	0.047	4.49	7.2×10^{-6}
rs12734617	31596033	*ZCCHC17*	Intron 7	G	1.26	2.8×10^{-5}	1.27	0.037	4.64	3.5×10^{-6}
rs12134484	31596328	*ZCCHC17*	Intron 7	G	1.26	2.8×10^{-5}	1.27	0.037	4.64	3.5×10^{-6}
rs4949389	31596610	*ZCCHC17*	Intron 7	G	1.26	2.3×10^{-5}	1.27	0.037	4.69	2.8×10^{-6}
rs2889730	31599746	*ZCCHC17*	Intron 7	C	1.26	2.5×10^{-5}	1.27	0.037	4.67	3.0×10^{-6}
rs12408524	31608440	*ZCCHC17*	Intron 7	A	1.25	3.3×10^{-5}	1.27	0.037	4.61	4.0×10^{-6}
rs2271072	31613013	*FABP3*	Intron 2	C	1.25	3.3×10^{-5}	1.27	0.037	4.61	4.0×10^{-6}
rs6670067	31615534	*FABP3*	Intron 1	C	1.26	2.8×10^{-5}	1.27	0.037	4.65	3.4×10^{-6}
rs10914368[a]	31620524	*Intergenic*	2 kb to 5′ of *FABP3*	A	1.25	3.1×10^{-5}	1.27	0.037	4.62	3.8×10^{-6}
rs4949393	31624462	*Intergenic*	5.9 kb to 5′ of *FABP3*	G	1.25	4.0×10^{-5}	1.26	0.046	4.53	5.9×10^{-6}
rs10914370	31627542	*Intergenic*	9 kb to 5′ of *FABP3*	T	1.25	5.3×10^{-5}	1.28	0.029	4.53	5.8×10^{-6}
rs4494186	31630434	*Intergenic*	11.9 kb to 5′ of *FABP3*	A	1.24	6.2×10^{-5}	1.27	0.037	4.47	7.7×10^{-6}
rs12563669	31630654	*Intergenic*	12.1 kb to 5′ of *FABP3*	A	1.24	6.2×10^{-5}	1.27	0.037	4.47	7.7×10^{-6}
rs12404861	31633858	*Intergenic*	15.3 kb to 5′ of *FABP3*	G	1.24	7.6×10^{-5}	1.27	0.037	4.43	9.6×10^{-6}
rs12129941	31644545	*Intergenic*	10.5 kb to 5′ of *SERINC2*	C	1.25	5.8×10^{-5}	1.25	0.049	4.45	8.8×10^{-6}
rs1039630[a]	**31654059**	*Intergenic*	940bp to 5′ of *SERINC2*	**C**	**1.32**	$\mathbf{2.6 \times 10^{-7}}$	**1.25**	**0.049**	**5.46**	$\mathbf{4.7 \times 10^{-8}}$
rs4478858[a]	**31656512**	*SERINC2*	Intron 2	**G**	**1.31**	$\mathbf{4.4 \times 10^{-7}}$	**1.26**	**0.021**	**5.53**	$\mathbf{3.1 \times 10^{-8}}$
rs12039509	31667998	*SERINC2*	Intron 5	A	1.23	5.6×10^{-4}	1.67	0.011	3.91	9.3×10^{-5}

Table 2 (*Continued*)

SNP	Position	Gene	Location	Risk allele	European-Americans		Australians		Meta-analysis	
					OR	*p*	OR	*p*	*Z*	*p*
rs2275436	**31671050**	*SERINC2*	Intron 8	**C**	**1.32**	**2.4×10^{-7}**	**1.34**	**0.043**	**5.50**	**3.8×10^{-8}**
rs2297884	31674239	*SERINC2*	Intron 10	G	1.22	4.7×10^{-4}	1.24	0.046	3.95	7.9×10^{-5}
rs10047085	31675005	*SERINC2*	Intron 12	G	1.20	7.9×10^{-4}	1.28	0.011	3.88	1.1×10^{-4}
rs6690908	31682676	*Intergenic*	2.6 kb to 3′ of *SERINC2*	T	1.31	7.8×10^{-6}	1.10	0.026	4.90	9.8×10^{-7}
rs6675883	31682789	*Intergenic*	2.7 kb to 3′ of *SERINC2*	C	1.34	2.0×10^{-6}	1.08	0.037	5.14	2.8×10^{-7}
rs6695167	31684674	*Intergenic*	4.6 kb to 3′ of *SERINC2*	A	1.32	4.5×10^{-6}	1.09	0.019	5.05	4.5×10^{-7}
rs6660687	31688988	*Intergenic*	8.9 kb to 3′ of *SERINC2*	A	1.28	3.1×10^{-5}	1.06	0.047	4.56	5.2×10^{-6}
rs6696700	31688998	*Intergenic*	8.9 kb to 3′ of *SERINC2*	C	1.28	3.1×10^{-5}	1.06	0.047	4.56	5.2×10^{-6}
rs6425751	31689945	*Intergenic*	9.8 kb to 3′ of *SERINC2*	T	1.32	4.0×10^{-6}	1.08	0.037	5.01	5.6×10^{-7}

The last 6 SNPs are located in LD Block 3; all other SNPs are located in Block 2. The non-imputed SNPs are underlined.

[a] Transcription factor binding sites.

Table 3
p-Values for risk expression quantitative trait loci (*cis*-eQTLs) in *NKAIN1–SERINC2* region.

	SNP[a]	Target	HapMap population (mRNA)			Europeans (Brain)		Europeans (PBMC)	
		Gene	CHB	JPT	YRI_parent	Transcript[*]	Exon-level[**]	Transcript[*]	Exon-level[**]
Block 1	rs6672056	*NKAIN1*	–	–	–	–	0.002	–	–
	rs7520468	*NKAIN1*	–	–	–	–	3.7×10^{-4}	0.025	0.003
	rs7544003	*NKAIN1*	–	–	–	–	1.4×10^{-4}	0.024	0.003
	rs2050815	*NKAIN1*	–	–	–	–	2.8×10^{-4}	–	–
	rs12087046	*SNRNP40*	–	0.005	–	–	–	–	–
	rs1107427	*SNRNP40*	0.006	–	–	–	–	–	–
	rs2377728	*SNRNP40*	0.024	0.003	–	–	–	0.029	–
Block 2	rs10798838	*SNRNP40*	–	0.003	–	–	–	–	–
	rs12121165	*SNRNP40*	–	–	–	0.012	0.001	0.028	0.002
	rs10753245	*SNRNP40*	–	0.003	–	–	–	–	–
	rs7519766	*SNRNP40*	–	0.007	–	–	–	–	–
	rs4949372	*SNRNP40*	–	–	–	0.012	0.001	0.026	0.002
	rs4949373	*SNRNP40*	–	0.006	–	–	–	–	–
	rs10914344	*SNRNP40*	–	0.007	–	–	–	–	–
	rs1566961	*ZCCHC17*	–	–	–	0.014	0.001	0.026	0.002
	rs7523438	*ZCCHC17*	–	–	–	0.014	0.001	0.026	0.002
	rs11576621	*ZCCHC17*	–	–	–	0.014	0.001	0.026	0.002
	rs4949391	*ZCCHC17*	–	–	–	–	3.1×10^{-4}	0.008	0.003
	rs6663779	*ZCCHC17*	–	–	–	–	2.0×10^{-4}	0.008	0.003
	rs7532845	*FABP3*	–	–	–	0.005	0.001	–	0.001
	rs11578034	*FABP3*	–	–	–	–	0.003	0.004	0.006
	rs951545	*FABP3*	–	–	–	–	0.002	–	–
	rs12562824	*FABP3*	–	–	–	–	3.1×10^{-4}	0.008	0.003
	rs1995585	*FABP3*	–	–	–	–	3.1×10^{-4}	0.008	0.003
	rs6659255	*SERINC2*	–	–	–	–	0.001	–	–
	rs4478858	*SERINC2*	–	–	–	0.024	0.002	0.026	0.005
	rs10047085	*SERINC2*	–	–	0.036	–	–	0.004	0.001
Block 3	rs7417775	*SERINC2*	–	–	0.024	–	–	–	–
	rs6688664	*SERINC2*	–	–	–	0.006	0.001	0.050	–
	rs6702129	*SERINC2*	–	–	–	0.019	–	–	–
	rs7548805	*SERINC2*	–	–	0.044	–	–	–	–
	rs6425751	*SERINC2*	–	–	0.044	–	–	–	–
	rs4261154	*SERINC2*	–	–	–	0.004	0.001	–	–

"–", $p > \alpha$.

[a] These SNPs are not imputed.

[*] The *p* values for transcript-level expression of local genes.

[**] The minimal *p* values for exon-level *cis*-acting regulatory effects on local gene expression (corrected for the number of exons: 6 exons of NKAIN1, 10 exons of SNRNP40, 8 exons of ZCCHC17, 4 exons of FABP3 and 10 exons of SERINC2).

Table 4
Correlation of distributions of −log(*p*) values for associations and *cis*-acting effects in *NKAIN1–SERINC2* region between different populations.

	Populations	Pearson correlation coefficients (*r*)							
		EA	Australian	CEU-Child	CEU-Parent	JPT	YRI-Child	Brain	PBMC
p-Values	EA		0.328	−0.300	−0.264	−0.399	–	–	–
	Australian	0.004		−0.363	–	–	–	–	0.402
	CEU-Child	0.031	0.008		–	–	−0.357	–	–
	CEU-Parent	0.030	–	–		–	–	–	–
	JPT	4.0×10^{-4}	–	–	–		0.378	–	–
	YRI-Child	–	–	0.005	–	0.002		–	0.597
	Brain	–	–	–	–	–	–		0.704
	PBMC	–	0.025	–	–	–	0.002	2.9×10^{-5}	

EA, European-American discovery samples. Brain, European Brain tissue sample; PBMC, European peripheral blood mononuclear cell (PBMC) samples. "–", $p > 0.05$.

top-ranked risk SNPs ($p < 10^{-5}$) in European-Americans, African-Americans, and the combined samples in that previous study (Bierut et al., 2010) were confirmed by our analysis (summarized in Zuo et al., 2012). In the present study, we found three top-ranked risk genes ($p < 5 \times 10^{-7}$ together with FDR < 0.05) for alcohol dependence (*SERINC2*, *KIAA0040* and *IPO11*) in European-American discovery sample. Two of these genes, i.e., *KIAA0040* and *IPO11*, were also among the top-ranked genes in the European-Americans reported previously (Bierut et al., 2010). However, the most significant gene (*SERINC2*) in the present study has not been reported before. It has been neglected because it was only suggestively significant in the separate SAGE and COGA datasets in previous GWASs. Here, by combining both datasets to increase statistical power (with overlapping excluded), stringently cleaning the phenotype and genotype data and controlling for various confounding effects, we discovered this risk gene locus. This locus was specific to alcohol dependence in subjects of European descent, but not associated with alcohol dependence in African-Americans and not with any other neuropsychiatric disorders examined.

A 246Kb region around *SERINC2* (i.e., *NKAIN1–SERINC2* region) was enriched with functional genetic SNPs with replicable associations with alcohol dependence across the European-American discovery sample, the Australians, four HapMap populations and two European samples. The association or functional peak SNPs in each of these populations were in high LD with each other or completely overlapped. In a word, the association and functional signals in this region were consistent across these eight samples.

These findings suggest that *NKAIN1–SERINC2* region might harbor a causal locus (or loci) for alcohol dependence and that the proteins encoded by *NKAIN1–SERINC2* might contribute directly to the vulnerability to alcohol dependence. First, *NKAIN1–SERINC2* region was the only association peak within a 10 Mb region in the European-American discovery sample (threshold $p = 10^{-4}$). It is, thus, highly likely that the putative causal locus for alcohol dependence is located within *NKAIN1–SERINC2*. Second, half of the unimputed risk SNPs in this region had significant *cis*-acting regulatory effects on *NKAIN1–SERINC2* mRNA expression, increasing the possibility that *NKAIN1–SERINC2* per se plays a direct functional role in the disorder. Third, the distributions of $-\log(p)$ values for either SNP-disease associations or for SNP-expression associations were consistent across at least 8 populations, suggesting that *NKAIN1–SERINC2* may contribute to the risk for alcohol dependence, and that the regulatory pathway via which these SNPs cause alcohol dependence may possibly be related to the *NKAIN1–SERINC2* proteins per se.

It is worth noting that the causal variant within the *NKAIN1–SERINC2* region may not be identical to the risk markers implicated in the current study, and therefore, may need to be identified by sequencing. First, none of the risk SNPs presented here are non-synonymous. Rather, they appear to have implications for risk and function by virtue of their being in LD with a putative causal variant and/or due to their location in regulatory regions (e.g., transcription factor binding sites, exonic splicing silencer or enhancer, or microRNA binding sites) that may in turn regulate transcription and translation of the causal variant. Second, the markers employed by GWAS are common, rather than rare, variants. Numerous studies have shown that many gene-disease associations are not due to a single common variant, but rather due to a constellation of more rare, regionally concentrated, disease-causing variants (Dickson et al., 2010). Thus, the signals of association credited to our common SNPs may be "synthetic associations" resulting from the contributions of multiple rare SNPs within the *NKAIN1–SERINC2* region. This issue can only be resolved by sequencing this region. Third, all five genes within *NKAIN1–SERINC2* were found to have significant association and functional signals in the present study (Tables 1 and 2); all have been associated with neuropsychiatric or neurological disorders before, including *NKAIN1* with Alzheimer disease (Li et al., 2008), *SNRNP40, ZCCHC17* and *FABP3* with narcolepsy (Miyagawa et al., 2008), *FABP3* with acute ethanol response (Kerns et al., 2005), and *SERINC2* with bipolar disorder (The Wellcome Trust Case Control Consortium, 2007) (summarized in Table S6[16]); and all have biological functions that might be related to human diseases (Inuzuka et al., 2005), as summarized in Table S6.[17] Thus, although *SERINC2* appears to be the most likely candidate, sequencing of this region is needed to identify the causal variant of alcohol dependence.

SERINC2 encodes serine incorporator 2 (Serinc2) that belongs to a cell membrane carrier protein family of Serinc1–5. High concentration of Serinc2 is seen in neurons of the hippocampus and cerebral cortex (Grossman et al., 2000). Serinc2 co-localizes with lipid biosynthetic enzymes (i.e., serine palmitoyltransferase) in endoplasmic reticulum membranes and interacts with an intracellular serine-synthesizing protein complex that included 3-phosphoglycerate dehydrogenase (Nedivi et al., 1993). It serves as an effector molecule that incorporates serine into membranes and facilitates the synthesis of two serine-derived membrane lipids, phosphatidylserine and sphingolipids (long-chain amino alcohols; Inuzuka et al., 2005). Phosphatidylserine is a phospholipid component of cell membranes, with the highest concentrations in the brain. At least 25 intervention studies suggested that consumption of phosphatidylserine supplement may reduce the risk of dementia and cognitive dysfunction in the elderly (Schreiber et al., 2000; Jorissen et al., 2001). Phosphatidylserine supplement has been approved by the U.S. Food and Drug Administration to treat memory deficit disorders such as Alzheimer's disease and other forms of dementia, to support healthy cognitive function during aging, and to remediate cognitive deficits as a result of heavy drinking and cigarette smoking. Furthermore, sphingolipids have the highest concentration in the brain too (Inuzuka et al., 2005). They associate with cholesterol molecules into lipid rafts that play a functional role in neural plasticity, signaling and axonal guidance (Nguyen et al., 1994; Tsui-Pierchala et al., 2002; Guirland et al., 2004). Alcohol consumption can increase sphingosine levels in the rat brains (Dasgupta et al., 2007). Activity of sphingolipid metabolism enzyme, i.e., acid sphingomyelinase (ASM), was also increased in alcohol-dependent patients (Reichel et al., 2011). The function of Serinc2 altered by the alleles of functional *SERINC2* variants may be implicated in the synthesis of phosphatidylserine and sphingolipids and might thus be relevant for the occurrence of alcohol dependence. Alternatively, Serinc2 might play a role in alcohol dependence by virtue of the glutaminergic pathway. This pathway is widely known to play important roles in alcohol intoxication and withdrawal (Krystal et al., 2003). Within the hippocampus, Serinc2 expression is increased following seizures induced by kainite, a glutamate agonist (Inuzuka et al., 2005). A drug that blocks kainite glutamate receptor function appears to decrease drinking (Johnson et al., 2007). This evidence supports the glutaminergic pathway hypothesis underlying the connection between Serinc2 and alcohol dependence.

NKAIN1–SERINC2 might influence alcohol dependence by interacting with other genes too. For instance, in the present study, we found that the transcript expression of *NKAIN1* was regulated by *TMEM178*, *CRY1*, *PSKH2* and *KDM4B*, and the transcript expression of *SERINC2* was regulated by *SORBS2*. Although the role of these regulatory genes in alcohol dependence is unknown, some of them were implicated in other mental illnesses (Miyagawa

[16] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

[17] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

et al., 2008), as summarized in Table S6.[18] Additionally, expression of *NKAIN1–SERINC2* transcripts was significantly correlated with expression of numerous alcoholism-related genes, including those in the dopaminergic, serotoninergic, cholinergic, GABAergic, glutamatergic, histaminergic, endocannabinoid, metabolic, neuropeptide and opoid systems (Chang et al., 2002; Oroszi et al., 2005; Ehringer et al., 2007; Zuo et al., 2007; Lind et al., 2009; Edenberg et al., 2010; McHugh et al., 2010). *NKAIN1–SERINC2* is 2.5 Mb from *OPRD1* and expression of their transcripts was significantly correlated (Table S4[19]) [A marker located between *NKAIN1–SERINC2* and *OPRD1*, i.e., rs1009080 at *PTPRU*, has been associated with schizophrenia ($p = 2.5 \times 10^{-6}$) by a recent GWAS (Ripke et al., 2011)]. These findings suggest that *NKAIN1–SERINC2* may also be implicated in alcohol dependence via the neurotransmitter systems or metabolic pathways, although the actual relationships between *NKAIN1–SERINC2* and those neurotransmitter systems and signaling pathways can only be delineated with analyses beyond simple pairwise correlations.

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Contributors

LZ and XL designed the study, analyzed the data, and drafted the manuscript. KW, XYZ, JHK, HZ, CSRL, and FZ provided critical comments, assisted in draft preparation and revision, and interpretation of the results. KW and FZ assisted in statistical analysis. All authors contributed to and have approved the final manuscript.

Conflict of interest

XL has read the journal's policy and have the following conflicts. Dr. Krystal has been a paid consultant for Aisling Capital, LLC, AstraZeneca Pharmaceuticals, Brintnall & Nicolini Inc., Easton Associates, Gilead Sciences Inc., GlaxoSmithKline, Janssen Pharmaceuticals, Lundbeck Research USA, Medivation Inc., Merz Pharmaceuticals, MK Medical Communications, F. Hoffmann-La Roche Ltd., SK Holdings Co. Ltd., Sunovion Pharmaceuticals Inc., Takeda Industries and Teva Pharmaceutical Industries, Ltd. He serves as a member of Scientific Advisory Boards for Abbott Laboratories, Bristol-Myers Squibb, Eisai Inc., Eli Lilly and Co., Forest Laboratories Inc., Lohocla Research Corporation, Mnemosyne Pharmaceuticals Inc., Naurex Inc., Pfizer Pharmaceuticals and Shire Pharmaceuticals. He is the Editor for Biological Psychiatry, a member of Board of Directors of Coalition for Translational Research in Alcohol and Substance Use Disorders, and the President Elect for American College of Neuropsychopharmacology. He also gets support from Tetragenex Pharmaceuticals. Other authors have no conflict of interest.

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Appendix A. Supplementary data

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[18] Supplementary material can be found by accessing the online version of this paper. Please see Appendix A for more information.

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