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Genome-wide significant association signals in *IPO11-HTR1A* region specific for alcohol and nicotine co-dependence

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Abstract

Background—Alcohol and nicotine co-dependence can be considered as a more severe subtype of alcohol dependence. A portion of its risk may be attributable to genetic factors.

Methods—We searched for significant risk genomic regions specific for this disorder using a genome-wide association study (GWAS). A total of 8,847 subjects underwent gene-disease association analysis, including (i) a discovery cohort of 818 European-American cases with alcohol and nicotine co-dependence and 1,396 European-American controls, (ii) a replication cohort of 5,704 Australian family subjects with 907 affected offspring, and (iii) a replication cohort of 449 African-American cases and 480 African-American controls. Additionally, a total of 38,714 subjects of European or African descent in 18 independent cohorts with 10 other non-alcoholism neuropsychiatric disorders were analyzed as contrast. Furthermore, 90 unrelated HapMap CEU individuals, 93 European brain tissue samples and 80 European peripheral blood mononuclear cell (PBMC) samples underwent *cis*-acting expression quantitative locus (*cis*-eQTL) analysis.

Results—We identified a significant risk region for alcohol and nicotine co-dependence between *IPO11* and *HTR1A* on chromosome 5q that was reported to be suggestively associated with alcohol dependence previously. In the European-American discovery cohort, 381 SNPs in this

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region were nominally associated with alcohol and nicotine co-dependence ($p < 0.05$); 57 associations of them survived region- and cohort-wide correction ($\alpha = 3.6 \times 10^{-6}$); and the top SNP (rs7445832) was significantly associated with alcohol and nicotine co-dependence at the genome-wide significance level ($p = 6.2 \times 10^{-9}$). Furthermore, associations for 34 and 11 SNPs were replicated in the Australian and African-American replication cohorts, respectively. Among these replicable associations, 4 reached genome-wide significance level in the meta-analysis of European-Americans and European-Australians: rs7445832 ($p = 9.6 \times 10^{-10}$), rs13361996 ($p = 8.2 \times 10^{-9}$), rs62380518 ($p = 2.3 \times 10^{-8}$) and rs7714850 ($p = 3.4 \times 10^{-8}$). *Cis*-eQTL analysis showed that many risk SNPs in this region had nominally significant *cis*-acting regulatory effects on *HTR1A* or *IPO11* mRNA expression. Finally, no markers were significantly associated with any other neuropsychiatric disorder examined.

Conclusions—We speculate that this *IPO11-HTR1A* region might harbor a causal variant for alcohol and nicotine co-dependence.

Keywords

GWAS; alcohol and nicotine co-dependence; *cis*-eQTL; *IPO11*; *HTR1A*

Introduction

Alcohol and nicotine are the most commonly misused substances in the United States. Nearly 20 million Americans are alcohol abusing or dependent, and almost 50 million Americans smoke cigarettes (Substance Abuse and Mental Health Services Administration (SAMHSA), 2010). Alcohol dependence and nicotine dependence frequently co-occur in the same individuals. Furthermore, nicotine dependent individuals are four times more likely than the general population to be alcohol dependent, and people who drink are three times more likely than the general population to smoke (Grant et al., 2004). Identical twins are twice as likely as fraternal twins to become alcohol and nicotine dependent if the other twin is dependent (Carmelli et al., 1993; Swan et al., 1997). Alcohol and nicotine may enhance motivation to use either drug by activating common brain targets that are responsible for their reinforcing effects. They may also exert synergistic effects on behaviors which may contribute to their concurrent use.

Alcohol and nicotine co-dependence may represent a more severe subtype of alcohol dependence. A large number of risk loci have been associated with both alcohol dependence and nicotine dependence by candidate gene approach, including many genes that are involved in the dopaminergic, serotonergic, GABAergic, glutamatergic, cholinergic, opioid and endocannabinoid systems. However, none of these genes have been confirmed by recent genome-wide association studies (GWASs) of alcohol dependence (Treutlein et al., 2009; Bierut et al., 2010; Edenberg et al., 2010; Heath et al., 2011; Johnson et al., 2011). Only two of them (*CHRNA6-CHRNA3* (Thorgeirsson et al., 2010) and *CHRNA5-CHRNA3-CHRNA4* (Liu et al., 2010)) were confirmed by meta-analysis GWASs of nicotine dependence. GWASs of alcohol dependence or alcohol consumption reported multiple other potential risk loci (Treutlein et al., 2009; Bierut et al., 2010; Edenberg et al., 2010; Heath et al., 2011; Schumann et al., 2011), so did most GWASs of nicotine dependence. To date, there has been only one GWAS (Lind et al., 2010) directly studying the phenotype of alcohol and nicotine co-dependence. That study identified three risk genes including *ARHGAP10*, *MARK1* and *DDX6*. However, those findings have not been replicated independently yet.

In the present study, we searched for significant risk genomic regions for alcohol and nicotine co-dependence using a GWAS. A European-American cohort was used as the discovery one, and a European-Australian cohort and an African-American cohort were used

as the replication ones. Additionally, we used three independent samples of European descent to detect expression quantitative trait locus (eQTL) signals in this risk genomic region, to see if the risk variants were functional. Finally, as contrast, we tested gene-disease associations in 18 additional independent cohorts with 10 other non-alcoholism neuropsychiatric disorders, to see whether the risk regions were specific for alcohol and nicotine co-dependence or not.

Materials and Methods

Subjects

A total of 8,847 subjects underwent gene-disease association analysis, including (i) a discovery cohort of 818 European-American cases with alcohol and nicotine co-dependence (476 males and 342 females; 38.3 ± 10.2 years) and 1,396 European-American controls (422 males and 974 females; 39.4 ± 10.4 years), (ii) a replication cohort of 5,704 European-Australian family subjects (1,856 families; 2,620 males and 3,084 females; 46.0 ± 10.0 years; 907 affected offspring with alcohol and nicotine co-dependence including 366 females), and (iii) a replication cohort of 449 African-American cases (260 males and 189 females; 40.3 ± 7.8 years) with alcohol and nicotine co-dependence and 480 African-American controls (170 males and 310 females; 39.6 ± 8.6 years). Additionally, a total of 38,714 subjects of European or African descent in 18 independent case-control or family-based cohorts with 10 other neuropsychiatric disorders were analyzed. These neuropsychiatric disorders included schizophrenia, autism, attention deficit hyperactivity disorder (ADHD), major depression, bipolar disorder, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), early onset stroke, ischemic stroke, and Parkinson's disease (Supplemental Tables S1a and S1b).

The European-American discovery cohort and the African-American replication cohort came from the dataset of the Study of Addiction - Genetics and Environment (SAGE) (dbGaP study accession phs000092.v1.p1) (Bierut et al., 2010), and the Australian replication cohort came from the dataset of the Australian twin-family study of alcohol use disorder (OZ-ALC) (dbGaP study accession phs000181.v1.p1) (Lind et al., 2010; Heath et al., 2011). (All subjects with alcohol and nicotine co-dependence in another dataset of the Collaborative Study on the Genetics of Alcoholism (COGA) (dbGaP: phs000125.v1.p1) (Edenberg et al., 2010) have been included in this SAGE dataset). These datasets were originally collected to study alcohol dependence alone. SAGE subjects were recruited from 8 different study sites in 7 states and the District of Columbia; the majority of subjects were recruited in Missouri (Bierut et al., 2010). All subjects were interviewed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994). Affected subjects met lifetime DSM-IV criteria for both alcohol and nicotine dependence (American Psychiatric Association, 1994). Affected subjects were excluded if they had schizophrenia or other psychotic illnesses. Controls were defined as individuals who had been exposed to alcohol and nicotine (and possibly to other drugs), but had never become dependent on these substances. Additionally, controls were also screened to exclude individuals with major axis I disorders, including schizophrenia, mood disorders, and anxiety disorders. The Australian subjects included twins and their parents, siblings, spouses, children and other family members. The index cases reported a history of alcohol dependence and nicotine dependence (DSM-IV). More detailed demographic information is available elsewhere (Edenberg et al., 2005; Bierut et al., 2010; Edenberg et al., 2010; Heath et al., 2011). The European-American discovery cohort and the African-American replication cohort were genotyped on the Illumina Human 1M beadchip and the Australian cohort was genotyped on the Illumina CNV370v1 beadchip.

Detailed demographic information, including sample sizes, ethnicity and diagnosis of the 18 cohorts with other neuropsychiatric disorders, is shown in Supplemental Table S1 or is available in dbGaP database (<http://www.ncbi.nlm.nih.gov/gap>). These subjects were genotyped on different Illumina or Affymetric microarray beadchip platforms. 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.

Imputation

After we identified a significant risk genomic region in the European-American discovery cohort, we imputed that entire region (1.5 Mb at Chr5: 61,708,573-63,257,546 from the transcript start site (TSS) of *IPO11* to the TSS of *HTR1A*) in all samples of 21 cohorts using the same strategy as previously (Zuo et al., 2012). Rare variants with minor allele frequencies (MAF) < 0.05 were excluded.

Data analysis

Before the association analysis, we strictly cleaned the phenotype and genotype data of all datasets (see the Supplemental Materials and Methods). We tested gene-disease associations in the European-American discovery cohort first, to identify the significant risk genomic regions at genome-wide significance level, and then we imputed and carefully examined this region across 21 cohorts.

- a. Genome-wide association tests in the European-American discovery cohort: The allele frequencies of all cleaned markers across the genome were compared between cases and controls using genome-wide logistic regression analysis implemented in the program PLINK (Purcell et al., 2007). Diagnosis served as the dependent variable, alleles served as the independent variables, and sex, age, the first 10 principal components and the most significant marker (i.e., rs7445832) served as the covariates. The principal component scores of our samples were derived from all autosomal SNPs across the genome using principal component analysis (PCA) implemented in the software package EIGENSTRAT (Price et al., 2006). Each individual received scores on each principal component. These principal components reflected the population structure of our samples. The first principal component (PC1) separated the self-identified European-American and African-American subjects very well, which was highly consistent with a previous report (Bierut et al., 2010). The second principal component (PC2) separated the self-identified Hispanic subjects from the non-Hispanic subjects. Other principal components also accounted for very small fractions of the total variance. The first 10 principal component scores accounted for >95% of variance. These PCs serving as covariates in the regression model can control for the population stratification and admixture effects on association analysis. The p-values derived from these association analyses are illustrated in Figure 1. Furthermore, similar association analysis was performed on the imputed data (see below). The top-ranked ($p < 10^{-5}$) risk markers are listed in Table 1. To mitigate false positive rates, genome-wide associations in the discovery cohort were corrected for multiple testing by Bonferroni correction ($\alpha = 5 \times 10^{-8}$).
- b. Association tests for the imputed genotype data in all samples in 21 cohorts: To analyze the associations between neuropsychiatric diseases and all imputed markers in the case-control samples, we used the logistic regression analysis described above. For the family samples, we tested associations using the program FBAT (Horvath et al., 2001). Association results were corrected for multiple comparisons by the effective number of SNPs within the *IPO11-HTR1A* region and the number

of cohorts examined (i.e., $n=21$). The effective marker numbers were calculated using the program SNPSpD (Li and Ji, 2005). In the present study, the effective genetic marker number was 669 in the *IPO11-HTR1A* region; thus, the region- and cohort-wide corrected α was set at 3.6×10^{-6} . The associations that were replicable between the discovery and replication cohorts are shown in Tables 2 and 3. Meta-analysis was performed on these replicable associations, to derive the combined p values using the program METAL.

- c. *Cis*-acting genetic regulation of expression analysis in the lymphoblastoid cell lines: To examine relationships between all available SNPs in the *IPO11-HTR1A* region and mRNA expression levels of local genes (i.e., *HTR1A* and *IPO11*) in the lymphoblastoid cell lines, we performed *cis*-acting expression quantitative locus (*cis*-eQTL) analysis. Expression array data of 14,925 transcripts (14,072 genes) in 90 unrelated HapMap CEU individuals were assessed (Stranger et al., 2005). Differences in the distribution of mRNA expression levels between SNP genotypes were compared using a Wilcoxon-type trend test. The risk SNPs that were associated with disease in the discovery cohort and had $p < 0.05$ in this *cis*-eQTL analysis are shown in the Supplemental Table S2.
- d. *Cis*-eQTL analysis on all available SNPs in the *IPO11-HTR1A* region in the brain tissue samples and the peripheral blood mononuclear cell (PBMC) samples: To examine whether the SNPs in the risk region influence the local gene expression changes, we also tested the associations between the genotypes and the expression levels of exons and transcripts of local genes (i.e., *HTR1A* and *IPO11*) in two additional European samples (Table S2). Expression array data in 93 autopsy-collected frontal cortical brain tissue samples with no defined neuropsychiatric condition and 80 PBMC samples collected from living healthy donors obtained from a study (Heinzen et al., 2008) at Duke University were evaluated. Each of these associations was analyzed using a linear regression model by correcting for age, sex, source of tissues, and principle component scores of ancestry. The expression array data have been confirmed by quantitative RT-PCR previously (Heinzen et al., 2008).

Results

We scanned the genome in the European-American discovery cohort and identified a significant risk region between *HTR1A* and *IPO11* on chromosome 5q at genome-wide significance level (Figures 1, 2 and 3), with the most significant SNP rs7445832 ($p=6.2 \times 10^{-9}$). We examined the 10Mb range surrounding this SNP, which covered the entire *IPO11-HTR1A* region (1.5Mb), in the discovery cohort, and found a total of 13 SNPs that had association signals for alcohol and nicotine co-dependence with $p < 10^{-4}$ (i.e., 6.2×10^{-9} p 9.1×10^{-5}). These SNPs were concentrated within a narrow region (0.5Mb) surrounding the most significant SNP between *IPO11* and *HTR1A* (Figure 2A).

We further examined the entire *IPO11-HTR1A* region (1.5Mb) in multiple populations, and detected many association and functional signals (Tables 1, 2, 3 and S2). In the European-American discovery cohort, among 2,726 SNPs including 261 originally-genotyped SNPs and 2,465 imputed SNPs, 381 SNPs were nominally associated with alcohol and nicotine co-dependence ($p < 0.05$) (Table 4); 57 SNPs were significantly associated with alcohol and nicotine co-dependence after region- and cohort-wide correction ($\alpha=3.6 \times 10^{-6}$). As mentioned, one of the SNP showed evidence for genome-wide significance (rs7445832; $p=6.2 \times 10^{-9}$). All risk alleles of these markers were minor alleles ($f < 0.5$). If conditional on the most significant SNP (i.e., rs7445832), all of the associations with other SNPs became less significant (all $p > 10^{-4}$; Figure 2C). In the Australian replication cohort, 100 SNPs were

nominally associated with alcohol and nicotine co-dependence ($0.001 \leq p \leq 0.049$; data not shown). Thirty-four associations in the discovery cohort ($6.2 \times 10^{-9} \leq p \leq 0.049$) were replicated in the Australian replication cohort ($0.001 \leq p \leq 0.049$) (Table 2 and Figure 2D), with the same directions of gene effects in both cohorts. Meta-analysis showed that all of these 34 replicable SNPs were associated with disease ($9.6 \times 10^{-10} \leq p \leq 0.021$; Table 2), including four genome-wide significant SNPs, i.e., rs7445832 ($p=9.6 \times 10^{-10}$), rs13361996 ($p=8.2 \times 10^{-9}$), rs62380518 ($p=2.3 \times 10^{-8}$) and rs7714850 ($p=3.4 \times 10^{-8}$). In the African-American replication cohort, 77 SNPs were nominally associated with alcohol and nicotine co-dependence ($0.002 \leq p \leq 0.049$; data not shown). Eleven risk SNPs in the discovery cohort ($8.1 \times 10^{-6} \leq p \leq 0.042$) were also risk SNPs in the African-American replication cohort ($0.032 \leq p \leq 0.049$) (Table 3). However, all of these 11 SNPs but one had opposite directions of gene effects between the discovery cohort and the African-American cohort. Meta-analysis showed that only this exceptional one SNP was associated with disease (rs10042968: OR=1.41, $p=8.1 \times 10^{-6}$ in European-Americans; OR=1.54, $p=0.041$ in African-Americans; OR=1.42, $p=8.0 \times 10^{-7}$ in meta-analysis; Table 3). Among these SNPs, rs690957 was a risk SNP across three cohorts ($p=0.008$, 0.004 and 0.047 in European-Americans, European-Australians and African-Americans, respectively). Rs690957 was also the most significant one in European-Australians (Table 4). In other 18 independent cohorts, 9-261 SNPs were nominally associated with diseases, but none of them survived region- and cohort-wide correction for multiple comparisons (Table 4).

Cis-eQTL analysis showed that, among the risk SNPs for alcohol and nicotine co-dependence, 30 SNPs had nominal *cis*-acting regulatory effects on expression of *HTR1A* or *IPO11* mRNA in the brain, PBMC or lymphoblastoid cell lines ($2.3 \times 10^{-13} \leq p \leq 0.05$); among all of the 65 SNPs within this region that were genotyped for eQTL analysis, 43 (66.2%) were risk markers for alcohol and nicotine co-dependence ($6.2 \times 10^{-9} \leq p \leq 0.048$) (Table S2). *Cis*-regulatory effects on *IPO11* expression were much stronger than those on *HTR1A* expression. All of the risk alleles for alcohol and nicotine co-dependence increased the expression of *HTR1A*. However, some of the risk alleles increased the expression of *IPO11* but the others decreased it.

Additionally, a total of 2,058 SNPs in *ARHGAP10*, *MARK1*, *DDX6*, *KIAA1409*, *CTBP2*, *GRM3*, *TBC1D2B*, *BACH2* and *CNTNA* that were significant risk genes for alcohol dependence, alcohol and nicotine co-dependence or nicotine dependence identified by Lind et al. (2010) were also tested in our samples. We listed all p values <0.01 in the Supplemental Table S3. We found that none of these markers were significantly associated with alcohol and nicotine co-dependence in our samples after Bonferroni correction.

Discussion

In the European-American population, we identified a genome-wide significant risk marker at the *IPO11-HTR1A* region specific for alcohol and nicotine co-dependence. The region surrounding this marker was enriched with many association signals and functional signals. We speculated that this region might harbor a causal variant for alcohol and nicotine co-dependence.

Several pieces of evidence supported our conclusion. First, within 10Mb range surrounding this genome-wide significant risk SNP, all association signals for alcohol and nicotine co-dependence with $p < 10^{-4}$ were concentrated within a narrow region surrounding this SNP. This region was completely located between *HTR1A* and *IPO11*. It is, thus, highly likely that the putative causal variant for alcohol and nicotine co-dependence was located within this region. Second, many risk SNPs in this region had significant *cis*-acting regulatory effects on mRNA expression both in the PBMC and in the brain, increasing the possibility

that the *IPO11-HTR1A* region plays a direct functional role in the disorder. Third, many associations discovered in European-Americans were replicated in European-Australians, and meta-analysis showed that four SNPs reached the genome-wide significance level. Some associations in European-Americans were also replicated in African-Americans. Finally, this region was specific for alcohol and nicotine co-dependence, not for any other non-alcoholism neuropsychiatric disorder examined. This region has been suggestively associated with alcohol dependence (75.6% nicotine dependence) in the same dataset before ($p=2.3\times 10^{-6}$ by Bierut et al. 2010; $p=2.8\times 10^{-7}$ by Zuo et al. 2011), but not genome-wide significant ($\alpha=5\times 10^{-8}$). The association was genome-wide significant only in the subgroup with alcohol and nicotine co-dependence ($p=6.2\times 10^{-9}$), which might suggest that this region is associated with a more severe subtype of alcohol dependence.

It is worth noting that the “causal” variants may not be identical to the “risk” markers, which is actually a common limitation of most association studies. There were other reasons for this inconsistency between the “causal” variants and the “risk” markers implicated in the current study. First, none of the risk SNPs presented here were non-synonymous. Rather, they appeared 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 the regulatory region that may in turn regulated transcription of the causal variant. Second, the SNPs employed by GWAS are common, but not 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. Thus, the signals of association credited to our common SNPs might be synthetic associations resulting from the contributions of multiple rare SNPs in the *IPO11-HTR1A* region, which needs to be identified by sequencing. Third, the associations in the European-American discovery cohort, the associations in the replication cohorts, and the functional signals in the eQTL analysis did not perfectly match, which was probably because these risk markers were not the causal variants *per se*, but rather in LD with a common putative causal variant. Fourth, current evidence, including the effect sizes and the significance strength of associations, was not sufficient to fine-map the putative causal variant to any one of the four genome-wide significant risk markers, although the most significant one (i.e., rs7445832) was most likely. Sequencing is warranted to detect the actual causal variant. Finally, after conditioning on rs7445832, all association signals for other markers were significantly reduced, which might suggest there exists only one putative causal locus in this region.

Our study is the first to detect the association between *HTR1A* and alcohol and nicotine co-dependence at a genome-wide significance level. *HTR1A* is located in 5q11.2-q13. It encodes the 5-HT_{1A} receptor that binds the endogenous neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). This receptor is a G protein-coupled receptor (GPCR) that is coupled to Gi/Go and mediates inhibitory neurotransmission. In the central nervous system, 5-HT_{1A} receptors exist in the cerebral cortex, hippocampus, septum, amygdala, and raphe nucleus in high densities. The activation of 5-HT_{1A} receptor has been shown to increase dopamine release in the medial prefrontal cortex, striatum, and hippocampus, to impair cognition, learning, and memory by inhibiting the release of glutamate and acetylcholine in various areas of the brain, or to increase impulsivity and inhibition of addictive behaviors. This activation is therefore likely to be related to the development of alcohol dependence or nicotine dependence. This is consistent with our findings that the risk alleles of the variants in the *IPO11-HTR1A* region for alcohol and nicotine co-dependence increased the expression of *HTR1A*. Additionally, a well-known and functional promoter SNP of *HTR1A*, C-1016G (rs6295), displays differential binding to repressors and affects transcription (Lemonde et al., 2003; Strobel et al., 2003). Its minor allele G has been reported to increase risk for alcohol dependence (Lee et al., 2009) or increase the relapse rate of alcohol

dependence (Wojnar et al., 2006), which is consistent with our conclusion that minor alleles in this region are risk alleles.

IPO11 is a flanking gene of *HTR1A*. It encodes the importin 11 that is a member of the karyopherin/importin-beta family of transport receptors. This receptor mediates nucleocytoplasmic transport of protein and RNA cargoes (Plafker and Macara, 2000). It has been reported that, in mice, *IPO11* expression was significantly regulated by ethanol in the prefrontal cortex (Kerns et al., 2005) and in the whole embryos (Zhou et al., 2011). In the present study, we found many alcohol and nicotine co-dependence-associated markers had significant *cis*-acting regulatory effects on *IPO11* mRNA expression both in the brain and the PBMC. Thus, *IPO11* might play important roles in alcohol and nicotine co-dependence too.

Among *CHRNA6-CHRNA3* and *CHRNA5-CHRNA3-CHRNA4* regions that have been widely associated to both alcohol and nicotine dependence before (Edenberg et al., 2004; Bierut et al., 2007; Saccone et al., 2007; Ray et al., 2009; Liu et al., 2010; Thorgeirsson et al., 2010), we only found that *CHRNA6*rs6474421 was modestly associated with alcohol and nicotine co-dependence in the European-American discovery cohort ($p=0.005$). Furthermore, this modest association was not replicated in the Australian and African-American replication cohorts, nor did the marker make the top-ranked gene list in the present study, consistent with previous results using the same SAGE dataset (Bierut et al., 2010; Wang et al., 2011). Additionally, the risk genes identified by Lind et al. (2010) were not significantly associated with alcohol and nicotine co-dependence in our samples after Bonferroni correction. Critical difference between the study of Lind et al. (2010) and ours might result from the sample heterogeneity. Finally, in the present study, only the region between the TSS of *IPO11* and the TSS of *HTR1A* was studied. The 5' regulatory regions, which boundaries are hard to defined, of both genes were excluded. Some information in these 5' regulatory regions might be lost.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Manhattan plot for the p-values in European-American discovery cohort
 [Y-axis: $-\log_{10} 0.05=1.3$; $-\log_{10} 10^{-5}=5$; $-\log_{10} (5 \times 10^{-8})=7.3$. X-axis: Chr1-22=Autosomes; X=ChrX; Y=ChrY; SNPs were ordered by physical distance within each chromosome/region]

Figure 2. Regional association plots

[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 cohort for a 10Mb region around the peak association SNP (rs7445832); (B) regional association plot in European-American discovery cohort for a 1Mb region around the peak association SNP (rs7445832) [without conditioning on rs7445832]; (C) regional association plot in European-American discovery cohort for a 1Mb region around the peak association SNP (rs7445832) [conditional on rs7445832]; (D) regional association plot in Australian replication cohort for a 1Mb region around the peak association SNP (rs7445832)]

Figure 3. QQ-plot for the p-values in European-American discovery cohort
 [X-axis: expected $-\log(P)$ values; Y-axis: observed $-\log(P)$ values; P-values correspond to associations between SNPs and alcohol and nicotine co-dependence; $\lambda=1.03$]

Table 1

P-values for top-ranked ($p < 10^{-5}$) risk SNPs in *IPO11-HTR1A* region in the discovery cohort

SNP	Position	risk allele	OR	p-values	SNP	Position	risk allele	OR	p-values
rs7445832	62622057	A	1.53	6.2×10^{-9}	rs6873148	62695568	A	1.52	1.0×10^{-6}
rs1494578	62622185	G	1.47	4.7×10^{-7}	rs6873152	62695579	A	1.52	1.0×10^{-6}
rs10471577	62623023	C	1.47	4.7×10^{-7}	rs10939966	62696069	C	1.37	6.8×10^{-6}
rs7702856	62630122	G	1.47	4.3×10^{-7}	rs10805382	62696495	C	1.38	5.1×10^{-6}
rs4700575	62631732	G	1.48	2.8×10^{-7}	rs10939967	62696691	C	1.52	1.0×10^{-6}
rs346425	62632000	T	1.36	3.2×10^{-6}	rs73119677	62698022	G	1.53	9.0×10^{-7}
rs73761305	62632087	T	1.43	4.1×10^{-6}	rs4590141	62698797	C	1.37	7.0×10^{-6}
rs1319474	62634947	G	1.46	7.4×10^{-7}	rs55701004	62699567	C	1.37	7.0×10^{-6}
rs1017776	62635216	G	1.47	6.7×10^{-7}	rs6860119	62700122	T	1.53	7.9×10^{-7}
rs1462460	62649397	T	1.53	8.3×10^{-6}	rs6860278	62700179	T	1.53	7.9×10^{-7}
rs7444332	62680629	C	1.47	5.9×10^{-7}	rs6860501	62700377	T	1.53	7.9×10^{-7}
rs4403132	62681092	T	1.39	4.8×10^{-6}	rs73119687	62701503	T	1.53	7.9×10^{-7}
rs7718679	62687582	C	1.54	6.7×10^{-7}	rs6893950	62704479	T	1.51	4.2×10^{-6}
rs73119652	62688162	T	1.54	5.1×10^{-7}	rs62380518	63020823	A	1.68	1.5×10^{-7}
rs57363006	62689707	G	1.50	2.8×10^{-6}	rs74829400	63027037	T	1.64	1.2×10^{-7}
rs57361220	62689740	T	1.50	2.8×10^{-6}	rs62380521	63028029	C	1.41	8.1×10^{-6}
rs59544801	62689880	T	1.52	1.1×10^{-6}	rs6887027	63046909	C	1.38	9.9×10^{-6}
rs7714594	62690438	A	1.53	6.1×10^{-7}	rs62380555	63070602	C	1.47	1.5×10^{-6}
rs7735086	62692417	A	1.52	1.0×10^{-6}	rs7714850	63072093	C	1.43	2.7×10^{-7}
rs7735004	62692558	G	1.38	6.2×10^{-6}	rs10042862	63072461	G	1.47	1.5×10^{-6}
rs7735451	62692702	A	1.52	1.0×10^{-6}	rs13354185	63073747	A	1.48	1.1×10^{-6}
rs4302532	62694427	C	1.55	4.4×10^{-7}	rs72766222	63074411	A	1.46	7.3×10^{-6}
rs4455508	62694538	A	1.54	5.7×10^{-7}	rs13361996	63075996	A	1.65	6.9×10^{-8}

All markers are in Hardy-Weinberg Equilibrium (HWE), common variants and ordered by chromosome position; all risk alleles are minor alleles. The markers underlined are non-imputed markers. The bold are the genome-wide significant markers with $p < 5 \times 10^{-8}$ in meta-analysis (see Table 2).

Table 2

P-values for replicable risk SNPs between European-American discovery and Australian replication cohorts

SNP	Position	Risk Allele	European-Americans		Australians		Meta-analysis	
			OR	p	OR	p	z	p
rs6861297	62212972	T	1.62	0.024	2.67	0.008	2.57	0.010
rs6884324	62213017	C	1.60	0.026	2.67	0.008	2.54	0.011
rs350306	62469018	T	1.38	0.012	1.56	0.005	3.18	0.001
rs1494622	62491395	T	1.27	0.021	1.17	0.009	2.92	0.004
rs1494623	62493473	C	1.20	0.037	1.18	0.004	2.82	0.005
rs691234	62496771	C	1.37	0.003	1.23	0.013	3.48	0.001
rs690957	62502634	C	1.38	0.008	1.67	0.001	3.20	0.001
rs681342	62507733	T	1.28	0.012	1.18	0.015	3.04	0.002
rs181156	62509099	A	1.39	0.002	1.23	0.007	3.66	2.6×10^{-4}
rs350309	62509354	T	1.38	0.002	1.23	0.007	3.66	2.6×10^{-4}
rs350311	62510417	C	1.39	0.003	1.17	0.019	3.43	0.001
rs350312	62511201	A	1.37	0.004	1.17	0.019	3.34	0.001
rs690816	62514088	G	1.38	0.003	1.17	0.020	3.43	0.001
rs114705639	62536627	A	1.35	0.022	1.46	0.019	2.71	0.007
rs17481124	62574713	G	1.23	0.048	1.21	0.048	2.30	0.021
rs72758793	62588647	A	1.37	0.018	1.80	0.046	2.62	0.009
rs55860379	62593868	A	1.36	0.010	1.78	0.034	2.85	0.004
rs72760718	62615268	G	1.35	0.016	1.80	0.027	2.67	0.008
rs7445832	62622057	A	1.53	6.2×10^{-9}	1.15	0.049	6.12	9.6×10^{-10}
rs9291778	62622713	A	1.51	4.0×10^{-5}	2.08	0.012	4.50	6.7×10^{-6}
rs56051136	62630434	A	1.41	0.003	3.33	0.001	3.51	4.6×10^{-4}
rs60685959	62630435	C	1.41	0.003	3.33	0.001	3.51	4.6×10^{-4}
rs73761304	62632086	T	1.37	4.7×10^{-4}	2.37	0.001	4.14	3.4×10^{-5}
rs6882265	62702209	T	1.28	0.001	1.58	0.026	3.82	1.3×10^{-4}
rs62380518	63020823	A	1.68	1.5×10^{-7}	1.33	0.028	5.59	2.3×10^{-8}
rs7714850	63072093	C	1.43	2.7×10^{-7}	1.27	0.042	5.52	3.4×10^{-8}
rs10042862	63072461	G	1.47	1.5×10^{-6}	1.24	0.039	5.18	2.2×10^{-7}

SNP	Position	Risk Allele	European-Americans		Australians		Meta-analysis	
			OR	p	OR	p	z	p
rs13354185	63073747	A	1.48	1.1×10^{-6}	1.33	0.034	5.21	1.9×10^{-7}
rs13361996	63075996	A	1.65	6.9×10^{-8}	1.78	0.002	5.76	8.2×10^{-9}
rs17180095	63104352	C	1.52	0.001	1.64	0.033	3.69	2.2×10^{-4}
rs10061598	63113748	T	1.14	0.048	1.59	0.033	2.51	0.012
rs989049	63114801	C	1.19	0.012	1.73	0.039	2.94	0.003
rs2202266	63117281	G	1.14	0.049	1.79	0.039	2.44	0.015
rs1478493	63119942	G	1.26	0.008	1.53	0.011	3.60	3.2×10^{-4}

The bold are the genome-wide significant markers with $p < 5 \times 10^{-8}$ in meta-analysis.

Table 3
P-values for replicable risk SNPs between European-American discovery and African-American replication cohorts

SNP	Position	Risk Allele	European-American		African-American		Meta-analysis	
			OR	P	OR	P	OR	P
rs10514949	62068672	C	1.15	0.042	0.78	0.049	1.05	0.376
rs58617906	62332704	A	1.24	0.020	0.61	0.032	1.12	0.185
rs690957	62502634	C	1.38	0.008	0.64	0.047	1.16	0.177
rs10042968	63069474	G	1.41	8.1×10^{-6}	1.54	0.041	1.42	8.0×10^{-7}
rs7700448	63124338	A	1.17	0.025	0.81	0.048	1.05	0.407
rs17795292	63125355	G	1.17	0.018	0.81	0.036	1.04	0.424
rs35393059	63125728	C	1.17	0.020	0.81	0.044	1.05	0.369
rs13159097	63125873	A	1.17	0.022	0.81	0.044	1.05	0.381
rs6876878	63127666	A	1.17	0.016	0.81	0.045	1.06	0.333
rs2365875	63128094	G	1.17	0.019	0.80	0.040	1.05	0.363
rs10939982	63128760	G	1.17	0.018	0.82	0.048	1.05	0.378

Table 4

Associations between IPO-11-HTR1A gene region and different neuropsychiatric disorders

Human Diseases	Dataset #	SNP # (total)	SNP # (p<0.05)	Minimal p value	Most sig. SNP	Gene	Minor allele frequency	
							Affected	Unaffected
AD+ND	1	2726	381	6.2×10^{-9}	rs7445832	<i>Sig. region</i>	0.289	0.211
AD+ND	2	2605	100	0.001	rs690957	<i>Intergenic</i>	0.084	0.076
AD+ND	3	2901	77	0.002	rs1353270	<i>Intergenic</i>	0.184	0.126
ADHD	4	2716	67	2.8×10^{-4}	rs1478498	<i>Intergenic</i>	0.229	0.236
Schizophrenia	5	2087	216	0.001	rs923963	<i>Intergenic</i>	0.372	0.326
Schizophrenia	6	1997	62	0.001	rs9283703	<i>Intergenic</i>	0.392	0.493
Schizophrenia	7	2013	126	0.001	rs7707596	<i>Sig. region</i>	0.059	0.094
Autism	8	2587	86	0.003	rs1319474	<i>Sig. region</i>	0.128	0.134
Major Depression	9	2743	143	0.005	rs16892399	<i>Intergenic</i>	0.255	0.226
Bipolar Disorder	10	2015	9	0.018	rs35509126	<i>Intergenic</i>	0.101	0.068
Bipolar Disorder	11	2015	164	0.001	rs347670	<i>Sig. region</i>	0.093	0.060
Bipolar Disorder	12	1948	95	0.002	rs260991	<i>Intergenic</i>	0.052	0.117
Alzheimer's Disease	13	2678	170	3.0×10^{-4}	rs4449492	<i>Intergenic</i>	0.473	0.457
Alzheimer's disease	14	1570	175	0.001	rs11160346	<i>Intergenic</i>	0.216	0.119
ALS	15	2492	143	0.002	rs1422301	<i>Sig. region</i>	0.197	0.281
Early Onset Stroke	16	2559	79	0.002	rs16891019	<i>Intergenic</i>	0.196	0.140
Early Onset Stroke	17	2817	144	0.001	rs56280615	<i>Sig. region</i>	0.054	0.118
Ischemic Stroke	18	2435	261	1.2×10^{-4}	rs13186191	<i>Intergenic</i>	0.319	0.184
Parkinson's Disease	19	2614	95	0.001	rs1851333	<i>Intergenic</i>	0.109	0.135
Parkinson's Disease	20	2572	149	3.1×10^{-4}	rs34606485	<i>Sig. region</i>	0.070	0.132
Parkinson's Disease	21	2683	95	0.004	rs6888308	<i>Intergenic</i>	0.324	0.278

AD + ND, alcohol and nicotine codependence

Only the most significant risk markers are listed. Dataset # refers to Table S1a. "Sig. Region", a 0.5Mb significant risk region for alcohol and nicotine co-dependence (see Figure 2A).