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Chen, C
Chen, C
et al.

Publication Date
2011

Addiction Biology

HUMAN GENETIC STUDY

doi:10.1111/j.1369-1600.2011.00348.x

Genetic variations in the dopaminergic system and alcohol use: a system-level analysis

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ABSTRACT

Alcohol use is highly heritable and has been associated with many gene variants, including those related to dopamine (DA). However, single gene association studies have shown inconsistent and small effects. Using a system-level approach, the current study aimed to estimate the overall effect of genetic variations in the DA system on alcohol use among male drinkers. One hundred seventy-six male college students who reported to have ever drunk alcohol were enrolled. Alcohol use was measured using the Alcohol Use Disorders Identification Test. Ninety-eight representative polymorphisms in all major DA neurotransmitter genes were genotyped. Using analysis of variance, we identified six single-nucleotide polymorphisms (SNP)s that made statistically significant contributions to alcohol use. Next, main effects and interactions of these SNPs were assessed using multiple regression. The final model accounted for approximately 20% of the variance for alcohol use. Finally, permutation analyses ascertained the probability of obtaining these findings by chance to be low, p ranging from 0.024 to 0.048. These results confirmed that DA-related gene variants made strong contributions to reported alcohol use and suggest that multiple regression can be a promising way to explore the genetic basis for multi-gene-determined human behaviors.

Keywords Alcohol use, dopamine genes, heritability, permutation, polygenetics, regression.

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INTRODUCTION

The history of alcohol use by humans can be traced back to at least the late Stone Age (McGovern 2003). Over the millennia, humans have used alcoholic beverages for multiple purposes, such as pleasure, rituals, medicine and nutrition. Alcohol, however, is a double-edged sword. It has nutritional and medicinal values, but it also has toxic and addictive effects. Biologically, after alcohol enters the human digestive system, ethanol (the most common type of alcohol found in alcoholic beverages) is metabolized into acetaldehyde. Acetaldehyde is toxic and has to be removed from the body by oxidation to acetate via a number of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes (Dick & Foroud 2003; Deitrich, Petersen & Vasiliou 2007). The speed at which these enzymes can convert ethanol tends to determine physiological effects of alcohol on individuals.

Many genes have been reported to show an effect on alcohol use. Liu *et al.* (2006) found that genes involved in myelination, ubiquitination, apoptosis, cell adhesion, neurogenesis and neural disease all showed altered expression levels in prefrontal cortex in alcoholics. A recent whole genome association study (Johnson *et al.* 2006) found 51 chromosome regions associated with alcohol dependence, although Bierut *et al.* (2010) found no significant regions with a denser single-nucleotide polymorphisms (SNP) array. All genome wide approaches, however, suffer from inadequate coverage of markers to assure strong linkage disequilibrium (LD) with all causative variants (Terwilliger & Hiekkalinna 2006; Saccone *et al.* 2009), now confirmed by direct DNA resequencing of numerous whole genomes (The 1000 Genomes Project Consortium 2010). This lack of adequate coverage in SNP arrays is one of many reasons why whole genome association studies often fail to detect causative genetic variants (Manolio *et al.* 2009). Reviews of previous research using a targeted gene approach (Dick & Foroud 2003; Köhnke 2008) have concentrated on the following alcoholism-related genes: (1) the

metabolizing enzymes *ADH* and *ALDH*; (2) genes involved in the dopamine (DA) system, such as DA receptors, especially *DRD2* and *DRD4*, transporters (*SLC6A3*, previously named *DAT1* or *DAT*), metabolizing enzymes (*DβH, COMT, MAO-A*); (3) GABA receptor genes (*GABAA* and *GABAB* receptors); (4) the glutamine system, especially N-methyl-daspartate receptor (*NMDAR*); (5) the serotonin system, especially the *5HTT*-LPR polymorphism; (6) the cholinergic system; (7) the opioid system; and (8) neuropeptide Y (*NPY*).

Of these, genes related to the DA system have attracted the greatest attention of researchers, perhaps because the DA-related 'reward system' plays a vital role in alcohol (and other drug) use (Muramatsu *et al.* 1996; Strat *et al.* 2008; Le Foll *et al.* 2009). The DA system has been proposed to be involved in the positive feelings associated with substance abuse, including alcohol consumption, and genes in this system are widely tested for their association with drug addiction (Le Foll *et al.* 2009). However, reported results have been mixed. For example, in terms of the DA receptor genes, there is convergent evidence of association between alcohol use and *DRD1* (Kim *et al.* 2007; Batel *et al.* 2008), while no association with alcoholism in *DRD3* (Wiesbeck *et al.* 2006; Kim *et al.* 2007) and *DRD5* (Kim *et al.* 2007), and inconsistent results in *DRD2* and *DRD4*. *DRD2* was found to be related to alcoholism in a number of studies (Huang *et al.* 2007; Hill *et al.* 2008; Namkoong *et al.* 2008; Smith *et al.* 2008; Esposito-Smythers *et al.* 2009), but not in several others (Wiesbeck *et al.* 2006; Haberstick *et al.* 2007). *DRD4* has been related to alcohol use in some studies (Mackillop *et al.* 2007; Grzywacz, Kucharska-Mazur & Samochowiec 2008; Namkoong *et al.* 2008; Ray *et al.* 2009, 2010; Vaughn *et al.* 2009), but not in Kim *et al.* (2007) and van den Wildenberg *et al.* (2007).

Studies on the DA transporter-, degradation- and synthesis-related genes have also been mixed. *SLC6A3* showed a protective role in alcohol abuse in two studies (Samochowiec *et al.* 2006; Lind, Eriksson & Wilhelmsen 2009), but not in a third (Preuss *et al.* 2007). In a study of treatment outcomes (Florez *et al.* 2008), *SLC6A3* was found to have no effect on treatment outcome. *COMT* was not associated with alcohol drinking nor smoking (Foroud *et al.* 2007), but had a main effect on alcohol dependence (Tiihonen *et al.* 1999) or ethanol consumption in mice (Tammimaki *et al.* 2008). Association of *MAOA* with alcohol use was also found in some studies (Contini *et al.* 2006; Nilsson *et al.* 2011) but not in others (Mokrovic *et al.* 2008). The *DβH* gene was reported to be associated with alcohol use in humans (La Grange *et al.* 1995; Köhnke *et al.* 2006) and rats (Casu *et al.* 2002), but negative results have also been reported (Köhnke *et al.* 2002; Freire, Hutz & Bau 2005). In their review paper, Le Foll *et al.* (2009) suggested that *DRD1* and *DRD2*, but not *DRD3* and *DRD5*, seem to influence alcohol dependence, but the results for *DRD4* have been mixed. In their meta-analytical review, Li & Burmeister (2009) concluded that *DRD2* and *SLC6A3* were related to alcohol dependence and cessation, but *DβH, DDC, DRD1, DRD3, DRD4, DRD5, COMT, MAOA*, and *TH* were related to alcohol dependence only in some studies.

All these divergent results make up a mixed picture about DA-related genes' contributions to alcohol use and abuse. One possible reason for these mixed findings is that these studies varied greatly in their samples and in the way they measured alcohol use. Furthermore, most studies focused on the contributions of single genes without knowing (or investigating) whether there were interactive effects of other genes or environmental factors. As Smith *et al.* (2008) suggested, it is not possible to solve this problem by focusing on single genes. Studies examining the combined contributions of multiple genes are needed. The combined effects may be additive or multiplicative. For example, Lee *et al.* (2009) found interactive effects between *ALDH2* and *MAOA*. Wang *et al.* (2007) also reported significant interactive effects of *DRD2* with *MAOA* on alcoholism, as well as on anxiety and depression. A focus on combined effects would also help explain more of the variance in alcohol use. Previous single-gene approaches typically accounted for less than 1% of the variance (Plomin & Davis 2009). Based on twins studies, the heritability index of alcohol use is estimated to be between 30 and 70% (Prescott *et al.* 1994; Kendler *et al.* 1997; McGue 1999; Goldman, Oroszi & Ducci 2005; Lin & Anthenelli 2005; Pagan *et al.* 2006; Fowler *et al.* 2007; Köhnke 2008; Poelen *et al.* 2008), which is far higher than what the molecular genetic data have been able to account for thus far.

In the current study, we selected 98 polymorphic loci [including 96 SNPs and 2 variable number tandem repeats (VNTR) polymorphisms] to cover a substantial portion (by LD) of the common variations within known genes of the DA system to estimate the additive and multiplicative contributions of these genes on alcohol use.

MATERIALS AND METHODS

Participants

Two hundred and seven healthy male Chinese college students, aged 20 (standard deviation = 1), were enrolled from Beijing Normal University, Beijing, China. All were in good health. A written consent form was obtained from each subject after a full explanation of the study procedure. Thirty-one of them reported having never drunk before and were excluded from further analyses. We excluded these individuals to minimize the effect

of known *ADH* and *ALDH* variants that cause alcohol toxicity at high frequency in Asian populations (Li *et al.* 2008, 2009). This left 176 remaining participants.

We studied healthy Chinese young adults for the following three reasons. First, using a population of similar geographic ancestry minimizes genetic heterogeneity present in many prior studies. Second, heritability of alcohol use is mostly estimated using young healthy twins (Han, McGue & Iacono 1999; Pagan *et al.* 2006; Fowler *et al.* 2007; Poelen *et al.* 2008), so we enrolled healthy college students in this study. Third, age has been a confounding factor in behavior genetic research, and we wanted to keep it a constant in this study. We also only selected male subjects for two reasons. First, Chinese males, for cultural reasons, usually drink more than females, and alcoholism is about 10 times more frequent in males than in females among the Han Chinese population (Hwu *et al.* 1988; Helzer *et al.* 1990). Second, the *MAOA* and *MAOB* genes are located on the X chromosome, so genotype distributions/dosage varies by sex and makes separate analysis for males and females imperative.

Behavior measure

The Alcohol Use Disorders Identification Test (AUDIT) (Saunders *et al.* 1993) was used to assess alcohol use. This test was developed by the World Health Organization and has been widely used in clinical practice and research. It asks 10 questions about alcohol consumption, alcohol dependence and alcohol-related problems, and uses the total score to identify persons with hazardous and harmful patterns of alcohol consumption.

Genetic analysis

Gene selection

We selected 16 genes in four subsystems of the DA system: (1) DA synthesis [tyrosine hydroxylase (*TH*), decarboxylase (*DDC*), DA beta-hydroxylase (*DβH*)]; (2) degradation/transport [catechol-O-methyl transferase (*COMT*), monoamine oxidase (MADA, MAOB), *SLC6A3*]; (3) DA receptor (*DRD1*, *DRD2*, *DRD3*, *DRD4*, *DRD5*); (4) DA modulation [four neurotensin genes (*NLN*, *NTS*, *NTSR1*, *NTSR2*)]. These genes represent all major genes involved in these four DA subsystems in humans (The International Human Genome Sequencing Consortium 2004).

DA synthesis involves converting the amino acid tyrosine (via TH) to levodopa, followed by subsequent decarboxylation (by DDC) to DA. Further conversion by DβH yields norepinephrine in some cells. For the degradation/transport subsystem, released DA is directly broken down at the synapse into inactive metabolites by two enzymes, COMT and MAO (including MAOA and MAOB). The DA transporter (SLC6A3), a membrane-spanning protein, pumps the neurotransmitter DA out of the synapse and into the pre-synaptic neuron for reutilization. For the receptor subsystem, we included all five genes for DA receptors. For the modulation subsystem, we focused on neurotensin genes, the only well characterized system, that has been implicated in the modulation of DA signaling.

To sample the genetic diversity of these 16 genes, we selected the tag SNPs (tSNPs) defined by the HapMap project [http://hapmap.ncbi.nlm.nih.gov/ (phase 3); The International HapMap Consortium 2007]. As defined by Hapmap, these tSNPs are the minimum set of SNPs needed to sample most genetic diversity through LD. The tSNPs were defined by HapMap in 2007 using the four populations investigated at that time (European ancestry, African–Yoruban ancestry, Chinese ancestry, Japanese ancestry), and used a general R^2 value of 0.8 for identification. Additional SNPs were added for some genes in regions of high LD uncovered in genomic searches for recent adaptive selection (Wang *et al.* 2006; Hawks *et al.* 2007). We chose SNPs that covered both coding and potential regulatory regions (for the latter up to l0 kb beyond the coding region).

Genotyping techniques

The SNPs were genotyped using the standard Illumina GoldenGate Genotyping protocol (see Illumina GoldenGate Assay Protocol for details, http://www.southgene.com.cn; Shanghai South Gene Technology Co., Ltd, Shanghai, China). In addition, three genetic markers (*DRD4* VNTR, *MAOA* VNTR, and *COMT* rs4680) were ascertained by standard polymerase chain reaction procedures (see Sabol, Hu & Hamer 1998; Qian *et al.* 2003, 2004; Wang *et al.* 2004).

Gene data preprocessing

As part of a larger project, the genetic data were screened for quality. In addition to automatic calling of genotypes, the Illumina genotyping platform supplied a quantitative quality measure known as the GenCall score. It measures how close a genotype is to the center of the cluster of other samples assigned to the same genotypes, compared with the centers of the clusters of the other genotypes. This measure ranges from 0 to 1, with a higher score indicating a more reliable result. The conventional cut-off point is 0.25 (Guan *et al.* 2009). Of the 95 SNPs of 176 subjects used in the current study, 23 genotypes (0.1%) were excluded because their GenCall was lower than 0.25. To examine sample representativeness, Hardy–Weinberg equilibrium (HWE) index was calculated using the chi square test by combining two homozygote groups

together and setting degrees of freedom (d.f.) to 1 (except for the *DRD4* VNTR because we combined it into three groups, see below for details). Five of the autosomal chromosome SNPs showed significant HW disequilibrium ($P < 0.05$). Finally, because of the inclusion of both tag SNPs and additional supplemental SNPs, there was high LD among a number of SNPs, as expected. Eleven SNPs were further excluded from multiple regression analysis because of their high LD with other adjacent SNPs ($R^2 > 0.8$, calculated with Plink (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell *et al.* 2007) using data of 478 subjects from our larger project). As a confirmation of our results, a preliminary analysis showed that these 'redundant' SNPs showed the same or almost the same results as the linked SNPs. Supporting Information Table S1 shows details about all 98 polymorphic loci (96 SNPs and two VNTRs) included in our study: location (rs number, chromosome, position), gene, DA subsystem, allele polymorphism and frequency, HWE, LD and deleted SNPs.

Data analysis

The total score of AUDIT was used as the index of alcohol use. Given that the distribution of the summarized score is skewed, a logarithmic transformation was applied.

The goal of the current study was to understand the relation between individual differences in alcohol use and genetic variations in the DA system in healthy male subjects. Moving beyond the single-gene or a small number of haplotypes approaches used in typical molecular behavior genetics research, this study examined contributions of the DA system (characterized by the major genes and their associated loci). Three major analyses were conducted in the present study. A series of analyses of variance (ANOVAs) were conducted to detect the loci with significant main effects on alcohol use. Next, multiple regression analyses were conducted to examine the overall contribution of those SNPs with significant main effects, the unique influence of loci and their interactions. Lastly, to assess the likelihood of false positives with the multiple regression approach, a series of permutation analyses were run on randomized data (by randomizing scores of alcohol use among participants).

A multiple regression procedure was used in this study for several reasons: (1) to generate the overall estimated contribution (R square) of multiple loci to the given behavior; (2) to detect the loci with unique contribution; and (3) to allow for gene–gene or SNP–SNP interactions. Results of multiple regression analyses are informative for separating the situations when multiple loci were significant because of their LD with one single action point from those when multiple loci indicated multiple action points; the former would involve one unique significant predictor, whereas the latter would involve multiple unique predictors.

In this study, we built two kinds of regression models. In model 1 (main effects), we included the loci with significant main effects based on the ANOVA results ($P < 0.05$). To run multiple regression analyses, all SNPs were coded in a linear way, i.e. the major homozygote, heterozygote, minor homozygote were coded as 1, 2, 3, respectively (SNPs on X chromosome were coded as 1 and 3 for major and minor allele). In addition, we separated the data of the *DRD4* VNTR into three dummy-coded groups: '4R/4R' and, 2 repeats '2R+' (e.g. 2R/2R, 2R/3R, 2R/4R, 2R/5R, 2R/6R) versus others. This was done because 4R is the major ancestral allele and 2R is of theoretical importance among Chinese (Ding *et al.* 2002; Wang *et al.* 2004). The *MAOA* VNTR was coded as 1 for the 3 repeat and 3 for the 4 repeat.

In model 2, we added interaction terms among those SNPs included in model 1. Multiplications of de-meaned codes of every pair of SNPs were used as interaction term. Forward stepwise regression was used to search for significant interactive effects among the large number of potential interactions. Model comparisons were made to ascertain the significance of interactive terms.

Finally, permutation analyses were conducted to assess the likelihood of obtaining our results under different assumptions. Basic multiple linear regressions assume linearity, normality, independence (or non-collinearity) among predictors, non-correlated errors, etc. Because these criteria are difficult to meet, the probability of significance we obtained for our results may be too liberal. To derive more stringent criteria, we did permutation analyses. We kept the genetic structure intact and randomized behavior data (alcohol use), then repeated the above process on the randomized data. Specifically, each permutation used all 98 loci to run ANOVAs on the randomized AUDIT data. We then selected SNPs with significant main effects ($P < 0.05$) on the randomized AUDIT scores (the number of significant SNPs varied across permutations) and used them in the regression models. Permutation was done 1000 times, to yield a distribution of R^2. Based on that distribution, the probability of obtaining the observed R^2 was determined.

RESULTS

Ninety-eight polymorphic loci (including 96 SNPs and two VNTR polymorphisms) were genotyped to cover a substantial portion (by LD) of the common variations within all known genes of the DA system (The International Human Genome Sequencing Consortium 2004). Specifically they include the following genes (and the number of polymorphic loci): *COMT* (7), *DβH* (9), *DDC*

(11), *DRD1* (5), *DRD2* (6), *DRD3* (4), *DRD4* (4 SNPs plus 1 VNTR), *DRD5* (9), *MAOA* (5 plus 1 VNTR), *MAOB* (3), *NLN* (10), *NTS* (4), *NTSR1* (5), *NTSR2* (1), *SLC6A3* (8) and *TH* (5). Details of these loci can be found in the Supporting Information Table S1.

The mean score of AUDIT was 4.8 [standard deviation (SD) = 3.5]. As discussed in the Materials and Methods section, 11 SNPs were excluded from further analysis because of high LD with adjacent SNPs, in order to prevent 'over-counting' of associative events. For the remaining 87 SNPs, six showed significant main effects with uncorrected $P < 0.05$ (rs77905, rs732833, rs2073837, rs165774, rs909525, rs4102942, see Table 1, and Supporting Information Table S2 for detailed information of all 98 loci). Their effects on reported alcohol use are also shown in Fig. 1. These SNPs were used in a regression analysis to build model 1 (main effects).

Table 2 shows the results of the multiple regression analysis. The six regressors accounting for 19% of the variance of alcohol use ($R^2 = 0.19$, and adjusted $R^2 = 0.16$), $F(6,168) = 6.6$, $P = 3*10^{-6}$. Variations in these SNPs made unique and additive contribution to alcohol use. Individuals homozygous for the major allele of rs165774 (*COMT*), rs909525 (*MAOA*) or rs4102942 (*DRD5*), heterozygous for rs77905 (*DBH*), or homozygous for the minor allele of rs732833 (*DBH*), or rs2073837 (*DBH*) tended to drink less than those with alternative alleles.

Permutation results are shown in Fig. 2. Based on 1000 permutations, the probability of attaining the R^2 or adjusted R^2 found in our model was 0.044 and 0.024, respectively.

In model 2, we added potential interactive effects to investigate whether additional variance in alcohol use can be accounted for by gene–gene interactions. In this analysis, we first entered all six regressors of the main effects, and then tested their one-to-one interactions using the stepwise procedure. For the six SNPs showing significant main effects, there were 15 potential interactions. Two of the interaction terms made significantly unique contributions to the model. The R^2 increased to 0.23, and adjusted R^2 increased to 0.19, $F(8,166) = 6.2$, $P = 4.6*10^{-7}$. Table 2 and Fig. 1 show the significant interactions. Compared with model 1, this model fit the data better, as indicated by a significant improvement of −2 log likelihood and a smaller Akaike's information criterion (Table 3), although Bayesian information criterion (an index that is more sensitive to the increased number of predictors) was slightly higher.

Permutation results again showed that our results were not likely because of chance or because of a large number of potential predictors. The probability of obtaining the R^2 we obtained in a randomized dataset was 0.048 and 0.040 for R^2 and adjusted R^2, respectively (Fig. 2).

Table 1 Means and standard deviations of Alcohol Use Disorders Identification Test score for each polymorphism, and main effects and *post hoc* comparisons of SNPs that showed significant main effects and used in subsequent multiple regression analysis.

SNP	*Subsystem*	*Gene*	*Maj*	*Mean*	*SD*	n	*Het*	*Mean*	*SD*	n	*Min*	*Mean*	*SD*	n	*F*	*P*	*mh*	*mm*	*hm*[a]
rs77905	Synthesis	*DBH*	GG	0.64	0.34	145	AG	0.43	0.32	31					9.37	< 0.01	[b]		
rs732833		*DBH*	GG	0.61	0.32	64	AG	0.65	0.35	86	AA	0.42	0.34	26	4.81	0.01	0.42	0.02	< 0.01
rs2073837		*DBH*	GG	0.62	0.31	43	AG	0.65	0.34	86	AA	0.49	0.35	47	3.25	0.04	0.64	0.08	0.01
rs165774	Degradation/transport	*COMT*	GG	0.57	0.35	140	AG	0.71	0.30	36					4.85	0.03	[b]		
rs909525		*MAOA*	GG	0.56	0.36	106	AG				AA	0.67	0.30	69	4.63	0.03	[b]		
rs4102942	Receptor	*DRD5*	GG	0.48	0.34	32	AG	0.62	0.35	129	AA	0.73	0.25	15	3.06	0.05	0.05	0.02	0.23

Note: An empty cell means no such genotype was found in our sample. [a]Results of *post hoc* comparison. mh = Maj versus Het, mm = Maj versus Min, hm = Het versus Min. [b]*Post hoc* comparison was not run because there were only 2 groups for this locus.

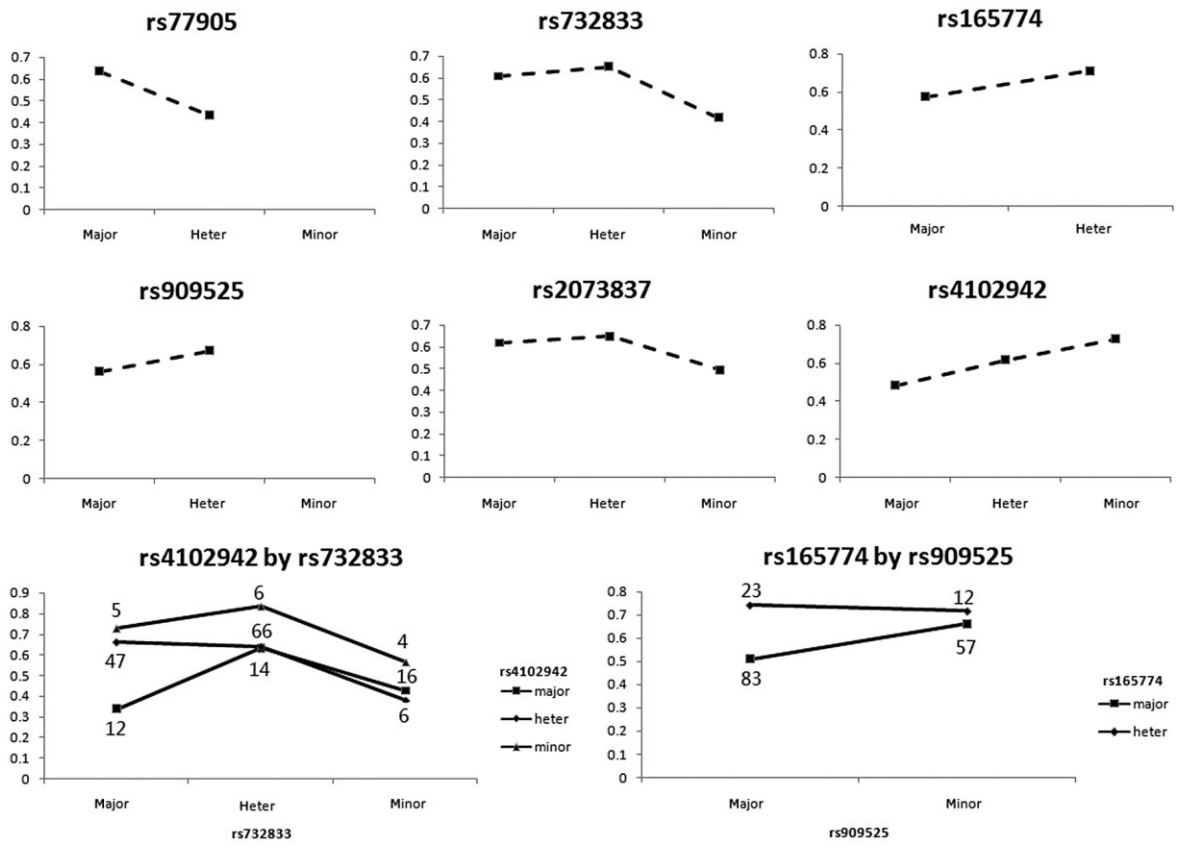

Figure 1 Main effects of six SNPs and two of their significant interactions on log-transformed Alcohol Use Disorders Identification Test score. Numbers in the interaction subplots were subject numbers for each cell

Table 2 Regression models. 'Gene1' and 'Gene2' are the corresponding genes for the SNPs; 'β' is the regression coefficient, 'T' and 'P' are *t*-test results.

			Model1			*Model2*		
Regressor	*Gene1*	*Gene2*	*β*	*T*	*P*	*β*	*T*	*P*
rs4102942	*DRD5*		0.15	3.09	0.00	0.15	3.17	0.00
rs77905	*DBH*		–0.16	–2.42	0.02	–0.19	–2.80	0.01
rs732833	*DBH*		–0.07	–1.70	0.09	–0.06	–1.48	0.14
rs2073837	*DBH*		–0.09	–2.23	0.03	–0.08	–2.02	0.04
rs165774	*COMT*		0.16	2.66	0.01	0.10	1.66	0.10
rs909525	*MAOA*		0.14	2.88	0.00	0.14	2.84	0.01
rs4102942-rs732833	*DRD5*	*DBH*				–0.13	–2.00	0.05
rs165774-rs909525	*COMT*	*MAOA*				–0.29	–2.32	0.02

DISCUSSION

By examining genes of the whole DA system and their association with alcohol use, we found that six SNPs of the DA-related genes together can explain about one fifth of individual differences in alcohol drinking. This result has two significant implications. First, it supports the idea that human traits may be determined by many loci, and only by summing up their overall (both main and interactive) effects can we understand the genetic basis of a trait. Most recently, researchers have estimated that multiple gene loci contribute to height (Yang *et al*. 2010) and sensation-seeking personality (Derringer *et al*. 2010). All these studies showed that by combining the effects of multiple loci, genes can account for a significant fraction of the variance in human traits, as suggested by behavioral genetic research. Second, and specific to alcohol use, the current study reveals a significant role for

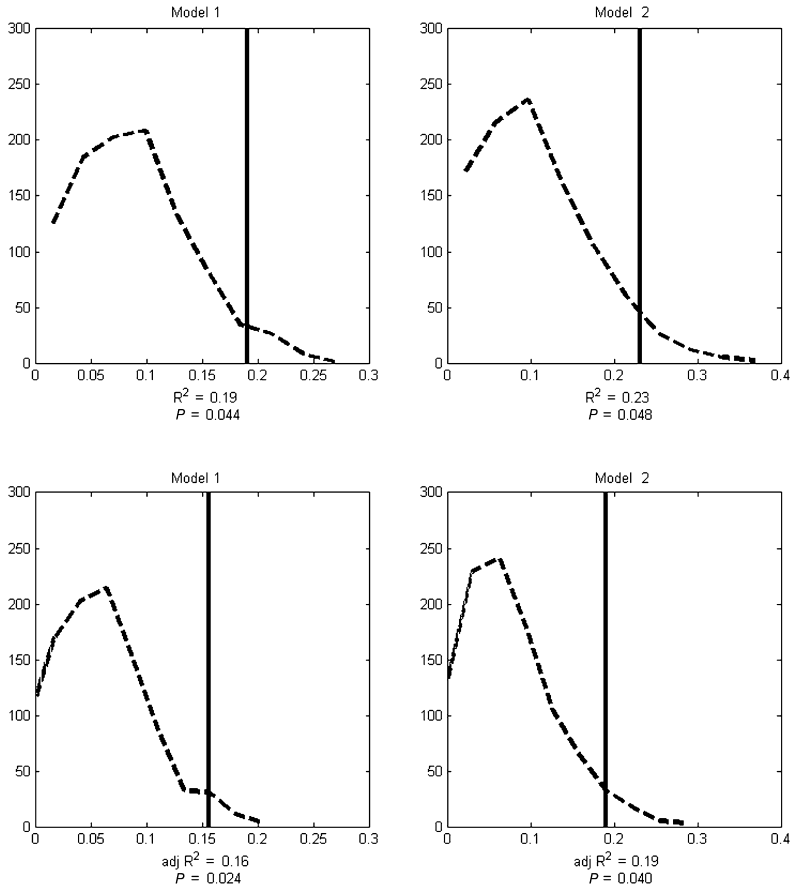

Figure 2 Permutation results. Dashed line represents distribution of R^2 obtained from randomized data and solid line represents the observed R^2. First column for model 1, second column for model 2, first row for R^2, second row for adjusted R^2

Table 3 Comparison of regression models

Models	R^2	ΔR^2	*−2LL*	*d.f.*	P	*AIC*	*BIC*
Model 1	0.19	–	83.2	6	–	95.2	114.2
Model 2	0.23	0.04	74.0	8	0.01	90.0	115.4

Note: R^2 are the proportion of variance explained by the models; ΔR^2 is the difference of R^2 between two models; −2LL is the log likelihood of the regression model multiplied by −2; *P* was calculated to estimate change in −2LL by Chi-square distribution with degrees of freedom (d.f.) equal the difference of d.f. between models. Akaike's information criterion (AIC) and the Bayesian information criterion (BIC) are information theoretic measures of goodness of model fit.

genes in the DA system. Although behavioral genetic studies have estimated a high heritability of alcohol use, single genes have been found to play only a small role. Our study also confirmed that single genes individually played a small role, which likely would not survive extreme statistical corrections applied to multiple comparisons conducted in whole genome analysis (Manolio *et al*. 2009). However, when examining the DA system as a whole, the DA-related genes were found to make a sizable contribution, explaining about 20% of individual differences. Considering that other genes and systems are also likely to make unique contributions to alcohol use (Dick & Foroud 2003; Köhnke 2008), the proverbial gap between genetic contributions estimated from twin studies (i.e. heritability) and molecular genetic research may be finally bridged.

We found that six SNPs of four genes (*DRD5*, *DβH*, *COMT*, *MAOA*) significantly contributed to alcohol use. As summarized in the introduction, previous research has already found evidence, although not all consistent, of association between each of these genes and alcohol use. However, the specific SNPs we identified have not been tested previously in alcohol use-related studies to the best of our knowledge. There have been a number of studies on *DβH*, *COMT* and *MAOA*, but the results have been inconsistent (see Köhnke 2008, for a review). As Köhnke summarized, *DβH*-1021C>T (rs1611115), *DβH* Taq I (rs1611128), *DβH**444GA (rs1108580), *COMT* rs4680 and *MAOA* uVNTR are polymorphic sites often studied. Of the SNPs identified in this study, *DβH* rs77905 is a synonymous mutation, and both rs732833 and rs2073837 are in intronic regions. LD between these three SNPs and previously studied *DβH* SNPs was calculated from HapMap CHB data (except for rs2073837, which was not available), and a maximum R^2 of 0.34 was found.

Similarly, rs165774 of *COMT* and rs909525 of *MAOA* are also in intronic regions. At present, the

functions of these SNPs are undetermined. They may play a role in splicing or simply implicate functional polymorphic loci nearby. In our study, we can rule out, however, two functional polymorphisms (*COMT* rs4680 and *MAOA* uVNTR) because they did not show significant associations. Foroud *et al.* (2007) tested 18 SNPs of the *COMT* gene, with three SNPs (rs5993883, rs740603 and rs4680) overlapping with our study, and two SNPs (rs737865 and rs4633) close (within 1 kb) to our data (rs737866 and rs2239393, respectively). Both their study and our study reported no significant association of these SNPs with alcohol use. Their study did not test rs165774, nor any SNPs in strong LD with rs165774, which was identified as associated with alcohol use in the current study.

DRD5 has not been studied extensively, with only one negative finding reported in a Korean population (Kim *et al.* 2007). Kim genotyped −226C>G (rs2076907 in the 5′ UTR region) and +324C>G (a novel SNP identified in Exon1), both of which were not tested in our study. The *DRD5* SNP associated with alcohol use in our study (rs4102942) is not in strong LD with the polymorphisms studied in Kim *et al.* 2007.

It should be noted that among the above genes, both *MAOA* and *DRD5* were identified in Wang *et al.* (2006) as having undergone strong recent selection, and, hence, likely to have variants that are associated with current behavioral differences. What had not been reported in previous studies are possible interactions among the SNPs. We found significant *DRD5*-by-*DBH* and *COMT*-by-*MAOA* interactions, suggesting complex relations between receptor-, synthesis- or degradation-related genes. This finding adds to the recent discussions and evidence of gene–gene (Skowronek *et al.* 2006; Huang *et al.* 2007; Wang *et al.* 2007; Karpyak *et al.* 2010) and gene–environment (Laucht *et al.* 2007; Lucht *et al.* 2007) interactions on alcohol use.

Several limitations of the current study need to be mentioned. First, this study adopts a system-level approach, which was recently developed (with several variations, see Derringer *et al.* 2010; Moore, Asselbergs & Williams 2010; Wu *et al.* 2010; Yang *et al.* 2010) and needs further methodological refinements. For example, we need to investigate whether permutation or adjustment for multiple comparisons would be a better choice to control for false positives and what is the best way to select SNPs to cover a particular system (DA, serotonin, GABA, etc.). Second, we focused only on healthy male Han Chinese college students, so these results need to be extended to other samples (females, clinical samples, other ethnic groups). Finally, we selected 98 polymorphism loci related to DA according to our previous study and HapMap information to cover variations of the major DA-related genes. A more extensive coverage may reveal more variants with significant effects. In addition, some of the genes we covered (i.e. *MAOA* and *COMT*) act not only on DA but also other neurotransmitters, so the biological mechanisms of their effects on alcohol use would need further investigation.

In conclusion, the current study used a system-level approach to examine DA-related genes and alcohol use. We found both main and interactive effects, which together accounted for a significant portion of variance in alcohol use. These results also suggest that the system-level approach may be a promising way to explore the genetic basis of human behavior.

Acknowledgements

This study was supported by the 111 Project of the Ministry of Education of China (B07008). We thank all graduate research assistants who helped with data collection.

Authors Contribution

Qi Dong, Chuansheng Chen and Robert Moyzis were responsible for the study concept and design. Chunhui Chen, Qinghua He, Jin Li, He Li and Bi Zhu contributed to data acquisition. Chunhui Chen, Chuansheng Chen and Hal Stern analyzed data. Chunhui Chen, Chuansheng Chen, Robert Moyzis and Jared Lessard wrote the manuscript. All authors critically reviewed content and approved the final version for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Detailed information of the SNPs used in this study

Table S2 Mean and standard deviations of AUDIT score for each polymorphism, and main effects and *post hoc* comparisons of each SNP.

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