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ORIGINAL INVESTIGATION

Association of the *HTR2A* gene with alcohol and heroin abuse

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Abstract Positive genetic associations of rs6313 (102T/C at exon 1) and rs6311 (−1438A/G) on the 5-hydroxytryptamine (serotonin) 2A receptor gene (*HTR2A* or *5-HT2A*) were reported for alcohol and drug abuse; however, other association studies failed to produce consistent results supporting the susceptibility of the two single nucleotide polymorphisms (SNPs). To clarify the associations of the *HTR2A* gene with substance use disorders, we performed a meta-analysis based on the genotypes from the available candidate gene association studies of the two SNPs with alcohol and drug abuse from multiple populations. Evidence of association was found for *HTR2A* rs6313 in all the combined studies (e.g., allelic $P = 0.0048$ and OR 0.86, 95 % CI 0.77–0.95) and also in the combined studies of alcohol dependence (abuse) (e.g., allelic $P = 0.0001$ and OR 0.71, 95 % CI 0.59–0.85). The same association trend was also observed in the Study of Addiction: Genetics and Environment datasets. The meta-analysis supports a contribution of the *HTR2A* gene to the susceptibility to substance use disorders, particularly alcohol dependence.

Introduction

Alcohol and drug abuse are chronic relapsing disorders characterized by compulsive seeking, abuse, tolerance and physical dependence on the substance in question. Studies have showed consistently that substance use disorders share some common genetic factors that play pivotal roles in the development of the disorders (Cadoret et al. 1995; Fu et al. 2002; True et al. 1999; Xian et al. 2008). Studies also demonstrated that drug dependence clusters in the families with drug-dependent probands (Bierut et al. 1998). For instance, among relatives of controls, the drug dependence rate was 3.5 %, whereas among relatives of opioid-dependent individuals, the rate was 20.5 % (Saxon et al. 2005). The genetic contribution to vulnerability to develop heroin addiction is 40–70 %, suggesting that addictions are multifactorial disorders caused by complex interaction of

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genetic and environmental factors (Gelernter and Kranzler 2009; Kendler et al. 2007; Uhl et al. 2008).

The 5-hydroxytryptamine (serotonin or 5-HT) 2A receptor is one of the subtypes of 5-HT receptors (HTR). The 5-HT 2A receptor (HTR2A or formally 5-HT2A) is a member of the G protein superfamily. Presynaptic HTR2A has been localized on dopaminergic neurons within the ventral tegmental area and nucleus accumbens, suggesting an important role within the brain reward system (Frazer and Hensler 1998). Postsynaptic activation of the receptor also plays a relevant role in substance dependence (Kreek et al. 2005). The HTR2A is related to affectivity, regulation and pharmacologic effects of antidepressant, anti-anxiety and antipsychotic medications. It may also play a role in cellular development and differentiation, while it is the action site of certain drugs and medications (Gelernter et al. 2006; Glatt et al. 2006; Kendler et al. 2003; Tsuang et al. 1998; van den Bree et al. 1998). It has also been shown that HTR2A and HTR2C antagonists cause attenuation of alcohol intake in both animals and humans (Pandey et al. 1995), and activation of HTR2A can reduce ethanol consumption (Maurel et al. 1999). These data suggested a role for the *HTR2A* gene as a candidate gene for ethanol and drug-related traits.

The *HTR2A* gene, which contains 62 kilobase pairs, is located on 13q14-q21, the long arm of chromosome 13. Previous association studies focused on two common single nucleotide polymorphisms (SNPs) which are in complete linkage disequilibrium in European populations (Arranz et al. 1998; Saiz et al. 2008). For the *MspI* polymorphic site at nucleotide 102, although the receptor protein remains unaltered, the SNP rs6313 (102T/C at exon 1) has been intensively studied among patients with alcohol and heroin addiction. Another SNP rs6311 (−1438A/G) is in the promoter region. Significant associations or noticeable effect sizes were described for the two SNPs with alcohol and (or) heroin dependence. However, other association studies failed to produce consistent results, e.g., some individual studies even reported opposite directions of the risk alleles. The nonreplicability of previous results made it necessary to clarify the contrary results among these individual association studies. Therefore, we combined all the available genotype data of *HTR2A* rs6313 and rs6311 of multiple populations from candidate gene association studies of substance use disorders via meta-analysis approaches.

Methods

Literature search

The studies included in the meta-analysis were selected from Scopus and the database of Chinese academic journals with keywords 'serotonergic receptor', '*HTR2A*', '*5-HT2A*', 'serotonin receptor', 'association', 'associated', 'drug', 'substance', 'alcoholism', 'alcohol', 'alcoholics', 'heroin', 'cocaine', 'opiate', 'opioid', 'opium' and other abbreviations of the gene (i.e., '*HTR*'). Both English and Chinese keywords were used for the Chinese journals. The references cited in these studies and in published reviews were examined to identify additional works not indexed by the databases. The analyzed data cover all identified publications in English and Chinese available up to July 2012.

Inclusion criteria

Eligible studies met all of the following criteria: they (1) were published in peer-reviewed journals and contained original data; (2) presented sufficient data to calculate the odds ratio (OR) with confidence interval (CI) and *P* value; (3) were association studies investigating one or two of the polymorphisms using either case–control or family-based approaches; (4) described or referenced appropriate genotyping primers, machines and protocols; (5) diagnosed the patients according to the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD), American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM) or Chinese Classification of Mental Disorders (CCMD) (Chen 2002) systems; and (6) used random population or healthy individuals as controls in case–control studies. Authors were contacted in cases where it would be useful to have additional information regarding their studies. The procedure of 'extended-quality score' (Li et al. 2006a) was applied to assist the assessment of quality of the association studies.

Statistical analyses

Studies were classified depending on whether they dealt with samples with European ancestries or those with Asian ancestries. For a study that contained data from multiple populations, each was considered effectively as an independent study if possible. Data from the case–control and haplotype relative risk (HRR) studies were summarized by two-by-two tables. The studies were statistically combined by the method described in our previous study of *HTR2A* with schizophrenia (Li et al. 2006b) to combine population-based and family-based studies into a single meta-analysis. In particular, from each table a log odds ratio and its sampling variance were calculated. The Cochran's χ^2-based Q statistic test was performed to assess the heterogeneity to ensure that each group of studies was suitable for meta-analysis. A test for funnel plot asymmetry, described by Egger et al. (1997), was used to assess evidence for publication bias. The test used a linear regression approach to

measure funnel plot asymmetry on the natural logarithm of the OR. The larger the deviation of each study from the funnel curve, the more pronounced was the asymmetry. Results from small studies will scatter widely at the bottom of the graph, with the spread narrowing among larger studies. The significance of the intercept was evaluated using the *T* test and the *P*(*T*) was used to estimate the significance of publication bias. The "Duval and Tweedie's Trim and Fill" procedure (Duval and Tweedie 2000) was used to impute the number of potentially missing studies for publication bias.

Odds ratios were pooled using the method of DerSimonian and Laird (1986), and 95 % CIs were constructed using Woolf's method (Woolf 1955). The significance of the overall OR was determined using the *Z* test. In addition, the studies were also subdivided and re-analyzed according to different populations (European vs. Asian) and different traits (alcohol vs. heroin dependence). Genotypic analyses were carried out under both dominant and recessive models. The classic fail-safe analysis (Rosenthal 1979) was adopted to evaluate the impact of publication bias on the results of meta-analysis. Retrospective analysis was performed to better understand the potential effect of year of publication upon the results. The type I error rate was set at 0.05. The tests were two tailed. Haplotype construction, counting and linkage disequilibrium (LD) block defining were performed separately using the HapMap CEPH and Asian samples, as described previously (Li et al. 2011).

Results

The combined search yielded 2,409 references. After discarding the references that clearly did not meet the criteria, the studies were then filtered to ensure conformity with our inclusion criteria. Two studies (Jakubczyk et al. 2011; Wrzosek et al. 2011) were excluded because no data of matched normal controls were described, and one study (Yang et al. 2012) was excluded because the genotype data were the same as that described in another study (Yang et al. 2010). Finally, 21 studies, composed of 20 case–control studies and 1 HRR study (Hill et al. 2002), met our criteria for inclusion. The 21 studies included 5 studies for European populations (Fehr et al. 2001; Hill et al. 2002; Parsian and Cloninger 2001; Wrzosek et al. 2012), 2 for Hispanic samples (Polina et al. 2009; Saiz et al. 2008) and 14 for Asian populations (Cao et al. 2002; Gao et al. 2011; Himei et al. 2000; Hwu and Chen 2000; Lee et al. 2009; Li et al. 2002; Nakamura et al. 1999; Shao et al. 2005; Terayama et al. 2003; Tsunoka et al. 2010; Wang et al. 2001; Yang et al. 2010). Among these studies, 8 (Cao et al. 2002; Gao et al. 2011; Li et al. 2002; Saiz et al. 2008; Shao et al. 2005; Wang et al. 2001; Yang et al. 2010) investigated heroin dependence or abuse, 1 (Tsunoka et al. 2010) investigated methamphetamine dependence and the other 12 (Fehr et al. 2001; Hill et al. 2002; Himei et al. 2000; Hwu and Chen 2000; Lee et al. 2009; Nakamura et al. 1999; Parsian and Cloninger 2001; Polina et al. 2009; Terayama et al. 2003; Wrzosek et al. 2012) investigated alcohol dependence or abuse. The 21 studies included 3,506 cases, 3,556 controls and 35 families. The demography of these studies is shown in supplementary Table 1. The results for each polymorphism are detailed below.

The rs6313 (102T/C) polymorphism

The T allele frequency varied across the samples, being abundant with an average percentage of 46 % (39–53 %) in European controls and 37 % (31–39 %) in cases, and less abundant with an average percentage of 56 % (51–71 %) in Asian controls and 55.9 % (51–70 %) in cases. In the 13 studies, 11 showed lower T allele frequency in cases than in controls (or less allele transmissions in families). The meta-analysis of all the combined studies produced an overall allelic *P* value of 0.0048 (OR 0.86, 95 % CI 0.77–0.95) with no evidence for heterogeneity between studies (Table 1). Evidence of significant association was also found in the combined European populations [allelic $P = 0.0074$ and OR 0.70 (95 % CI 0.54, 0.91)]. The combined studies of alcohol dependence (abuse) showed more significant association [allelic $P = 0.0001$ and OR 0.71 (95 % CI 0.59, 0.85)]. Evidence of association was also observed under the dominant [(TT + TC)/CC) and recessive (TT/(TC + CC)] models, e.g., $P = 0.0008$ and OR 0.61 (0.46, 0.81) for the combined studies of alcohol dependence (abuse) under the recessive model. The same genetic association trend was also observed in the Study of Addiction: Genetics and Environment (SAGE) datasets of European and African American subjects. The findings are shown for the allelic and genotypic analyses in Table 1 and supplementary Table 2, respectively. The allele frequencies and forest plots of rs6313 are shown in Figs. 1 and 2, respectively.

The rs6311 (−1438A/G) polymorphism

The A allele is the minor allele with an average percentage of 43 % (39–45 %) in the European and Hispanic normal samples and 51 % (50–55 %) in cases, but is the major allele with an average percentage of 57 % (53–64 %) in the Asian normal populations and 56 % (49–61 %) in cases. Of the 12 case–control studies, 7 showed higher A allele frequency in cases than in controls. No evidence of significant association was found when all the studies were combined under the random effects model with heterogeneity between studies. Evidence of heterogeneity was

Table 1 Results of the overall and sub-group allelic analyses

SNPs/groups	Studies[a]	Samples[b]	LnOR (95 % CI)	OR (95 % CI)	*P*(*Z*)	*P*(*Q*)
HTR2A rs6313 (102T/C)						
All the studies	13	1,407/1,908/35	−0.16 (−0.26, −0.05)	0.86 (0.77, 0.95)	**0.0048**	0.23
Europeans	4	326/155/35	−0.35 (−0.61, −0.09)	0.7 (0.54, 0.91)	**0.0074**	0.34
Asians	9	1,081/1,753	−0.11 (−0.23, 0.01)	0.89 (0.79, 1.01)	0.062	0.34
Alcohol dependence/abuse	9	715/503/35	−0.34 (−0.52, −0.17)	0.71 (0.59, 0.85)	**0.0001**	0.47
Alcohol dependence/abuse (Asians)	5	389/348	−0.34 (−0.58, −0.1)	0.71 (0.56, 0.91)	**0.0063**	0.37
All the studies and SAGE[c]	15	3,098/3,620/35	−0.11 (−0.19, −0.04)	0.89 (0.83, 0.96)	**0.0023**	0.29
Europeans and SAGE[c]	5	1,440/1,437/35	−0.11 (−0.22, −0.01)	0.89 (0.8, 0.99)	**0.0338**	0.12
Alcohol dependence/abuse and SAGE[c]	11	2,406/2,215/35	−0.14 (−0.23, −0.05)	0.87 (0.8, 0.95)	**0.0013**	0.15
HTR2A rs6311 (−1438A/G)						
All the studies	12	2,760/3,056	0.06 (−0.06, 0.19)	1.07 (0.94, 1.21)	0.3263	0.0071
Europeans and hispanic	3	359/623	0.42 (0.22, 0.61)	1.51 (1.24, 1.85)	$\mathbf{4 \times 10^{-5}}$	0.98
Asians	9	2,401/2,433	−0.05 (−0.13, 0.04)	0.95 (0.87, 1.04)	0.2957	0.41
Heroin dependence/abuse	7	1,770/1,578	0.08 (−0.03, 0.18)	1.08 (0.97, 1.2)	0.149	0.21
Alcohol dependence/abuse	4	794/676	0.12 (−0.27, 0.51)	1.13 (0.76, 1.66)	0.5478	0.0016
Alcohol dependence/abuse (Europeans)	2	246/203	0.42 (0.15, 0.68)	1.52 (1.16, 1.98)	**0.0023**	0.83
Alcohol dependence/abuse (Asians)	2	548/473	−0.22 (−0.4, −0.04)	0.81 (0.67, 0.96)	**0.0176**	0.56
All the studies and SAGE[c]	14	4,451/4,768	0.03 (−0.08, 0.13)	1.03 (0.93, 1.14)	0.6283	0.0063

P(*Z*) *Z* test used to determine the significance of the overall OR. The *P* values <0.05 are indicated in boldfaces

P(*Q*) Cochran's χ^2-based *Q* statistic test used to assess the heterogeneity

P(*T*) *T* test used to estimate the significance of publication bias (not shown)

[a] The number of studies included are indicated

[b] The number of cases/controls/families. The 35 families included 116 subjects

[c] 1,691 cases and 1,712 controls from the Study of Addiction: Genetics and Environment (SAGE) datasets (European and African Americans) were included in the meta-analysis

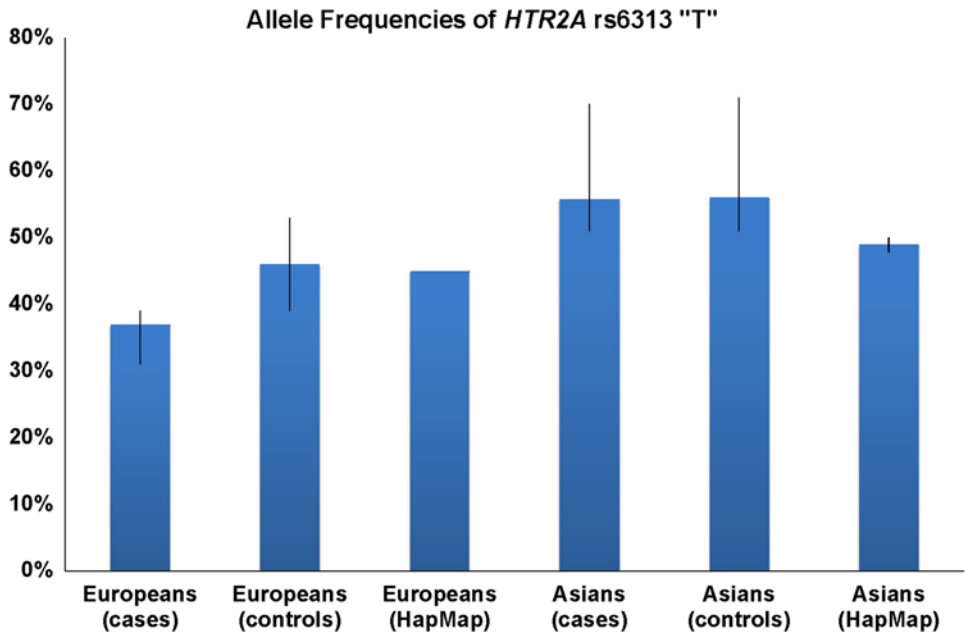

Fig. 1 Frequencies of *HTR2A* rs6313 T allele. The *error bar* shows the highest and lowest frequencies in the individual studies analyzed in the meta-analysis

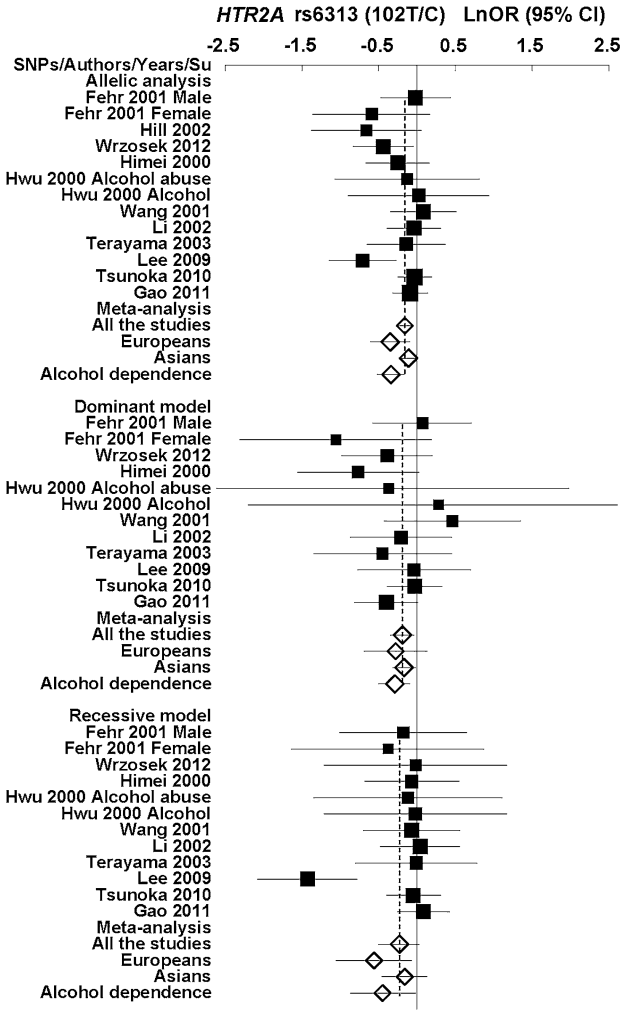

Fig. 2 Forest plots of ln(OR) and overall ln(OR) with 95 % CI of the allelic and genotypic analyses for *HTR2A* rs6313. *Black squares* indicate the ln(OR), with the size of the square inversely proportional to its variance, and *horizontal lines* represent the 95 % CIs. The overall ln(OR) are indicated by the *unshaded black diamond*

further observed between European and Asian populations ($P = 3 \times 10^{-5}$) and, therefore, we also analyzed the two populations separately. The combined studies of European populations showed an allelic *P* value of 4×10^{-5} [OR 1.51 (1.24, 1.85)], which was also significant under the dominant [(AA + AG)/GG) and recessive (AA/(AG + GG)] models. However, it should be noted that the sample size of the subgroup analysis of Europeans was small. The findings are shown in Table 1 and supplementary Table 2. The allele frequencies and forest plots of rs6311 are shown in Fig. 3 and supplementary Figure 1, respectively.

Publication bias and fail-safe analyses

In the present meta-analysis, no evidence of significant publication bias was found ($P(T) > 0.05$). For rs6313, the classic fail-safe analysis showed that at least 23 and 22 assumed non-significant association studies would be required to bring the *P* values to >0.05 for the meta-analysis of all the combined studies and for the meta-analysis of alcohol dependence (abuse), respectively. This finding further supported the significant association observed in rs6313. The funnel plots of standard error by log odds ratio of rs6313 are shown for all the combined studies and the combined studies of alcohol dependence (abuse) in Fig. 4 and supplementary Figure 2, respectively.

Sensitivity analyses

The results of sensitivity analysis showed that no individual study included in the meta-analysis biased the overall significant results significantly. For example, for rs6313, meta-analysis showed consistency, regardless of the data set removed, and the *P* values were never >0.035 or >0.0051 for all the combined studies and for those of alcohol dependence (abuse), respectively. For rs6311, the *P* values were never >0.0023 for the Europeans studies, regardless of the data set removed. The results are shown in supplementary Table 3.

Retrospective analyses

Meta-analysis based on publication years showed that the cumulative results, e.g., asymptote lines, were not stable for rs6313; therefore, more replication studies are still necessary to confirm the current findings. The plots of rs6313 are shown for all the combined studies and those of alcohol dependence (abuse) in supplementary Figures 3 and 4, respectively.

Discussion

Alcohol and drug abuse constitutes major public health problems. The cost of drug abuse has grown to approximately one trillion dollars per year in the USA (Califano 2007). In this meta-analysis, evidence of association was found for *HTR2A* rs6313 in all the combined studies ($P = 0.0048$ and OR 0.86, 95 % CI 0.77–0.95) as well as in the combined studies of alcohol dependence (abuse) ($P = 0.0001$ and OR 0.71, 95 % CI 0.59–0.85). For *HTR2A* rs6311, evidence of association was observed in the combined studies of European populations (allelic $P = 4 \times 10^{-5}$ and OR 1.51, 95 % CI 1.24–1.85); however, considering the limited sample size of the European subjects, more replication association studies are necessary to fully demonstrate the role of rs6311 in substance abuse.

The *HTR2C* gene was also reported frequently as a candidate gene for ethanol-related traits. The potential

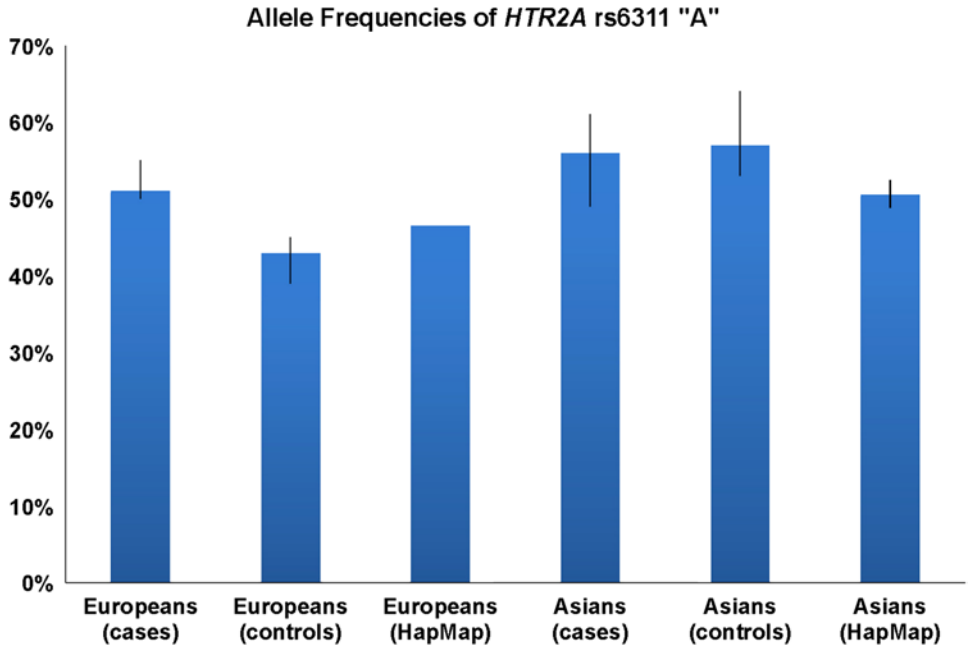

Fig. 3 Frequencies of *HTR2A* rs6311 A allele

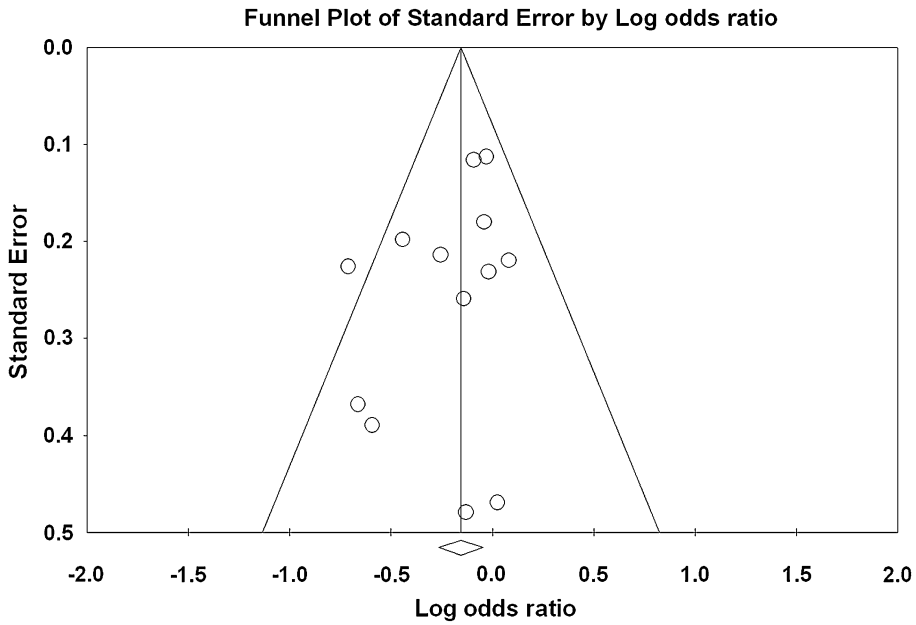

Fig. 4 Egger's funnel plots of all the combined studies for *HTR2A* rs6313

association of 23Cys/Ser polymorphism (HinfI) of *HTR2C* with alcoholism was also analyzed in the present meta-analysis. Our results showed evidence of marginal association ($P = 0.045$) based on the available genotypes. HTR2A has attracted more attention because it appears to be an important active site for atypical antipsychotic agents (Schmidt et al. 1995), hallucinogens (Marek and Aghajanian 1996) and selective serotonin reuptake inhibitors (Roth et al. 1998). Prior findings suggested that rs6311 might have functional effects on HTR2A expression in the brain and could be responsible for the association of rs6311 and its strongly linked rs6313 with many neuropsychiatric disorders (Lesch et al. 1996; Myers et al. 2007).

Although most disease-associated genes show similar effect sizes in different populations, there are still many loci that have unique effects in specific ethnic groups, and those loci identified in one population fail to be replicated in other populations. The population-specific effects could be attributable to different genetic background, unique LD structure or different environmental factors of those ethnic groups. This meta-analysis showed different levels of association results between the two ethnicity populations,

e.g., Europeans and Asians for rs6311 (Table 1). The allele frequencies varied among the investigated populations, such as, the rs6311 A allele showed lower frequency in Europeans than Asians. It is well known that gene–trait associations largely depend on the ethnic background of investigated patients and matched controls. Sampling methods and sample heterogeneity may differentiate the results. The power to detect the putative vulnerability alleles might be diminished due to the quality of the samples. For instance, one of the studies (Hill et al. 2002) analyzed in the meta-analysis included a small proportion of Hispanic and African-American subjects.

Substance use disorders are often comorbid with psychiatric disorders, for instance, co-occurrence of alcohol dependence and behavior disorders may differentiate the findings. Some psychiatric disorders and addiction may not have mutual pathogenesis associated with the *HTR2A* gene, and thus the potential comorbidity might have significantly impaired the possibility to detect strong association signals. Diagnostic classification and ascertainment of the patients and stages of the disorders (Kreek et al. 2005) may further differentiate the association levels, e.g., two studies investigated the diagnostic category of alcohol abuse (Hwu and Chen 2000) and heroin abuse (Cao et al. 2002) other than "dependence". In addition, small sample size may exaggerate population variations. The ethnic and genetic heterogeneity may also explain the underlying population-specific gene pathways observed in each population.

It is possible that rs6313 and rs6311 may not contribute directly to the etiology of alcohol and heroin dependence, but it could be assumed that a variant in LD with the SNPs may be responsible for the vulnerability effect. The supplementary Figures 5 and 6 show the LD plots of the *HTR2A* gene for the European and Asian ancestries, respectively (the pair-wise LD measures (D' and r^2 values) are shown in supplementary Table 4). Overall, the Asian samples showed a relatively weak LD than the European samples. Because addiction has a complex inheritance mode in which multiple genes exert small effects, the possibility that other genetic variants on this gene or another gene that is in LD with the *HTR2A* variants should also be investigated. For example, for the two *HTR2A* SNPs, Thr25Asn and His452Tyr, which cause amino acid replacement, Thr25Asn is located within the same haplotype block as rs6313 and rs6311 and His452Tyr maps in another dense haplotype block. The *ESD* gene, a genetic marker for retinoblastoma and Wilson's disease, is very close to the *HTR2A* gene.

This study also has some limitations. For example, small sample size may not have sufficient power to detect risk variants, particularly when the effects of variants are small. The sample size in this meta-analysis was moderate or small, particularly for subgroup analyses, and therefore the subgroup results should be interpreted cautiously, since they could be influenced by factors such as random genotyping errors from individual association studies. Secondly, two SNPs and multiple phenotype and ethnicity subgroups were analyzed in this meta-analysis (Table 1). Although the subgroup analyses were not independent, and the two SNPs were in very strong LD, multiple comparisons were still noted. Some less significant associations might not survive when stringent Bonferroni correction is applied.

To conclude, evidence of association was found at the *HTR2A* gene, particularly for rs6313 with alcohol and heroin dependence. The vulnerability effect of rs6311 warrants further investigation in larger samples. Our findings support a contribution of the *HTR2A* gene to the susceptibility to alcohol and heroin dependence.

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Conflict of interest None.

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