A Meta-Analysis of the Associations between *SLC6A4* Promoter Polymorphism (5-HTTLPR) and Risk of Alcohol Dependence

Short running title: 5HTTLPR and Alcohol Dependence

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ABSTRACT

Serotonin reuptake variation is linked to a functional polymorphism in the promoter region of the *SLC6A4* gene on chromosome 17. It is plausible that variations in genetically determined *SLC6A4* activity may modify the risk of alcohol dependence. To determine whether this allele is associated with alcohol dependence, the authors conducted a systematic review and a meta-analysis. Twenty five studies with 8,885 participants were included. The meta-analysis was conducted using a random-effects model. Overall, the results did not support the association between alcohol dependence and *SLC6A4* promoter polymorphism for the dominant, recessive and additive genetic risk models respectively (ORs = 0.99 [95% CI: 0.83, 1.18], 0.86 [95% CI: 0.71, 1.03], and 0.88 [95%CI: 0.69, 1.13]). When effect modification was tested for gender, race/ethnicity, presence/absence of a psychiatric disorder, year of publication, and diagnostic criteria, none of the factors were significantly associated with alcohol dependence. The findings in this meta-analysis suggest that *SLC6A4* promoter polymorphism is not associated with alcohol dependence.

Key Words:

5HTTLPR polymorphism; *SLC6A4* gene; meta-analysis; alcohol dependence

INTRODUCTION

Alcohol dependence is a complex trait, with multigenic etiology influenced by environmental and genetic factors where heritability varies between 40% to 60% (Kimura, 2011; Sander et al., 1997). The process of addiction at the molecular level involves networks in which hundreds of genes may be involved. However, only a few functional genes moderating vulnerability to alcohol dependence have been identified. Alcohol dependence is characterized by obsessive, compulsive and uncontrolled consumption of alcohol associated with behavior of maladaptation and emotional disturbance (Grant et al., 2004; Oroszi, 2004).

Several studies have demonstrated that the serotonin (5-hydroxytryptamine: 5-HT) system is associated with alcohol dependence (Gorwood et al.,2000; Lesch, 2005). Consequently, genetic analyses of 5-HT signaling in alcohol dependence have focused on the serotonin transporter gene *SLC6A4* due to its main role in the adjustment of serotonergic neurotransmission (Lesh et al., 1996; Matsushita et al., 2001). Serotonin reuptake variation is linked to a 44 base-pair deletion/insertion polymorphism, customarily called 5-HTTLPR, in the promoter region of the *SLC6A4* gene on chromosome 17q11.1-q12 (Lesh et al., 1996). This polymorphism affects serotonergic neurotransmission by reuptake of synaptic serotonin, ending neurotransmission (Sander et al., 1997). The short allele is associated with lower transcriptional activity, lower serotonin binding and uptake in platelets and lymphoblasts compared with the long allele yielding differential expressions of *SLC6A4* (Kranzler et al., 2002; Matsushita et al., 2001). Evidence suggests that the *SLC6A4* promoter polymorphism may play an important role in the pathogenesis of alcohol dependence (Dick et al., 2007; Lesch, 2005).

Furthermore, the *SLC6A4* gene may regulate the duration and amplitude of serotonin response to alcohol dependence (Kenna et al., 2012).

A number of studies and previous reviews have reported an association between *SLC6A4* promoter polymorphism and alcohol dependence (Choi et al., 2006; Hallikainen et al., 1999; Hammoumi et al., 1999). In particular, studies showed an increased frequency of the homozygous SS genotype in alcohol dependent individuals compared to controls (Hammoumi et al., 1999; Lichtermann et al., 2000). However, the genetic effect of the *SLC6A4* promoter polymorphism was more pronounced in patients with a history of withdrawal syndromes including seizures and/or delirium; suicide attempts; and depression (Gokturk et al., 2008; Marques, 2006; Preuss, 2001; Sander et al., 1997).

Outcomes that were reported in several case-control studies analyzing the risk of the *SLC6A4* promoter polymorphism with alcohol dependence were inconsistent. Earlier studies reported an association with the short allele in alcohol dependent individuals compared with non-alcohol dependent controls; others have reported an association with the long allele; whereas some studies did not find an association with either allele (Choi et al., 2006; Gokturk et al., 2008; Hallikainen et al., 1999; Ishiguro et al., 1999; Kweon, 2005; Matsushita et al., 2001; Thompson, 2010). In addition, several studies have also investigated the association between *SLC6A4* promoter polymorphism and psychiatric disorders in adults, including both internalizing disorders such as depression and anxiety and externalizing disorders such as antisocial personality disorder (ASPD), conduct disorder (CD) and attention deficit hyperactivity disorder (ADHD). However, results were mixed (Lotrich, 2004; Schinka, 2004).

MATERIALS AND METHODS

Objective

The aim of this meta-analysis was to systematically review available evidence from case-control studies examining the association of the *SLC6A4* promoter polymorphism with alcohol dependence.

Inclusion Criteria

Studies were selected if they evaluated an association between *SLC6A4* promoter polymorphism and alcohol dependence and included non-alcohol dependent control groups. Studies included were case-control studies, published in peer-reviewed journals, contained original and independent data, described or referenced appropriate genotyping methods and protocols and published only in the English language. We identified eligible studies by searching Medline (National Library of Medicine, Bethesda Maryland), BIOSIS (Thompson Scientific, Stanford, Connecticut) and ISI Web of Science databases for those published before November 1, 2013. The MEDLINE search strategy was organized using Medical Subjects Headings (MeSH), as follows: 5-HTTLPR polymorphism, *SLC6A4* gene or genetic or genotype or polymorphism or allele or haplotype or genes or genetic polymorphism or genotype and alcohol abuse, or chronic alcoholic or alcoholism or alcoholic intoxication or alcohol dependence, alcoholism or alcohol and dependence or alcohol dependence. The searches in BIOSIS and ISI Web of Science were performed using the same strategy but adjusting the search terms to adjust the differing structure of the MeSH tree.

Data Extraction

Two investigators K.V. and J.A. independently extracted data on: study design; population characteristics (sample size, country of origin, ethnicity, age, sex) selection of diagnostic criteria used to classify participants as alcohol dependent group; selection and categorization strategy for the control group; inclusion or exclusion of psychiatric disorders; genotyping method; blinding of genotyping; and genotype and allelic frequencies. Reference lists of all relevant papers were cross-checked for possible inclusion. Studies were categorized based on place of origin and were divided in three categories: a) Asian including China, Japan and Korea; b) European including France, Finland, Germany, Croatia, Italy, Poland and Spain; c) Hispanic including Mexico.

Data Analysis

The data on the association between *SLC6A4* promoter polymorphism and alcohol dependence were pooled by means of weighted average to generate summary odds ratios using a random-effects model. This way, each study was weighted by the inverse of its variance, taking into account within/between-studies variances. Since previous studies found a significant effect on both the S and the L alleles with *SLC6A4* promoter polymorphism on the risk of alcohol dependence, we used the dominant (LL vs. SS + SL), the recessive (LL + LS vs. SS) and additive genetic risk models treating the S allele as the risk allele for this meta-analysis. In addition, effect modification was analyzed using multivariate meta-regressions with both the log effect sizes and corresponding standard errors as outcomes running Monte Carlo permutations (Iterations=1000) to reduce chances of type I error (Higgins and Thompson,

2004). Factors that were considered included gender, race/ethnicity, presence/absence of a psychiatric disorder, year of publication, and diagnostic criteria. Publication and small-studies effect biases (studies more likely to be published because of statistical significance or size) were evaluated by creating a funnel plot and employing the Egger linear regression test. Sensitivity analysis was done by investigating the influence of each study on the pooled estimate to identify the ones that exerted a disproportionate impact. All analyses were performed in STATA (Version 11; Stata Corporation, College Station, TX, USA) and a *P* value of <0.05 was considered to be statistically significant for the associations between *SLC6A4* promoter polymorphism and alcohol dependence.

RESULTS

Description of Quality of Included Studies

We reviewed 971 titles and abstracts and obtained 175 full-text papers. We identified 25 case-control studies that met inclusion criteria with a total of 8,885 participants. Characteristics of included studies are listed numerically and summarized in *Table 1*. **Five** studies (Edenberg et al., 1998; Lichtermann et al., 2000; Hill et al., 2013, Samochwiec et al., 2006 and Dick et al., 2007) were not included in the analysis because they used the transmission disequilibrium (TDT) and Pedigree Disequilibrium test (PDT) methodology which may compromise the ease of comparison to the majority of studies using case-control methods. Two other studies (Nelissery et al., 2003 and Philibert et al., 2008) were also excluded because of insufficient publicly available data as shown in *Figure 1*.

All studies reported genotype and/or allelic frequencies except for study 11 which reported only allele frequencies. Determination of alcohol dependence was established using several diagnostic criteria: the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised; Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; International Statistical Classification of Diseases and Related Health Problems, Tenth Revision; Alcohol Use Disorders Identification Test; International Classification of Disease; European Addiction Severity Index and Michigan Alcohol Screening Test. Only study 2 used autopsy-confirmed cases of alcohol-related pathology to identify cases of alcohol dependence. Cases were classified as alcohol dependent without psychiatric disorders and alcohol dependent with psychiatric disorders. Controls were classified as non-alcohol dependent. The majority of studies did not screen for psychiatric disorders in controls (references 2, 3, 4, 5, 6, 7, 10, 11, 14, 15, 17, 18, 20, 22, 23, and 24 in Table 1). Eleven studies analyzed alcohol dependence and psychiatric disorders including, major depression, suicide attempts and antisocial personality (references 3, 4, 7, 8, 12, 14, 15, 16, 17, 19, and 23 in Table 1). Matching for sex or age was included in studies 7 and 17, while only studies 12, 18, and 23 reported blinding of clinical investigators. Finally, statistically significant departures from Hardy-Weinberg Equilibrium in the controls were detected in studies 1 and 3.

Association between *SLC6A4* promoter polymorphism and alcohol dependence

Twenty five studies, including a total of 4388 cases and 4497 controls, reported genotype frequencies for *SLC6A4* promoter polymorphism and alcohol dependence. For all studies combined, when a dominant, recessive or additive model was assumed, non significant

associations were found between *SLC6A4* promoter polymorphism and alcohol dependence for all genetic risk models respectively (ORs = 0.99 [95% CI: 0.83, 1.18], 0.86 [95% CI: 0.71, 1.03], and 0.88 [95%CI: 0.69, 1.13]). Similar results were found for allele frequency (OR = 0.96, 95 % CI: 0.86, 1.07). Significant heterogeneity between studies was observed (Cochran Q test *p*-value < 0.10; I^2 ranging from 41.3% to 61.8%) as shown in Figures 2-5. When effect modification was tested, none of the factors included in the meta-regression analysis significantly moderated the association between *SLC6A4* promoter polymorphism and alcohol dependence in any of the models (Table 2). There was a subtle asymmetry on visual observation of funnel plots for the comparison between the dominant, recessive and additive genetic risk models suggesting publication bias. However, the Egger tests for small-studies effect were not significant. When the influence analysis test was performed, no study omitted significantly changed the overall estimates.

DISCUSSION

This meta-analysis did not find an overall association between *SLC6A4* promoter polymorphism and alcohol dependence. Substantial variations between studies were observed. The observed heterogeneity could be due to a difference in how the samples were selected and screened or to methods of genotyping or interaction with other risk factors. We tested whether genotype frequencies in the control groups were in agreement with Hardy Weinberg equilibrium because a departure from it may point to genotyping errors resulting in misleading results. However, exclusion of the two studies with significant deviation from Hardy-Weinberg

equilibrium did not account for the heterogeneity nor significantly changed the pooled estimates.

All of the studies had one or more methodological limitations including: 1) inconsistent screening for control groups for alcohol dependence and/or other psychiatric disorders; 2) lack of matching for or adequate control group; 3) insufficient description of genotyping methods; 4) potential disparity in case definition due to the different diagnostic criteria used; and 5) ambiguity in the severity of alcohol dependence, including how much alcohol was consumed and for how long. Selection of the control groups was especially problematic since many studies included convenience samples rather than population-based controls. These factors may have led to the observed heterogeneity in our meta-analysis.

To further understand the variance that was unaccounted for by *SLC6A4* promoter polymorphism and alcohol dependence, potential effect modifiers were examined in the meta-analysis. However, gender, race/ethnicity, year of publication, presence/absence of psychiatric disorders, and diagnostic criteria did not moderate the overall effect. Results from this meta-analysis differed from Feinn et al (2005), where studies with a co-occurring clinical feature moderated the association with alcohol dependence (Feinn, Nellissery,and Kranzler, 2005). Our results may differ because in addition to the four studies from Feinn et al, (Gorwood et al., 2000; Johann et al., 2003; Sander et al., 1997; and Stoltenberg et al., 2002), we added, Preuss et al., 2001; Marques et al., 2006; Matsushita et al., 2001; Ishiguro et al., 1999; and Gokturk et al., 2008, generating a larger estimate. The heterogeneity of psychiatric disorders in the studies

may have contributed to the negative results. However, grouping them by psychiatric disorder as suggested by Fenn and Colleagues was not possible due to the limited number of studies.

Even though considerable efforts were made to locate all studies, certain limitations in this meta-analysis should be noted. First, only published studies were included in the review and other potentially eligible, but unpublished studies with either positive or negative effects could not be included. Regardless, no publication bias was observed. Second, differences in the methodologies used by the selected studies did not permit for a combination of case-control and family-based studies. Last, overestimation of the odds ratio cannot be ruled out since the modest strength of association found in this study is similar to reported odd ratios by other meta-analysis with similar associations.

Authors of previous reviews, meta-analyses and primary studies have presented contrasting findings on the role of *SLC6A4* promoter polymorphism and alcohol dependence. A recent meta-analysis by Cao et al. (2013) where case-control and family-based studies were evaluated for the association between *SLC6A4* promoter polymorphism (5HTTLPR) and alcohol, heroin, cocaine and methamphetamine abuse found a significant association with alcohol dependence (Cao, 2013). Our meta-analysis excluded family-based studies that used PDT, TDT and haplotype relative risk methodologies, which may have been a contributing factor for the null finding. Alternately, a meta-analysis of case-control studies by McHugh et al. (2010), which excluded PDT, and TDT methods and assessed publication bias, determined that the year of publication and study sample size acted as moderators, making the results non-significant when nine unpublished studies with null results were added.(McHugh, 2010) Consistent with the

findings from McHugh et al., our research added six studies that were not included in their meta-analysis (references 13, 18, 19, 21, 23, and 25 from Table 1), where four of the studies did not find an association between *SLC6A4* promoter polymorphism and alcohol dependence. Alcohol dependence is a complex disorder with multiple subtypes and clinical phenotypes, it may be relevant to identify genetic susceptibility variants affecting variability in serotonin pathway. This may hold the potential for premorbid risk assessment, preventive strategies and treatment individualization for individuals genetically predisposed to alcohol dependence.

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Figure 1. Study selection process

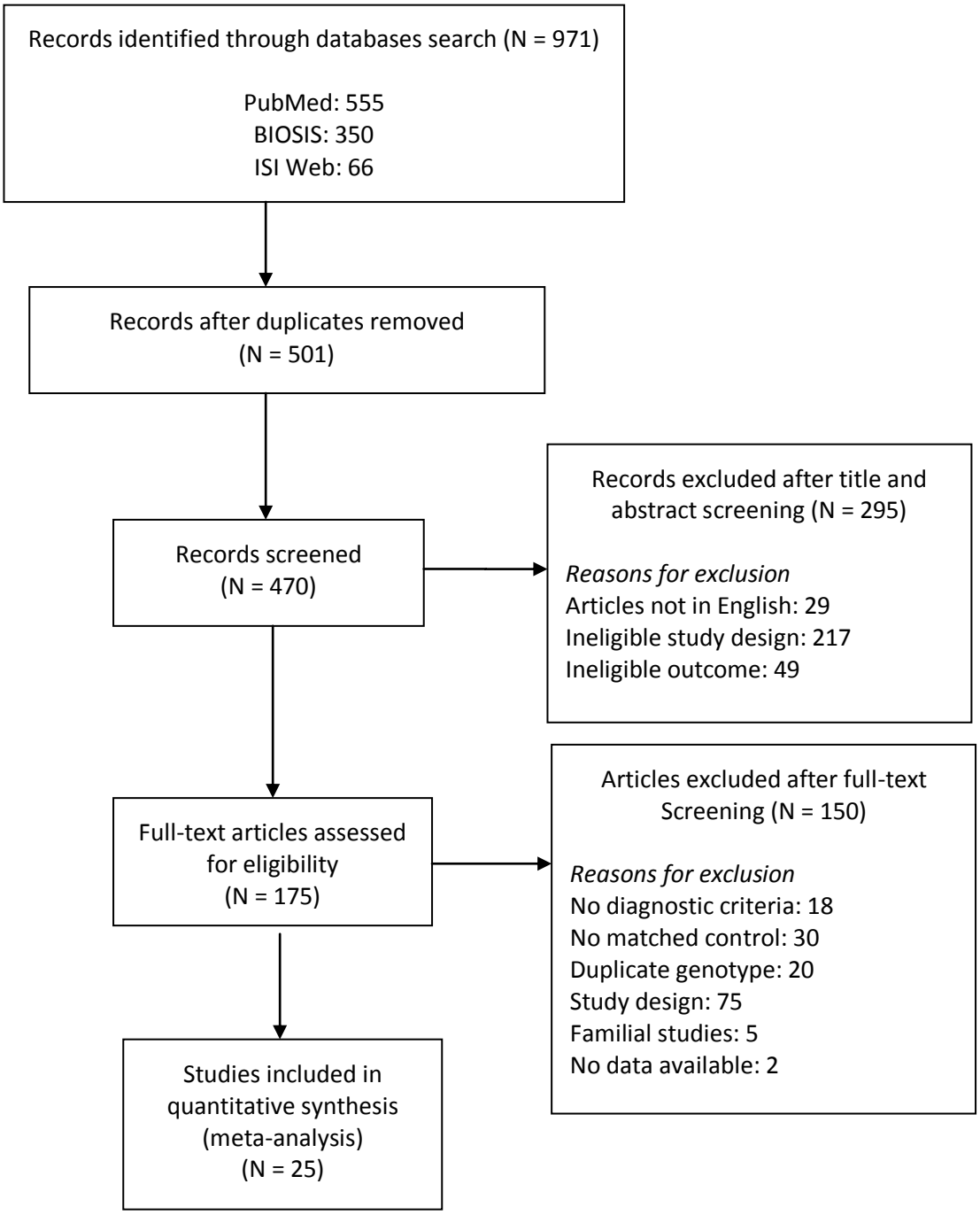

Figure 2. Forest plot showing the association between 5HTTLPR polymorphism and alcohol dependence assuming additive model

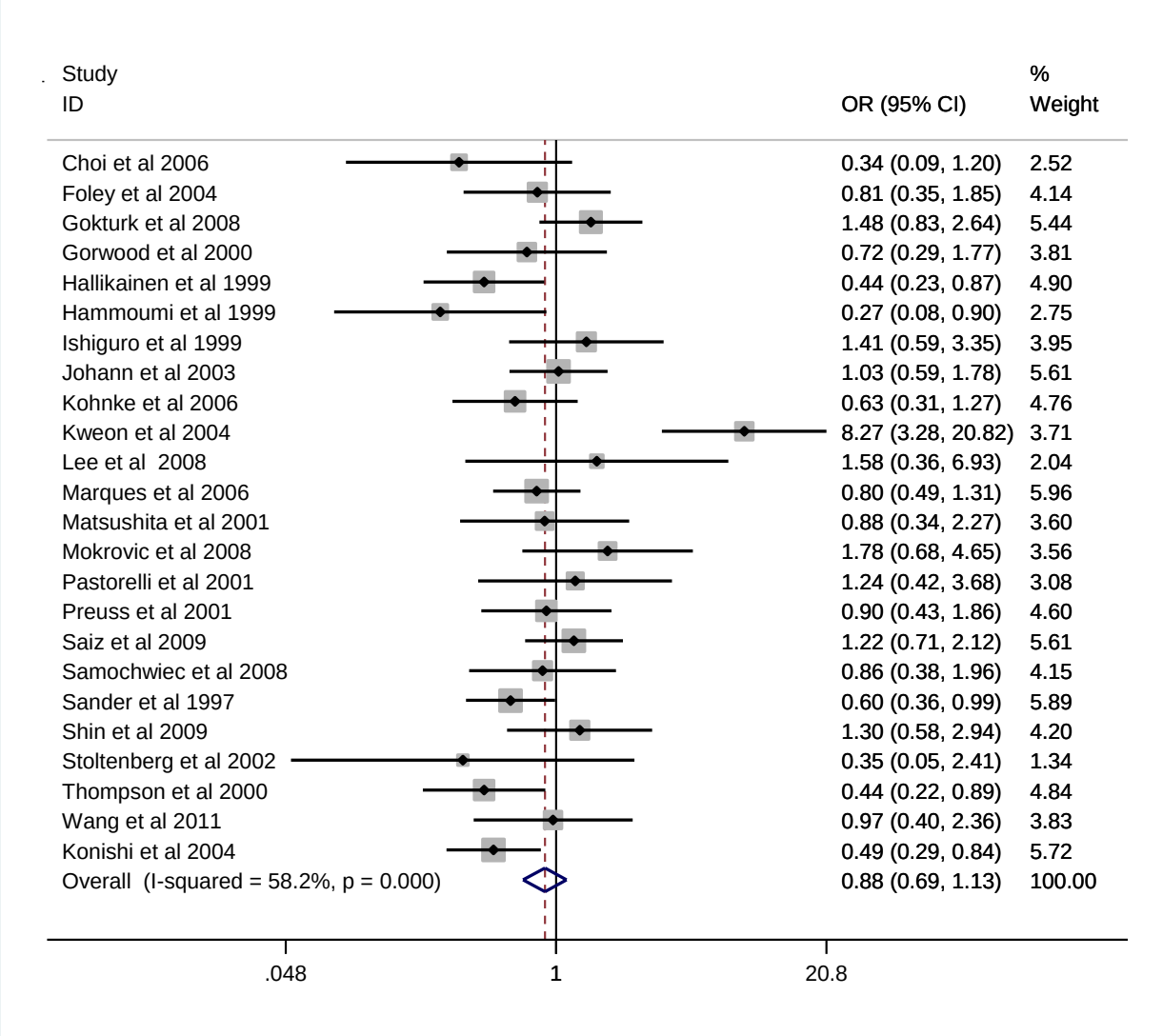

Figure 3. Forest plot showing the association between 5HTTLPR polymorphism and alcohol dependence assuming a dominant (LL vs. SS + SL) model

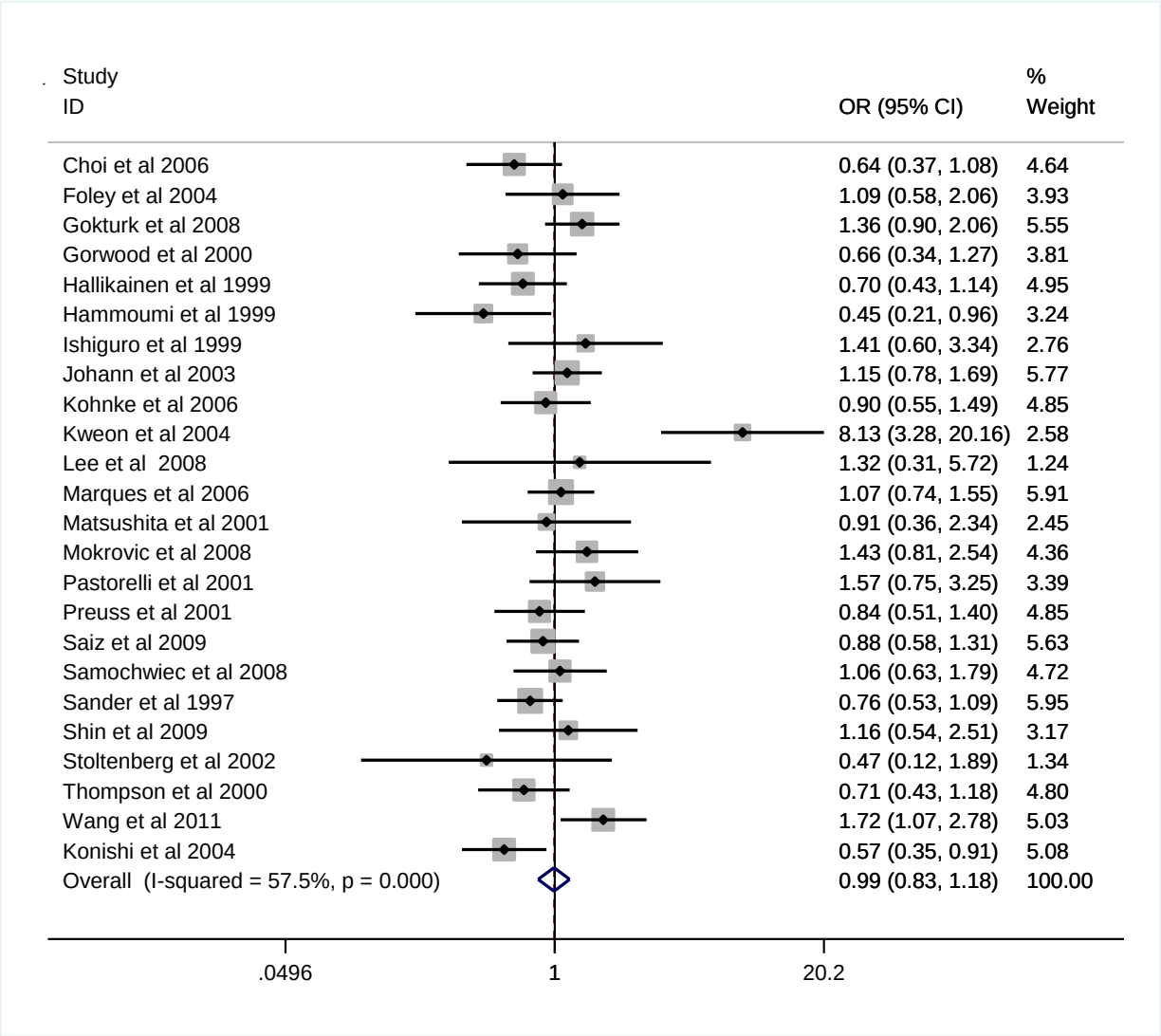

Figure 4. Forest plot showing the association between 5HTTLPR polymorphism and alcohol dependence assuming a recessive (LL + LS vs. SS) model

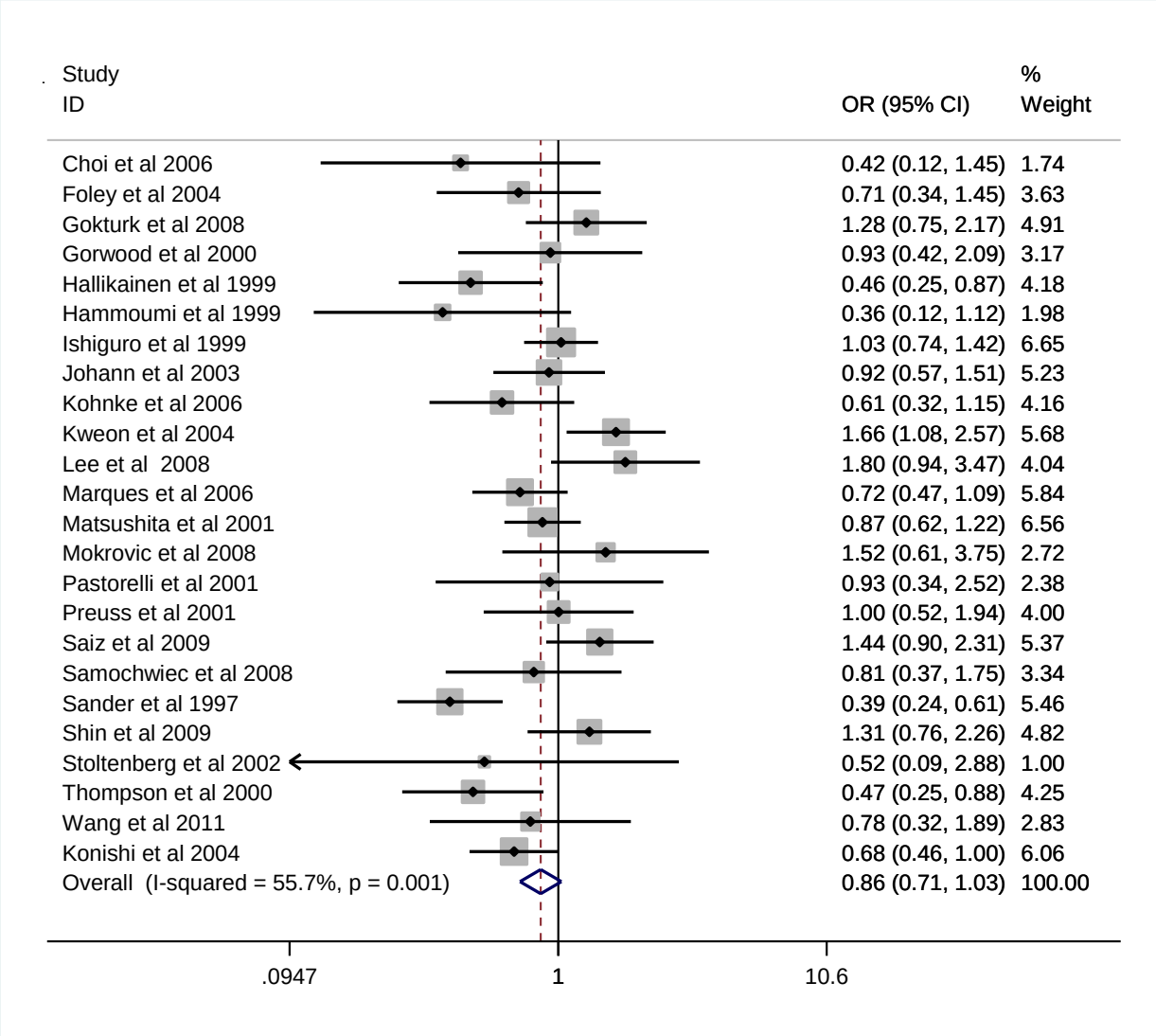

Figure 5. Forest plot of association between 5HTTLPR and alcohol dependence, comparing allele frequency

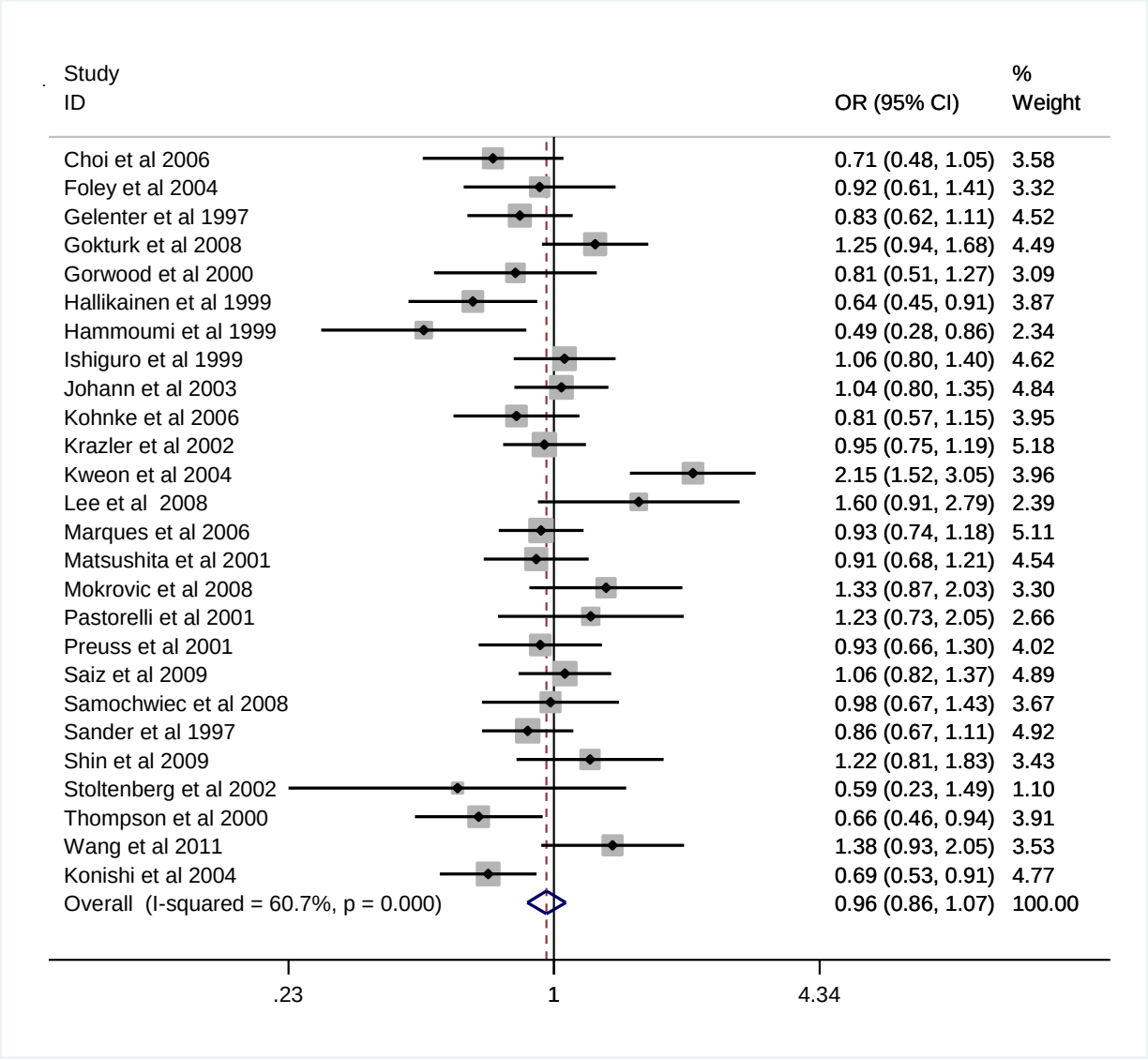

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Table 1. Characteristics of included studies

		Participants characteristics			Genotype Frequencies						ORhet (95% CI)	ORhom (95% CI)	ORAdd (95% CI)
					Cases			Controls					
		Cases	Controls		L/L	L/S	S/S	L/L	L/S	S/S			
1	Choi 2006	Korean alcoholic subjects; DSM-IV and AUDIT criteria; with and without family history of alcohol dependence and without major mental disorder; males; n = 111; mean age 43.5 years	Korean non-alcoholics; without major psychological disorders; males; n = 123; mean age 45.5	Familial	10	27	4	55	64	4	2.3 0.98, 5.84	5.5 0.85, 33.9	2.5 1.1, 5.6
				Non familial	27	37	4				1.2 0.61, 2.3	2.0 0.35, 11.7	1.2 0.76, 2.23
2	Foley 2004	Brain tissue from Caucasian alcoholics; with alcohol related brain damage; n= 74	Non alcoholic brain tissue; with non alcohol related brain damage; n= 108		24	32	18	34	55	19	1.6 0.75, 3.54	1.3 0.58, 0.78	1.5 0.72, 3.1
3	Gokturk 2008	Sweden alcoholic subjects with and without anxiety and/or depressive disorder; ICD10 criteria; interviewed on the history of alcohol abuse and psychiatric illness; n = 110; females; age range 18-75	Sweden non-alcoholic controls from the Survey of Adolescent Life in Vestmanland-2006; n = 631; females; age range 17-18	With Anx /dep	5	13	4	213	285	133	1.5 0.44, 4.73	0.8 0.25, 2.94	1.2 0.43, 3.74
				Without anx/dep	40	33	15				1.0 0.56, 1.94	1.6 0.89, 3.16	1.3 0.70, 2.36
4	Gorwood 2000	French alcoholic subjects with major depressive disorder, antisocial personality disorder, pathological gambling, agoraphobia, and other addictions; DSM-III-R criteria; excluded dementia, schizophrenia, bipolar maniac-depressive disorder; interview; n = 110 males; mean age 43.6	French non-alcoholics without any substance dependence; n = 61 males; age > 35 years old	With suicide	12	30	13	24	26	11	1.4 0. 49, 3.08	1.2 0.52, 3.59	1.2 0.52, 3.28
				Without suicide	21	26	8				0.9 0.48, 2.74	0.4 0.24, 2.79	0.7 0.51, 2.33
5	Hallikainen 1999	Finnish alcoholics subjects, type 1 and type 2; DSM-III-R criteria, MAST test; excluded major mental disorder; n = 166; 114 type 1, 51 type 2; mean age 43.8	Finnish non-alcoholic; interview and screened for alcohol dependence; n = 54 mean age 44.1	Type I	50	37	27	26	20	8	0.96 0.44, 2.1	1.7 0.65, 5.10	1.2 0.62, 2.35
				Type II	15	18	18				1.6 0.58, 4.23	3.9 1.22, 12.8	2.3 0.99, 4.98
6	Hammoumi 1999	French alcoholic subjects; depression disorder excluded; MAST criteria; interview for alcohol	French non-alcoholic controls from a regional transfusion center; n = 38		34	43	25	20	14	4	4.2 0.74, 4.46	3.7 1.03, 16.4	2.2 1.04, 4.74

		dependence; n = 104												
7	Ishiguro 1999	Japanese alcoholic subjects with history of withdrawal or delirium; DSM-IV criteria; major psychiatric disorder or substance dependence other than alcohol or nicotine excluded; interviewed on history of alcohol abuse; n = 166; 153 men, 13 women; mean age 52.2	Japanese non-alcoholics n = 213		16	114	286	201	81	8	0.70 0.25, 1.84	0.71 0.25, 1.80	1.0 0.65, 1.67
8	Johann 2003	German alcoholic subjects with and without ADHD; DSM-IV , ICD-10 criteria; major psychiatric disorder or substance dependence other than alcohol or nicotine excluded; n = 314; 262 men, 52 women; mean age 43.1 years old	German non-alcoholic without addictive or major psychiatric disorder; interview; n = 220	With ADHD	23	34	10	69	116	35	0.88 0.46, 1.7	0.86 0.33, 2.13	0.87 0.49, 1.56
				Without ADHD	85	120	42				0.84 0.55, 1.29	0.97 0.54, 1.75	0.87 0.59, 1.28
9	Kohnke 2006	German alcoholic subjects with no history or family history of psychiatric disorders; DSM-IV criteria; n = 215	German non-alcoholic subjects with no history or family history or psychiatric disorders or addition except for nicotine n=94		75	89	51	35	44	15	1.2 0.67, 2.06	4.1 2.03, 8.16	1.9 1.13, 3.21
10	Konishi 2004	Mexican American alcoholic subjects; DSM-IV criteria; without history of psychiatric disorders. n = 130 males; mean age 38.2 years old	Mexican American non-alcoholic; n = 251; 105 men, 146 females; mean age 32.5 years old		32	90	78	63	112	76	1.6 0.98, 2.58	2.1 1.08, 4.19	1.8 1.09, 2.82
11	Kranzler 2002	European American, African American alcoholic subjects; DSM-III-R criteria; with cocaine and opiod dependence; n = 471, 363 EA, 108 AA; 353 males, 108 females	European American, African American controls; screened for alcohol dependence; n = 235, 192 EA, 43 AA; 138 males, 97 females										
12	Kweon 2004	Korean alcoholic subjects; DSM-IV criteria; semi-structured interview for aggressive behavior; major psychiatric disorder or substance	Korean non-alcoholics; interviewed for psychiatric conditions or neurological disease; family history of		29	40	76	6	65	130	1.0 0.36, 1.75	8.3 3.16, 25.2	1.7 1.07, 2.47

		dependence other than alcohol or nicotine were excluded; n = 145 males	alcoholism or major psychiatric or neurological diseases were excluded; n = 201											
13	Lee 2009	Chinese alcoholic subjects; DSM-IV criteria; clinical structured interview for family alcohol history and psychiatric information; major psychiatric disorder or substance dependence other than alcohol or nicotine were excluded; n = 119	Chinese non-alcoholics; interviewed for psychiatric conditions; family history of alcoholism or major psychiatric or neurological diseases were excluded; n = 76		59	33	5	56	17	3	1.7 0.92, 3.7	1.6 0.37, 6.91	1.8 0.93, 3.46
14	Matsushita 2001	Japanese alcoholic subjects with antisocial personality; DSM-III-R criteria; structure interview for family alcohol history; n = 679 males; mean age 50.5	Japanese non-alcoholics; KAST assessment; n = 270; mean age 50.0		8	214	443	13	84	173	4.1 1.65 , 10.3	4.2 1.71, 10.5	4.0 1.72, 9.87
15	Marques 2006	European Brazilian alcoholic subjects with drug abuse, antisocial personality, major depressive disorder, suicide attempts and ideation and nicotine addiction; DSM-III-R criteria; Semi-Structured Assessment for the Genetics of Alcoholism interview used; males n=114	European Brazilian non-alcoholics; blood donors; interviewed for alcohol and nicotine abuse; CAGE questionnaire used to measure exposure to alcohol; n = 218	Antisocial Personality	8	8	4	24	46	24	0.7 0.28, 1.31	0.5 0.13, 1.84	0.5 0.18, 1.51
				Major depression	8	17	16	24	36	12	1.4 0.53, 3.83	4.0 1.34, 11.9	2.0 0.83, 5.15
				Suicide attempt	13	34	16	56	74	38	1.9 0.96, 4.10	1.8 0.78, 4.20	1.9 0.96, 3.83
16	Mokrovic 2008	Croatian type 2 alcoholics with PTSD, personality disorder, anxiety/depression; DSM-IV criteria; n = 59 males; mean age 32 years	Croatian non-alcoholic blood donors; no personal or family history of alcohol dependence or psychiatric disorder; n = 282; mean age 42 years		26	26	6	102	138	42	1.3 0.50, 3.41	1.7 0.68, 4.53	1.5 0.61, 3.75
17	Pastorelli 2001	Italian alcoholic subjects; seeking treatment or admitted for medical reasons; 80% smokers; 27% polysubstance abusers; interview; n = 60; 45 males, 15 females; mean age, 46.4 years	Italian blood donors matched on sex and age; non-alcoholics; 33% smokers; 0 % polysubstance abusers; interview; n = 64; 51 males, 13 females; mean age, 45 years		26	25	9	21	34	9	0.7 0.25, 2.38	1.2 0.41, 3.77	0.9 0.34, 2.51

18	Samochwiec 2008	Polish alcoholic subjects; ICD-10 criteria; interview; n = 122; 99 males,23 females; mean age 39 years old	Polish non-alcoholic; questionnaires to exclude drug and alcohol dependence; n = 150; 120 males, 30 females; mean age 35 years old		45	43	12	41	48	11	0.82 0.37, 2.05	0.9 0.38, 2.17	0.9 0.38, 2.16
19	Preuss 2001	German alcoholic subjects with suicide attempts; ICD-10, DSM-IV criteria; semi-structured interview; n = 163; 131 males, 32 females	German non-alcoholic controls; psychiatric, mental and substance abuse disorders excluded; n = 117; 56 males, 61 females	With suicide	11	30	11	41	58	18	0.8 0.41, 1.91	0.4 0.74, 1.18	0.6 0.21, 1.43
				Without suicide	40	57	14				1.3 0.52, 2.96	1.2 0.55, 2.84	1.3 0.62, 2.65
20	Saiz 2009	Spanish alcoholic subjects; substance dependence other than nicotine excluded; interview; EuropASI criteria; n = 165; mean age 47.8	Spanish non-alcoholic controls; interview; n = 420; mean age 31.6		44	93	27	124	203	93	1.5 0.94, 2.73	0.8 0.45, 1.46	1.1 0.72, 1.72
21	Sander 1997	German alcoholic subjects; International Classification of Disease criteria; interviewed on the history of alcohol abuse; n = 315; 271 males, 44 females; mean age 42.2	German non-alcoholics; blood donors and other were interviewed for absence of addictive disorder or previous psychiatric treatment; n = 216		32	44	27	81	100	35	1.7 0.98, 3.20	0.3 0.0 7, 2.91	0.9 0.68, 1.67
22	Shin 2009	Chinese alcoholic subjects; DSM-IV criteria; semi structured interview; n = 68 males only; mean age 72.5	Chinese non-alcoholics; semi-structured interview; n = 232 males only; mean age 72.5		10	26	32	30	77	125	1.2 0.71, 2.46	1.3 0.52, 2.95	1.1 0.74, 2.28
23	Stoltenberg 2002	Caucasian biological family (50 families) alcohol dependents; DSM-III-R criteria; NOE-FFI personality test; n = 31	Caucasian biological family non-alcohol dependents; n = 13	AAL	3	10	4	5	6	2	1.1 0.36, 23.9	3.3 0.24, 55.3	1.8 0.57, 5.81
				NAAL	4	6	4				1.2 0.16, 9.89	2.5 0.19, 39.5	1.4 0.42, 4.67
24	Thompson 2000	Blood samples from volunteers from the CAMH; DSM-IV criteria;	Blood samples from non-alcoholic volunteers from		43	52	36	51	55	19	1.1 0.62, 2.03	2.2 1.07, 4.76	2.2 1.20, 4.45

		psychiatric disorders excluded; n = 131	CAMH; n = 125											
25	Wang 2011	Chinese alcoholic subjects; DSM-IV criteria; major psychiatric disorder or substance dependence other than alcohol or nicotine excluded;	Non-alcoholic controls; without major psychiatric disorder or substance dependence ; n = 214; average	83	26	9	124	77	13	0.5 0.29, 0.87	1.0 0.37, 2.7	0.5 0.36, 0.94		

CI = confidence interval; ALL = antisocial alcoholic; NAAL = nonalcoholic; ORhet = odds ratio of heterozygotes vs. homozygotes for the major allele; ORhom = odds ratio of homozygotes for the minor allele vs. homozygotes for the major allele; ORadd = odds ratio of each increased r-fold for heterozygotes and 2r-fold for homozygotes allele

Table 2: Analysis of effect modification by study characteristics

Characteristics	Allele frequency		Recessive model		Dominant model		Additive model	
	B (95% CI)	P[†]	B (95% CI)	P[†]	B (95% CI)	P[†]	B (95% CI)	P[†]
Publication year	0.02 (-0.01, 0.05)	.12	0.02 (-0.01, 0.06)	.21	-0.03 (-0.08, 0.02)	.22	-0.06 (-0.25, 0.13)	.44
Gender	-0.07 (-0.33, 0.18)	.56	-0.09 (-0.39, 0.20)	.50	0.07 (-0.40, 0.53)	.77	-0.19 (-1.01, 0.62)	.57
Race/ethnicity[††]	-0.06 (-0.32, 0.19)	.63	-0.02 (-0.32, 0.27)	.87	0.26 (-0.21, 0.73)	.43	-0.14 (-1.56, 1.28)	.81
Psychiatric condition	-0.26 (-0.58, 0.07)	.12	-0.10 (-0.37, 0.16)	.41	0.54 (-0.10, 1.18)	.09	1.49 (-2.08, 5.05)	.33
Diagnostic criteria								
ICD-10	0.11 (-0.32, 0.55)	.59	0.07 (-0.46, 0.60)	.79	-0.32 (-1.13, 0.50)	.26	-0.94 (-5.46, 3.58)	.62
DSM IV	0.36 (-0.03, 0.76)	.07	0.33 (-0.14, 0.81)	.16	-0.62 (-1.36, 0.12)	.10	-1.45 (-5.29, 2.39)	.44

[†] Based on 1000 Monte Carlo simulations.

[††] Race/ethnicity dichotomized in European vs. Non-Europeans

Analysis for effect modification performed using multivariate meta-regressions with log effect sizes and its standard error as outcomes and characteristics as independent variables.