ORIGINAL RESEARCH

Association of metabotropic glutamate receptor 3 gene polymorphisms with schizophrenia risk: evidence from a meta-analysis

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Abstract: To date, the role of metabotropic glutamate receptor 3 (GRM3) rs274622, rs1468412, rs917071, rs6465084, and rs2299225 polymorphisms in schizophrenia remains controversial. To provide a clearer picture for the effect of the five most studied GRM3 polymorphisms on risk of schizophrenia, this meta-analysis with eligible data from published studies was performed. Relevant case–control studies were retrieved by literature search and selected according to established inclusion criteria. Odds ratios with 95% confidence intervals were used to assess the strength of association. A total of 33 individual studies were identified and included in our meta-analysis: nine for rs1468412, with 5,314 cases and 6,147 controls; six for rs917071, with 2,660 cases and 3,517 controls; seven for rs274622, with 3,820 cases and 4,015 controls; five for rs2299225, with 3,492 cases and 3,735 controls; and six for rs6465084, with 4,960 cases and 5,613 controls. However, no significant association was found between these GRM3 polymorphisms and schizophrenia in the overall population. With respect to rs1468412 polymorphism, a finding of very borderline statistical significance emerged in dominant comparison model for non-Asian populations, calling for large-scale verification to assess the marginally elevated risk of schizophrenia. In conclusion, these GRM3 polymorphisms have limited effect on the risks of schizophrenia. Further large and well-designed studies are needed to confirm this conclusion.

Keywords: schizophrenia, GRM3, SNP, meta-analysis

Introduction

Schizophrenia is one of the most detrimental psychiatric disorders, with high heritability[1] and a median incidence of 15.2/100,000 persons worldwide.[2] Plenty of efforts have been made to understand its pathological mechanisms, and now it is suggested that genetic vulnerability with nongenetic factors can act in combination to lead to the development of schizophrenia.[3] In recent years, with increased knowledge of human gene functions and architecture, it has become clear that differences in genetic backgrounds, such as single nucleotide polymorphisms (SNPs), could substantially influence schizophrenia risk.[3–5]

Glutamate has been implicated as the most ubiquitous excitatory neurotransmitter in the central nervous system. The glutamatergic neurotransmission plays important roles in various brain functions, including motor control, learning and memory, cognition, and neural development.[6] Moreover, evidence has indicated that hypofunction of glutamatergic neurotransmission may be important in the etiology and pathophysiology of schizophrenia.[7–9] As a member of the group II metabotropic glutamate receptors (mGluR), mGluR3 (encoded by the *GRM3* gene) functions as the regulator of glutamate neurotransmission and synaptic plasticity.[10,11] Given the prior evidence,

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GRM3, along with other glutamatergic genes, is a candidate gene for schizophrenia.

The human GRM3 gene rs274622, rs917071, rs1468412, rs6465084, and rs2299225 loci are five commonly studied polymorphisms for schizophrenia. The rs274622 locus, along with other polymorphisms, might be useful as a predictor of negative-symptom improvement in persons with schizophrenia treated with olanzapine.[12] In combination with the rs757656 polymorphism, the rs917071 locus has been associated with alcohol dependence,[13] which could have substantial influence on schizophrenic exacerbation.[14] As for the rs1468412 locus, it has been suggested to be associated with worsened cognitive and symptom response to antipsychotic therapy for schizophrenia.[15] For the rs2299225 locus, several genetic epidemiological studies have been performed to assess its association with schizophrenia risk.[16–18] rs6465084 has been described as a functional polymorphism that may influence glutamate concentrations, and the minor allele of this SNP was associated with poor performance on several cognitive test.[19,20] As well as helping verify whether these SNPs might contribute etiologically to the schizophrenia, rather than just pathophysiologically or therapeutically, several genetic association studies have been performed to identify common risk variants at the GRM3 gene locus. However, the evidence for the association between GRM3 polymorphisms and schizophrenia risk is still controversial. The most likely reasons for the inconsistencies among these studies could be tremendous differences in sample size, diverse ethnic background, incompatible statistical methods, and possible sampling bias. To provide a clearer picture for the effect of these five GRM3 polymorphisms on the risk of schizophrenia, we conducted this meta-analysis with eligible data from published studies.

Materials and methods
Search strategy
Systematic searches of the PubMed database (to November 2, 2014) were performed using the search terms: "GRM3", "metabotropic glutamate receptor 3", "polymorphism", "SNP", "variant", and "schizophrenia". The search was limited to human studies. All eligible studies were retrieved, and their bibliographies were checked for other relevant research. When the data set for the same patient population was used in several publications, only the most recent or complete study was included for further analysis. An additional manual search was also conducted to add any recently published studies.

Inclusion criteria
The following criteria were used for the study selection: 1) evaluate the association between five GRM3 polymorphisms (rs1468412, rs274622, rs917071, rs2299225, and rs6465084) and schizophrenia; 2) case–control studies; 3) sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI); and 4) studies with full-text articles in English.

Data extraction
Data were carefully evaluated and extracted from all eligible studies by two investigators independently according to the inclusion criteria listed above. When disagreement occurred, the third investigator was invited to discuss and check the data until a consensus was reached. The following elements were collected from eligible studies: first author's name, year of publication, country, ethnicity, diagnostic criteria of schizophrenia cases, genotype frequency of cases and controls, and the results of Hardy–Weinberg equilibrium (HWE) test.

Statistical analysis
Departure from the HWE for the control group in each study was assessed with Pearson's goodness-of-fit χ^2 test with 1 *df* by an online program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl), and violation of the HWE was determined using a threshold of $P<0.05$. The strength of association between GRM3 polymorphisms and schizophrenia risk was measured by ORs with 95% CIs. Pooled ORs were calculated for homozygote comparison (aa versus AA, a being the minor allele and A the major allele), heterozygote comparison (Aa versus AA), dominant model (aa+Aa versus AA), and recessive model (aa versus Aa+AA), respectively. Studies that have no events in either arms (schizophrenia and control groups) were excluded from the corresponding comparison model. Heterogeneity among pooled studies was investigated by the χ^2-based Cochran's Q test[21] and I^2 statistics.[22] To be more conservative, heterogeneity was considered to be present when the Cochran Q test P-value was less than 0.1, then a random-effect model (DerSimonian–Laird method)[23] was utilized; otherwise, a fixed-effect model (Mantel–Haenszel method)[24] was utilized. Inconsistency across studies was accessed by the I^2 statistic, and $I^2 <25\%$, 25%–75%, and $>75\%$ were considered to represent low, moderate, and high degree of heterogeneity, respectively.[22] To explore the source of heterogeneity among the studies of this meta-analysis, a Galbraith plot was generated to detect the potential outliers, which could largely contribute to the heterogeneity.[25] Stratification analyses were performed to quantify the

different effects of ethnicity. Sensitivity analysis was carried out through omitting individual study in turn to check the consistency of the results. Publication bias was evaluated by visual inspection of funnel plots and Egger's test ($P<0.05$ was considered a significant publication bias).[26]

All statistical tests were performed with metafor (version: 1.9-4)[27] and meta (version: 3.8-0; http://cran.r-project.org/web/packages/meta/) packages of R (version 3.1.1).

Results

Study characteristics

The initial literature search through the PubMed database yielded 42 published articles. When reviewed in full-text, eight had no usable reported data, two was not case–control studies, 12 were not concerned with schizophrenia risk, three were reviews, two were not GRM3 research, two were not concerned with these five GRM3 polymorphisms, and two

were not in English. All these articles were excluded. Among the remaining eleven articles, studies presented separate ORs by different polymorphisms, ethnicity, or cohort, and each of them was considered separately for pooling analysis. Therefore, nine studies for rs1468412 polymorphism (5,314 cases and 6,147 controls),[16–18,28–32] seven studies for rs274622 (3,820 cases and 4,015 controls),[16–18,28,29,32] six studies for rs917071 (2,660 cases and 3,517 controls),[16,28–32] six studies for rs6465084 (4,960 cases and 5,613 controls),[20,30–34] and five studies for rs2299225 (3,492 cases and 3,735 controls)[16–18,32] were included eventually. A flowchart of study selection is shown in Figure 1, and the main characteristics of eligible studies are summarized in Table 1.

Quantitative synthesis

The associations between these GRM3 polymorphisms and schizophrenia risks were analyzed and summarized ORs for
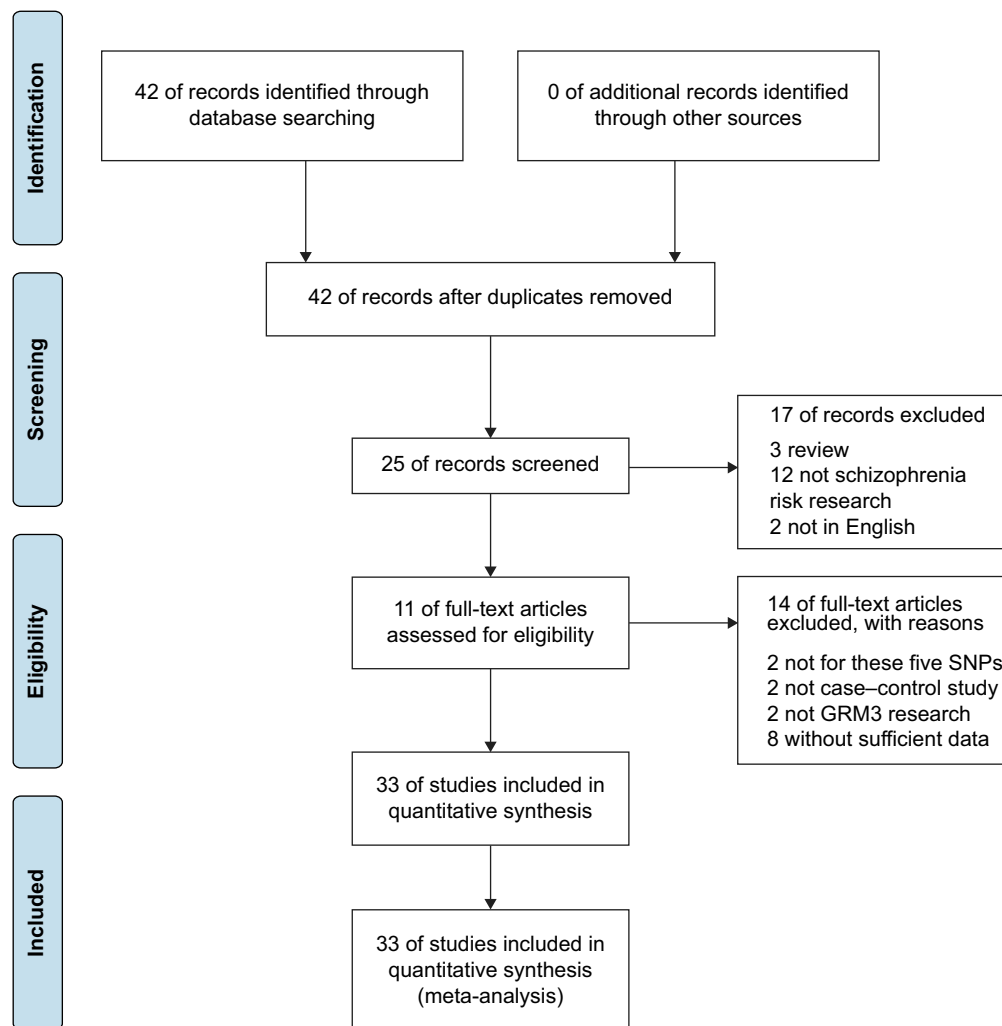

Figure 1 Flowchart of study selection in this meta-analysis.
Abbreviation: SNPs, single-nucleotide polymorphisms.

Table I Characteristics of the studies included in this meta-analysis

Author	Year	SNP	Country	Ethnicity	Diagnostic criteria of cases	Case (AA/Aa/aa)	Control (AA/Aa/aa)	HWE
Jia et al[16]	2014	rs1468412	PRC	Asian	*DSM-IV*	278/131/24	405/188/26	0.48
Jönsson et al[30]	2009	rs1468412	Denmark, Sweden, and Norway	Caucasian	ICD-10	413/352/69	796/567/103	0.88
Bishop et al[28]	2007	rs1468412	USA	Mixed	CASH	125/87/20	92/55/18	0.03
Albalushi et al[17]	2008	rs1468412	Japan	Asian	*DSM-IV*	319/142/18	293/163/21	0.78
Albalushi et al[17]	2008	rs1468412	Japan	Asian	*DSM-IV*	924/457/55	935/445/52	0.92
Tochigi et al[32]	2006	rs1468412	Japan	Asian	*DSM-IV*	265/113/24	297/145/15	0.60
Norton et al[31]	2005	rs1468412	Germany	Caucasian	*DSM-IV*	345/264/54	375/274/49	0.91
Chen et al[18]	2005	rs1468412	PRC	Asian	*DSM-IV*	485/222/28	503/208/22	0.93
Fujii et al[29]	2003	rs1468412	Japan	Asian	*DSM-IV*	71/25/4	54/37/9	0.47
Jia et al[16]	2014	rs274622	PRC	Asian	*DSM-IV*	297/109/24	459/142/16	0.21
Bishop et al[28]	2007	rs274622	USA	Mixed	CASH	92/113/27	71/75/19	0.90
Albalushi et al[17]	2008	rs274622	Japan	Asian	*DSM-IV*	312/150/13	311/152/14	0.37
Albalushi et al[17]	2008	rs274622	Japan	Asian	*DSM-IV*	999/403/37	1,013/390/35	0.73
Tochigi et al[32]	2006	rs274622	Japan	Asian	*DSM-IV*	286/103/13	318/137/13	0.70
Chen et al[18]	2005	rs274622	PRC	Asian	*DSM-IV*	557/175/10	562/177/11	0.49
Fujii et al[29]	2003	rs274622	Japan	Asian	*DSM-IV*	74/23/3	78/19/3	0.19
Jia et al[16]	2014	rs917071	PRC	Asian	*DSM-IV*	259/150/24	374/211/34	0.56
Jönsson et al[30]	2009	rs917071	Denmark, Sweden, and Norway	Caucasian	ICD-10	412/353/69	769/588/111	0.92
Bishop et al[28]	2007	rs917071	USA	Mixed	CASH	115/96/21	87/63/15	0.47
Tochigi et al[32]	2006	rs917071	Japan	Asian	*DSM-IV*	233/144/25	251/181/36	0.67
Norton et al[31]	2005	rs917071	Germany	Caucasian	*DSM-IV*	337/272/50	373/278/46	0.55
Fujii et al[29]	2003	rs917071	Japan	Asian	*DSM-IV*	58/39/3	57/37/6	0.99
Mounce et al[33]	2014	rs6465084	USA	Mix	*DSM-IV-TR*	33/34/7	51/31/4	0.79
Jönsson et al[30]	2009	rs6465084	Denmark, Sweden, and Norway	Caucasian	ICD-10	468/317/49	874/511/76	0.91
Mössner et al[20]	2008	rs6465084	Germany	Caucasian	ICD-10	381/224/26	279/212/28	0.13
Nunokawa et al[34]	2008	rs6465084	Japan	Asian	*DSM-IV*	2,045/305/8	2,132/289/12	0.51
Tochigi et al[32]	2006	rs6465084	Japan	Asian	*DSM-IV*	353/38/6	349/53/4	0.22
Norton et al[31]	2005	rs6465084	Germany	Caucasian	*DSM-IV*	375/246/45	406/255/47	0.42
Jia et al[16]	2014	rs2299225	PRC	Asian	*DSM-IV*	393/31/0	564/48/0	0.31
Albalushi et al[17]	2008	rs2299225	Japan	Asian	*DSM-IV*	388/83/8	362/105/10	0.47
Albalushi et al[17]	2008	rs2299225	Japan	Asian	*DSM-IV*	1,110/309/18	1,101/310/22	0.97
Tochigi et al[32]	2006	rs2299225	Japan	Asian	*DSM-IV*	312/81/8	373/85/5	0.95
Chen et al[18]	2005	rs2299225	PRC	Asian	*DSM-IV*	662/83/6	685/63/2	0.67

Abbreviations: SNP, single-nucleotide polymorphism; HWE, Hardy–Weinberg equilibrium (significant at the 0.05 level); *DSM, Diagnostic and Statistical Manual of Mental Disorders*; ICD, International Classification of Diseases; CASH, comprehensive assessment of symptoms and history; PRC, People's Republic of China.

schizophrenia risk of these five polymorphisms are shown in Table 2. The overall ORs by the fixed/random-effect model showed no significant association between GRM3 rs274622, rs917071, rs1468412, rs2299225 or rs6465084 polymorphism and schizophrenia risk (Figure 2). When stratified according to ethnicity, no significant association with increased schizophrenia risk was observed in all subgroups for rs274622, rs917071, and rs6465084 polymorphisms. As for rs1468412, only the dominant model for the non-Asian subgroup showed a slightly significant association (OR: 1.15; 95% CI: 1.01–1.30; *P*=0.04), while the other comparison

models showed no relationship with schizophrenia risk for the Asian or the non-Asian populations (Table 2).

Heterogeneity analysis

Galbraith plots were created to graphically assess the sources of heterogeneity in the comparison models for GRM3 polymorphisms. For the dominant model of rs1468412 (Figure 3A), rs2299225 (Figure 3B), and rs6465084 (Figure 3C), when the most obvious outliers were excluded, heterogeneity significantly decreased (dominant model for rs1468412: *P*=0.37, I^2=7.8%; dominant model for rs2299225: *P*=0.23, I^2=30.9%;

Table 2 Main results of this meta-analysis

Comparison	n[a]	Case/control	Homozygote model			Heterozygote model			Dominant model			Recessive model		
			OR (95% CI)	P	P[b] (I²)	OR (95% CI)	P	P[b] (I²)	OR (95% CI)	P	P[b] (I²)	OR (95% CI)	P	P[b] (I²)
rs274622														
Total	7	3,820/4,015	1.19 (0.92–1.55)	0.19	0.53 (0.0%)	1.03 (0.93–1.14)	0.57	0.72 (0.0%)	1.04 (0.95–1.15)	0.38	0.53 (0.0%)	1.17 (0.90–1.51)	0.25	0.56 (0.0%)
Asian	6	3,588/3,850	1.21 (0.91–1.61)	0.19	0.41 (0.8%)	1.02 (0.92–1.13)	0.68	0.65 (0.0%)	1.04 (0.94–1.15)	0.47	0.43 (0.0%)	1.20 (0.90–1.60)	0.21	0.46 (0.0%)
Non-Asian	1	232/165	1.10 (0.57–2.13)	0.79	NA	1.16 (0.76–1.78)	0.49	NA	1.15 (0.77–1.72)	0.50	NA	1.01 (0.56–1.89)	0.97	NA
rs917071														
Total	6	2,660/3,517	1.05 (0.86–1.29)	0.63	0.65 (0.0%)	1.05 (0.95–1.17)	0.34	0.73 (0.0%)	1.05 (0.95–1.17)	0.33	0.58 (0.0%)	1.03 (0.84–1.25)	0.80	0.77 (0.0%)
Asian	3	935/1,187	0.84 (0.58–1.21)	0.35	0.55 (0.0%)	0.95 (0.79–1.14)	0.60	0.63 (0.0%)	0.94 (0.79–1.12)	0.46	0.56 0.0%	0.86 (0.60–1.23)	0.40	0.59 (0.0%)
Non-Asian	3	1,725/2,330	1.16 (0.91–1.48)	0.23	0.96 (0.0%)	1.10 (0.97–1.27)	0.12	0.96 (0.0%)	1.12 (0.99–1.27)	0.08	0.98 (0.0%)	1.11 (0.88–1.40)	0.39	0.93 (0.0%)
rs1468412														
Total	9	5,314/6,147	1.16 (0.98–1.37)	0.09	0.35 (10.2%)	1.03 (0.95–1.12)	0.45	0.12 (37.1%)	**1.02 (0.92–1.14)**	**0.69**	**0.07 (45.1%)**	1.13 (0.96–1.34)	0.13	0.47 (0.0%)
Asian	6	3,585/3,818	1.13 (0.89–1.42)	0.32	0.19 (32.9%)	0.98 (0.88–1.08)	0.62	0.13 (41.4%)	**0.96 (0.83–1.12)**	**0.59**	**0.06 (52.5%)**	1.14 (0.91–1.44)	0.26	0.29 (19.1%)
Non-Asian	3	1,729/2,329	1.19 (0.94–1.51)	0.16	0.50 (0.0%)	1.14 (0.99–1.30)	0.06	0.66 (0.0%)	1.15 (1.01–1.30)	0.04	0.64 (0.0%)	1.13 (0.89–1.42)	0.32	0.50 (0.0%)
rs6465084														
Total	6	4,960/5,613	1.04 (0.82–1.31)	0.77	0.31 (16.2%)	**1.01 (0.86–1.20)**	**0.90**	**0.03 (60.0%)**	**1.02 (0.86–1.20)**	**0.85**	**0.02 (63.3%)**	1.02 (0.81–1.29)	0.86	0.55 (0.0%)
Asian	2	2,755/2,839	0.90 (0.44–1.84)	0.76	0.34 (0.0%)	**0.93 (0.61–1.41)**	**0.73**	**0.07 (69.7%)**	1.03 (0.88–1.21)	0.70	0.13 (56.6%)	0.90 (0.44–1.85)	0.77	0.31 (3.6%)
Non-Asian	4	2,205/2,774	1.05 (0.82–1.35)	0.68	0.18 (38.5%)	**1.04 (0.82–1.31)**	**0.74**	**0.03 (67.4%)**	**1.05 (0.82–1.35)**	**0.69**	**0.01 (73.5%)**	1.04 (0.81–1.32)	0.78	0.42 (0.0%)
rs2299225*														
Total	5	3,492/3,735	1.04 (0.67–1.62)	0.86	0.26 (24.7%)	1.00 (0.88–1.14)	0.98	0.12 (44.7%)	**1.02 (0.83–1.25)**	**0.86**	**0.06 (55.2%)**	1.05 (0.67–1.63)	0.84	0.30 (17.5%)

Notes: [a]Number of studies; [b]P-values for heterogeneity from Q test; *all these included studies were for Asian populations. Bold font indicates where random-effect model was used.
Abbreviations: CI, confidence interval; NA, not applicable; OR, odds ratio.
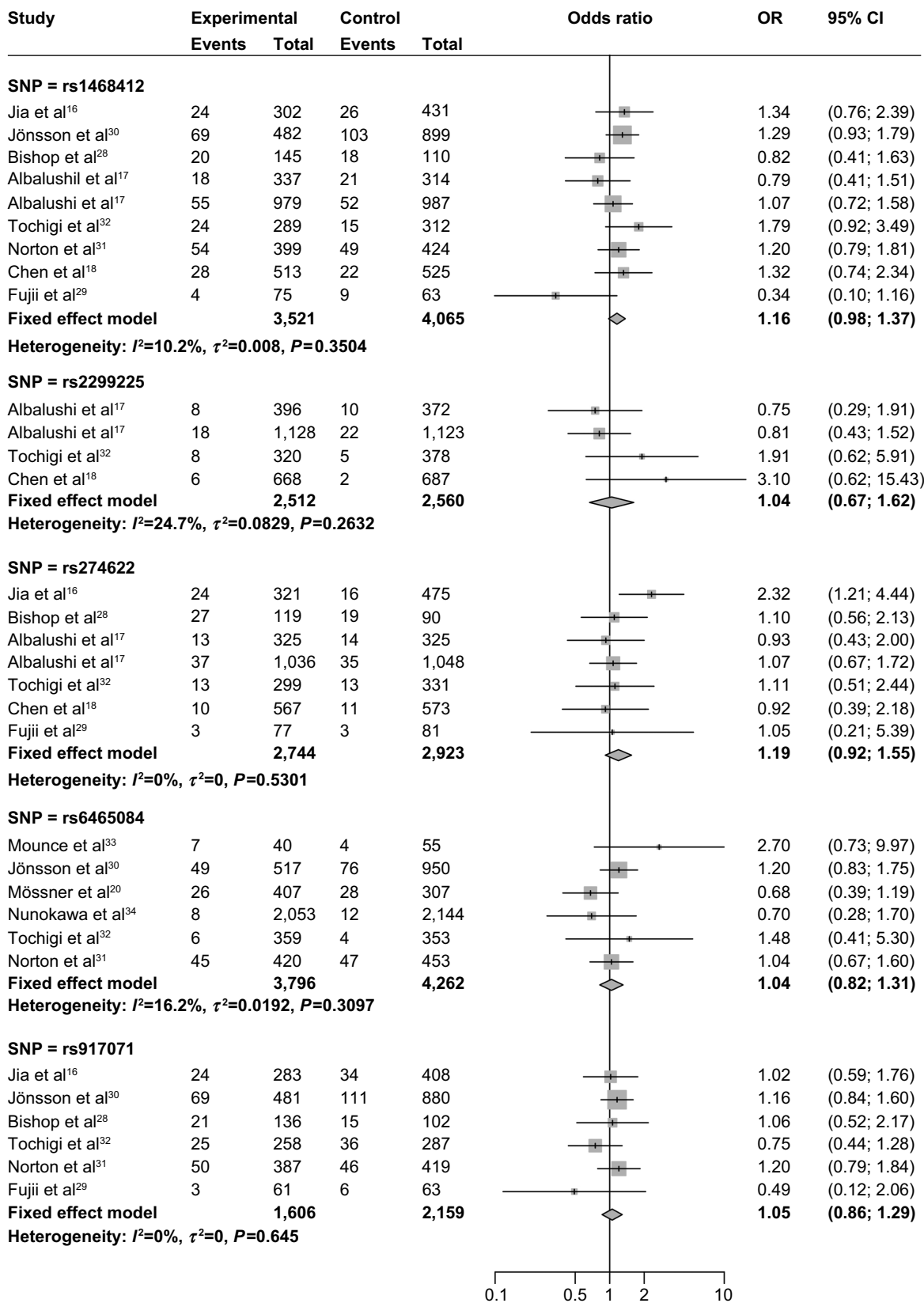
Study	Experimental		Control		Odds ratio	OR	95% CI
	Events	Total	Events	Total			
SNP = rs1468412							
Jia et al[16]	24	302	26	431		1.34	(0.76; 2.39)
Jönsson et al[30]	69	482	103	899		1.29	(0.93; 1.79)
Bishop et al[28]	20	145	18	110		0.82	(0.41; 1.63)
Albalushil et al[17]	18	337	21	314		0.79	(0.41; 1.51)
Albalushi et al[17]	55	979	52	987		1.07	(0.72; 1.58)
Tochigi et al[32]	24	289	15	312		1.79	(0.92; 3.49)
Norton et al[31]	54	399	49	424		1.20	(0.79; 1.81)
Chen et al[18]	28	513	22	525		1.32	(0.74; 2.34)
Fujii et al[29]	4	75	9	63		0.34	(0.10; 1.16)
Fixed effect model		**3,521**		**4,065**		**1.16**	**(0.98; 1.37)**
Heterogeneity: I^2=10.2%, τ^2=0.008, P=0.3504							
SNP = rs2299225							
Albalushi et al[17]	8	396	10	372		0.75	(0.29; 1.91)
Albalushi et al[17]	18	1,128	22	1,123		0.81	(0.43; 1.52)
Tochigi et al[32]	8	320	5	378		1.91	(0.62; 5.91)
Chen et al[18]	6	668	2	687		3.10	(0.62; 15.43)
Fixed effect model		**2,512**		**2,560**		**1.04**	**(0.67; 1.62)**
Heterogeneity: I^2=24.7%, τ^2=0.0829, P=0.2632							
SNP = rs274622							
Jia et al[16]	24	321	16	475		2.32	(1.21; 4.44)
Bishop et al[28]	27	119	19	90		1.10	(0.56; 2.13)
Albalushi et al[17]	13	325	14	325		0.93	(0.43; 2.00)
Albalushi et al[17]	37	1,036	35	1,048		1.07	(0.67; 1.72)
Tochigi et al[32]	13	299	13	331		1.11	(0.51; 2.44)
Chen et al[18]	10	567	11	573		0.92	(0.39; 2.18)
Fujii et al[29]	3	77	3	81		1.05	(0.21; 5.39)
Fixed effect model		**2,744**		**2,923**		**1.19**	**(0.92; 1.55)**
Heterogeneity: I^2=0%, τ^2=0, P=0.5301							
SNP = rs6465084							
Mounce et al[33]	7	40	4	55		2.70	(0.73; 9.97)
Jönsson et al[30]	49	517	76	950		1.20	(0.83; 1.75)
Mössner et al[20]	26	407	28	307		0.68	(0.39; 1.19)
Nunokawa et al[34]	8	2,053	12	2,144		0.70	(0.28; 1.70)
Tochigi et al[32]	6	359	4	353		1.48	(0.41; 5.30)
Norton et al[31]	45	420	47	453		1.04	(0.67; 1.60)
Fixed effect model		**3,796**		**4,262**		**1.04**	**(0.82; 1.31)**
Heterogeneity: I^2=16.2%, τ^2=0.0192, P=0.3097							
SNP = rs917071							
Jia et al[16]	24	283	34	408		1.02	(0.59; 1.76)
Jönsson et al[30]	69	481	111	880		1.16	(0.84; 1.60)
Bishop et al[28]	21	136	15	102		1.06	(0.52; 2.17)
Tochigi et al[32]	25	258	36	287		0.75	(0.44; 1.28)
Norton et al[31]	50	387	46	419		1.20	(0.79; 1.84)
Fujii et al[29]	3	61	6	63		0.49	(0.12; 2.06)
Fixed effect model		**1,606**		**2,159**		**1.05**	**(0.86; 1.29)**
Heterogeneity: I^2=0%, τ^2=0, P=0.645							

0.1   0.5   1   2   10

Figure 2 Forest plots for GRM3 polymorphisms and schizophrenia risk.
Notes: ORs were estimated under the homozygote comparison, using a fixed-effect model. One study was not included in homozygote comparison for rs2299225,[16] because no minor-allele homozygote carrier was detected in either the schizophrenia or control groups.
Abbreviations: OR, odds ratio; CI, confidence interval; SNP, single-nucleotide polymorphism; GRM3, metabotropic glutamate receptor 3.
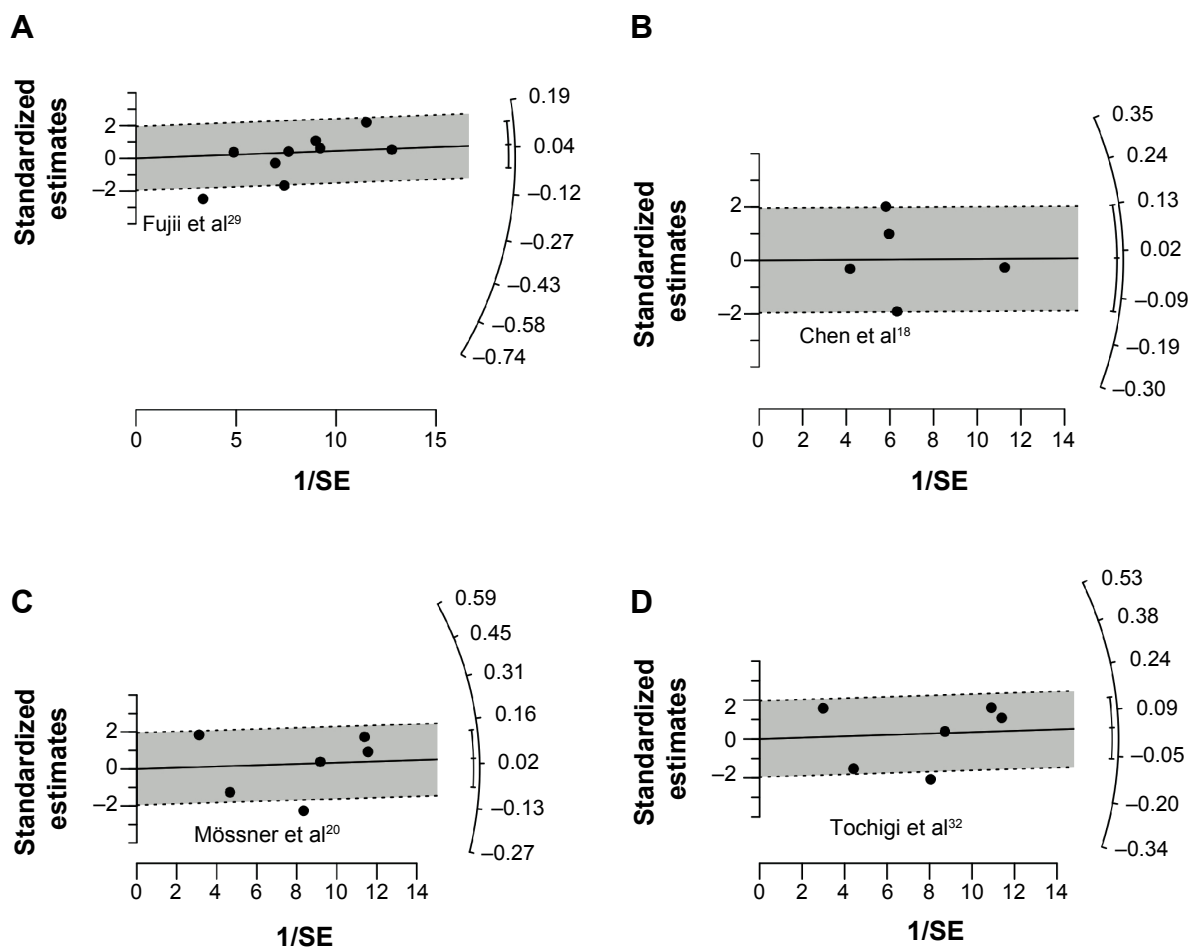
A

B

C

D

Figure 3 Galbraith plot analysis for the source of heterogeneity.
Notes: (**A**) rs1468412 polymorphism under dominant comparison model; (**B**) rs2299225 polymorphism under dominant comparison model; (**C**) rs6465084 polymorphism under dominant comparison model; (**D**) rs6465084 polymorphism under heterozygote comparison model.
Abbreviation: SE, standard error.

dominant model for rs6465084: $P=0.20$, $I^2=33.3\%$), and the results remained stable for both overall and subgroup analyses (data not shown). As for the heterozygote model of the rs6465084 polymorphism (Figure 3D), after removing the outlier, the heterogeneity among studies was also alleviated, although still existed (heterozygote model of rs6465084: $P=0.05$, $I^2=58.1\%$).

Publication bias and sensitivity analysis

Begg's funnel plot and Egger's test were done to assess the publication bias of studies. The shape of the funnel plots (Figure 4) did not show any obvious asymmetry. The statistical results of Egger's test still did not reveal publication bias for rs274622 polymorphism (homozygote model, $P=0.794$; heterozygote model, $P=0.680$; dominant model, $P=0.656$; recessive model, $P=0.830$), rs917071 polymorphism (homozygote model, $P=0.185$; heterozygote model, $P=0.682$;

dominant model, $P=0.470$; recessive model, $P=0.215$), rs6465084 (homozygote model, $P=0.678$; heterozygote model, $P=0.882$; dominant model, $P=0.624$; recessive model, $P=0.701$) and rs2299225 (homozygote model, $P=0.088$; heterozygote model, $P=0.822$; dominant model, $P=0.893$; recessive model, $P=0.094$). For the rs1468412 polymorphism, the heterozygote model ($P=0.026$) and dominant model ($P=0.013$) showed publication bias, but not the homozygote model ($P=0.120$) or recessive model ($P=0.285$). However, when the outlier detected by the Galbraith plot (Figure 3A) was omitted, Egger's test P-value turned to no significance (heterozygote model, $P=0.300$; dominant model, $P=0.267$).

The influences of each individual study on the overall ORs for the five polymorphisms were evaluated. The results showed the pooled ORs of these polymorphisms were not materially altered by the omission of any individual study (Figure 5).
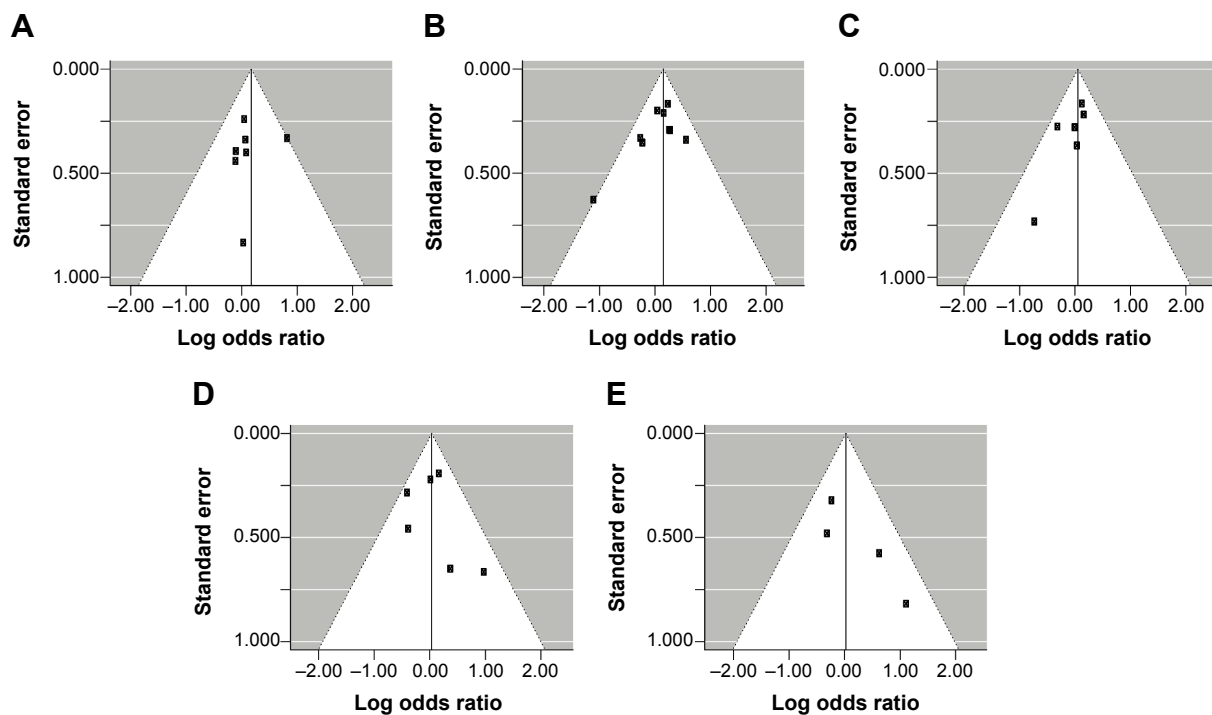
Figure 4 Funnel plot for publication bias in studies on GRM3 polymorphisms and schizophrenia risk.
Notes: The odds ratio was estimated under the homozygote comparison. (**A**) rs274622; (**B**) rs1468412; (**C**) rs917071; (**D**) rs6465084; (**E**) rs2299225. One study was not included in homozygote comparison for rs2299225,[16] because no minor-allele homozygote carriers were detected in either the schizophrenia or control groups.
Abbreviation: GRM3, metabotropic glutamate receptor 3.

Discussion

Common genetic polymorphisms or mutation in the glutamatergic neurotransmission may lead to profound influence to most aspects of normal brain function and involvement in many neuropathologic conditions. In recent years, interest in the genetic susceptibility to neuropathogenesis has led to a growing attention to the study of polymorphisms of genes involved in schizophrenia. To date, many molecular epidemiological studies have been performed to explore the role of GRM3 polymorphisms on schizophrenia susceptibility, but the results are still far from conclusive. Moreover, several studies were plagued by their limited sample size, which subsequently led to statistical power too low to unveil the effects that may exist. Therefore, we performed this meta-analysis with eligible studies in order to improve the statistical power and accurately determine the effect size of the association between GRM3 polymorphisms and schizophrenia risk.

Our results showed that GRM3 rs1468412, rs274622, rs917071, rs2299225, and rs6465084 polymorphisms were not associated with schizophrenia risk when all studies were pooled together. Ethnicity is usually regarded as a potential factor that may influence the risk of common disease by different genetic backgrounds and environmental exposures, while the stratified analyses in the present study allowed us to infer the ethnic specificity of the association between these

SNPs and schizophrenia. In further subgroup analysis stratified by ethnicity, none of the GRM3 rs274622, rs917071, or rs6465084 polymorphisms showed any significant association with schizophrenia risk. With respect to rs1468412, although an increased risk was observed in the dominant model for non-Asian populations, given the limited studies and sample sizes for this subgroup, the borderline statistical significance (P=0.04) in only one single comparison model is not enough to indicate any underlying association. Further large and well-designed studies are needed to confirm this.

Heterogeneity between studies should be noted, because this may potentially affect the strengths of the meta-analysis. In the present study, heterogeneity was found in the heterozygote comparison model for the rs6465084 polymorphism, and in the dominant comparison model for the rs1468412, rs6465084, and rs2299225 polymorphisms. A Galbraith plot was utilized to identify which of the included studies may be sources of heterogeneity. After removal of those identified outliers, the heterogeneity among studies was obviously alleviated, while the main conclusions remained stable. Furthermore, the publication bias for the heterozygote model and dominant model of rs1468412 significantly decreased after removing the outlier. The sensitivity analysis did not show significant alteration of our meta-analyses for any of the five polymorphisms, indicating the robustness of our results.
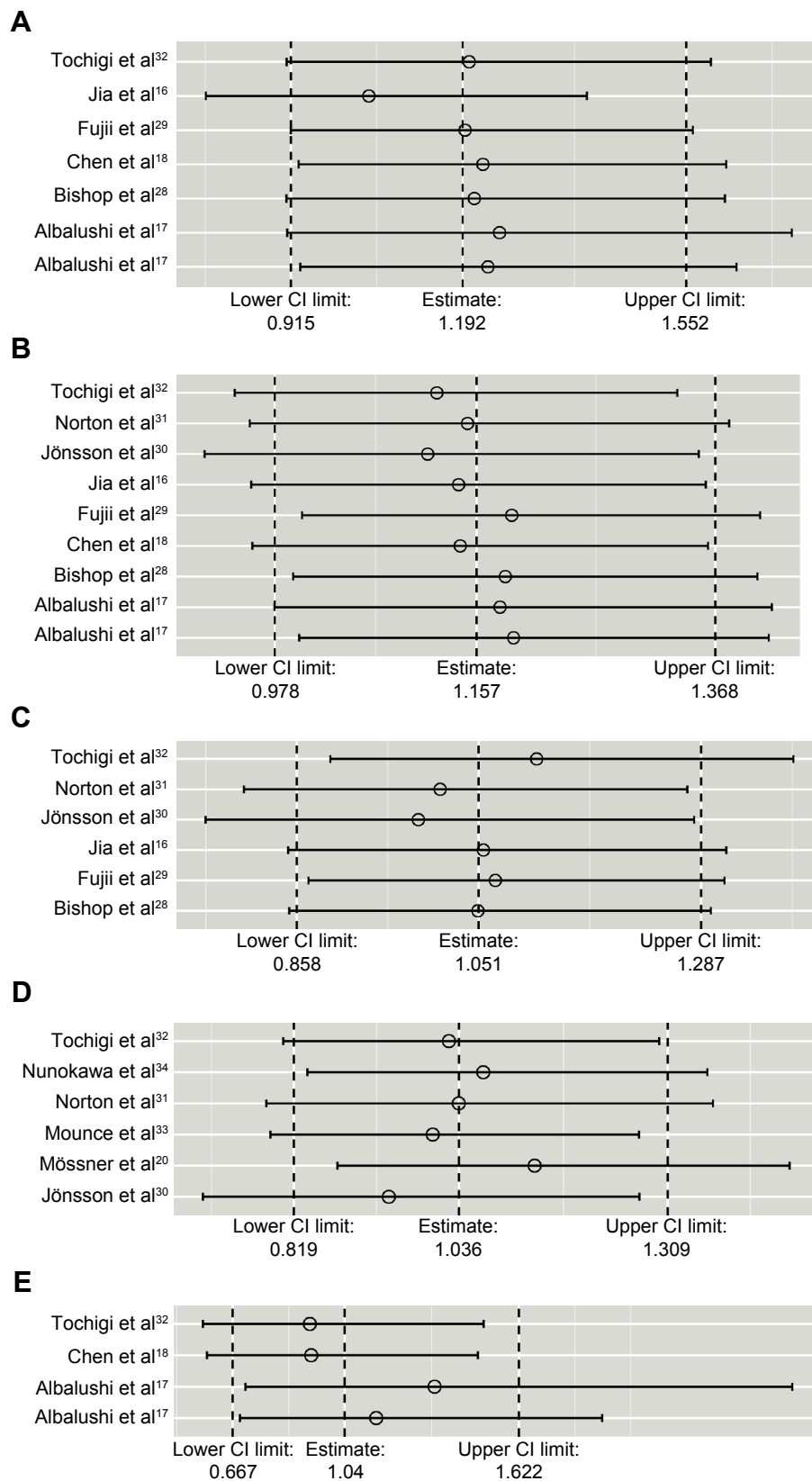
Figure 5 Sensitivity analyses of the summary odds ratios of the association between GRM3 polymorphisms and schizophrenia risk.
Notes: Odds ratios were estimated under the homozygote comparison, using a fixed-effect model. (**A**) rs274622; (**B**) rs1468412; (**C**) rs917071; (**D**) rs6465084; (**E**) rs2299225. One study was not included in homozygote comparison for rs2299225,[16] because no minor-allele homozygote carriers were detected in either the schizophrenia or control groups.
Abbreviations: CI, confidence interval; GRM3, metabotropic glutamate receptor 3.

Taken together, these results strengthened the reliability of the conclusions from this meta-analysis.

Although comprehensive analysis provided pooled data on a substantial number of cases and controls and objectively evaluated the influence of five GRM3 polymorphisms on the risk of schizophrenia, there are still some limitations inherited from the involved studies and our analysis strategies. First of all, the number of studies and subjects included in the current meta-analysis were still relatively small and most of the published studies were performed in Asian-descent and Caucasian-descent populations. It is critical that larger and well-designed multiethnic studies with detailed subtyping data should be performed to reevaluate the association and further explore the possible ethnic specificity. In addition, our analysis did not consider the possibility of gene–environment interactions or the linkage disequilibrium between gene polymorphisms, due to lack of original data. Finally, although all cases and controls of each study were recruited with similar inclusion criteria, some other potential factors not taken into account, such as lifestyle, may have biased our results. Owing to these limitations listed, further rigorous multicentric studies with large samples, multiple races and ethnicities, standardized unbiased genotyping methods, and well-matched controls are needed to validate the findings. In addition, other information, including but not limited to gene–gene interaction, gene–environment interaction, and lifestyle factors, should also be taken into account to evaluate the combined effect on schizophrenia.

Conclusion

In conclusion, this meta-analysis suggests that there is lack of evidence proving the association of GRM3 rs1468412, rs274622, rs917071, rs6465084, and rs2299225 polymorphisms with increased risk of developing schizophrenia.

Acknowledgments

Author contributions

Xiaoqin Yang and Xia Yue contributed to the design of the project, and wrote the manuscript; Xiaoqin Yang and Guiping Wang conducted the research; Guiping Wang and Yaodong Wang contributed to the design of the project and data acquisition; all authors critically revised the manuscript and approved the final version to be published.

Disclosure

The authors report no conflicts of interest in this work.

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