ORIGINAL RESEARCH

Glutathione S-Transferase M1/T1 Polymorphisms and Schizophrenia Risk: A New Method for Quality Assessment and a Systematic Review

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Background: *GST* genes were reported to be involved in susceptibility to mental disorder. The results between deletions of *GST* genes and schizophrenia were inconclusive and confusing. Therefore, we performed this updated meta-analysis to outline the association using a new method for quality assessment.

Methods: Sixteen reported studies were selected, and the overall OR and 95% CI were calculated and analyzed by Review Manager 5.4 and STATE 12. The Newcastle-Ottawa Quality Assessment Scale (NOS) for case–control studies was rewritten to evaluate the quality of published studies, as there was no "Exposure" in these studies and other factors should be suggested to assess the quality.

Results: There was no significant association between deletions of *GST* genes and SZ risk ($p > 0.05$ in Random model). We also failed to find a significant relation between null genotypes and SZ risk in East Asian population. Based on further analysis of PCR methods, *GSTM1* null was weakly associated with SZ risk in 8 studies using multiplex PCR (OR = 1.17, 95% CI = 1.00–1.37, $p = 0.05$), but *GSTT1* null was a protective factor for SZ risk (OR = 0.73, 95% CI = 0.56–0.94, $p = 0.02$). When stratified by rewritten NOS stars and deductions, *GSTM1* null was significantly associated with SZ risk in 9 studies with high quality (OR = 1.24, 95% CI = 1.08–1.43, $p = 0.002$), and in 10 studies with no deductions (OR = 1.20, 95% CI = 1.05–1.38, $p = 0.007$).

Conclusion: *GSTM1* null genotype may be a genetic risk factor for SZ in studies using multiplex PCR and high-quality studies. However, *GSTT1* null might be a protective factor. Besides, we provided a new method for quality assessment and it was useful and should be promoted in further analysis.

Keywords: *glutathione S-transferase T1*, *GSTM1*, polymorphism, schizophrenia, meta-analysis

Introduction

Schizophrenia (SZ) is a severe psychiatric disorder characterized by abnormal behavior and altered reality.[1] The chronic psychotic symptoms have a profound impact on patients with SZ, and the average life expectancy is 10–20 years shorter than the general population.[2] Despite the efforts of researchers, treatment for SZ remains limited, and the therapeutic targets have not expanded for decades.[3] Our poor understanding of the neurobiological underlines of mental disorder results in limited treatment. Although many studies suggested that both genetic polymorphisms and environmental factors were implicated in the susceptibility and etiology of SZ, SZ was a highly heritable disease.[4–6] Some GWAS studies with large participants probed that some genetic variants were related to SZ risk, including copy number variation (CNV) at 22q11.2.[7,8] Therefore, null genotypes of *glutathione S-transferases* (*GST*) genes might be well suited for exploring the association with SZ.

Glutathione S-transferases (GSTs) are a family of Phase II metabolic isozymes, which code by glutathione S-transferase mu 1 (*GSTM1*), glutathione S-transferase theta-1 and theta-2 (*GSTT1* and *T2*), glutathione S-transferase pi-1 (*GSTP1*), and glutathione S-transferase omega-1 (*GSTO1*).[5] *GSTM1* is located at 1p13.3, and *GSTT1* is situated at 22q11.23. Deletion of the entire gene results in a lack of enzymatic activity and may cause upregulation of oxidative

stress.[4] Homozygous deletions of *GSTM1* or *GSTT1* have also been reported in various diseases, including SZ, cancer, and vascular disease.[6,9,10]

They are one of the key enzymes in the converting toxic compounds into hydrophilic metabolites for detoxification, and they play a crucial role in protecting neural cells from oxidative stress.[6] GSTs are regarded as neuro-protective antioxidants, and some reported results showed that the GSTs levels were decreased in cerebrospinal fluid of SZ patients.[11] This suggested that GSTs might be involved in the susceptibility and progression of SZ. Currently, a number of studies had found *GSTM1* and *GSTT1* polymorphisms to be associated with SZ risk, but some other studies were failed to find this association.[12–27] Antioxidants impairment and oxidative damage could account for the positive results, while other enzymes completely reducing the effects of GST deletion might be responsible for the negative results. However, some studies showed that *GSTM1* null genotype was reduced in SZ patients, and a protective effect of *GSTT1* null genotype had also been observed. These results were confusing and required to re-analysis. Besides, case–control study about genetic polymorphisms needed to be assessed for quality using new methods. Therefore, we performed this meta-analysis on case–control studies using a new method for quality assessment. This was the first attempt to assess the quality of genetic study.

Materials and Methods

Search Strategy

PubMed and Chinese national knowledge infrastructure (CNKI) were selected and the keywords "schizophrenia", "GSTT1", "GSTM1", "glutathione S-transferase", "glutathione S-transferase T1", "glutathione S-transferase M1", and "polymorphism" were used for searching. All relevant studies from 1990 to March 2022 were included with no restriction.

Inclusion and Exclusion Criteria

All the studies should be complied with the following criteria: (1) The patients of studies must be schizophrenia; (2) The literatures should be the association studies included risk of SZ and *GST* genes; (3) Only case–control studies were considered; (4) The papers should provide sample size, OR values, and 95% CI or provide the related information such as genotype frequencies. Other studies would be excluded: (1) no healthy controls or case-only studies; (2) overlapped studies; (3) the articles are reviews; (4) studies without useful data, including genotype frequencies.

Study Selection and Data Extraction

According to pre-established criteria of inclusion and exclusion, a double-check procedure was performed to make sure the accuracy of the data entry. The following information was extracted from the studies and listed in Table 1: first author, published year, population, the data of total and exposure number in case and control groups. Other information was also collected and assessed by rewritten NOS in Supplementary Table S1, consisting of PCR method and PCR control, source of the controls, diagnostic criteria, subgroup analysis, age and gender of cases and controls, age of onset years, Hardy-Weinberg equilibrium (HWE) tests of CNVs, and deductions. Two reviewers were invited, and a standardized procedure was performed independently. Scores of studies were summarized in Supplementary Table S1.

Quality Assessment and Rewritten NOS

All studies were assessed using Newcastle-Ottawa quality assessment scale (NOS) rewritten by us in the Supplementary Table S2. The "Exposure" was deleted and items of "Methodology" and "Deductions" were added in rewritten NOS (reNOS). PCR methods, PCR controls, subgroup analysis, Hardy-Weinberg equilibrium (HWE), and age of onset years were included in item of "Methodology". Deductions were defined by inconsistent data or incorrect descriptions, which would affect the quality score of studies. Studies with 6–10 stars or no deduction were indicated high-quality studies. The detail information and obtained stars of 16 studies were shown in the Supplementary Table S1.

Table 1 Data Extraction of Eligible Studies Included in the Meta-Analysis

Authors, Year, and Reference[12–28]	Country	Schizophrenia						Control					
		GSTM1		GSTT1		GSTM1 +GSTT1		GSTM1		GSTT1		GSTM1 +GSTT1	
		Null	Total	Null	Total	Null	Total	Null	Total	Null	Total	Null	Total
Ansari-Lari (2021)[12]	Iran	41	47	12	78	ND	ND	47	91	23	91	ND	ND
Nakamura (2015)[13]	Japan	303	640	273	640	ND	ND	337	622	268	622	ND	ND
Pejovic-Milovancevic (2016)[14]	Serbia	28	49	9	49	5	49	137	278	65	278	23	278
Pinheiro (2017)[15]	Brazil	28	54	13	54	9	54	36	78	10	78	3	78
Yan (2020)[16]	China	177	379	158	379	109	379	151	415	161	415	83	415
Zhang (2020)[17]	China	208	386	176	386	93	386	150	264	133	264	78	264
Rodríguez-Santiago (2009)[18]	Spain	243	594	142	645	ND	ND	289	585	105	593	ND	ND
Gravina (2011)[19]	Italy	82	138	25	138	10	138	70	133	30	133	21	133
Harada (2001)[20]	Japan	57	87	ND	ND	ND	ND	87	176	ND	ND	ND	ND
Kashani (2012)[21]	Iran	15	93	6	93	3	93	26	99	2	99	2	99
Matsuzawa (2009)[22]	Japan	129	214	88	214	ND	ND	119	220	80	220	ND	ND
Pae (2004)[23]	South Korea	70	111	ND	ND	ND	ND	61	130	ND	ND	ND	ND
Raffa (2013)[24]	Tunisia	79	138	59	138	39	138	63	123	67	123	30	123
Saadat (2007)[25]	Iran	ND	ND	52	292	ND	ND	ND	ND	99	292	ND	ND
Saruwatari (2013)[26]	Japan	77	154	68	154	40	154	99	203	99	203	45	203
Watanabe (2010)[27]	Japan	336	627	ND	ND	ND	ND	323	620	ND	ND	ND	ND
Kordi-Tamandani (2014)[28]	Iran	It was excluded due to methylation analysis of GSTT1											
Total	——	1873	3711	1081	3260	308	1391	1995	4037	1142	3411	285	1593

Abbreviations: ND, no data; *GSTM1*, glutathione S-transferase mu 1; *GSTT1*, glutathione S-transferase theta 1.

Statistical Analysis Methods

Statistical analysis was done by using Review Manager 5.4 and STAT 12. Total OR and 95% CI were calculated to explore the strength of association between *GSTM1/T1* null genotypes and schizophrenia. Q test was performed to check heterogeneity, and the heterogeneity was considered significant when $p<0.10$.[4] The fixed effect model was used when $p>0.10$; otherwise, a random effect model was selected. Subgroup analysis was used to evaluate the source of bias. The funnel plot was used to detect publication bias. Egger's test and Begg's test were also selected to test publication bias. The sensitivity analysis was performed by deleting studies to assess the pooled results. All the tests were two-sided, and a p value of 0.05 for any test or model was considered to be statistically significant.

Results
Characteristics of Studies

According to the search strategy, 16 papers are selected in Figure 1. Fifteen studies included 3742 cases and 4037 controls were selected in *GSTM1*. Thirteen studies related to *GSTT1* included 3260 cases and 3411 controls. The data extraction of studies is shown in Table 1 and Supplementary Table S1. Stratification of studies by ethnicity, PCR methods, total stars and deductions based on the rewritten NOS is summarized in Table 2.

Overall Results of Meta-Analysis

The overall results of heterogeneity assessed with Q test and I^2 statistic are shown in Figure 2. The random-effect methods were selected due to $p < 0.05$ of Q test or $I^2 > 50\%$. As shown in Figure 2, there were no significant associations between deletions of *GSTM1/T1* and SZ. Publication bias was investigated using funnel plot, and the shape of the funnel plot was symmetrical. The result of Egger's test was $p = 0.117$, and Begg's test was $p = 0.073$. No publication bias was observed in this meta-analysis. The sensitivity analysis performed by deleting studies showed that there were no changes in the overall results. This indicated that the bias had few effects on the overall results and the conclusion was robust.

Subgroup Results of Ethnicity

Stratification of studies by ethnicity is performed in Table 3. The results of East Asian were (OR = 1.19, 95% CI = 0.94–1.49, $p = 0.15$) for *GSTM1* null, (OR = 0.99, 95% CI = 0.87–1.13, $p = 0.89$) for *GSTT1* null and (OR = 1.16, 95% CI = 0.71–1.89, $p = 0.56$) for both deletions of *GSTM1* and *GSTT1* as shown in Table 3. No significant association was found in subgroup analysis of ethnicity. This result changed the inherent thinking that different races might provide diversity and race might not play an important role in this meta-analysis. Funnel plot and Egger's test were used for evaluation of the publication bias, and no publication bias was observed ($p > 0.05$).

Subgroup Results of PCR Methods

PCR method was not the criteria for quality assessment, but it was interesting to perform a sub-analysis due to judgement of genotype with same methods. However, the results should be interpreted carefully. Stratification of studies by PCR methods is performed in Table 4. When stratified for PCR methods of Multiplex PCR as shown in Table 4, *GSTM1* null genotype showed weak association with risk of SZ (OR = 1.17, 95% CI = 1.00–1.37, $p = 0.05$). $p = 0.05$ should be interpreted with caution. Usually, we should re-analyze with a larger sample size, but this was a meta-analysis and difficult to re-analyze. We preferred to find a weak association in this section, and the positive results were more interesting. Surprisingly, there was a significant association between *GSTT1* null genotype and the decreased risk of SZ (OR = 0.73, 95% CI = 0.56–0.94, $p = 0.02$). These results indicated that the *GST* genes played different roles in SZ patients and combination of *GSTM1* null and *GSTT1* present should be further researched. Besides, double null showed no relation with SZ in subgroup analysis of multiplex PCR ($p > 0.05$), but significant association was observed in subgroup analysis of other methods (OR = 1.73, 95% CI = 1.27–2.36, $p = 0.0006$). This positive result should be carefully interpreted due to only 3 included studies, and it might not be representative. As mentioned above, no publication bias was observed ($p > 0.05$).

Subgroup Results of NOS Stars and Deductions

Meta-analysis was an evidence-based method using reported studies, and study quality was a very important factor for making a conclusion. Therefore, we re-written the NOS scale for quality assessment and conducted the sub-analysis. The subgroup results of studies with 6–10 stars are listed in Table 5. *GSTM1* null genotype showed a significant association with risk of SZ (OR = 1.24, 95% CI = 1.08–1.43, $p = 0.002$). This result revealed that we should pay more attention to
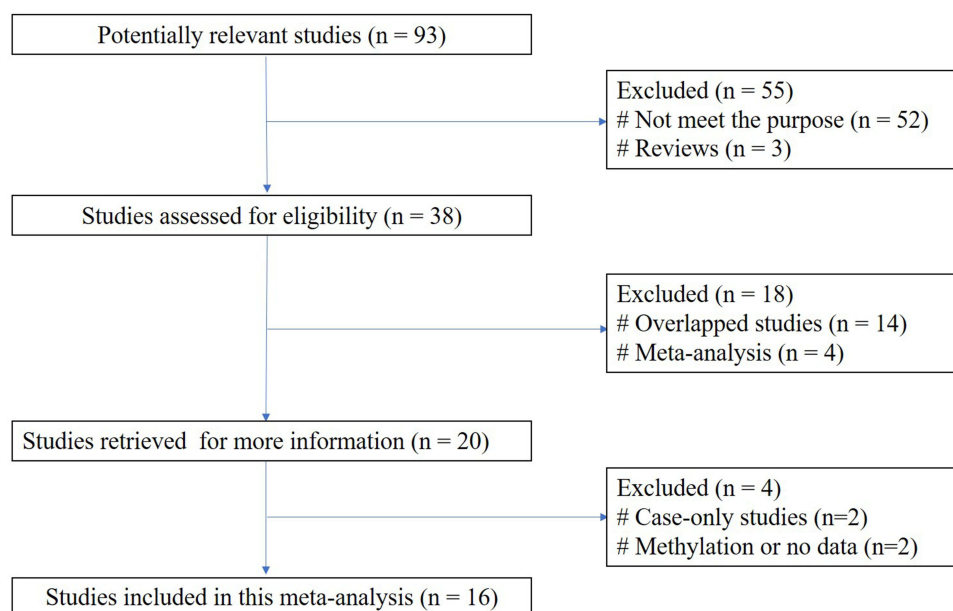

Figure 1 A flow diagram of the study selection process.

Table 2 Stratification of Studies by Ethnicity, PCR Methods, Total Stars and Deductions Based on the Rewritten NOS

Authors	Population	Ethnicity	PCR Methods	Deductions	Total Stars
Ansari-Lari (2021)[12]	Iran	Caucasian	Multiplex PCR	No deduction	7
Nakamura (2015)[13]	Japan	East Asian	TaqMan qRT-PCR	One deduction	5
Pejovic-Milovancevic (2016)[14]	Serbia	Caucasian	Multiplex PCR	One deduction	5
Pinheiro (2017)[15]	Brazil	Caucasian	Multiplex RT-PCR	No deduction	7
Yan (2020)[16]	China	East Asian	Ordinary PCR	No deduction	6
Zhang (2020)[17]	China	East Asian	Multiplex PCR	No deduction	8
Rodríguez-Santiago (2009)[18]	Spain	Caucasian	MLPA	Two deductions	3
Gravina (2011)[19]	Italy	Caucasian	Multiplex PCR	No deduction	7
Harada (2001)[20]	Japan	East Asian	PCR/long fragment PCR	One deduction	7
Kashani (2012)[21]	Iran	Caucasian	Ordinary PCR	No deduction	5
Matsuzawa (2009)[22]	Japan	East Asian	PCR	No deduction	7
Pae (2004)[23]	South Korea	East Asian	PCR	No deduction	5
Raffa (2013)[24]	Tunisia	Caucasian	Multiplex PCR	No deduction	6
Saadat (2007)[25]	Iran	Caucasian	PCR	No deduction	6
Saruwatari (2013)[26]	Japan	East Asian	PCR	No deduction	7
Watanabe (2010)[27]	Japan	East Asian	TaqMan RT-PCR	One deduction	4

Abbreviations: PCR, polymerase chain reaction; qRT-PCR, quantitative real-time PCR; MLPA, multiplex ligation dependent probe amplification; GSTM1/T1, glutathione S-transferase mu 1 / theta 1; NOS, Newcastle-Ottawa quality assessment scale.

the quality of studies and new assessment scale was needed for evaluation. However, there was no significant association between *GSTT1* null genotype and SZ risk ($p > 0.05$), and double null genotypes also had no association with SZ ($p > 0.05$).

When stratified by no deductions as shown in Table 6, *GSTM1* null genotype also showed significant association with risk of SZ (OR = 1.20, 95% CI = 1.05–1.38, $p = 0.007$). This result was consistent with subgroup analysis of high-quality studies in Table 5. Not surprisingly, *GSTT1* null genotype was not related with SZ ($p > 0.05$), and double null genotypes were also not found to be associated with SZ ($p > 0.05$). Moreover, no publication bias was observed ($p > 0.05$).

Discussion

In our study, no significant association was observed between *GSTM1/T1* null genotypes and SZ risk using a meta-analysis of total population. Further stratified analysis by ethnicity also suggested that deletions of *GSTM1/T1* had no clear association with increased susceptibility to SZ. However, subgroup analysis of multiplex PCR revealed that *GSTM1* null genotype had a weak association with risk of SZ. Interestingly, *GSTT1* null genotype significantly reduced SZ risk in studies using multiplex PCR. When stratified by re-NOS stars and deductions, *GSTM1* null was significantly associated with risk of SZ in high-quality studies. These results indicated that the quality of studies and methods of research should be paid more attention in further meta-analysis.

Up to now, some studies have evaluated the association between genetic deletion of *GST* genes and risk of SZ using meta-analysis.[4–6,27] However, these published results remain conflicting and an updated meta-analysis is necessary for further assessment. Watanabe et al found a weak significant association between *GSTM1* null genotype and SZ risk in Asian population.[27] A significant association was also reported by Cai et al between *GSTM1* null genotype and risk of SZ, but *GSTT1* null genotype was related to the decreased risk of SZ.[5] In another meta-analysis reported by Kim et al, they failed to find the association between *GST* genes and SZ. Subgroup analysis based on ethnicity showed that *GSTM1* polymorphism had a weak association with SZ risk in East Asian population.[4] Huang et al also reported that *GSTM1* deletion was significantly associated with SZ.[6] However, the literature included and data extraction were not consistent in these reported studies. Especially, the quality of included studies was not assessed. To identify them, we performed this updated meta-analysis using new method to re-analyze the association and provide new insight and improving perspective for further research.
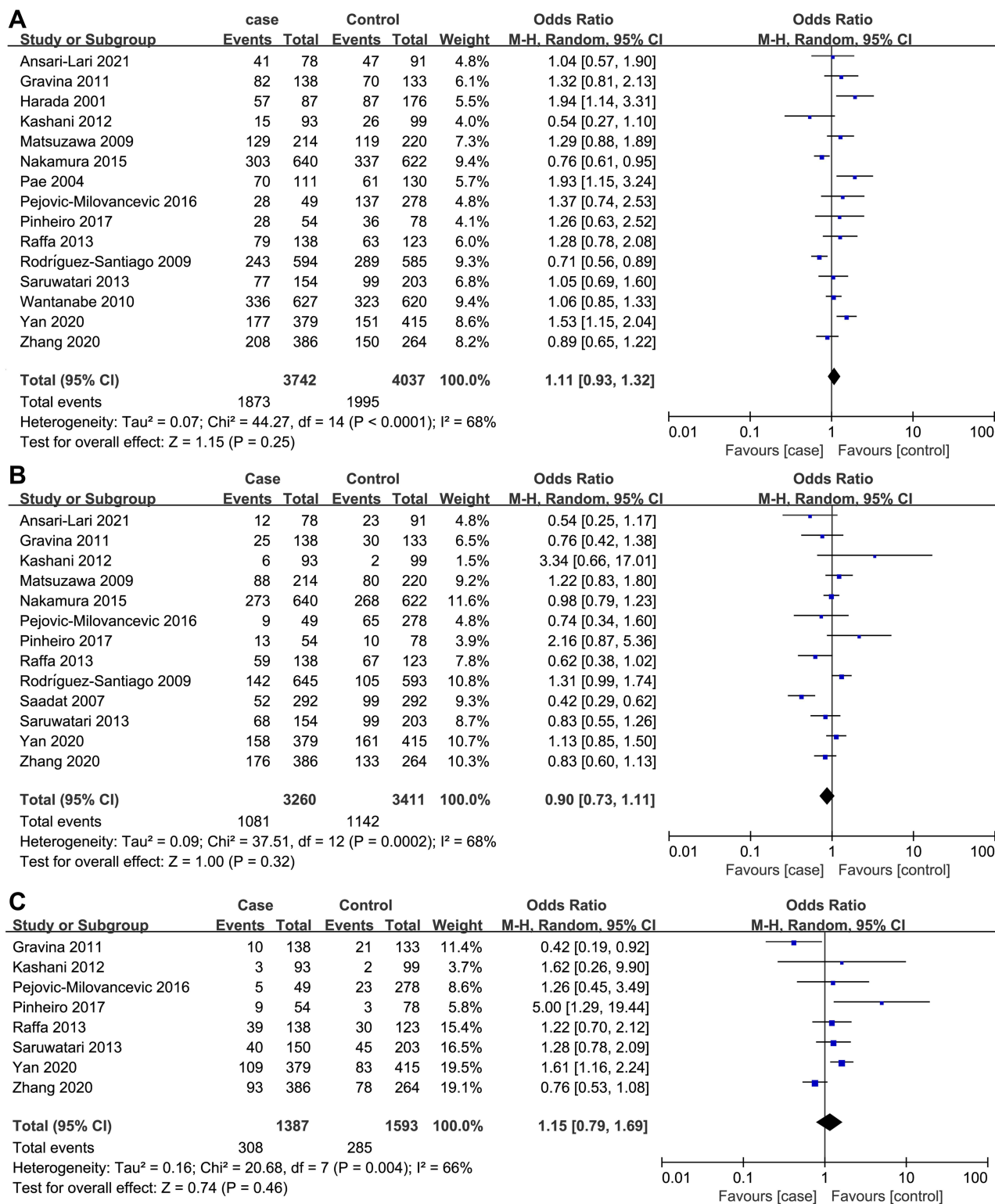
A

Study or Subgroup	case Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ansari-Lari 2021	41	78	47	91	4.8%	1.04 [0.57, 1.90]
Gravina 2011	82	138	70	133	6.1%	1.32 [0.81, 2.13]
Harada 2001	57	87	87	176	5.5%	1.94 [1.14, 3.31]
Kashani 2012	15	93	26	99	4.0%	0.54 [0.27, 1.10]
Matsuzawa 2009	129	214	119	220	7.3%	1.29 [0.88, 1.89]
Nakamura 2015	303	640	337	622	9.4%	0.76 [0.61, 0.95]
Pae 2004	70	111	61	130	5.7%	1.93 [1.15, 3.24]
Pejovic-Milovancevic 2016	28	49	137	278	4.8%	1.37 [0.74, 2.53]
Pinheiro 2017	28	54	36	78	4.1%	1.26 [0.63, 2.52]
Raffa 2013	79	138	63	123	6.0%	1.28 [0.78, 2.08]
Rodríguez-Santiago 2009	243	594	289	585	9.3%	0.71 [0.56, 0.89]
Saruwatari 2013	77	154	99	203	6.8%	1.05 [0.69, 1.60]
Wantanabe 2010	336	627	323	620	9.4%	1.06 [0.85, 1.33]
Yan 2020	177	379	151	415	8.6%	1.53 [1.15, 2.04]
Zhang 2020	208	386	150	264	8.2%	0.89 [0.65, 1.22]
Total (95% CI)		3742		4037	100.0%	1.11 [0.93, 1.32]
Total events	1873		1995			

Heterogeneity: Tau² = 0.07; Chi² = 44.27, df = 14 (P < 0.0001); I² = 68%
Test for overall effect: Z = 1.15 (P = 0.25)

B

Study or Subgroup	Case Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ansari-Lari 2021	12	78	23	91	4.8%	0.54 [0.25, 1.17]
Gravina 2011	25	138	30	133	6.5%	0.76 [0.42, 1.38]
Kashani 2012	6	93	2	99	1.5%	3.34 [0.66, 17.01]
Matsuzawa 2009	88	214	80	220	9.2%	1.22 [0.83, 1.80]
Nakamura 2015	273	640	268	622	11.6%	0.98 [0.79, 1.23]
Pejovic-Milovancevic 2016	9	49	65	278	4.8%	0.74 [0.34, 1.60]
Pinheiro 2017	13	54	10	78	3.9%	2.16 [0.87, 5.36]
Raffa 2013	59	138	67	123	7.8%	0.62 [0.38, 1.02]
Rodríguez-Santiago 2009	142	645	105	593	10.8%	1.31 [0.99, 1.74]
Saadat 2007	52	292	99	292	9.3%	0.42 [0.29, 0.62]
Saruwatari 2013	68	154	99	203	8.7%	0.83 [0.55, 1.26]
Yan 2020	158	379	161	415	10.7%	1.13 [0.85, 1.50]
Zhang 2020	176	386	133	264	10.3%	0.83 [0.60, 1.13]
Total (95% CI)		3260		3411	100.0%	0.90 [0.73, 1.11]
Total events	1081		1142			

Heterogeneity: Tau² = 0.09; Chi² = 37.51, df = 12 (P = 0.0002); I² = 68%
Test for overall effect: Z = 1.00 (P = 0.32)

C

Study or Subgroup	Case Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Gravina 2011	10	138	21	133	11.4%	0.42 [0.19, 0.92]
Kashani 2012	3	93	2	99	3.7%	1.62 [0.26, 9.90]
Pejovic-Milovancevic 2016	5	49	23	278	8.6%	1.26 [0.45, 3.49]
Pinheiro 2017	9	54	3	78	5.8%	5.00 [1.29, 19.44]
Raffa 2013	39	138	30	123	15.4%	1.22 [0.70, 2.12]
Saruwatari 2013	40	150	45	203	16.5%	1.28 [0.78, 2.09]
Yan 2020	109	379	83	415	19.5%	1.61 [1.16, 2.24]
Zhang 2020	93	386	78	264	19.1%	0.76 [0.53, 1.08]
Total (95% CI)		1387		1593	100.0%	1.15 [0.79, 1.69]
Total events	308		285			

Heterogeneity: Tau² = 0.16; Chi² = 20.68, df = 7 (P = 0.004); I² = 66%
Test for overall effect: Z = 0.74 (P = 0.46)

Figure 2 Forest plot of overall studies. (**A**) Analysis of null genotype vs present genotype of *GSTM1*. P = 0.25 [Overall OR = 1.11, 95% CI = (0.93–1.32)]. (**B**) Analysis of null genotype vs present genotype of *GSTT1*. P = 0.32 [Overall OR = 0.90, 95% CI = (0.73–1.11)]. (**C**) Analysis of null genotype vs present genotype of *GSTM1 + GSTT1*. P = 0.46 [Overall OR = 1.16, 95% CI = (0.79–1.69)].

Table 3 Overall Analysis and Subgroup Analysis of Ethnicity

Gene Symbols	Comparsions	Ethnicity	No. of Studies	Heterogeneity		Model	OR	95% CI	p
				p	I^2				
GSTM1	Null type vs Present type	All	15	<0.0001	68%	Random	1.11	0.93–1.32	0.25
		Asian	8	0.0003	74%	Random	1.19	0.94–1.49	0.15
		Caucasian	7	0.04	55%	Random	1.00	0.75–1.33	0.98
GSTT1	Null type vs Present type	All	13	0.0002	68%	Random	0.90	0.73–1.11	0.32
		Asian	5	0.42	0%	Fixed	0.99	0.87–1.13	0.89
		Caucasian	8	<0.0001	78%	Random	0.85	0.55–1.31	0.46
GSTM1+GSTT1	Double null vs Present type	All	8	0.004	66%	Random	1.15	0.79–1.69	0.46
		Asian	3	0.008	79%	Random	1.16	0.71–1.89	0.56
		Caucasian	5	0.03	63%	Random	1.22	0.59–2.51	0.60

Abbreviations: GSTM1/T1, glutathione S-transferase mu 1/theta 1; OR, odds ratio; CI, confidence interval.

Table 4 Overall Analysis and Subgroup Analysis of PCR Methods

Gene Symbols	Comparsions	PCR Methods	No. of Studies	Heterogeneity		Model	OR	95% CI	p
				p	I^2				
GSTM1	Null type vs Present type	All	15	<0.0001	68%	Random	1.11	0.93–1.32	0.25
		Multiplex PCR	8	0.35	10%	Fixed	1.17	1.00–1.37	**0.05**
		Others	7	<0.0001	81%	Random	1.02	0.76–1.36	0.91
GSTT1	Null type vs Present type	All	13	0.0002	68%	Random	0.90	0.73–1.11	0.32
		Multiplex PCR	8	0.02	58%	Random	0.73	0.56–0.94	**0.02**
		Others	5	0.18	36%	Fixed	1.14	0.98–1.32	0.08
GSTM1+GSTT1	Double null vs Present type	All	8	0.004	66%	Random	1.15	0.79–1.69	0.46
		Multiplex PCR	5	0.09	50%	Fixed	0.90	0.71–1.14	0.39
		Others	3	0.28	21%	Fixed	1.73	1.27–2.36	**0.0006**

Notes: p > 0.05, the difference is not statistically significant; p < 0.05, indicated in bold, the difference is statistically significant; p = 0.05, bold, the difference tends to be statistically significant, but need to further analysis.
Abbreviations: GSTM1/T1, glutathione S-transferase mu 1/theta 1; PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval.

Table 5 Overall Analysis and Subgroup Analysis of Stars Obtained from NOS

Gene Symbols	Comparsions	Stars of Rewritten NOS	No. of Studies	Heterogeneity		Model	OR	95% CI	p
				p	I^2				
GSTM1	Null type vs Present type	All	15	<0.0001	68%	Random	1.11	0.93–1.32	0.25
		6–10 stars	9	0.25	22%	Fixed	**1.24**	**1.08–1.43**	**0.002**
		<=5 stars	6	0.001	76%	Random	0.95	0.72–1.25	0.70
GSTT1	Null type vs Present type	All	13	0.0002	68%	Random	0.90	0.73–1.11	0.32
		6–10 stars	9	0.0007	70%	Random	0.82	0.63–1.08	0.16
		<=5 stars	4	0.15	43%	Fixed	1.09	0.92–1.29	0.30
GSTM1+GSTT1	Double null vs Present type	All	8	0.004	66%	Random	1.15	0.79–1.69	0.46
		6–10 stars	6	0.001	76%	Random	1.13	0.73–1.75	0.58
		<=5 stars	2	0.81	0%	Fixed	1.34	0.56–3.24	0.51

Notes: p > 0.05, the difference is not statistically significant; p < 0.05, indicated in bold, the difference is statistically significant.
Abbreviations: GSTM1/T1, glutathione S-transferase mu 1/theta 1; OR, odds ratio; CI, confidence interval; NOS, Newcastle-Ottawa quality assessment scale.

Table 6 Overall Analysis and Subgroup Analysis of Deductions Assessed Using Rewritten NOS

Gene Symbols	Comparsions	Deductions	No. of Studies	Heterogeneity		Model	OR	95% CI	p
				p	I²				
GSTM1	Null type vs	All	15	<0.0001	68%	Random	1.11	0.93–1.32	0.25
	Present type	No deductions	10	0.08	42%	Fixed	**1.20**	**1.05–1.38**	**0.007**
		Deductions	5	0.001	78%	Random	1.00	0.75–1.34	0.99
GSTT1	Null type vs	All	13	0.0002	68%	Random	0.90	0.73–1.11	0.32
	Present type	No deductions	10	0.0005	70%	Random	0.85	0.65–1.12	0.26
		Deductions	3	0.18	42%	Fixed	1.08	0.91–1.28	0.39
GSTM1+GSTT1	Double null vs	All	8	0.004	66%	Random	1.15	0.79–1.69	0.46
	Present type	No deductions	7	0.002	71%	Random	1.15	0.76–1.74	0.51
		Deductions	1	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

Notes: p > 0.05, the difference is not statistically significant; p < 0.05, the difference is statistically significant. Not applicable: only one studies is not suitable for analysis.
Abbreviations: GSTM1/T1, glutathione S-transferase mu 1/theta 1; OR, odds ratio; CI, confidence interval; NOS, Newcastle-Ottawa quality assessment scale;

As we all known, the distribution of *GSTM1/T1* deletion is various in different populations. Nelson et al found that the frequency of *GSTT1* null genotype was 64% in Asian, 28% in Caucasian, and 22% in African American population.[28,29] Therefore, stratification of studies by ethnicity was usually used in meta-analysis. However, we failed to find the association between *GST* genes and risk of SZ in Asian population. These results suggested that subgroup analysis of ethnicity might be out of magic and other assessment methods of studies should be promoted. In the present studies, we performed stratified analysis of studies by PCR methods and rewritten NOS. When subgroup analysis of multiplex PCR, *GSTM1* null showed a weak association with SZ risk, but *GSTT1* null was a protective factor. These inconsistent results were interesting and needed to be interpreted with caution. The reason might be that the same PCR method used in the studies would be comparable and the judgement of genotype was less arbitrariness.

The quality assessment of reported studies was the highlights of this study. As shown in Supplementary Table S2, the NOS was rewritten and some important criteria were added for evaluation, including methodology domain and deduction domain. Deductions were defined by inconsistent data or incorrect descriptions, which would affect the quality score of studies. The detailed information was listed in the Supplementary Table S1. Meta-analysis was an evidence-based method using reported studies, and study quality was very important for making a conclusion. Therefore, we re-written the NOS scale for quality assessment and conducted the sub-analysis. The subgroup analysis of studies obtained 6–10 stars revealed that *GSTM1* null genotype was significantly associated with risk of SZ. The result indicated that *GSTM1* null was related to SZ risk in high-quality studies. Moreover, in studies with no deductions, *GSTM1* null also showed a significant association with risk of SZ. The same conclusions were reached, and the consistent results were also indicated that study quality was very important and needed to be assessed. However, quality assessment of studies was missing and ignored in the reported meta-analysis. These results were very interesting and suggested that the included studies needed to be assessed for quality in further meta-analysis. Our study provided a new quality assessment method with the ability to discover potential associations.

The deficiency of GST enzymes causes by deletions of *GSTM1/T1* could lead to the reduction of Glutathione (GSH).[30–32] GSH is an important antioxidant, which detoxifies reactive oxygen species (ROS) in the central nervous system, and thus plays an important role in protecting neural tissues.[33,34] GSH levels were significantly decreased in cerebrospinal fluid of SZ patients and proton magnetic resonance spectroscopy revealed a significant reduction of GSH in the medial prefrontal cortex of schizophrenic patients, suggesting that GSH-related enzyme deficiency might play a vital role in risk and etiology of schizophrenia.[11,32] Besides, dysregulation of glutathione metabolism was reported to be associated with brain inflammation in psychiatric disorders.[35,36] *GSTM1* null genotype might increase risk of SZ through brain inflammation induced by enzyme inactivity.[35–37]

GST genes were widely present in human body and were abundant in the kidney, liver, and lung.[36–38] In addition, GSTM1 and GSTP1 were detected in both human and rodent brains.[39] Notably, GSTM1 was also suggested that it is one of the most abundant proteins in astrocytes.[40] However, the result showed that *GSTT1* null might be a protective factor. This result should be interpreted with caution and the reason should be further research. It indicated that *GST* genes played different roles in SZ patients and combination of *GSTM1* null and *GSTT1* present should be further discussed.

There are some limitations in this meta-analysis. First, only case–control studies were included in this meta-analysis, which would cause publication bias. However, funnel plot, Egger's test and Begg's test showed that publication bias was negligible. Second, *GSTM1* null and *GSTT1* null were antithetical in subgroup analysis of multiplex PCR and should be interpreted with caution. Some detailed information might be important for this conflicted result. However, it was hard for collecting the information. Third, heterogeneity is difficult to rule out and risk of bias is difficult to assess. It may be determined by confounding factors, such as control selection, methods, and missing data (arising from reporting biases). However, the information is hard to evaluate completely.

This study is a meta-analysis to investigate the relationship between *GSTM1/T1* deletions and susceptibility to SZ. We found that *GSTM1* null was associated with SZ risk in studies using multiplex PCR. The significant association was also observed between *GSTM1* null and SZ risk in high-quality studies. Besides, we provided a new method for quality assessment and it should be promoted in further analysis. To confirm this result, further studies with larger sample size are required to provide more precise evidence.

Data Sharing Statement

All the data used to support the findings of this study are included within the article.

Registration Information

The review was not registered.

Review Protocol

A protocol was not prepared.

PRISMA 2020 Statement

This systematic review adherence to the PRISMA 2020 guidelines.

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Disclosure

The authors declare no conflict of interest.

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