Association between Common Genetic Variants in *ESR1* and Stroke Risk: A Systematic Review and Meta-Analysis

Rong Fu, MD, Yin Shen, MD, and Jin Zheng, MD

Objectives: The associations between estrogen receptor alpha (*ESR1*) polymorphisms and stroke risk have been investigated in various studies, but remain controversial. The aim of this meta-analysis was to determine the relationships between *ESR1* rs2234693 and rs9340799 polymorphisms and the risk of stroke. *Methods:* Electronic databases of PubMed, Embase, Cochrane Library, CNKI, VIP database, and Wan-Fang database were searched for eligible studies up to March 2019. The pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the strength of associations under different genetic models. *Results:* Ten independent case-control studies including 2151 stroke cases and 6378 control subjects were enrolled in this meta-analysis. The meta-analysis results indicated that *ESR1* rs2234693 polymorphism was associated with an increased risk of stroke in recessive model (OR, 1.20; 95%CI, 1.04-1.38) and homozygous model (OR, 1.18; 95%CI, 1.00-1.38). Subgroup analysis on stroke subtype showed that rs2234693 polymorphism was connected with ischemic stroke, but not hemorrhagic stroke. The further subgroup analyses on ethnicity and gender suggested that the association between rs2234693 polymorphism and stroke risk was significant in Caucasian population and in the male population. No positive associations between rs9340799 polymorphism and stroke risk were detected in 4 genetic models. *Conclusions:* The results of this meta-analysis suggest that *ESR1* rs2234693 polymorphism is significantly correlated with an increased risk of stroke, especially ischemic stroke. There was no evidence of a significant association between *ESR1* rs9340799 polymorphism and stroke risk.

Key Words: Stroke—ESR1—rs2234693—polymorphisms—meta-analysis

Introduction

Stroke is a leading cause of mortality and disability around the world. In 2016, 5.5 million deaths and 116.4 million disability-adjusted life-years were due to stroke, and although age-standardized mortality rates have decreased sharply from 1990 to 2016, the reduction in age-standardized incidence has been less steep, indicating that the burden of stroke is likely to remain high.[1] Stroke is considered to be a complex multifactorial and polygenic disease, which is closely related to environmental factors and genetic factors. Established risk factors for stroke comprise advanced age, smoking, drinking, obesity, hypertension, hyperlipidemia, diabetes, and so on,[2] and in recent years, increasing evidence support that genetic factors also play a crucial role in the pathogenesis of stroke.[3,4]

Epidemiological and laboratory studies have revealed that estrogen has a protective effect on the cardiovascular system, which protects young women from cardiovascular diseases.[5,6] Estrogen exerts its biological effects mainly by binding to 2 specific estrogen receptors (ERs), ER-α and ER-β, which were encoded by *ESR1* and *ESR2*, respectively.[6] Studies have proved that *ESR1* genetic variants might affect the expression of *ESR1* and further influence the effect of estrogen.[6] *ESR1*, located on chromosome 6, comprises 7 introns and 8 exons. There are 2 common site polymorphisms in the first intron, located at the recognition sites of the restriction enzymes and known as *Pvu*II or

From the Department of Neurosurgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

Received April 22, 2019; revision received July 14, 2019; accepted August 14, 2019.

Address correspondence to Jin Zheng, MD, Department of Neurosurgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Street, Jianghan District, Wuhan 430022, China. E-mail: 2012xh0948@hust.edu.cn.

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.104355

rs2234693 and *Xba*I or rs9340799. Previous studies have suggested that *ESR1* rs2234693 and rs9340799 polymorphisms were related to various diseases, such as breast cancer,[7] hypertension,[8] and coronary artery disease.[9]

Recently, the relationships between *ESR1* rs2234693 and rs9340799 polymorphisms and stroke risk have been explored in various observational studies. However, the results of these studies were greatly inconsistent. A few studies[10,11] have demonstrated that rs2234693 and rs9340799 polymorphism was correlated with stroke risk, but some other studies[12-20] did not discover the association. Hence, we conducted this meta-analysis by systematically reviewing the current evidence to clarify the relationships between *ESR1* rs2234693 and rs9340799 polymorphisms and stroke risk, and further evaluated the effect of study characteristics on the relationships.

Materials and Methods

Study Selection

We searched the electronic databases of PubMed, Embase, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI), VIP database, and Chinese WanFang database (update until for March 8, 2019) for eligible studies with the following keywords and subject terms ("estrogen receptor" or "estrogen receptor alpha" or "ESR1" or "ESRα"), ("stroke" or "cerebrovascular disease"), and ("SNP" or "mutation" or "variation" or "polymorphism" or "variant").

Two investigators (R. Fu and Y. Shen) independently reviewed and screened all relevant articles by the following inclusion criteria: (1) cohort study or case-control study investigating the relationship of *ESR1* rs2234693 and rs9340799 polymorphisms with stroke; (2) clear definition of stroke; (3) more than 10 cases of stroke were reported; (4) sufficient data on the frequencies of SNPs were provided; (5) articles were published in English or Chinese language and in peer-reviewed journals. If multiple studies were published with the same study population, only the study reporting the larger number of participants was included. Disagreements were adjudicated by consensus.

Data Extraction

Two researchers (R Fu and Y Shen) independently abstracted relevant data from all included studies, and disagreements were resolved by consensus. The following data were extracted from each study: first author, year of publication, region, ethnicity, sample size, age of case and control, stroke type, genotyping method, SNP type, evidence of Hardy-Weinberg equilibrium in control subjects, and allele and genotype distributions of *ESR1* rs2234693 and rs9340799. A well-defined diagnosis of stroke was defined as 1 validated by computed tomography, magnetic resonance imaging, or cerebral angiography. All data were extracted from the included studies, and we did not contact the authors for additional data.

Quality Assessment

Two researchers (R. Fu and Y. Shen) independently assessed the methodological quality of the included studies using the Newcastle-Ottawa scale (NOS).[21] The NOS evaluates quality of observational study based on 3 aspects: selection, comparability, and ascertainment of exposure and outcomes. Three aspects assigns a maximum score of 4, 2, and 3, respectively, and the assessment score for each study ranges from 0 to 9. Studies with a NOS score of 7 or more were regarded as high-quality study. Any disagreements were settled by consensus.

Statistical Analysis

To investigate the relationships of *ESR1* rs2234693 and rs9340799 polymorphisms with stroke risk, we conducted the meta-analyses using a series of genetic models, including allele model (C versus T for rs2234693 and G versus A for rs9340799), homozygous model (CC versus TT for rs2234693 and GG versus AA for rs9340799), dominant model (TC/CC versus TT for rs2234693, and AG/GG versus AA for rs9340799), and recessive model (CC versus TT/TC for rs2234693 and GG versus AA/AG for rs9340799). Besides theses, subgroup analyses were carried out according to stroke subtype, ethnicity, gender, and sample size. The strength of correlation between *ESR1* variants and stroke was measured by odds ratios (ORs) and the corresponding 95% confidence intervals (CIs). Between-study heterogeneity was evaluated by the χ^2-based Q test and I^2 statistics. *P* value of Q test < .10 and I^2 > 50% indicated evidence of heterogeneity, and then we used a random-effect model to count the summary risk estimate; otherwise, we chose a fixed-effect model. Sensitivity analysis was performed by removing 1 study at a time and calculating the rest repeatedly to evaluate the stability of the overall results. Begg's funnel plot and Egger's test were used to estimate potential publication bias, and *P* value of Egger's test <.05 indicated the existence of publication bias. All above statistical analyses were performed using Stata 14.0, and *P* values were 2-sided with a statistical significance level of .05, except for tests of heterogeneity where a level of .10 was used.

Results

Characteristics of Studies

We identified a total of 234 relevant articles through searching electronic databases. Of these, 24 duplicate articles were removed, 190 articles were excluded by screening title and abstract, and a further 10 articles were excluded based on reviewing full text. Finally, 10 independent case-control studies meeting all inclusion criteria were included in this meta-analysis for testing the relationship between *ESR1* variants and stroke risk (Fig 1).

Among the included studies, 4 studies were conducted in Caucasian population and 6 studies in Asian

population. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or TaqMan was used for genotyping of *ESR1* rs2234693 and rs9340799. Two studies reported outcomes on stroke in which 1 study distinguished ischemic stroke and hemorrhagic stroke, 7 studies only reported ischemic stroke, and 1 study only reported hemorrhagic stroke. Genotype frequencies of control subjects were all in agreement with Hardy-Weinberg equilibrium (all $P > .05$). All included studies have 7-8 points according to the NOS, which indicates that these studies have high quality and might be subject to low risk of bias. The detailed characteristics and methodological quality of the included studies are summarized in Table 1.

Association of ESR1 rs2234693 Polymorphism and Stroke Risk

Ten studies with 2151 stroke cases and 6378 controls were pooled to examine the association of *ESR1* rs2234693 polymorphism with stroke risk. Positive associations were found in recessive model (OR, 1.20; 95%CI, 1.04-1.38) and homozygous model (OR, 1.18; 95%CI, 1.00-1.38), whereas no evidence of correlation was discovered in allele model (OR, 1.07; 95%CI, .99-1.16) and dominant model (OR, 1.03; 95%CI, .92-1.16). No significant heterogeneity among these studies were detected in the 4 genetic models (All $I^2 < 50\%$ and P for heterogeneity >.10) (Fig 2).

We also conducted subgroup analyses on stroke type, ethnicity, gender, and sample size. Subgroup analysis of stroke type indicated that rs2234693 polymorphism was associated with ischemic stroke (Recessive model: OR, 1.19; 95%CI, 1.02-1.39), but not with hemorrhagic stroke. Subgroup analysis of ethnicity showed that the positive association between rs2234693 polymorphism and stroke risk was present in Caucasian population (Recessive model: OR, 1.27; 95%CI, 1.02-1.57), not in Asian population or Chinese population. In the subgroup analysis of gender, the results suggested that rs2234693 polymorphism was related to an increased risk of stroke in the

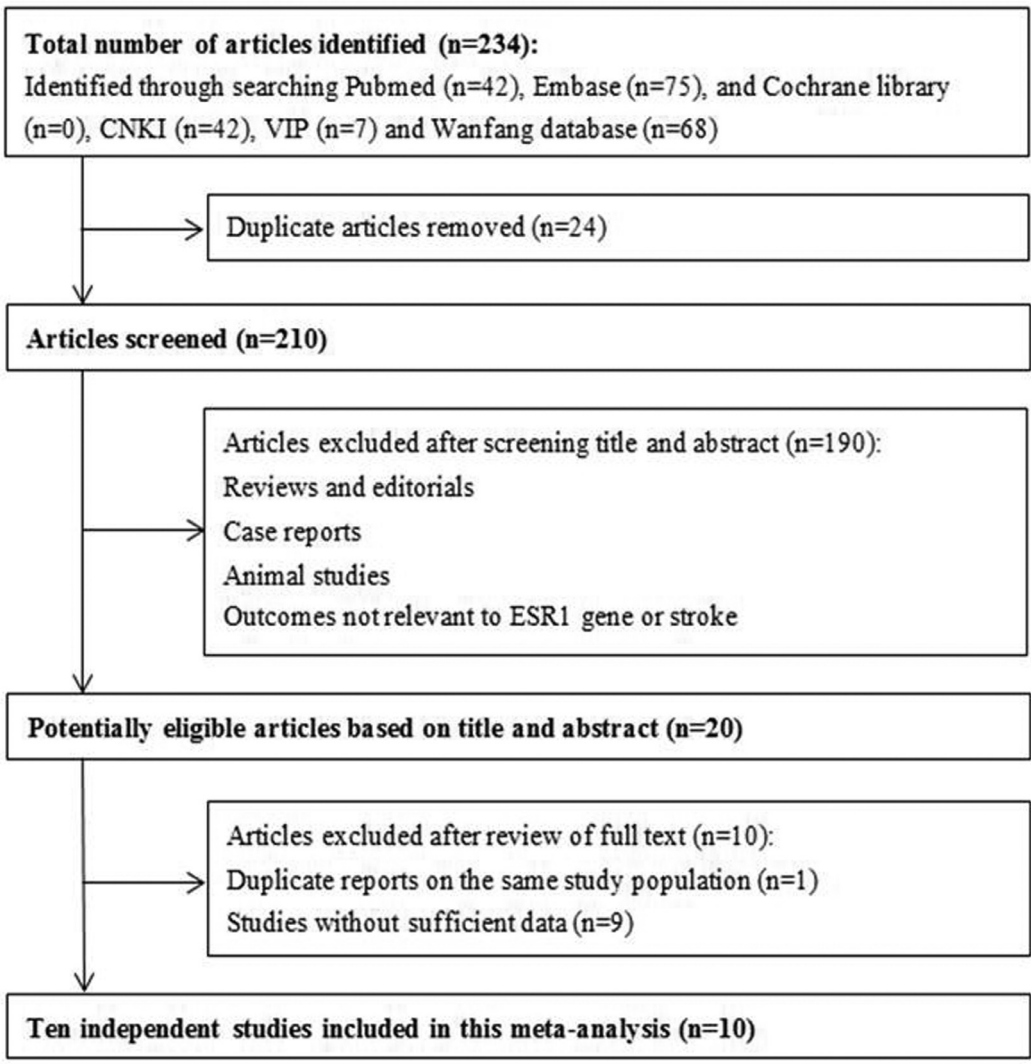

Figure 1. *Flowchart of the selection of eligible studies.*

Table 1. *Basic characteristics of the studies included for this meta-analysis*

Study	Region (Eethnicity)	Sample size (Ccase/Ccontrol)	Age* (Ccase/Ccontrol)	Gender		Stroke type	Genotyping method	SNP type	HWE test	NOS score
				Case (Mmale/ Ffemale)	Control (Mmale/ Ffemale)					
Zhang, 2002	China (Asian)	234/259	63.9 ± 10.3/59.2 ± 9.2	140/94	91/168	Ischemic stroke	PCR-RFLP	rs2234693	.09	7
								rs9340799	.23	
Zhang, 2004	China (Asian)	63/160	61.8 ± 7.7/58.8 ± 8.7	0/63	0/160	Hemorrhagic stroke	PCR-RFLP	rs2234693	.06	7
Shearman, 2005	UK (Caucasian)	55/2654	57.5 ± 3.1/56.0 ± 3.4	55/0	2654/0	Stroke	PCR-RFLP	rs2234693	.23	8
Kjaergaard, 2007	Denmark (Caucasian)	311/1239	64 (57−70)/64(57−70)†	187/124	743/496	Ischemic stroke	TaqMan	rs2234693	.82	8
Molvarec, 2007	Hungary (Caucasian)	198/180	68 (56−75)/52(49−56)†	96/102	81/99	Ischemic stroke	PCR-RFLP	rs2234693	.55	7
								rs9340799	.86	
Lazaros, 2008	Greece (Caucasian)	130/240	59.2 ± 8.6/58.4 ± 8.3	84/46	100/140	Ischemic stroke	PCR-RFLP	rs2234693	.77	7
								rs9340799	.55	
Wang, 2009	China (Asian)	40/39	69.9 ± 8.6/66.7 ± 9.6	40/0	39/0	Ischemic stroke	PCR-RFLP	rs2234693	.92	7
Munshi, 2010	India (Asian)	400/380	49.3 ± 17.3/47.0 ± 16.8	285/115	278/102	Ischemic stroke	PCR-RFLP	rs2234693	.30	8
								rs9340799	.20	
Hsieh, 2012	China (Asian)	305/309	43.7 ± 6.0/43.6 ± 5.6	217/88	221/88	Ischemic stroke	PCR-RFLP	rs2234693	.12	8
								rs9340799	.05	
Huang, 2015	China (Asian)	453/919	58.2 ± 8.9/58.2±8.8	0/453	0/919	Stroke (Ischemic/Hemorrhagic stroke)	TaqMan	rs2234693	.72	8
								rs9340799	.36	

Abbreviations: HWE, Hardy-Weinberg equilibrium; NOS, Nowcastle-Ottawa scale; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

*Mean ± standard deviation.

†Median (minimum-maximum).

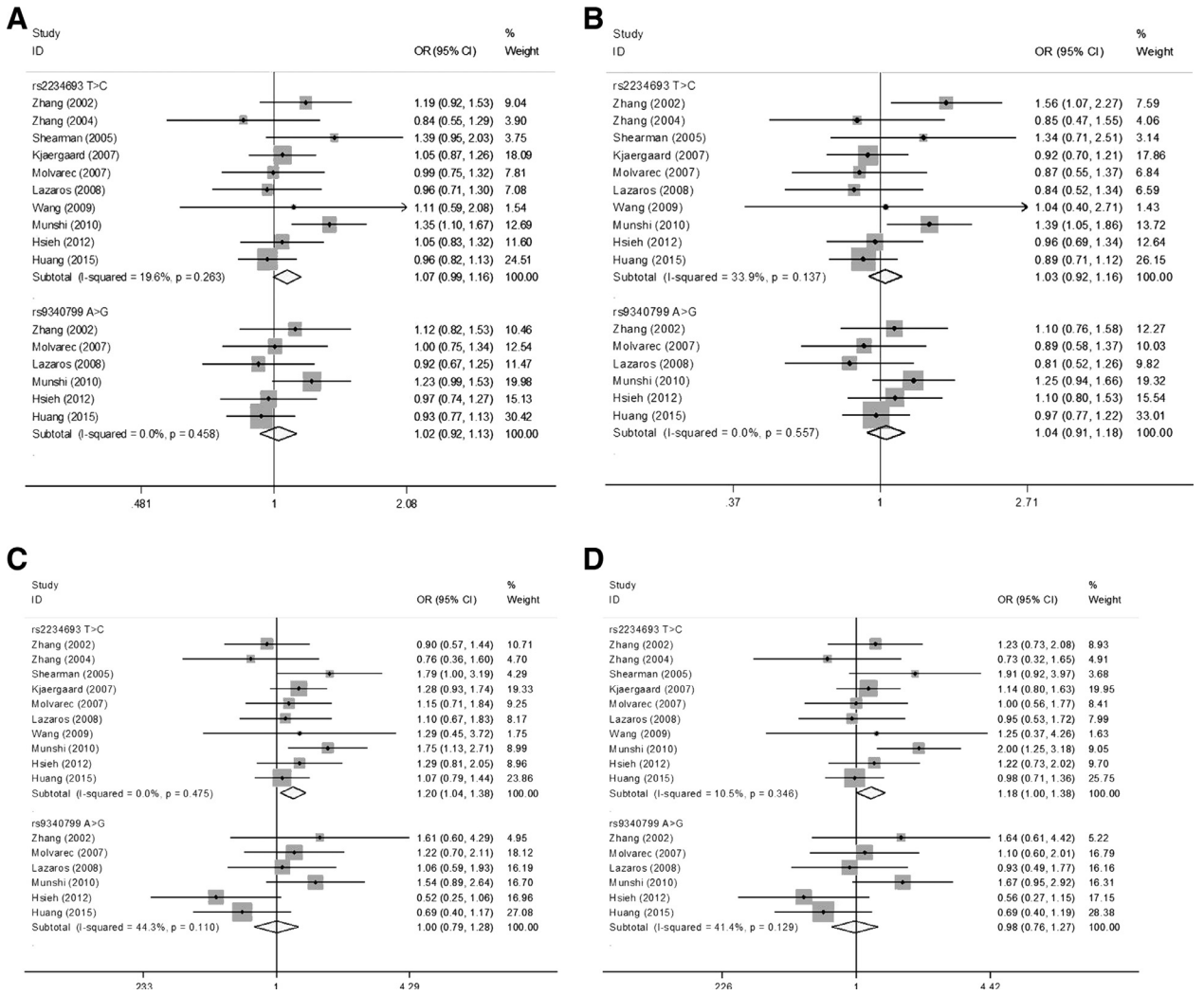

Figure 2. *Forest plots for the relationships between* ESR1 *genetic polymorphisms and stroke risk.* ***(A)*** *allele model: rs2234693 (C verusus T); rs9340799 (G versus A).* ***(B)*** *dominant model: rs2234693 (TC + CC versus TT); rs9340799 (AG + GG versus AA).* ***(C)*** *recessive model: rs2234693 (CC versus TT + TC); rs9340799 (GG versus AA + AG).* ***(D)*** *homozygous model: rs2234693 (CC versus TT); rs9340799 (GG versus AA).*

male population (Recessive model: OR, 1.38; 95%CI, 1.06-1.80), but not in the female population. We further conducted subgroup analysis of gender on ischemic stroke, and we detected similar findings that the relationship between rs2234693 polymorphism and increased risk of ischemic stroke was also significant for male population, not female population. Further subgroup study based on sample size showed significant relationship between rs2234693 polymorphism and stroke risk in the group of larger sample size ($n \geq 500$) (recessive model: OR, 1.30; 95%CI, 1.10-1.54; homozygous model: OR, 1.25; 95%CI, 1.03-1.51) (Table 2).

Association of ESR1 rs9340799 Polymorphism and Stroke Risk

Six studies including 1716 stroke cases and 2287 controls were combined to test the association between *E*SR1 rs9340799 polymorphism and stroke risk. However, we did not detect any positive association between them in 4 genetic models and no significant heterogeneity among these studies were found in the 4 genetic models (Fig 2).

Further subgroup analyses on stroke type, ethnicity, gender, and sample size also did not find any significant relationship between *E*SR1 rs9340799 polymorphism and stroke risk (Table 3).

Sensitivity Analysis and Publication Bias

Sensitivity analyses was performed based on data of recessive genetic model, and the results indicated that removing each separate study did not significantly affect the overall pooled ORs and the results of the meta-analysis on association of *ESR1* rs2234693 and rs9340799 polymorphisms with stroke risk were stable and reliable (Fig 3).

Publication bias was qualitatively estimated by the Begg's funnel plots and quantitatively examined using

Table 2. *Stratified meta-analyses of the correlation between rs2234693 polymorphism and stroke risk*

Study characteristics	No. of studies	Sample size (case/control)	Allele model (C versus T)		Dominant model (TC/CC versus TT)		Recessive model (CC versus TT/TC)		Homozygous model (CC versus TT)	
			OR (95%CI)	I^2(%)	OR (95%CI)	I^2(%)	OR (95%CI)	I^2(%)	OR (95%CI)	I^2(%)
Total	10	2151/6378	1.07 (.99−1.16)	19.6	1.03 (.92−1.16)	33.9	**1.20 (1.04−1.38)**	0	**1.18 (1.00−1.38)**	10.5
Stroke subtype										
Ischemic stroke	8	1865/3564	1.05 (.97−1.15)	40.3	1.01 (.82−1.23)	59.2	**1.19 (1.02-1.39)**	.0	1.14 (.96-1.36)	22.1
Hemorrhagic stroke	2	228/1079	.91 (.74−1.13)	.0	.84 (.63−1.13)	.0	.98 (.67−1.43)	.0	.89 (.59−1.34)	.0
Ethnicity										
Caucasian	4	660/4313	1.05 (.93−1.20)	.0	.93 (.76−1.14)	.0	**1.27 (1.02−1.57)**	.0	1.14 (.89−1.47)	.0
Asian	6	1491/2065	1.09 (.98−1.20)	40.9	1.09 (.95−1.25)	52.0	1.16 (.96−1.39)	21.0	1.20 (.98−1.47)	33.2
Chinese	5	1091/1685	1.02 (.91−1.14)	.0	1.01 (.86−1.18)	39.8	1.05 (.85−1.29)	.0	1.05 (.84−1.32)	.0
Gender (stroke)										
Male	5	631/1194	1.08 (.93−1.26)	.0	.96 (.77−1.19)	.4	**1.38 (1.06−1.80)**	.0	1.25 (.93−1.68)	.0
Female	7	982/2072	.95 (.79−1.14)	53.1	.90 (.67−1.21)	60.4	.97 (.79−1.19)	35.6	.95 (.75−1.19)	39.3
Gender (ischemic stroke)										
Male	5	631/1194	1.08 (.93−1.26)	.0	.96 (.77−1.19)	.4	**1.38 (1.06−1.80)**	.0	1.25 (.93−1.68)	.0
Female	6	751/1912	1.06 (.84−1.35)	66.4	1.11 (.75−1.65)	74.4	1.03 (.82−1.29)	42.2	1.03 (.80−1.33)	52.1
Sample size										
Large (n ≥ 500)	5	1486/5500	1.09 (.99−1.20)	52.3	1.02 (.89−1.17)	44.7	**1.30 (1.10−1.54)**	14.4	**1.25 (1.03−1.51)**	46.8
Small (n < 500)	5	665/878	1.03 (.89−1.19)	.0	1.06 (.85−1.33)	36.3	1.01 (.79−1.31)	.0	1.02 (.76−1.37)	.0

Abbreviations: CI, confidence interval; OR, odds ratio.

Begg's test and Egger's test based on recessive genetic model data. The Begg's funnel plots showed the symmetry of patterning by visual inspection, which suggested no evidence of publication bias, and the further results of Begg's test and Egger's test also certified the outcomes (All $P > .05$) (Fig 4).

Discussion

Recently, numerous studies exploring the relationship between *ESR1* genetic variants and stroke risk have been conducted, but the results were discordant. In the present meta-analysis, 10 case-control studies with a total of 2151 stroke cases and 6378 controls were included to assess the relationships of *ESR1* rs2234693 and rs9340799 polymorphisms with stroke risk, and our study demonstrated that *ESR1* rs2234693 polymorphism was associated with an increased risk of stroke under recessive and homozygous models. We did not observe any positive association between rs9340799 polymorphism and stroke risk in the 4 genetic models.

We have conducted subgroup analysis on stroke subtype, and found that *ESR1* rs2234693 polymorphism was related to an increased risk of ischemic stroke. Our result was significantly consistent with the finding of a previous meta-analysis by Li et al published in 2012,[22] which only included 5 studies and also detected an elevated risk of ischemic stroke connected with rs2234693 polymorphism, not with rs9340799 polymorphism. We did not observe positive relationship between rs9340799 polymorphism and hemorrhagic stroke, whereas the result was reached based on very limited data of 2 studies, which required

Table 3. *Stratified meta-analyses of the correlation between rs9340799 polymorphism and stroke risk*

Study characteristics	No. of studies	Sample size (case/control)	Allele model (G versus A)		Dominant model (AG/GG versus AA)		Recessive model (GG versus AA/AG)		Homozygous model (GG versus AA)	
			OR (95%CI)	I^2(%)	OR (95%CI)	I^2(%)	OR (95%CI)	I^2(%)	OR (95%CI)	I^2(%)
Total	6	1716/2,287	1.02 (.92−1.13)	.0	1.04 (.91−1.18)	.0	1.00 (.79−1.28)	44.3	.98 (.76−1.27)	41.4
Stroke subtype										
Ischemic stroke	6	1548/2,287	1.03 (.92−1.14)	.0	1.33 (.90−1.98)	86.7	.78 (.43−1.40)	80.3	1.04 (.80−1.35)	28.3
Hemorrhagic stroke	1	165/919	.98 (.74−1.30)	—	.92 (.66−1.30)	—	1.23 (.65−2.36)	—	1.18 (.61−2.28)	—
Ethnicity										
Caucasian	2	328/420	.96 (.78−1.19)	.0	.85 (.63−1.16)	.0	1.15 (.77−1.71)	.0	1.01 (.65−1.58)	.0
Asian	4	1388/1867	1.04 (.93−1.17)	26.0	1.08 (.94−1.25)	.0	.94 (.54−1.64)	63.8	.99 (.56−1.74)	64.2
Chinese	3	988/1487	.98 (.85−1.12)	.0	1.03 (.87−1.22)	.0	.72 (.49−1.06)	41.5	.74 (.50−1.10)	35.7
Gender (stroke)										
Male	3	438/412	1.00 (.80−1.24)	20.5	.99 (.75−1.30)	.0	1.04 (.63−1.71)	67.6	.97 (.58−1.64)	62.9
Female	5	795/1417	1.06 (.77−1.47)	71.2	1.07 (.89−1.28)	62.9	.85 (.59−1.24)	57.6	.81 (.34−1.95)	68.0
Gender (ischemic stroke)										
Male	3	438/412	1.00 (.80−1.24)	20.5	.99 (.75−1.30)	.0	1.04 (.63−1.71)	67.6	.97 (.58−1.64)	62.9
Female	5	627/1417	1.06 (.76−1.48)	71.2	1.50 (.83−2.69)	84.9	.56 (.19−1.65)	79.1	.87 (.37−2.04)	64.3
Sample size										
Large (n ≥ 500)	3	1154/1608	1.03 (.91−1.17)	47.4	1.08 (.93−1.26)	.0	.84 (.44−1.59)	71.3	.88 (.45−1.71)	72.2
Small (n < 500)	3	562/679	1.01 (.85−1.20)	.0	.95 (.75−1.20)	.0	1.20 (.83−1.75)	.0	1.10 (.73−1.64)	.0

Abbreviations: CI, confidence interval; OR, odds ratio.

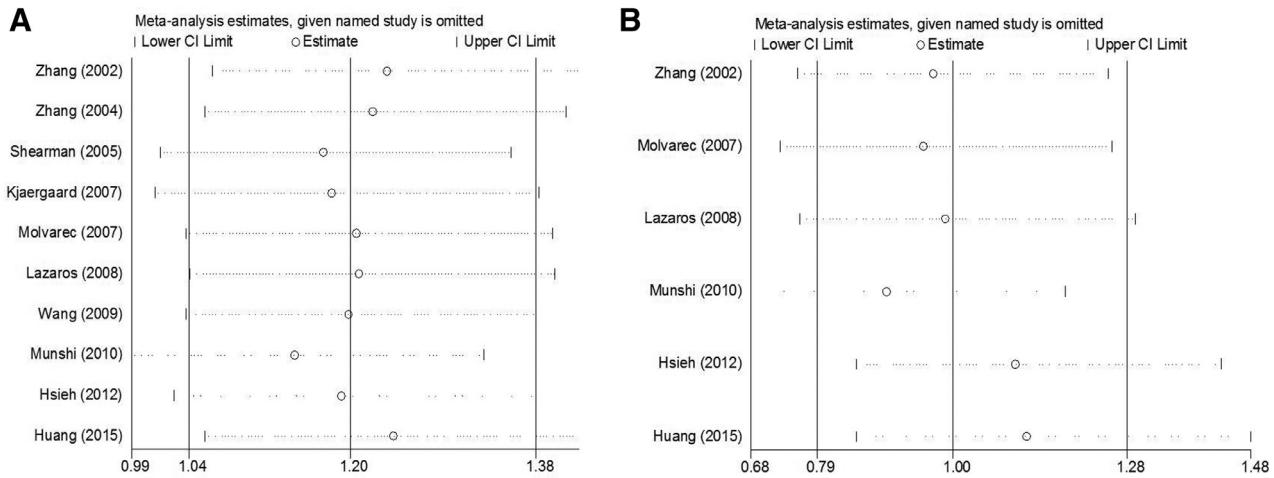

Figure 3. *Sensitivity analyses of the pooled ORs on the relationships between* ESR1 *genetic polymorphisms and stroke risk under recessive model. (**A**) rs2234693 (CC versus TT + TC); (**B**) rs9340799 (GG versus AA + AG).*

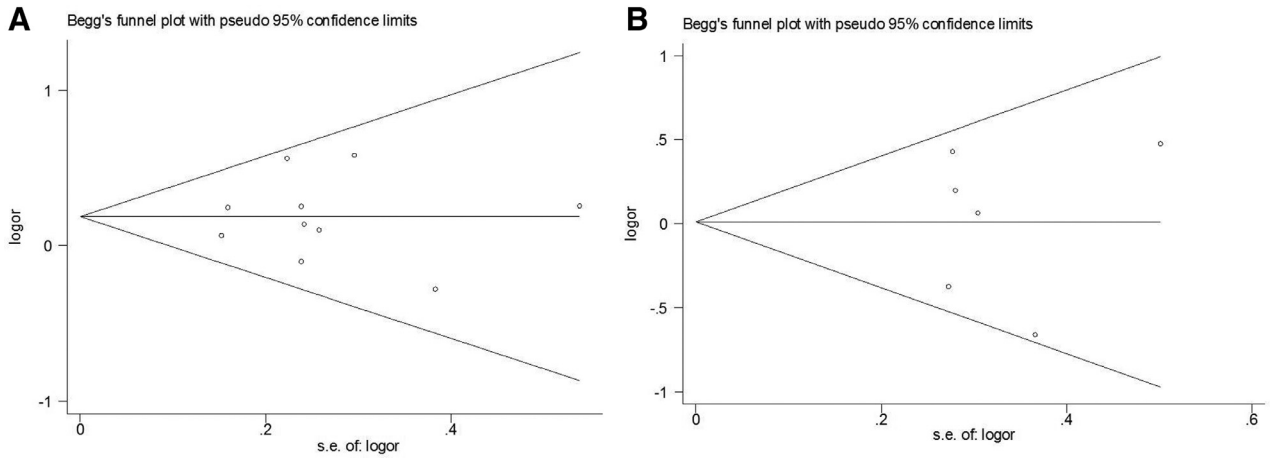

Figure 4. *Funnel plots of publication biases on the relationships between* ESR1 *genetic polymorphisms and stroke risk under recessive model.(**A**) rs2234693 (CC versus TT + TC); (**B**) rs9340799 (GG versus AA + AG).*

more investigations to clarify the relationship. Subgroup analysis of gender indicated that rs2234693 CC genotype could significantly increase the risk of stroke compared with TT/TC genotypes for males, and no statistically association between rs2234693 polymorphism and stroke risk was found for females. Wei et al[9] before have also discovered a positive correlation of rs2234693 polymorphism with coronary heart disease for men, not for women. These similar findings hint that rs2234693 polymorphism may play a vital role in the development of cardiovascular diseases for male population. However, currently it is hard to explain potential biological mechanism for the sex-specific finding, which still needs further study. Subgroup analysis on ethnicity demonstrated that the association between rs2234693 polymorphism and stroke risk was positive in Caucasians. However, no significant relationships were observed in Asian or Chinese population. The difference in stroke susceptibility may attribute to racial difference. Besides, it is well known that stroke is a complicated disease resulting from genetic and environmental factors as well as gene-environment interactions, and the differences in environment factors, such as lifestyles and climate, may affect the role of genes.

To the best of our knowledge, the present meta-analysis is the first meta-analysis to evaluate the relationships between *ESR1* genetic variants and stroke risk. Our meta-analysis results showed that rs2234693 polymorphism was associated with risk of stroke, especially ischemic stroke, suggesting that the polymorphism was significantly involved in the development of stroke. Although the exact function of *ESR1* gene in the pathogenesis of stroke is not fully elucidated, a potential explanation might be that rs9340799 polymorphism in the *ESR1* gene could affect the expression and regulation of ERα, which further influence blood pressure and/or lipid metabolism[6] and thereby influence the risk of stroke.

There are several limitations to this meta-analysis. Firstly, stroke is a complex multifactorial disease generated by the synthesized effect of environmental and genetic risk factors.[23] The effects of gene-environmental

and gene-gene interactions were not evaluated in this meta-analysis because of lacking of original data. Secondly, the results of this meta-analysis were based on unadjusted estimates, and the confounding factors, such as dyslidemia, hypertension, and diabetes[2], failed to be controlled for. Thirdly, only 10 studies with limited sample size were included in this meta-analysis, which limited the ability to make more solid conclusions.

In conclusion, results of this meta-analysis suggested that *ESR1* rs2234693 polymorphism is associated with an increased risk of stroke, especially in Caucasian population and male population. However, no evidence of positive correlation between *ESR1* rs9340799 polymorphism and stroke risk was detected. Further large-scale studies evaluating gene-gene and gene-environment interactions are needed.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments: Thanks to my wife, for supporting my work and encouraging me. Also we would like to express our gratitude to all those who helped me during the writing of this thesis, especially our superior Prof. Zhao Wohua.

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