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Association between the polymorphism (rs17222919, −1316T/G) of 5-lipoxygenase-activating protein gene (ALOX5AP) and the risk of stroke

A meta analysis

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Abstract

Background: The objective of this study was to evalutate the relationship between 5-lipoxygenase-activating protein gene (ALOX5AP) -rs17222919-1316T/G polymorphisms and the risk of stroke.

Methods: Relative studies were searched in January 2018. Case–control studies with extractable data were selected. Four gene models were analyzed including, allele genetic model (G vs T), additive genetic model (GG vs TT, GT vs TT), recessive genetic model (GG vs GT + TT), and dominant genetic model (GG + GT vs TT). Effect sizes included odds ratio (OR) and 95% confidence interval (CI). Heterogeneity was assessed by using Q test and I^2 test. Publication bias was evaluated by using Egger method. The reliability of the results was assessed with sensitivity analysis. All the data analysis was performed with R 3.10 software.

Results: A total of 5 studies inclusing 8492 patients were included. There were significant relationship between ALOX5AP-rs17222919-1316T/G polymorphisms and stroke under all models ($P < .05$) except the additive genetic model GT versus TT ($P > .05$). No publication bias was noted. Sensitivity analysis indicated that the results were not stable.

Conclusion: This meta-analysis indicates that ALOX5AP-rs17222919-1316T/G may be a protective factor aginst stroke.

Abbreviations: ALOX5AP = 5-lipoxygenase-activating protein gene, IS = ischemic stroke.

Keywords: ALOX5AP protein, human, meta-analysis, polymorphism, single nucleotide, stroke

Key Points

- This is meta-analysis of *ALOX5AP* polymorphism -rs17222919-1316T/G and stroke.
- A total of 5 studies inclusing 8492 patients were included.
- Signifiant relationship between ALOX5AP-rs17222919-1316T/G polymorphism was found.
- No publication bias was noted.
- Sensitivity analysis indicated that the results were not stable.

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1. Introduction

Stroke is an acute cerebrovascular disease that occurs when the blood supply to brain neurons is disrupted by either blockage (ischemic stroke, IS) or a bleeding (hemorrhagic stroke).[1] It is the second most common life-threaten disease worldwide.[2] The incidence of IS is higher than hemorrhagic stroke, accounting for 80% of the total stroke.[2] In recent years, the relationship of stroke susceptibility and genetic tendency gradually attracted people's attention.

The pathophysiology of stroke is complex and involves excitotoxicity mechanisms, inflammatory pathways, oxidative damage, ionic imbalances, apoptosis, angiogenesis, and neuroprotection.[3] In genetic test, arachidonate 5-lipoxygenase-activating protein gene (ALOX5AP) is found to be involved in the stroke. It encodes 5-lipoxygenase, an arachidonic acid metabolites which is critical for inflammatory responses.[4,5] Currently, a large amount of literatures have reported the relationship between stroke and ALOX5AP by clinical trials[6–14] and meta-analysis,[15,16] focusing on SG13S25G (rs17222814, promoter, G/A), SG13S114T (rs1050739), and other sites. However, the results about the site of rs17222919 were controversial, and no study evaluated the site of rs17222919 by meta-analysis. Therefore, we conducted this meta-analysis to explore the relationship between rs17222919 and stroke by combining the published results.

2. Materials and methods

2.1. Data sources

Search strategies were as follows: English electronic literatures were searched in PubMed (http://www.ncbi.nlm.nih.gov/

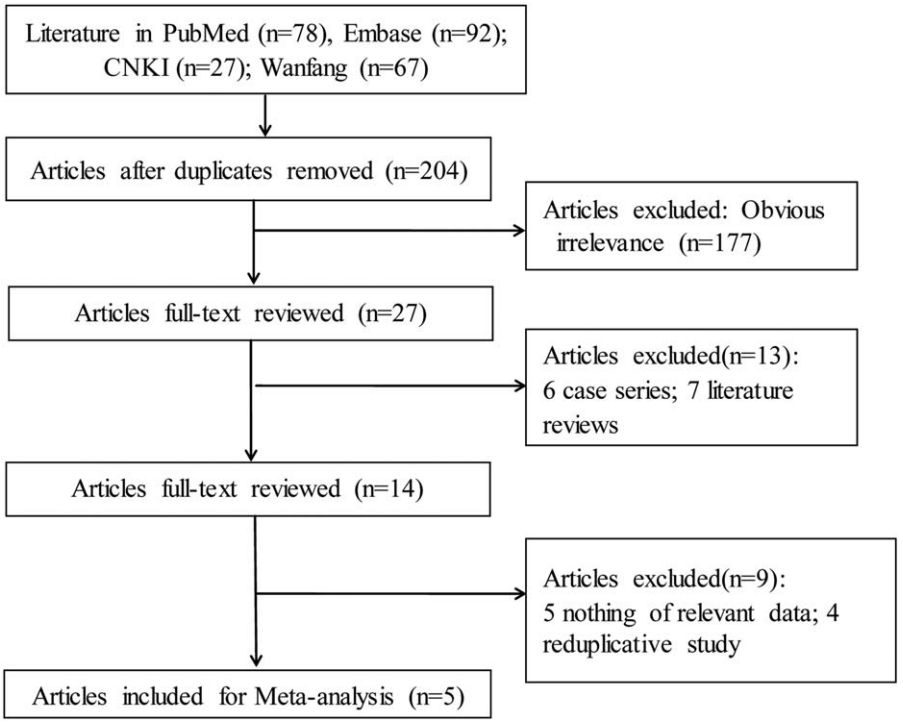

Figure 1. Flowchart of study selection.

Table 1

The basic characteristics of the selected studies.

Author	Public year	Location	Study year	NOS score	IS subjects			Control subjects		
					N	Male/female	Age, y (mean ± SD)	N	Male/female	Age, y (mean ± SD)
Fan	2015	China	NA	7	910	479/431	56.1 ± 10.6	925	478/447	55.3 ± 10.3
Fan	2015	China	NA	7	1003	542/461	60.5 ± 7.8	889	458/431	59.6 ± 7.4
Kim	2011	Korea	2007.10–2009.12	7	117	64/53	65.5 ± 12.1	398	194/204	53.8 ± 15.2
Wang	2012	China	2008–2011	8	658	392/266	69.42 ± 10.58	704	388/316	69.22 ± 9.04
Yang	2016	China	NA	6	810	416/394	57.7 ± 8.6	825	428/397	55.3 ± 7.2
Wang	2013	China	2010.9–2011.12	7	622	314/308	56.8 ± 10.2	631	321/310	55.9 ± 10.7

IS = ischemic stroke, NA = not available, NOS = Newcastle-Ottawa scale, SD = standard deviation.

Table 2

The distribution of genotypes.

Author	Public year	IS subjects				Control subjects				HWE in control		
		TT	TG	GG	T	G	TT	TG	GG	T	G	*P*
Fan	2015	593	288	29	1474	346	550	327	48	1427	423	.947
Fan	2015	658	312	33	1628	378	529	313	47	1371	407	.937
Kim	2011	234	145	19	613	183	71	40	6	182	52	.906
Wang	2012	417	219	22	1053	263	469	207	26	1145	259	.600
Yang	2016	525	258	27	1308	312	486	296	43	1268	382	.811
Wang	2013	415	189	18	1019	225	379	221	31	979	283	.867

It was evaluated using the likelihood-ratio chi-square test, *P*-values were presented. $P < .05$ was considered representative of a departure from HWE.
HWE = Hardy–Weinberg equilibrium, IS = ischemic stroke.

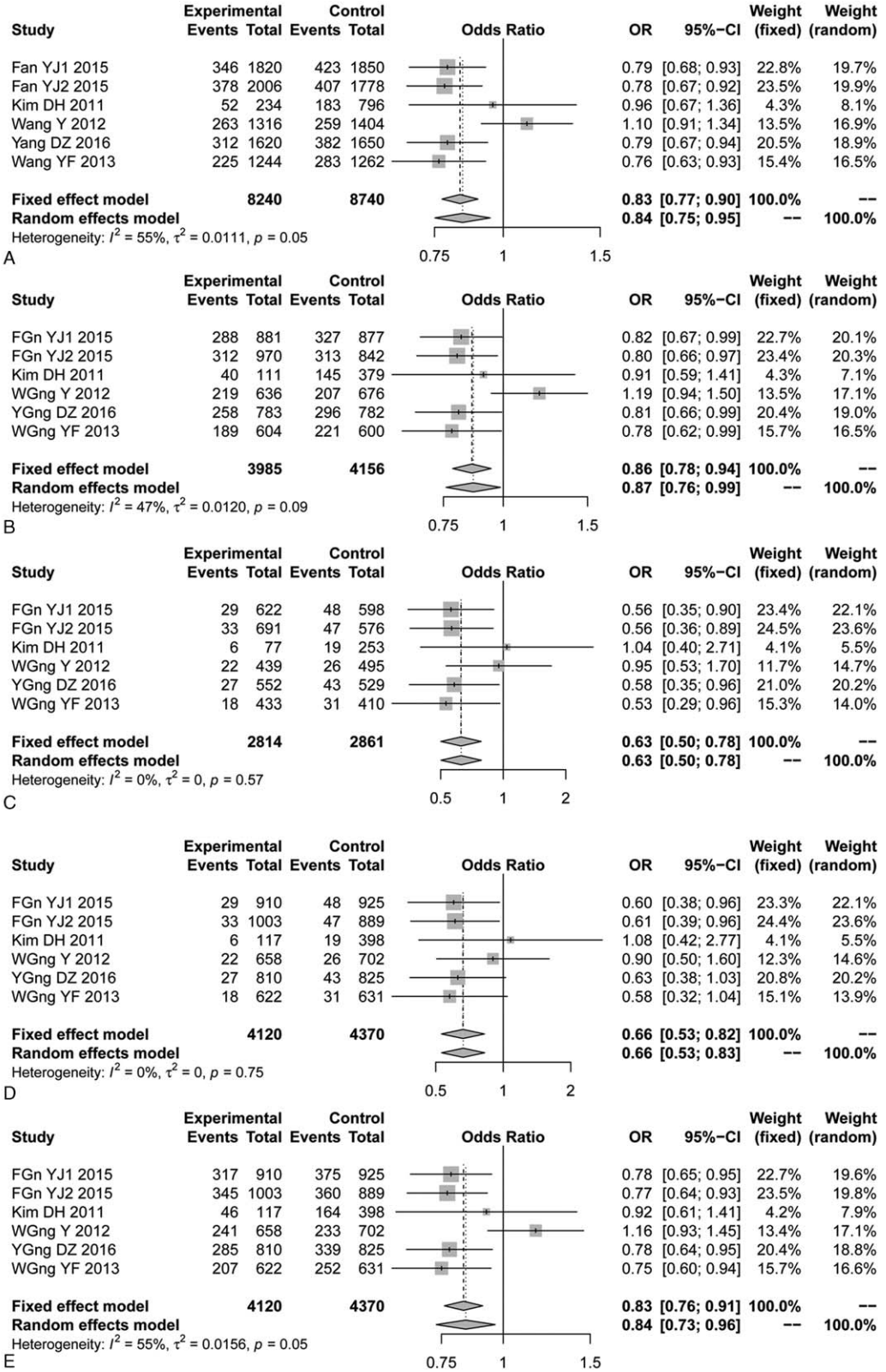

Figure 2. Forest plots of all models. (A) G versus T; (B) GT versus TT; (C) GG versus TT; (D) GG versus GT+TT; (E) GG+GT versus TT.

pubmed) and Embase (http://www.embase.com); Chinese electronic literatures were searched in Wanfang and China National Knowledge Infrastructure databases. The keywords included stroke or "cerebral apoplexy," ALOX5AP gene, rs17222919 (rs17222919, -1316T/G). The retrieval deadline was January 2018. This study was a meta-analysis of previous studies on IS patients and did not involve animal experiments; therefore, no ethical review was needed.

Table 3

Meta-analysis results of all models.

	Total gene		Test of association			Model	Test of heterogeneity*,†			Egger test‡	
Type	Cases	Control	OR (95%CI)	Z	P		Q	P	I^2, %	T	P
G vs T	8240	8740	0.8436 [0.7515; 0.9469]	2.88	0.0039	Random	11.08	0.0498	54.9	0.8834	.4269
GT vs TT	3985	4156	0.8601 [0.7842; 0.9432]	3.20	0.0300	Fixed	9.39	0.0945	46.8	0.4899	.6498
GG vs TT	2814	2861	0.6265 [0.5012; 0.7831]	4.11	<0.0001	Fixed	3.86	0.5703	0	1.9531	.1225
GG vs GT+TT	4120	4370	0.6614 [0.5302; 0.8249]	3.67	0.0002	Fixed	2.65	0.7534	0	2.3549	.0781
GG+GT vs TT	4120	4370	0.8396 [0.7320; 0.9631]	2.50	0.0125	Random	11.13	0.0488	55.1	0.6418	.5559

CI = confidence interval, OR = odds ratio.

* Random-effects model was used when the *P* for heterogeneity test < .05, otherwise the fixed-effect model was used.

† $P < .05$ is considered statistically significant for Q statistics.

‡ Egger test to evaluate publication bias, $P < .05$ is considered statistically significant.

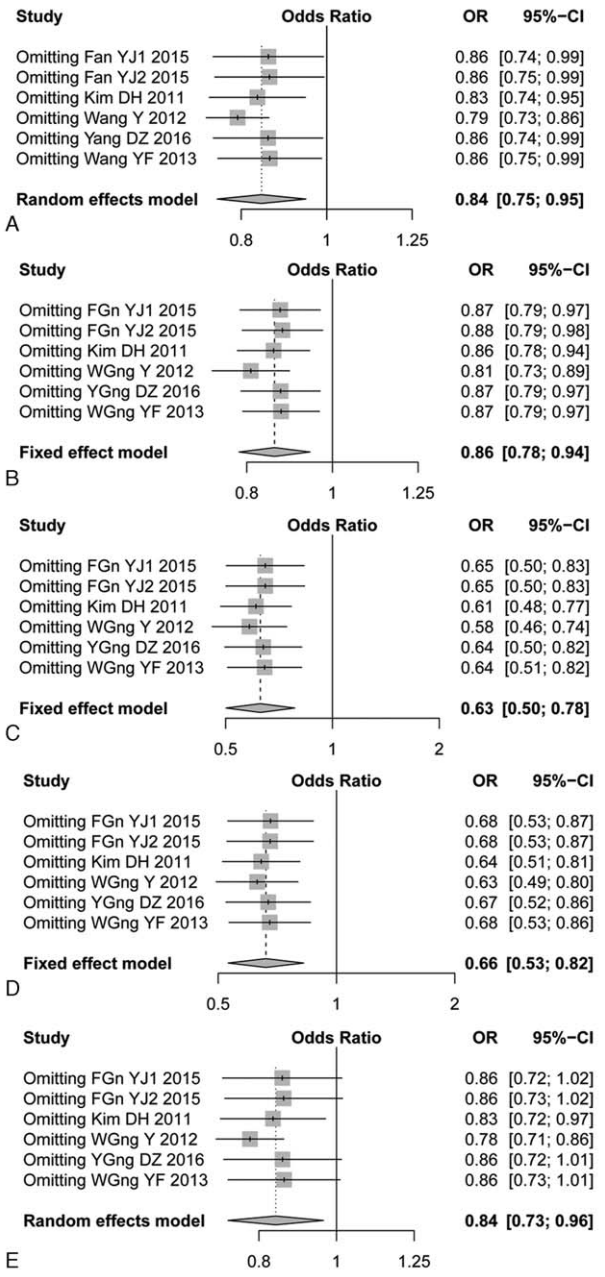

Figure 3. Sensitivity analysis results. (A) G versus T; (B) GT versus TT; (C) GG versus TT; (D) GG versus GT+TT; (E) GG+GT versus TT.

2.2. Document selection criteria

Literature were selected based on the following criteria: a case–control study with stroke patients as case group and healthy subjects as control group; study about the relationship between ALOX5AP gene polymorphism on -rs17222919 site and stroke; written in English; and with reports of the number of cases and controls, genotypes, and alleles. Review, report, comments, and letters were excluded.

2.3. Literature data extraction and quality assessment

Two authors (HY and ZC) independently extracted the following data: the first author, year of publication, study countries, research time, number of genotypes in case and control groups, as well as demographic data characteristics (e.g., gender and age composition, etc.). If there was different data extraction, consistent results were obtained through panel discussions with a third author (XL). Quality assessment was performed by using the United States Agency for Healthcare Research and Quality recommended the Newcastle-Ottawa scale[17]; the evaluation includes 3 aspects with a total of 9 points, wherein the subject selection 4 points, comparability 2 points, and exposure assessment 3 points.

2.4. Statistical analysis

This meta-analysis observed the LOX5AP gene polymorphism on -rs17222919 loci and stroke based on the mutant allele (G) and wild type (T). Four gene models were analyzed including, allele genetic model (G vs T), additive genetic model (GG vs TT, GT vs TT), recessive genetic model (GG vs GT+TT), and dominant genetic model (GG+GT vs TT).

First, Hardy–Weinberg equilibrium test (HWE tests) was conducted on subjects in the control group.[18] In order to ensure that our research quality study that the control group did not comply with HWE ($P < .05$) would be rejected.[19]

Meta-analysis was performed by using the meta-package of R 3.10 software. Effect sizes included odds ratio (OR) and 95% confidence interval (CI). Q test based on χ^2[20] and I^2 statistics were used for heterogeneity assessment. If heterogeneity was statistically significant ($P < .05$, $I^2 > 50\%$), the merged effect sizes were calculated under the random effects model, otherwise, under the fixed effect model.[21] Egger method was applied for publication bias detection. Finally, exclusion method was used for sensitivity analysis test by ignoring a study each time to observe the effect of this study on the overall OR.

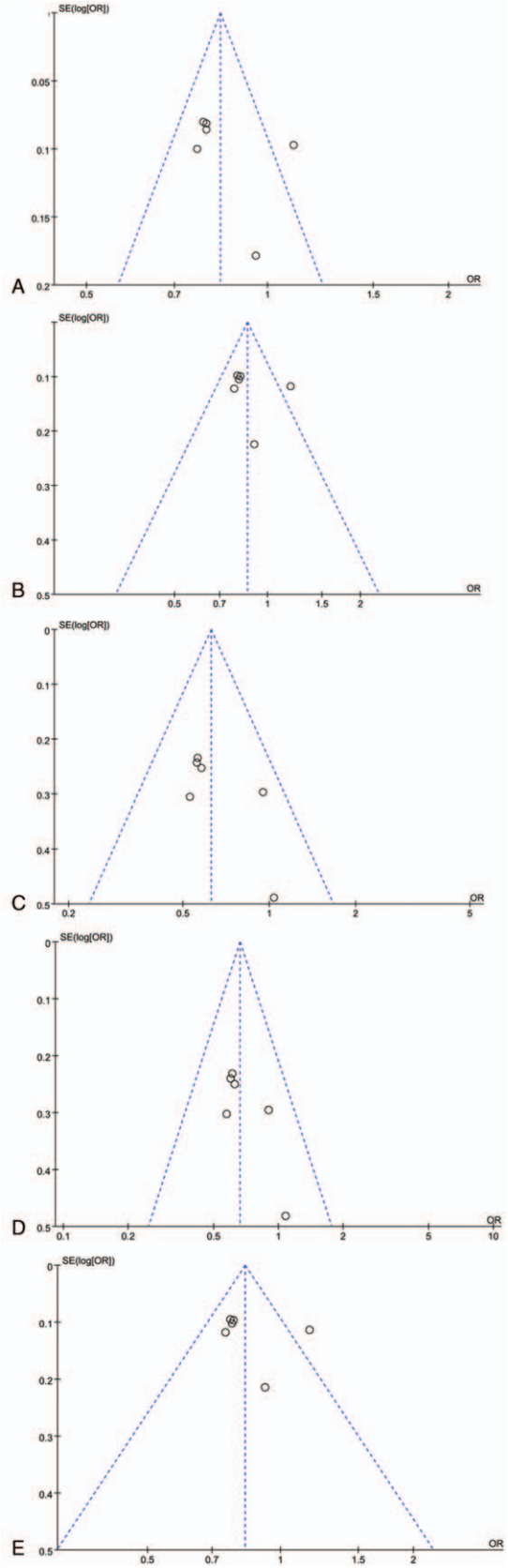

Figure 4. Funnel plots of all models. (A) G versus T; (B) GT versus TT; (C) GG versus TT; (D) GG versus GT + TT; (E) GG + GT versus TT.

3. Results

3.1. General characteristics of the selected literature

Literature search results and literature selection process are shown in Fig. 1. Firstly, 358 documents were searched and 144 repeated documents were excluded. By screening of the title and summary, 177 documents those obviously not meet the inclusion criteria were removed. Then in the remaining 27 documents, 22 were excluded, including 7 reviews, 6 cases reports, 4 studies with repeated crowd, and 5 studies with unacquirable data. Finally, 5 documents[22–26] were included in this meta-analysis.

The general characteristics of the selected are shown in Table 1. A total of 8492 cases were included, with 4120 cases in the case group and 4372 cases in the control group. The selected documents were published between 2011 and 2016. Basic demographic characteristics: average age 55 to 69 years old without statistical difference between the 2 groups; more male than female (case group 2207/1913, control group 2267/2105); subjects came from China and Korean. Quality assessment showed that all selected documents had high quality (Newcastle-Ottawa scale ranged of 6–8 points). Table 2 shows that all the controls in the selected studies accorded to HWE.

3.2. Quantitative data consolidation

The heterogeneity test showed that for the models G versus T and GG + GT versus TT, the heterogeneity was statistically significant ($P < .05$, $I^2 > 50\%$), so the random effects were used, while, for GT versus TT, GG versus TT, and GG versus GT + TT, the fixed effect model was chosen due to the homogeneity ($P > .05$, $I^2 < 50\%$).

Meta-analysis showed except under the additive genetic model GT versus TT (OR = 0.8601, 95% CI: 0.7842–0.9432, $P > .05$), there were significant relationships between ALOX5AP -rs17222919 polymorphism and stroke under all models (Fig. 2): allelic genetic model (G vs T, OR = 0.8436, 95% CI: 0.7515–0.9469, $P < .05$), additive genetic model (GG vs TT, OR = 0.6265, 95% CI: 0.5012–0.7831, $P < .0001$), recessive genetic model (GG vs GT + TT, OR = 0.6614, 95% CI: 0.5302–0.8249, $P < .05$), and dominant genetic model (GG + GT vs TT, OR = 0.8396, 95% CI: 0.7320–0.9631, $P < .05$). The results were summarized in Table 3.

3.3. Sensitivity analysis

The sensitivity analysis showed that for the dominant genetic model (GG + GT vs TT), the OR values undergone different changes, while for allele genetic model (G vs T), additive genetic model (GT vs TT), additive genetic model (GG vs TT), and recessive genetic model (GG vs GT + TT), the OR values did not reversed (Fig. 3). The studies Kim 2011[23] and Wang 2012[24] had significant influence in the overall ORs.

3.4. Assessment of publication bias

Egger method showed no publication bias exists, indicating that the results were reliable (Table 3, Fig. 4).

4. Discussion

Our study was a meta-analysis of previous studies on IS patients. To our knowledge, this is the first meta-analysis on the

relationship between ALOX5AP-rs17222919 and stroke. Basing on large sample size (8492 cases), this study makes a reliable conclusion that LOX5AP -rs17222919 gene is a protective gene for stroke.

There are previous meta-analyses on the relationship. Zintzaras et al[15] found the no evidence of relationship between ALOX5AP gene and stroke by a meta-analysis focusing on HapA haplotype, HapB haplotype, and SG polymorphisms. However, Sophie et al found that in the Iberian population, the SG13S114 (HapB haplotype) variant is an independent risk factor for IS.[16] Based on the ALOX5AP-rs17222919, the present meta-analysis with all subjects in Asian indicated a strong relationship between ALOX5AP gene and stroke.

The heterogeneity of some models was significant. From the forest plot, the study of Kim 2011[23] and Wang 2012[24] contribute the high heterogeneity. Except Kim 2011, the subjects of all other studies were IS. The difference in mechanisms of hemorrhagic stroke and IS can lead to different genetic characteristic. The sensitivity analysis also hints the influence of these 2 studies. The heterogeneity can come from other aspects like racial differences in country areas. The subjects of Kim 2011[23] came from Korean while those of other studies from Chinese. Other confounding factors, age, sex, living habits, and cultural exchange may also affect the results. However, due to the limited data in the original study, subgroup analysis could not be conducted.

As reported in the original study, ALOX5AP mRNA levels were not compared between cases and controls,[22,25] which might also be a limitation of this study. Other limitations are listed as follows. Firstly, the potential confounding factors those may affect the meta-analysis were not corrected due to the incomplete data. Secondly, this study only focused on gene polymorphism of 1 gene site rs17222919, which may lead to misjudgment on the overall relationship. Thirdly, sensitive analysis hints that part of the results is not stable. The studies of Kim[23] and Wang[24] had significant influence in the overall ORs.

In short, the present meta-analysis showed that rs17222919 genetic polymorphism is a protective factor for stroke. Of course, the conclusion of this study still needs verification by more large-scale association analysis or larger sample size study updated meta-analysis.

Author contributions

Lifa Huang and Hui Ye conceived and designed the research. Tiehui Zhang and Chao Yang acquired the data. Xin Zhang, Zupeng Chen, and Xu Li performed the statistical analysis. Lifa Huang drafted the manuscript. All the authors have revised and approved the final version.
Data curation: Tiehui Zhang.
Formal analysis: Chao Yang.
Investigation: Xin Zhang.
Methodology: Zupeng Chen.
Software: Xu Li.
Writing – original draft: Hui Ye.
Writing – review & editing: Lifa Huang.

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