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ORIGINAL ARTICLES

Association between cyclooxygenase gene rs20417 polymorphism and aspirin resistance: a meta-analysis

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Abstract

Background: Although the association of single nucleotide polymorphisms (SNPs) of cyclooxygenase (*COX*) genes and the risk of aspirin resistance (AR) has been extensively studied, the results remain conflicting. The majority of studies have focused on the role of rs20417 (*COX-2* -G765C) in AR. To derive a more comprehensive and accurate evaluation of this association, we performed a meta-analysis including the most recent studies. *Methods:* Relevant studies published up to October 2018 were identified by searching the PubMed, EMBASE, Web of Science, Cochrane, China Nation Knowledge Infrastructure Platform, Wanfang, and VIP databases, and by manual searching reference lists of the retrieved articles. Odds ratios (ORs) and 95% confidence intervals (CIs) were applied to assess the strength of associations. Sensitivity and subgroup analyses were performed to explore the stability of results and between-study heterogeneity, respectively. *Results:* A total of 18 studies on rs20417 were pooled into the meta-analysis. Rs20417 was found to be associated with an increased risk of AR (C vs. G: OR = 1.43, 95% CI = 1.10–1.86, $p < 0.05$; GC+CC vs. GG: OR = 1.54, 95% CI =1.15–2.05, $p < 0.05$). These associations were stronger in Chinese participants and in patients with ischemic stroke in subgroup analyses.
Conclusion: The presence of rs20417 indicates an increased risk of AR, especially in Chinese participants and patients with ischemic stroke. This association could help to improve personalized medicine and initiate appropriate treatment as necessary. Further large-scale studies are warranted to confirm our findings.

Keywords: Cyclooxygenase, COX-2, polymorphism, aspirin resistance, meta-analysis

INTRODUCTION

Aspirin is prescribed as a clinical anti-thrombotic medication, which functions through inhibition of cyclooxygenase (COX) enzymes, leading to reduction of thromboxane A2 biosynthesis to significantly reduce the risk and incidence of vascular events.[1] However, some patients are not responsive to the antithrombotic action of aspirin, and this condition, known as "aspirin resistance" (AR), has been suggested to be associated with genetic factors.[2] Owing to the central roles of COX in the mechanism of aspirin antiplatelet aggregation, single nucleotide polymorphisms (SNPs) of *COX* genes, especially rs20417, have been the most widely studied variants to understand the mechanism of AR. Nonetheless, there has been no consensus reached to date about an association of rs20417 with AR risk.

METHODS

Therefore, we conducted a comprehensive meta-analysis by searching the PubMed, EMBASE, Web of Science, Cochrane, China Nation Knowledge Infrastructure Platform, Wanfang, and VIP databases up to October 2018. The inclusion criteria were: (1) evaluation of the association of rs20417 with AR defined by laboratory tests; (2) case-control studies with sufficient original data for estimating odds ratios (ORs) and 95% confidence intervals (CIs); and (3) original articles published in English or Chinese. When separate studies included patient datasets of overlapping

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time periods at the same institution, the study of better quality or a larger sample was selected for inclusion in the analysis.

Heterogeneity among eligible studies was evaluated by Cochran's Q test and the I^2 statistic. Pooled ORs were calculated by a random-effects model depending on the presence of heterogeneity between studies ($P_Q < 0.10$ and an $I^2 > 50\%$). Several of the included studies[3-5] only provided genotypic data of carriers and non-carriers of the variant allele (GC+CC vs. GG), without data on homozygotes for the major allele, heterozygotes, and homozygotes for the variant allele, respectively. Thus, the genotypic frequencies of carriers and non-carriers of the variant allele (i.e., the dominant model) were calculated for eligible studies, and the allelic frequencies were only calculated for studies with complete data. All meta-analyses were performed using STATA 12.0 software.

RESULTS

Eighteen studies[3-20] with 1416 aspirin-resistant or semi-resistant patients and 3771 controls were included in the meta-analysis. Figure 1 shows the flow chart of the selection process of included studies. Significant associations were found between the rs20417G/C variant and risk of AR in the allele frequency comparison. (Figure 2)

Potential publication bias was revealed in the rs20417 allelic model by visual inspection of the asymmetric Begg's funnel plot, and was detected in the dominant model (GC+CC vs. GG) by Begg's funnel plots, Begg's test (P = 0.044), and Egger's regression tests (P = 0.020). Thus, sensitivity analysis was performed based on selected studies of high quality (Newcastle Ottawa Scale score ≥ 7) and showing Hardy-Weinberg equilibrium (P > 0.05). The corresponding results were stable and reliable. The trim-and-fill method was also used for sensitivity analysis. A significant association was maintained for the allelic comparison (C vs. G, OR = 1.38, 95% CI: 1.01–1.74; P = 0.040), whereas no association was detected in the dominant model (GC+CC vs. GG, OR = 1.30, 95% CI: 0.97–1.74; P = 0.076) with pooled analysis incorporating hypothetical studies, indicating that the results were unstable, and may be affected by underlying factors. (Figure 3, 4)

Given the significant heterogeneity observed in both genetic models, subgroup analyses were performed (Table 1). Overall, the corresponding

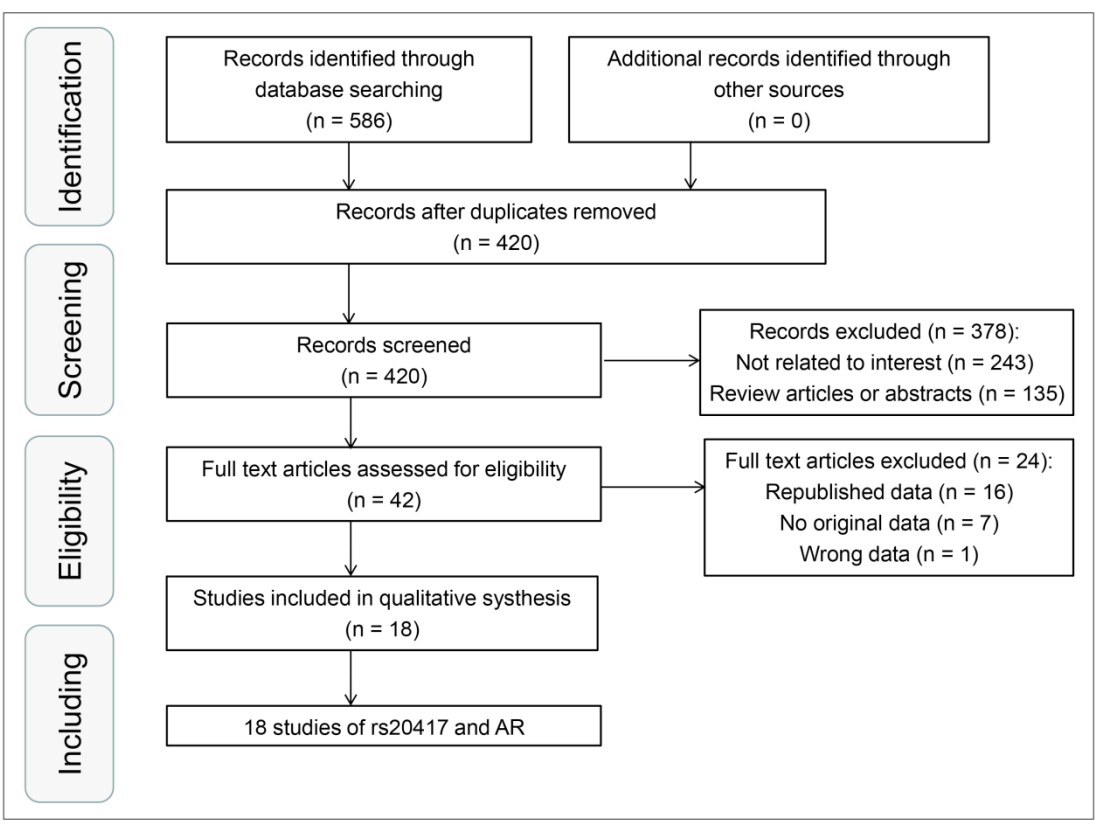

Figure 1. Flow chart of the selection process of included studies.

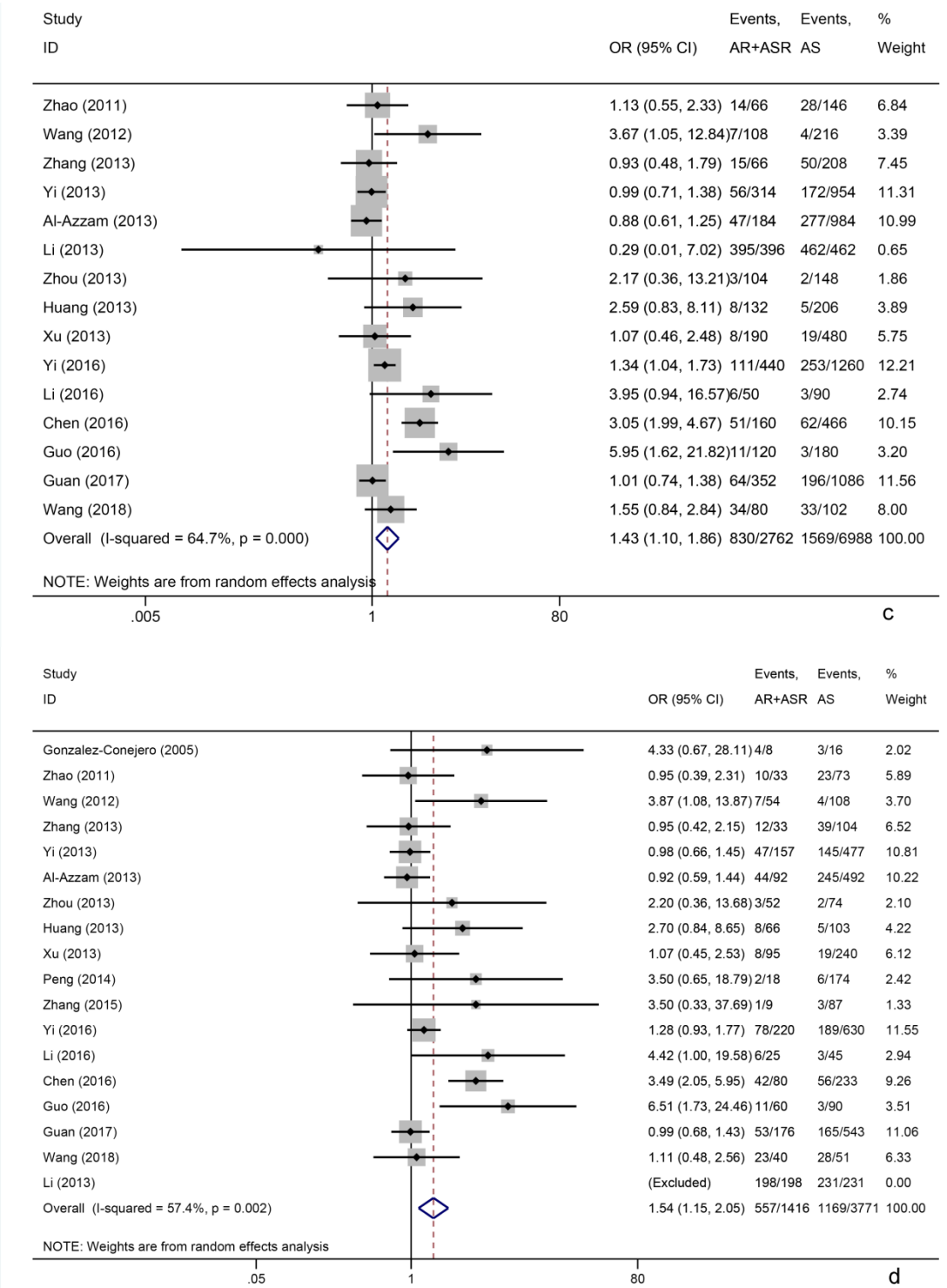

Figure 2. Forest plots of the relationship between *COX* gene SNP with AR risk in the genetic comparisons of rs20417 C allele vs. G allele and rs20417 GC+CC vs. GG.

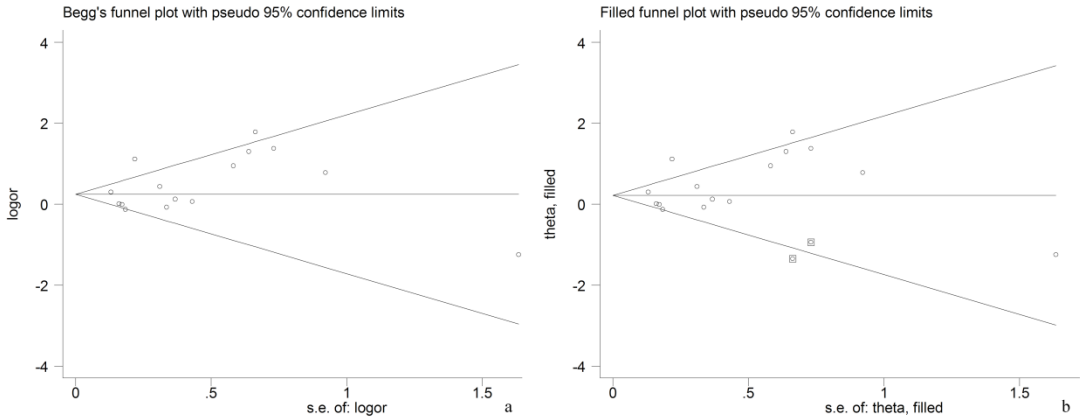

Figure 3. Begg's Funnel Plots of the relationship between *COX* gene SNP with AR risk in the genetic comparisons of rs20417 C allele vs. G allele Without (a) and With (b) Trim and Fill.

ORs of both genetic models slightly increased, indicating that the G/C polymorphism in the Chinese population and in patients with ischemic stroke may contribute to an increased AR risk for both the allele model and dominant model. This may be attributed to the greater number of Chinese participants overall and the greater prevalence of rs20417 in individuals with a higher risk of AR, who are more likely to experience ischemic stroke. Table 2 lists the main characteristics of studies included in the meta-analysis. Table 3 lists the genotype and allele distribution for the rs20417 polymorphisms in subjects.

DISCUSSION

Some limitations of the study should be addressed. First, the risk effect may have been influenced by the interaction with other confounding risk factors such as blood pressure, serum cholesterol, and environmental factors. These factors may also modulate the development of AR and thus could have influenced the estimates of the association. Second, potentially high-quality studies published in languages other than English and Chinese were not included. Third, most of the study participants were Chinese, which may have caused bias to influence the overall results. Nevertheless, given the strict and standardized protocol applied, including study selection, data identification, and statistical analysis to reduce potential bias throughout the process, the results are considered to be objective and reliable.

Overall, further large-scale studies including various ethnicities and more refined sub-group analyses considering potential confounding risk factors are needed to confirm these associations, and the mechanism by which *COX-2* polymorphisms influence the risk of AR should be further elucidated. Exploration of more functional

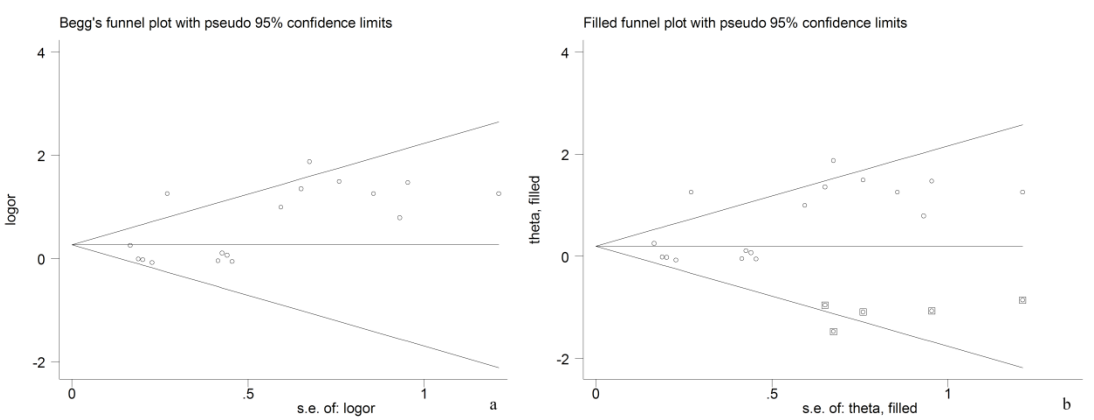

Figure 4. Begg's Funnel Plots of the relationship between *COX* gene SNP with AR risk in the genetic comparisons of rs20417 GC+CC vs. GG Without (a) and With (b) Trim and Fill.

Table 1: Subgroup analyses for the association between the *COX*-2 rs20417 polymorphism and aspirin resistance

Categories	Subgroups	Allele model comparisons					Dominant model comparisons				
		No. of studies	OR (95% CI)	P_{OR}	I^2	P_Q	No. of studies	OR (95% CI)	P_{OR}	I^2	P_Q
Overall	-	15	1.43 (1.10, 1.86)	0.008	64.7%	0.000	17	1.54 (1.15, 2.05)	0.003	57.4%	0.002
Quality	NOS ≥ 7	13	1.42 (1.06, 1.90)	0.018	69.1%	0.000	15	1.54 (1.12-2.11)	0.007	63.4%	0.001
HWE status	P > 0.05	10	1.91 (1.22, 3.00)	0.005	69.3%	0.001	-[a]	-	-	-	-
Ethnicity	Chinese	14	1.52 (1.15, 2.02)	0.004	62.6%	0.001	15	1.60 (1.17, 2.19)	0.003	58.2%	0.002
	Non-Chinese	1	0.88 (0.61, 1.25)	0.469	-	-	2	1.52 (0.37, 6.23)	0.564	59.7%	0.115
Type of diseases	Ischemic stroke	10	1.58 (1.14, 2.19)	0.006	70.9%	0.000	12	1.67 (1.17, 2.39)	0.005	63.3%	0.002
	Other*	5	1.08 (0.72, 1.64)	0.703	26.1%	0.248	5	1.27 (0.76, 2.13)	0.362	38.5%	0.165

P_{OR} = P-value of the odds ratio; I^2 = statistic of heterogeneity; P_Q = P-value of Cochran's Q test for heterogeneity; HWE, Hardy-Weinberg equilibrium

[a] P-value of HWE was not available due to lack of complete original data.

* Healthy individuals or patients with other diseases or complication.

Table 2: Main characteristics of studies included in the meta-analysis

Author	Year	Location	Initial dosage (mg/d)	Type of disease	Mean age	No. of patients (male)	AR+ASR	AS	HWE P-value	NOS score
Gonzalez-Conejero[4]	2005	Spanish	100	Healthy subjects	35.6±5.9	24 (13)	8	16	NA	6
Zhao[14]	2011	Chinese	100	Elderly patients with metabolic syndrome	72.82±6.16	106 (64)	33	73	0.08	8
Wang[15]	2012	Chinese	100	Elderly inpatients	68.95±14.75	162 (125)	54	108	0.84	8
Zhang[16]	2013	Chinese	100	IS	59.07±8.36	137 (75)	33	104	<0.05	8
Yi[13]	2013	Chinese	200	IS	AS 69.3±10.22 AR+ASR 70.2±10.51	634 (332)	157	477	<0.05	8
Al-Azzam[17]	2013	Jordanian	100	Inpatients in department of cardiology	AR 61.79±11.79 AS 61.37±10.65	584 (355)	92	492	0.12	8
Li[12]	2013	Chinese	75-160	Elderly patients	AR 76.33±8.85 ASR 74.02±8.03 AS 73.88±8.00	431 (283)	198	231	NA	7
Zhou[18]	2013	Chinese	100	IS	72.5±1.7	126 (77)	52	74	0.91	6
Huang[19]	2013	Chinese	100	IS	72.30±10.54	169 (111)	66	103	0.80	8
Xu[20]	2013	Chinese	100	IS, CHD	IS 67.1±10.1 CHD 63.1±10.9	335 (213)	95	240	0.52	8
Peng[5]	2014	Chinese	100	IS	AR61.13±13.76 AS64.35±11.88	192 (124)	18	174	NA	7
Zhang[3]	2015	Chinese	100	IS	AR61.13±13.76 AS64.35±11.88	96 (62)	9	87	NA	8
Yi[10]	2016	Chinese	200	IS	AR+ASR 70.8±12.76 AS 70.01±11.35	850 (443)	220	630	<0.05	8
Li[7]	2016	Chinese	100	IS	57.73±10.35	70 (48)	25	45	0.82	8
Chen[8]	2016	Chinese	100	IS	67±11	313 (192)	80	233	0.29	8
Guo[9]	2016	Chinese	100	IS	AR68.60±11.48 AS67.01±9.86	150 (85)	60	90	0.87	8
Guan[11]	2017	Chinese	200	IS	70.1±10.5	719 (376)	176	543	<0.05	8
Wang[6]	2018	Chinese	100	IS	NA	91 (NA)	40	51	0.83	6

AR: aspirin resistance; AS: aspirin sensitive; ASR, aspirin semi-resistant; TIA: transient ischemic attack; ACD: acute coronary disease; IS: ischemic stroke; CHD: coronary heart disease; HWE: Hardy-Weinberg equilibrium; NA: not available

Table 3: Genotype and allele distribution for the rs20417 polymorphisms in subjects

Study	Genotype distribution						Allele distribution			
	AR+ASR, N (%)			AS, N (%)			AR+ASR, N (%)		AS, N(%)	
	G/G	G/C + C/C	Total	G/G	G/C + C/C	Total	G	C	G	C
Gonzalez-Conejero 2005[4]	4(50.0)	4(50.0)	8	13(81.2)	3(18.8)	16	NA	NA	NA	NA
Zhao 2011[14]	23(69.7)	6(18.2)+4(12.1)	33	50(68.5)	18(24.7)+5(6.8)	73	52(78.8)	14(21.2)	118(80.8)	28(19.2)
Wang 2012[15]	47(87.0)	7(13.0)+0(0)	54	104(96.3)	4(3.7)+0(0)	108	101(93.5)	7(6.5)	212(98.1)	4(1.9)
Zhang 2013[16]	21(63.6)	9(27.3)+3(9.1)	33	65(62.5)	28(26.9)+11(10.6)	104	51(77.3)	15(22.7)	158(76.0)	50(24.0)
Yi 2013[13]	110(70.1)	38(24.2)+9(5.7)	157	332(69.6)	118(24.7)+27(5.7)	477	258(82.2)	56(17.8)	782(82.0)	172(18.0)
Al-Azzam 2013[17]	48(52.2)	41(44.6)+3(3.2)	92	247(50.2)	213(43.3)+32(6.5)	492	137(74.5)	47(25.5)	707(71.8)	277(28.2)
Li 2013[12]	0(0)	1(0.5)+197(99.5)	198	0(0)	0(0)+231(100)	231	1(0.3)	395(99.7)	0(0)	462(100)
Zhou 2013[18]	49(94.2)	3(5.8)+0(0)	52	72(97.3)	2(2.7)+0(0)	74	101(97.1)	3(2.9)	146(98.6)	2(1.4)
Huang 2013[19]	58(87.9)	8(12.1)+0(0)	66	98(95.1)	5(4.9)+0(0)	103	124(93.9)	8(6.1)	201(97.6)	5(2.4)
Xu 2013[20]	87(91.6)	8(8.4)+0(0)	95	221(92.1)	19(7.9)+0(0)	240	182(95.8)	8(4.2)	461(96.0)	19(4.0)
Peng 2014[5]	16(88.9)	2(11.1)	18	168(96.6)	6(3.4)	174	NA	NA	NA	NA
Zhang 2015[3]	8(88.9)	1(11.1)	9	84(96.6)	3(3.4)	87	NA	NA	NA	NA
Yi 2016[10]	142(64.5)	45(20.5)+33(15.0)	220	441(70.0)	125(19.8)+64(10.2)	630	329(74.8)	111(25.2)	1007(79.9)	253(20.1)
Li 2016[7]	19(76.0)	6(24.0)+0(0)	25	42(93.3)	3(6.7)+0(0)	45	44(88.0)	6(12.0)	87(96.7)	3(3.3)
Chen 2016[8]	38(47.5)	33(41.3)+9(11.2)	80	177(76.0)	50(21.4)+6(2.6)	233	109(68.1)	51(31.9)	404(86.7)	62(13.3)
Guo 2016[9]	49(81.7)	11(18.3)+0(0)	60	87(96.7)	3(3.3)+0(0)	90	109(90.8)	11(9.2)	177(98.3)	3(1.7)
Guan 2017[11]	123(69.9)	42(23.9)+11(6.2)	176	378(69.6)	134(24.7)+31(5.7)	543	288(81.8)	64(18.2)	890(82.0)	196(18.0)
Wang 2018[6]	17(42.5)	12(30.0)+11(27.5)	40	23(45.1)	23(45.1)+5(9.8)	51	46(57.5)	34(42.5)	69(67.6)	33(32.4)

NA: not available

variants and the possible role of other risk factors in AR is also necessary to clarify the complete mechanism contributing to AR.

In conclusion, the present meta-analysis demonstrates a significant association of the *COX* SNP rs20417 with an increased risk of AR, especially in the Chinese population and for patients with ischemic stroke. This association could allow for selection of patients with a high risk of AR to provide timely and appropriate management. In particular, detection of an at-risk genotype would be appropriate for individuals with this condition, enabling a change to another effective antiplatelet therapy as necessary, which may provide a new basis for diagnosis and personalized medicine.

DISCLOSURE

Conflict of interest: None.

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