$P2Y_{12}$ receptor gene polymorphism and the risk of resistance to clopidogrel: A meta-analysis and review of the literature

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Abstract

A number of investigators have evaluated the association between T744C, G52T and C34T polymorphisms in the $P2Y_{12}$ receptor gene and clopidogrel resistance (CR), but the results of their research are controversial. To quantify the evidence addressing this issue, we performed a meta-analysis of all available studies to evaluate the above association between the 3 different $P2Y_{12}$ genotypes and CR in patients suffering from cardiovascular diseases. This study included articles up to October 2015. We performed a systematic search of PubMed, Embase, Web of Science, Cochrane database, China National Knowledge Infrastructure (CNKI) and WanFang database. Articles meeting the inclusion criteria were included and accumulated by meta-analysis including 5769 participants from 15 individual studies. For G52T polymorphism, a significant relationship between the $P2Y_{12}$ receptor gene and CR was found under the dominant genetic model ($p < 0.05$). There was a clear positive correlation between the C34T polymorphism and CR under the dominant, recessive, additive genetic models, respectively ($p < 0.05$). The evidence from the present meta-analysis indicates that $P2Y_{12}$ receptor gene C34T and G52T polymorphism might be a risk factor for the poor response to the platelet in patients on clopidogrel therapy, whereas a lack of association was found for T744C polymorphism examined by various genetic models.

Key words: polymorphism, cardiovascular diseases, resistance, clopidogrel, $P2Y_{12}$

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Increased platelet activation and thrombus formation are involved in the development and progression of cardiovascular diseases (CVDs).[1] Current oral anti-platelet agent thienopyridine clopidogrel inhibits adenosine diphosphate (ADP) binding to platelet ADP receptor $P2Y_{12}$ on the platelet surface, and thus inhibits platelet aggregation. $P2Y_{12}$ has been shown to trigger platelet activation when stimulated in this receptor. Clopidogrel is effective in decreasing platelet activation and the subsequent risk of atherosclerosis-related CVDs including myocardial infraction, coronary heart disease and ischemic stroke.[2] However, in recent years, the concept of clopidogrel resistance (CR) or poor responsiveness, is increasingly evoked in the cardiac literature.[3]

The mechanisms of CR have not been fully characterized but are likely to be multifactorial, involving possible genetic polymorphisms, drug interaction and variable absorption or metabolism.[4] Five polymorphisms (T744C, C34T G52T, ins801A and C139T) of the $P2Y_{12}$ gene have been identified by Fontana et al.[5] It has been proposed that genetic polymorphisms of the platelet surface receptor affect the responsiveness to clopidogrel. Some have suggested that polymorphisms of T744C and C34T G52T contribute to CR.[6–8] However, this proposal is controversial, because other studies have shown that the correlations between the mutational statuses and poor response to clopidogrel therapy were not statistically significant.[7,9] Thus, in the present study, a meta-analysis including 5769 patients with CVDs was performed to clarify whether or not clopidogrel response to the platelet may be affected by $P2Y_{12}$ receptor gene polymorphism in patients with various types of CVDs. This study may help to identify the correct therapeutic approach for each individual patient with CVDs in order to maximize the therapeutic effect.

Methods

Search strategies

Published studies on the association between $P2Y_{12}$ receptor gene polymorphism and CR were retrieved by searching the following English and Chinese bibliographic databases: PubMed, Embase, Web of Science, Cochrane database, China National Knowledge Infrastructure (CNKI) and WanFang database. The search strategy was based on the following keywords: "$P2Y_{12}$" or "T744C" or "C34T" or "G52T" and "polymorphism" or "mutation" or "genotype" or "allele" and "clopidogrel" and "resistance" or "response". The literature search was updated to October 2015.

Study selection

Studies included in the current meta-analysis had to be consistent with these criteria: a) the association of $P2Y_{12}$ gene polymorphism and CR was included; b) provides the genotype frequency in the target population; c) was a case-control study; d) all patients had received clopidogrel therapy. Studies were excluded if they were: (1) duplicates of previous publications; (2) based on incomplete data; (3) meta-analyses, letters, reviews, or editorial articles; (4) publications with non-English abstracts. If more than 1 study by the same author using the same case series was published, only the study with the largest sample size or the most recent publication was included.

Data extraction

Two reviewers independently extracted the information, including the name of first author, year of publication, country, disease name, diagnostic standard of CR and genotypes and the frequency in patients with and without CR. In cases of conflicting evaluations, disagreements were resolved through discussions among all of the 5 authors.

Statistical analysis

The strength of the association between $P2Y_{12}$ gene polymorphism and CR was represented by odds ratios (OR) and 95% confidence intervals (CI). The OR and 95% CI were calculated according to 3 genetic models of inheritance: dominant (heterozygotes + homozygotes vs wild-type homozygotes), recessive (wild-type homozygotes + heterozygotes vs homozygotes), and additive (heterozygotes + homozygotes vs wild-type homozygotes + + heterozygotes). Heterogeneity between the results of different studies was examined using a χ^2 test. A 2-side value of $p < 0.05$ was considered statistically significant as previously described.[10] A fixed effects model was used when $p < 0.05$, and a random effects was used when $p > 0.05$. All analyses were performed by RevMan, v. 5.2 for Windows (Cochrane Collaboration, Oxford, UK) using our previously described method.[11]

Results

Study selection and characteristics

As shown in Fig. 1, 297 relevant studies were identified using the key words by a computerized search of PubMed, Embase, Web of Science, Cochrane database, CNKI and WanFang database. According to the selection criteria as described in methods, 14 studies were included for meta-analysis. Of the studies, there were 9 comparisons for T744C polymorphism, 7 and 6 comparisons for G52T and C34T, respectively.[6,8,9,12–22] The studies were conducted in France, the United States, China, Croatia, Egypt, and the Czech Republic. The characteristics of the studies included in this meta-analysis are presented in Table 1.

Table 1. Characteristics of the studies included in the meta-analysis

Position	First author	Year	Country	Disease	Dose (mg)	Definition of CR	CR		NCR	
T744C							CC + CT	TT	CC + CT	TT
	Cuisset[13]	2007	France	ACS	600	HPPR = ADP-induced aggregation > 70%	106	347	32	112
	Lev[14]	2007	USA	PAD	300	percent inhibition of ADP ≤ 10%	6	23	27	64
	Wang[18]	2009	China	CHD	300	percent inhibition of ADP ≤ 10%	36	57	67	87
	Sun[17]	2011	China	CAD	300	HPPR = ADP-induced aggregation > 70%	53	93	178	291
	Galic[6]	2013	Croatia	CHD	300	percent inhibition of ADP ≤ 10%	3	11	13	23
	Zoheir[8]	2013	Egypt	ACS	NA	HPPR = ADP-induced aggregation > 70%	1	23	10	6
	Shi[16]	2013	China	cerebral infarction	300	percent inhibition of ADP ≤ 10%	7	5	22	69
	Chen[12]	2014	China	ischemic stroke	75	percent inhibition of ADP ≤ 10%	5	26	25	40
	Shi[15]	2014	China	cerebral infarction	75	percent inhibition of ADP ≤ 10%	16	20	27	58
G52T							TT + GT	GG	TT + GT	GG
	Wang[18]	2009	China	CHD	300	percent inhibition of ADP ≤ 10%	41	50	53	96
	Bonello[19]	2010	France	CAD	600	percent inhibition of ADP ≤ 10%	0	10	6	27
	Sun[17]	2011	China	CAD	300	percent inhibition of ADP ≤ 10%	37	109	95	374
	Liu[9]	2011	China	CHD	300	percent inhibition of ADP ≤ 10%	9	26	13	61
	Li[20]	2014	China	CAHD	75	percent inhibition of ADP ≤ 10%	52	48	100	170
	Chen[12]	2014	China	ischemic stroke	75	percent inhibition of ADP ≤ 10%	4	28	17	47
	Zhao[21]	2015	China	ACS	75	HPPR = ADP-induced aggregation > 70%	10	16	10	48
C34T							TT + CT	CC	TT + CT	CC
	Wang[18]	2009	China	CHD	300	percent inhibition of ADP ≤ 10%	58	35	56	98
	Sun[17]	2011	China	CAD	600	percent inhibition of ADP ≤ 10%	91	55	230	239
	Galic[6]	2013	Croatia	CHD	300	percent inhibition of ADP ≤ 10%	8	6	18	18
	Tang[22]	2013	China	ACS	300	percent inhibition of ADP < 30%	53	55	167	302
	Li[20]	2014	China	CAHD	75	percent inhibition of ADP ≤ 10%	74	26	84	186
	Zhao[21]	2015	China	ACS	75	HPPR = ADP-induced aggregation > 70%	12	14	19	39

CR – clopidogrel resistance; NCR – non-clopidogrel resistance; ACS – acute coronary syndrome; HPPR – high post-treatment platelet reactivity; PAD – peripheral arterial disease; CHD – coronary heart disease; CAD – coronary artery disease; AMI – acute myocardial infarction; NA – not applicable; CAHD – coronary atherosclerotic heart disease.

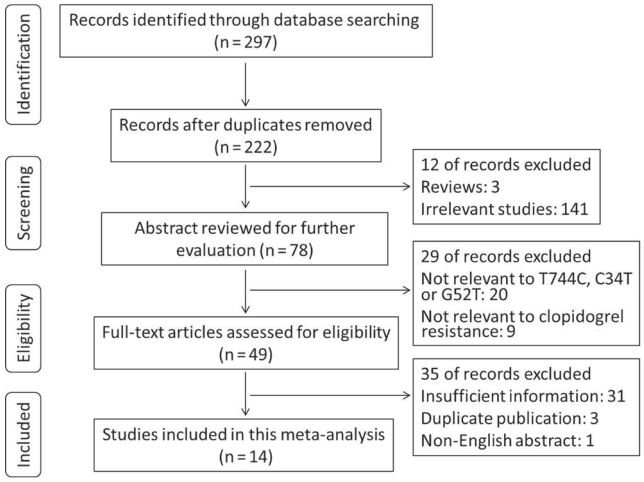

Fig. 1. Flow diagram of study selection procedure

Quantitative data synthesis

The conclusions based on the included studies showed that: (1) $P2Y_{12}$ receptor gene T744C polymorphism had no association with CR (OR: 0.88, 95% CI: 0.58–1.33, p = 0.54), (2) CVD cases with CR had a significantly higher frequency of CT and TT genotypes (OR: 1.45, 95% CI: 1.14–1.85, p = 0.003) than the CC (wild type) genotype of G52T under the dominant genetic model, (3) an association between $P2Y_{12}$ C34T polymorphism and CR was detected under the recessive (OR: 2.19, 95% CI: 1.44–3.34, p = 0.0003), dominant (OR: 2.30, 95% CI: 1.50–3.51, p = 0.0001) and additive (OR: 0.57, 95% CI: 0.47–0.71, p < 0.00001) genetic models. These results revealed that mutant genotypes (G52T/C34T) of the $P2Y_{12}$ receptor gene might be associated with an increased risk of CR.

Subgroup analysis and sensitivity analysis

For T744C and C34T polymorphisms, high heterogeneity was observed (I^2 = 63%) according to the reported quantifying heterogeneity approach.[10] Subgroup analysis of the T744C genetic polymorphism was performed to determine the potential sources of the heterogeneity (Table 2). We classified the studies based on the geographic region (European and American, Asian). In the results, whether geographic region was adjusted or not, this association did not change (p > 0.05). For the G52T polymorphism, we did not perform a subgroup analysis due to the limited number of included studies (only 2 studies included from European and American regions).

We removed one study by Galic et al. in the review due to T744C genotype distribution in the control groups of these studies deviating from the Hardy-Weinberg equilibrium (HWE) and found that the association (OR= 1.17, 95% CI: 0.86–1.60, p = 0.32) was not significantly altered after exclusion of the study, indicating that the result of the meta-analysis was stable.[6] In addition, potential publication bias or heterogeneity was detected using visual as-

Table 2. Subgroup analyses of the association between $P2Y_{12}$ receptor gene T744C polymorphism and CR according to region

Subgroup	Pooled OR (95% CI)	z-value	p-value	Study number	$P_{heterogeneity}$ (I^2%)
European and American participants	0.93 (0.62–1.37)	0.38	0.70	3	0.41 (0%)
Asian participants	1.22 (0.98–1.53)	1.75	0.08	6	0.04 (57%)

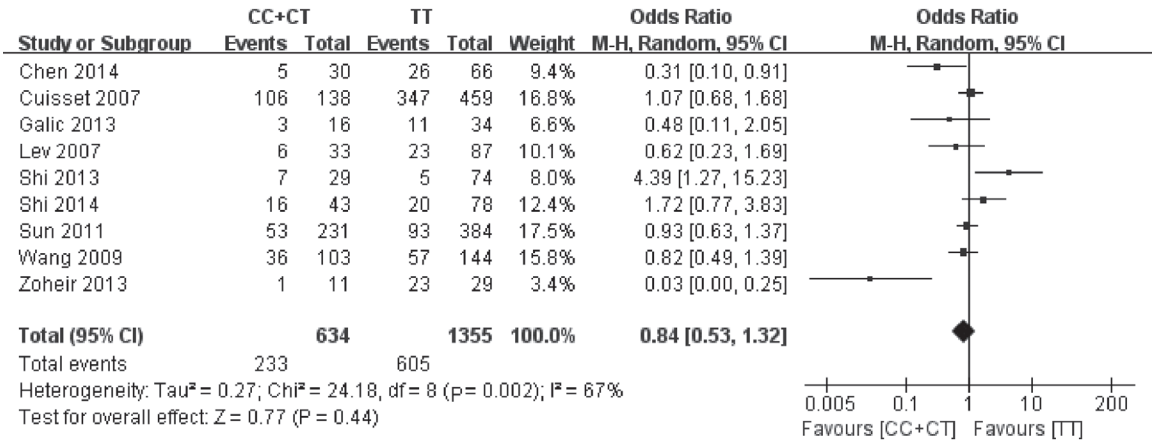

Fig. 2. Forest plot of OR of CR associated with $P2Y_{12}$ receptor gene T744C polymorphism in the dominant genetic model. Comparison: CC + CT vs TT

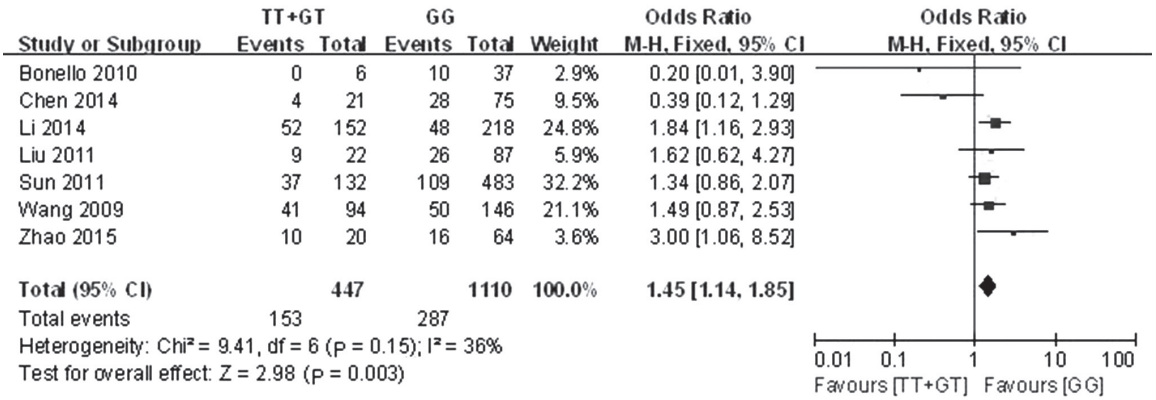

Fig. 3. Forest plot of OR of CR associated with $P2Y_{12}$ receptor gene G52T polymorphism in the dominant genetic model. Comparison: TT + GT vs GG

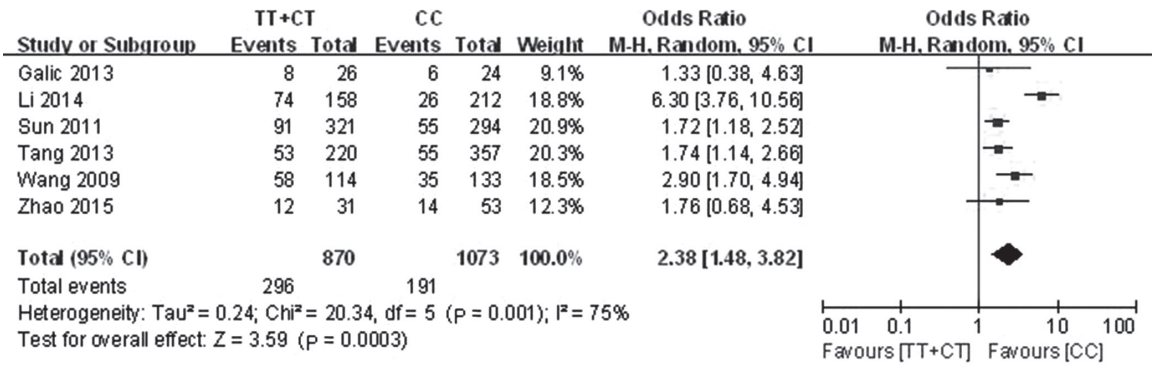

Fig. 4. Forest plot of OR of CR associated with $P2Y_{12}$ receptor gene C34T polymorphism in the dominant genetic model. Comparison: TT + CT vs CC

sessment of the Begg's funnel plot calculated by RevMan analyses. Funnel plots (Fig. 5) display symmetrical distribution of OR estimations, suggesting no publication bias.

Discussion

The strength of our study is the statistically most extensive studies to date addressing whether the risk of CR is associated with the frequencies of $P2Y_{12}$ receptor gene T744C, C34T and G52T genotypes in patients with CVDs. In the current review, 15 independent studies were included, comprised of 1829 patients with CR and 3940 patients without CR. For the first time, we used meta-analysis to summarize that there was probably a significant association between $P2Y_{12}$ receptor gene G52T/C34T polymorphism and CR.

The $P2Y_{12}$ receptor, activated by ADP, plays a critical

Table 3. Summary of meta-analysis of association of $P2Y_{12}$ receptor gene polymorphism and CR using recessive genetic and additive models

Model	Pooled OR (95% CI)	z-value	p- value	Study number	$P_{heterogeneity}$ (I^2%)
Recessive genetic model					
T744C (CC vs TT + TC)	1.08 (0.57–2.05)	0.23	0.82	4	0.13 (48%)
G52T (TT vs GG + GT)	2.32 (0.58–9.28)	1.18	0.24	3	0.48 (0%)
C34T (TT vs CC + CT)	2.19 (1.44–3.34)	3.64	0.0003*	4	0.20 (36%)
Additive genetic model					
T744C (T vs C)	1.01 (0.83–1.24)	0.12	0.90	5	0.49 (0%)
G52T (G vs T)	0.78 (0.58–1.04)	1.67	0.09	3	0.41 (0%)
C34T (C vs T)	0.57 (0.47–0.71)	5.29	< 0.00001*	4	0.14 (45%)

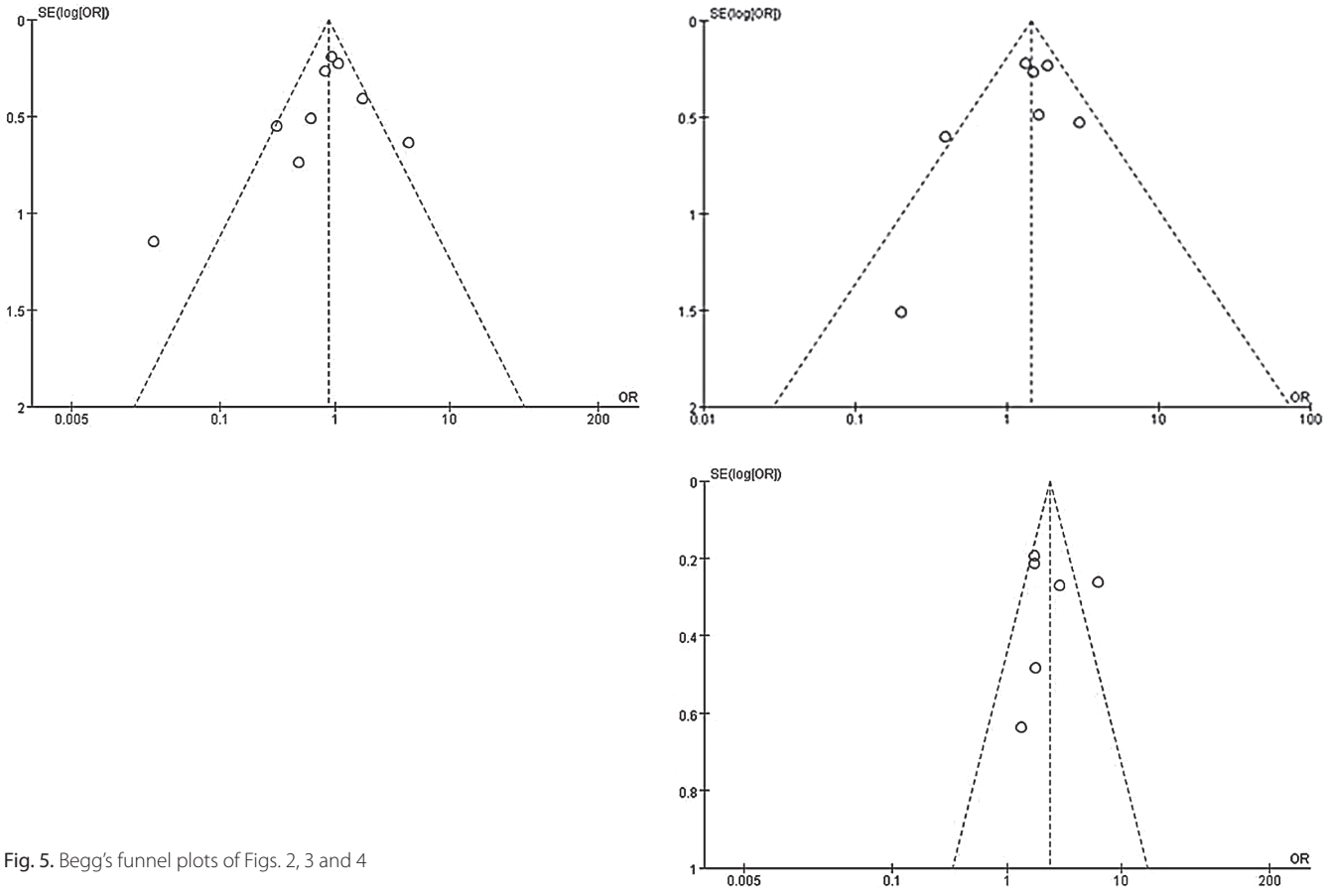

Fig. 5. Begg's funnel plots of Figs. 2, 3 and 4

role in platelet aggregation and is a target of antiplatelet therapeutic agents that has proven therapeutic value.[23] One of the first successful drugs is clopidogrel. Clopidogrel is a prodrug and its metabolite inhibits ADP receptor-mediated platelet aggregation. Clopidogrel is a milestone in the development of antiplatelet therapy and entails a reduction in the risk of atherothrombotic event, which is the leading cause of CVDs.[24,25] However, the concept of CR or poor responsiveness is increasing evoked in the cardiac literature in antiplatelet response.[3] Although numerous association studies reflecting the influence of $P2Y_{12}$ receptor gene polymorphisms on CR have been published, the results are controversial, possibly because of variations in heterogeneous population, sample sizes and other issues. A meta-analysis by systematically combining all the results of individual studies increases the power to detect an association.[26]

In the current meta-analysis, we did not find a significant association between the T744C polymorphism and CR in various genetic models. This finding suggests that the T744C polymorphism may be not susceptible to CR in patients with CVDs. In contrast, we also found that patients with a higher frequency of homozygous and heterozygous (TT + GT) genotypes of G52T had a higher risk of CR than wild-type (GG) genotypes (p = 0.003). For C34T polymorphism, despite the statistical heterogeneity of the studies associating $P2Y_{12}$ receptor gene C34T polymorphism under the dominant genetic model (p = 0.002,

I^2 = 71%), all of the included studies suggested a positive association between the risk of the CR and C34T polymorphism. We pooled these studies with a random-effects model and the result was consistent with the individual study (OR: 2.30, 95% CI: 1.50–3.51, p = 0.0001).

Variability in the definitions of CR and different regions of population possibly contributed to the heterogeneity in the comparisons. The use of antiplatelet therapy, including differing doses and lengths of the treatment, may also result in the heterogeneity. Antiplatelet drugs could influence the association of the $P2Y_{12}$ gene polymorphisms with clopidogrel resistance as this might affect clopidogrel and ADP binding.[27] In addition, an association was also found for the C34T gene polymorphism determined under the dominant, additive, and genetic models ($p < 0.05$). Conversely, in a previous study excluded in this meta-analysis, it was shown that there is lack of association between the frequency of C34T polymorphism and the antiplatelet aggregation response of clopidogrel.[28] Our results clearly indicate augmented CR for C34T polymorphism.

Although considerable effects have been found in the current study, there are some inherent limitations. First, the sample size is still relatively small in the current meta-analysis. Although 5489 total participants from 14 individual studies were included in the analysis, < 1600 participants were analyzed in the G52T polymorphism group. Fewer case subjects were included in some studies. Of the included studies, 3 had less than 100 participants.[6,8,19] Second, 3 different definitions of CR were adopted by researchers since a standard definition of CR was not available. This may slightly weaken the comparability of the data. Third, the overall ORs were calculated according to the unadjusted estimates. It has been reported that drug-drug interaction or coexisting polymorphisms of $P2Y_{12}$ and other genes may influence CR.[22,29] This may exist in the included studies and could interfere with the meta-analysis. Fourth, different clopidogrel doses (75–600 mg) and various times after initial therapy (6 h–7 days) were used in the included studies. Those may be a confounder in the analysis of the platelet aggregation test. Such confounding factors could not be extracted from the included studies due to a lack of information, but they limit the potential to draw robust conclusions.

In summary, this is the first meta-analysis to provide evidence that $P2Y_{12}$ G52T/C34T polymorphism is related to a poor response of clopidogrel in patients, reflected by platelet function assay. In contrast, a lack of association between T744C polymorphism and CR was found. This finding warrants further studies of larger sample sizes and multiple countries for a more sensitivity analysis. Furthermore, this study raises concerns about future studies addressing individual alternative clopidogrel-based antiplatelet therapy for patients with $P2Y_{12}$ C34T and/or G52T polymorphism, and encourages a precision medicine approach to the treatment of CVDs.

References

1. Hansson GK, Klareskog L. Pulling down the plug on atherosclerosis: Cooling down the inflammasome. *Nat Med.* 2011;17(7):790–791.
2. Behan MW, Storey RF. Antiplatelet therapy in cardiovascular disease. *Postgrad Med J.* 2004;80(941):155–164.
3. Hurst NL, Nooney VB, Raman B, et al. Clopidogrel "resistance": Pre- vs post-receptor determinants. *Vascul Pharmacol.* 2013;59(5–6):152–161.
4. Siller-Matula J, Schror K, Wojta J, et al. Thienopyridines in cardiovascular disease: Focus on clopidogrel resistance. *Thromb Haemost.* 2007;97(3):385–393.
5. Fontana P, Dupont A, Gandrille S, et al. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation.* 2003;108(8):989–995.
6. Galic E, Vrbanic L, Kapitanovic S, et al. P2RY12 gene polymorphisms and effect of clopidogrel on platelet aggregation. *Collegium antropologicum.* 2013;37(2):491–498.
7. Staritz P, Kurz K, Stoll M, et al. Platelet reactivity and clopidogrel resistance are associated with the H2 haplotype of the P2Y12-ADP receptor gene. *Int J Cardiol.* 2009;133(3):341–345.
8. Zoheir N, Abd Elhamid S, Abulata N, et al. P2Y12 receptor gene polymorphism and antiplatelet effect of clopidogrel in patients with coronary artery disease after coronary stenting. *Blood Coagul Fibrinolysis.* 2013;24(5):525–531.
9. Liu YM, Liu NF. Evaluating association of P2Y12 gene polymorphisms with clopidogrel response variability in Chinese patients undergoing PCI. *Journal of Southeast University.* 2011;30(3):449–453.
10. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ.* 2003;327(7414):557–560.
11. Zhang SY, Chan SW, Zhou X, et al. Meta-analysis of association between ALDH2 rs671 polymorphism and essential hypertension in Asian populations. *Herz.* 2015;40:203–208.
12. Chen XM, Jin J, Huang M, et al. Association between CYP2C19, P2Y12 genetic polymorphisms and clopidogrel resistance in ischemic stroke patients. *China Pharmaci.* 2014;17(6):885–888.
13. Cuisset T, Frere C, Quilici J, et al. Role of the T744C polymorphism of the P2Y12 gene on platelet response to a 600-mg loading dose of clopidogrel in 597 patients with non-ST-segment elevation acute coronary syndrome. *Thromb Res.* 2007;120(6):893–899.
14. Lev EI, Patel RT, Guthikonda S, et al. Genetic polymorphisms of the platelet receptors P2Y(12), P2Y(1) and GP IIIa and response to aspirin and clopidogrel. *Thromb Res.* 2007;119(3):355–360.
15. Shi HT, Zhou BR, Wang R, et al. Analysis of clinical characteristics of P2Y12 and CYP2C19 mutation genes and their relationship with the incidence of dynamic clopidogrel resistance in patients after cerebral infarction. *J Apoplexy and Nervous Diseases.* 2014;31(10):9–13.
16. Shi ST, Li YJ, Zhang MJ, et al. Related factors of clopidogrel resistance in Han patients with cerebral infarction. *J Shanxi Med Univ.* 2013;44(12):953–956.
17. Sun Y, Han YL, Zhang XL, et al. Association of T744C polymorphism of the P2Y12 gene with clopidogrel resistance in North Chinese patients of coronary artery disease. *China Medical Engineering.* 2011;19(04):44–47.
18. Wang JJ. Influences of P2Y12 (C34T, G52T, iT744C). Polymorphisms on clopidogrel resistance in patients with coronary heart disease. *Fujian Medical University.* 2009:1–51.
19. Bonello L, Bonello-Palot N, Armero S, et al. Impact of P2Y12-ADP receptor polymorphism on the efficacy of clopidogrel dose-adjustment according to platelet reactivity monitoring in coronary artery disease patients. *Thromb Res.* 2010;125(4):167–170.
20. Li XJ, Chen XM. Association between clopidogrel resistance and polymorphism of platelet adenosine diphosphate receptor in patients with coronary atherosclerotic disease. *Journal of Zhejiang University-Science B.* 2014;43(3):333–338.
21. Zhao M, Chen BX, Du FH. Association between P2Y12 gene C34T and G52T platelet receptor polymorphisms and clopidogrel efficacy. *Lab Med Clin.* 2015;12(5).
22. Tang XF, Zhang JH, Wang J, et al. Effects of coexisting polymorphisms of CYP2C19 and P2Y12 on clopidogrel responsiveness and clinical outcome in patients with acute coronary syndromes undergoing stent-based coronary intervention. *Chin Med J (Engl).* 2013;126(6):1069–1075.

23. Dorsam RT, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. *J Clin Invest*. 2004;113(3):340–345.
24. Bhatt DL, Fox KA, Hacke W, et al. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med*. 2006;354(16):1706–1717.
25. Viles-Gonzalez JF, Fuster V, Badimon JJ. Atherothrombosis: A widespread disease with unpredictable and life-threatening consequences. *Eur Heart J*. 2004;25(14):1197–1207.
26. Ioannidis JP, Ntzani EE, Trikalinos TA, et al. Replication validity of genetic association studies. *Nat Genet*. 2001;29(3):306–309.
27. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Variability in individual responsiveness to clopidogrel: Clinical implications, management, and future perspectives. *J Am Coll Cardiol*. 2007;49(14):1505–1516.
28. Chen BL, Li Q, Xie W, et al. P2Y12 gene polymorphism in Chinese healthy population and patients with coronary artery disease and the association with effect of clopidogrel. *Chinese Journal of New Drugs*. 2008;17(16):1435–1439.
29. Lau WC, Waskell LA, Watkins PB, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: A new drug-drug interaction. *Circulation*. 2003;107(1):32–37.