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SYSTEMATIC REVIEW

The association of *CYP2C19* LoF alleles with adverse clinical outcomes in stroke patients taking clopidogrel: An updated meta-analysis

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Abstract

The aggregated risk of recurrent stroke in stroke/transient ischemic attack (TIA) patients carrying *CYP2C19* LoF alleles who take clopidogrel has not been investigated recently, and the available research is limited. This study aimed to perform an updated meta-analysis to assess the association between *CYP2C19* LoF alleles and the risk of recurrent stroke in patients taking clopidogrel. Databases were searched for the literature on eligible studies. The end points were recurrent stroke, composite vascular events, and bleeding events. Odds ratios (ORs) were calculated using RevMan software, where $p < 0.05$ was considered statistically significant. Patients carrying *CYP2C19* LoF alleles who were treated with clopidogrel had a significantly increased risk of recurrent ischemic stroke compared with non-carriers (OR 2.18, 96% CI 1.80–2.63; $p < 0.00001$). The risk of recurrent stroke was only significantly different in Asian patients (OR 2.29, 96% CI 1.88–2.80; $p < 0.00001$) but not in patients of other ethnicities; however, there were a limited number of studies in other ethnic groups. Both observational studies (OR 2.83, 96% CI 2.20–3.65; $p < 0.00001$) and RCTs (OR 1.48, 96% CI 1.10–1.98; $p = 0.009$) found associations with a significantly increased risk of recurrent ischemic stroke. Asian stroke patients or TIA patients carrying *CYP2C19* LoF alleles and taking clopidogrel were at a significantly higher risk of recurrent ischemic stroke than non-carriers. Significantly increased risk of recurrent ischemic stroke was found in both observational studies and RCTs.

INTRODUCTION

In patients suffering from acute ischemic stroke or transient ischemic attack (TIA), clopidogrel has been proven to reduce the occurrence of new stroke events; therefore, it is considered as the first-line therapy in managing such neurological disorders.[1,2] However, high inter-individual variabilities have been found with regard to clopidogrel responses, and a substantial number of adverse clinical outcomes, for example, recurrent strokes, have been reported in spite of clopidogrel treatment, although some were treated with dual antiplatelet agents (i.e., clopidogrel

with aspirin).[2,3] Previous analyses established very strong associations for those carrying *CYP2C19* loss-of-function (LoF) alleles, which encode a reduced function of the CYP2C19 enzyme, with a significantly increased risk of major adverse cardiovascular events (MACEs) in patients with either stable coronary artery disease (CAD) or acute coronary syndrome (ACS) treated with clopidogrel.[4–6] The clinical pharmacogenetic implementation consortium (CPIC) guidelines, first published in 2011 and updated in 2013, only considered a limited number of *CYP2C19* genetic variants to generate pharmacogenomics-based dosing guidelines.[7,8] The guidelines were updated again in 2022 with consideration of some additional *CYP2C19* genetic variants, as well as other genetic variants.[9] Most importantly, these guidelines are only effective and applicable to ACS patients; stroke patients were not included in these recommendations. In addition, the FDA also updated the label information of clopidogrel with a suggestion to consider an alternative antiplatelet for patients identified as poor metabolizers of CYP2C19.[10]

Yet, while some previous studies reported that stroke patients with *CYP2C19* LoF alleles had an increased risk of recurrent ischemic stroke,[11,12] other studies did not find such associations, a result which presents a challenge for clinicians in rationalizing antiplatelet therapy in patients with *CYP2C19* LoF alleles.[13,14] To date, only one well-established meta-analysis is available for patients with stroke/TIA through which to assess such associations.[2] Although a very recent meta-analysis is also available, the study has serious limitations.[15] For example, the recent meta-analysis conducted by Wang et al., in 2021, only included prospective cohort studies, mostly from China, and included some studies that we did not find in our literature search through PubMed or even in a Google search, that is, Fukuma (2017), LiNa Qiu (2014), and many more, as we did not check all of them. Another very recent meta-analysis was published to validate the associations between carriers of *CYP2C19* LoF alleles and the risk of recurrent stroke in non-East Asian populations but this analysis did not consider other populations.[16]

Thus, the only available well-established meta-analysis was published in 2017, which included 15 studies with 4762 stroke/TIA patients from all ethnic groups, including Asians, Europeans, Africans, and other ethnicities.[2] Although it was a well-established analysis, since then, many more studies have appeared in the literature; therefore, there is a need to update the meta-analysis of the literature. Additionally, the previous meta-analysis did not investigate the differences in the impacts on *CYP2C19* LoF allele carriers and non-carriers between observational and randomized controlled trials (RCTs), respectively. Since there are methodological differences between observational studies and RCTs, it is important to assess the risk differences encountered when comparing these two study types.

With the recent completion of the available literature, we aimed to conduct a systematic review and meta-analysis through which to assess the association between genetic polymorphisms, especially in patients carrying or not carrying *CYP2C19* LoF alleles, and the occurrences of recurrent ischemic stroke, composite vascular events, and bleeding events in clopidogrel-treated patients suffering from ischemic stroke or TIA.

METHODS

The literature was searched systematically in different databases, that is, PubMed, ScienceDirect, Scinapse, and 1000 Genomes, in order to select articles using different combinations of search keywords, following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines, from the inception of the database to May 31, 2023.[17] After completing all keyword searches, all of the search histories were combined and imported into Rayyan QCRI software.[18,19] The studies were included if they fulfilled the following criteria: (i) Antiplatelets (clopidogrel, with or without aspirin) were prescribed for stroke/TIA patients. (ii) Outcomes were reported for at least one of the two clinical end points, that is, efficacy or safety, for which efficacy represents either the occurrence of recurrent stroke or composite vascular events, including ischemic or hemorrhagic stroke, myocardial infarction (MI), or vascular death. Safety represents bleeding events, for which bleeding was defined by the Global Use of Strategies to Open Occluded Arteries (GUSTO) as clinically significant bleeding.[20] (iii) Clinical outcomes were reported for at least two treatment groups of patients, in which one group of patients carried *CYP2C19* LoF alleles (*2, *3, *4, *5, *6, *8) and the other group of patients carried the *CYP2C19* wild-type genotype, that is, *CYP2C19*1/*1*. Studies were excluded based on the following criteria: (i) The study had only one group of treated patients, with either clopidogrel or alternative antiplatelets, without any studied *CYP2C19* genetic effects. (ii) The study did not clearly report the outcomes for the two selected treatment groups of patients, described above. (iii) The article was a review, perspective, editorial, case report, or letter to the editor.

The studies were selected using Rayyan QCRI software, following the inclusion and exclusion criteria, as described above. The full texts of all the preliminarily selected studies were downloaded and checked extensively to determine the final selection of the studies. Two independent investigators were involved in the study selection process. Any disagreement between these two investigators was

resolved by the senior authors via mutual discussion. The quality levels of the included studies were assessed as follows: Newcastle–Ottawa Scale (NOS) guidelines were used to assess the quality levels of observational studies.[21] In contrast, a 5-point Jadad Scale (JS) was used to assess the quality levels of RCTs.[22] Pooled odds ratios (ORs) and a 95% confidence interval (CI) were calculated using Review-Manager software (RevMan version 5.3 Windows; The Cochrane Collaboration, Oxford, UK), following the random- or fixed-effect models, based on the levels of heterogeneity in the included studies (I^2 statistics). The decisions on the publication bias of the included studies were made based on the visual inspection of the distribution of the ORs of recurrent stroke in a funnel plot. A p-value <0.05 was considered statistically significant when analyzing all data.

RESULTS

General characteristics and quality of the included studies

The complete process for the identification of the included studies is shown in Figure S1. Altogether, 28 studies, comprising 9443 stroke or TIA patients (mean age 65.7 ± 11.1; 34% female), were included in this meta-analysis, of which 22 studies[11–13,23–41] were observational cohort studies, and six studies were RCTs.[14,42–46] The follow-up period for collecting outcome data for the included studies ranged from 5 days to 54 months. In total, 16 studies reported that the patients were taking clopidogrel during the follow-up period, whereas 12 studies reported that the patients were taking clopidogrel and aspirin during the follow-up period. The important baseline characteristics of the included studies are summarized in Table 1. The quality of the majority of the included observational studies, as assessed with the Newcastle–Ottawa scale, was high (score ranges between 6 and 9), and the RCTs were also of high quality, as assessed with the Jadad scale (Tables S1 and S2, respectively).

Clinical outcomes for *CYP2C19* LoF allele carriers vs. non-carriers

As shown in Figure 1, the results of this meta-analysis after pooled estimation indicated that patients carrying *CYP2C19* LoF alleles who were treated with clopidogrel had a significantly increased risk of recurrent ischemic stroke compared with non-carriers (OR 2.18, 96% CI 1.80–2.63; $p < 0.00001$). The occurrence of composite vascular events was also significantly different between *CYP2C19* LoF allele carriers and non-carriers (OR 1.43, 96% CI 1.05–1.94; $p = 0.02$), as shown in Figure 2.

Comparison of clinical outcomes in observational studies vs. RCTs for *CYP2C19* LoF allele carriers and non-carriers

The risk of recurrent ischemic stroke was significantly different in both observational studies (OR 2.83, 96% CI 2.20–3.65; $p < 0.00001$) and RCTs (OR 1.48, 96% CI 1.10–1.98; $p = 0.009$) for the patients carrying *CYP2C19* LoF alleles, as compared to the non-carriers, as shown in Figure 3. However, the risk was striking in the observational studies, as compared to the RCTs (OR 2.83 vs. OR 1.48).

Comparison of clinical outcomes in Asian vs. Other ethnicities for *CYP2C19* LoF allele carriers compared with non-carriers

The current analysis also investigated the impacts of *CYP2C19* LoF alleles on Asian patients compared with patients of other ethnicities. The risk of recurrent ischemic stroke was only significantly different in Asian patients (OR 2.29, 96% CI 1.88–2.80; $p < 0.00001$) and not in patients of other ethnicities (Europeans: OR 1.47, 96% CI 0.71–3.04, $p = 0.30$; Africans OR 1.93, 96% CI 0.58–6.43, $p = 0.29$; others OR 0.22, 96% CI 0.02–2.32, $p = 0.21$), as shown in Figure 4.

Bleeding events

Out of the 28 studies included in this analysis, 13 studies assessed the bleeding events associated with carrying *CYP2C19* LoF alleles. It was found that bleeding events were not significantly different between patients taking clopidogrel who carry *CYP2C19* LoF alleles and those who do not (OR 0.86, 96% CI 0.62–1.19; $p = 0.37$), Figure 5.

Heterogeneity, sensitivity analysis, and publication bias

A moderate level of heterogeneity was found in this study when comparing the risk of recurrent ischemic stroke between the two treatment groups (I^2=42%); therefore, a sensitivity analysis was conducted. This analysis was carried out by removing studies, one by one, in chronological order, and we did not find any difference in the clinical outcomes. Sensitivity analysis was also carried out between the observational studies and RCTs, which also did not affect

TABLE 1 Baseline characteristics of included studies.

Author, Year	Site(s)	Study design	Clinical diagnosis	Drug regimen	Age ± SD; % of male	Sample size	*CYP2C19* Non-LoF carriers		*CYP2C19* LoF carriers		Clinical outcomes measured	Follow-up, months/days
							n (%)	Allele	*n* (%)	Allele		
Al-Rubaish et al., 2021 (PMID: 35385889)	Saudi Arabia	Prospective study	IS	Clopidogrel (75 mg) + Aspirin (50–325 mg)	61 ± 12.5; 64.9	256	202 (78.9)	*1, *17	54 (21.1)	*2	Recurrent stroke, MI, death	6 months
Fang et al., 2015 (PMID: 26663068)	China	Cohort study	Acute IS	Clopidogrel (NR)	66.0 ± 10.4; 75.4	114	39 (34.2)	*1, *17	75 (65.8)	*2, *3	Recurrent stroke	12 months
Han et al., 2015 (PMID: 26177117)	China	Observational study	Acute IS	Clopidogrel (75 mg)	68.1 ± 11.5; 67.8	345	144 (40.7)	*1, *17	201 (59.3)	*2, *3	Ischemic events (MACE, recurrent stroke, TIA), composite of vascular death, bleeding events	12 months
Han et al., 2017 (PMID: 29037010)	South Korea	RCT	IS	Clopidogrel (75 mg)	61 ± 10.9; 66.7	393	149 (37.9)	*1, *17	244 (62.1)	*2, *3	Recurrent stroke, major vascular events (stroke, MI, or vascular death), bleeding events	31 months
Hoh et al., 2016 (PMID: 26587656)	USA	Cohort study	Stroke or TIA attributable to ICAD	Clopidogrel (75 mg) + Aspirin (100 mg)	67.0 ± 12.2; 63.3	188	137 (72.9)	*1, *17	51 (27.1)	*2, *3, *8	Composite vascular events (Recurrent stroke, TIA, MI, death)	12 months
Jeong et al., 2015 (PMID: 25529343)	South Korea	Retrospective study	IS	Clopidogrel (75 mg) + Aspirin (100 mg)	61.6 ± 12.9; 75	76	45 (59.2)	*1, *17	31 (40.8)	*2, *3	Recurrent stroke	5 days
Jia et al., 2013 (PMID: 23640828)	China	Observational study	Acute IS	Clopidogrel (75 mg)	66.5 ± 11.8;64.4	259	99 (38.2)	*1	160 (61.8)	*2, *3	Recurrent stroke	6 months
Li et al., 2016 (PMID: 26870959)	China	Cohort study	IS undergoing stenting	Clopidogrel (75 mg) + Aspirin (100 mg)	63.0 ± 9.0; 85.1	268	150 (56.0)	*1, *17	118 (44.0)	*2, *3	Ischemic events (recurrent stroke/TIA, MI), vascular death	12 months
Lin et al., 2014 (PMID: 24330577)	China	Retrospective study	Stroke/TIA	Clopidogrel (75 mg) + Aspirin (100 mg)	66.8 ± 9.6; 78.6	90	46 (51.1)	*1	44 (48.9)	*2, *3	Composite vascular events, recurrent stroke, deaths, bleeding events	54 months
Lin et al., 2018 (PMID: 29804161)	China	Prospective observational study	IS	Clopidogrel (75 mg) + Aspirin (200 mg)	69 ± 12.5; 64.5	375	153 (40.8)	*1	222 (59.2)	*2	Composite of recurrent IS, MI, death, bleeding events	8 months

TABLE 1 (Continued)

Author, Year	Site(s)	Study design	Clinical diagnosis	Drug regimen	Age ± SD; % of male	Sample size	*CYP2C19* Non-LoF carriers		*CYP2C19* LoF carriers		Clinical outcomes measured	Follow-up, months/ days
							n (%)	Allele	*n* (%)	Allele		
Lin et al., 2021 (PMID: 35016407)	China	Retrospective observational study	IS	Clopidogrel (75 mg)	65.1 ± 13.2; 57.3	89	38 (42.7)	*1	51 (57.3)	*2, *3	Recurrent stroke, bleeding events	12 months
Liu et al., 2020 (PMID: 32176040)	China	Retrospective study	IS	Clopidogrel (75 mg)	66.6 ± 10.9; 58.1	289	130 (45.0)	*1	159 (55.0)	*2, *3	Recurrent IS, MI, vascular death	6 months
Lv et al., 2022 (PMID: 34596891)	China	Prospective observational study	Acute IS	Clopidogrel (75 mg)	68.1 ± 11.5; 67.8	314	127 (40.4)	*1, 17	187 (59.6)	*2, *3	Composite of recurrent IS/TIA, MI, vascular death	54 months
McDonough et al., 2015 (PMID: 26019129)	USA	RCT	Small subcortical stroke/ TIA	Clopidogrel (75 mg) + Aspirin (325 mg)	62.5 ± 10.5; 61.9	493	386 (78.3)	*1, *17	107 (21.7)	*2, *3	Recurrent ischemic or hemorrhagic stroke, MI, vascular death, bleeding events	40 months
Meschia et al., 2020 (PMID: 32568642)	USA	RCT	Stroke/TIA	Clopidogrel (75 mg) + Aspirin (50–325 mg)	63.0 ± 9.5; 56.9	932	667 (71.6)	*1, *17	265 (28.4)	*2, *3	Recurrent IS, MI, ischemic vascular death, bleeding events	90 days
Qiu et al., 2015 (PMID: 25489921)	China	Observational study	Acute IS	Clopidogrel (75 mg)	67.1 ± 12.6; 55.0	211	82 (38.9)	*1	129 (61.1)	*2, *3	Composite recurrent IS, MI, and death	6 months
Spokoyny et al., 2014 (PMID: 23849748)	USA	Retrospective cohort study	Stroke and TIA	Clopidogrel (75 mg)	69.6 ± NR; 53.4	53	36 (67.9)	*1, *17	17 (32.1)	*2, *3	Recurrent IS/TIA	NR
Sun et al., 2015 (PMID: 25207801)	China	Cohort study	IS	Clopidogrel (75 mg)	61.6 ± 12.2; 74.4	625	234 (37.4)	*1, *17	391 (62.5)	*2, *3	Composite of vascular death, recurrent non-fatal IS, MI, bleeding events	12.7 months.
Tanaka et al., 2019 (PMID: 31006731)	Japan	Cohort study	IS/TIA	Clopidogrel / 75 mg	68.0 ± NR; 72.7	501	425 (84.8)	*1, *17	76 (15.2)	*2, *3	Composite vascular events (recurrent IS/TIA, AMI, CV death), bleeding events	728 days
Tomek et al., 2018 (PMID: 29509167)	Czech Republic	Retrospective cohort study	IS	Clopidogrel (75 mg)	64.5 ± 13.9; 60	130	86 (66.2)	*1, *17	44 (33.8)	*2	Composite vascular events (recurrent IS/TIA, MI, or vascular death)	14.9 months
Tornio et al., 2018 (PMID: 28653333)	UK	Cohort study	Stroke	Clopidogrel (75 mg)	74.0 ± NR; 61.7	94	27 (73.3)	*1	67 (26.7)	*2	Recurrent stroke	24 months

(Continues)

TABLE 1 (Continued)

Author, Year	Site(s)	Study design	Clinical diagnosis	Drug regimen	Age ± SD; % of male	Sample size	*CYP2C19* Non-LoF carriers		*CYP2C19* LoF carriers		Clinical outcomes measured	Follow-up, months/ days
							n (%)	Allele	*n* (%)	Allele		
Wang et al., 2016 (PMID: 27348249)	China	RCT	Stroke/TIA	Clopidogrel (75 mg) + Aspirin (75 mg)	62.8 ± 10.6; 67.2	1463	609 (41.6)	*1, *17	854 (58.4)	*2, *3	Composite vascular events (IS, MI, vascular death), bleeding events	90 days
Wang et al., 2019 (PMID: 31171523)	China	RCT	Stroke/TIA	Clopidogrel (75 mg) + Aspirin (100 mg)	60.5 ± 9.0; 73.5	319	133 (41.7)	*1, *17	186 (58.3)	*2, *3	Composite vascular events (recurrent stroke/TIA, MI, vascular death), bleeding events	90 days
Yi et al., 2016 (PMID: 26961113)	China	Prospective cohort study	Acute IS	Clopidogrel (75 mg)	68.5 ± 11.7; 66.7	363	148 (40.8)	*1	215 (59.2)	*2, *3	Composite of recurrent IS, MI, vascular death, bleeding events	6 months
Yi et al., 2018 (PMID: 27637911)	China	Prospective cohort study	IS	Clopidogrel (75 mg)	69.1 ± 12.8; 64.3	502	221 (44.0)	*1	281 (56.0)	*2, *3	Composite of recurrent IS, MI, vascular death, bleeding events	12 months
Zhang et al., 2014 (PMID: 25457586)	China	Prospective cohort study	IS	Clopidogrel (75 mg)	66.0 ± 10.0; 65.3	95	42 (44.2)	*1	53 (55.8)	*2, *3	Composite vascular events, recurrent stroke	6 months
Zhou et al., 2021 (PMID: 33411687)	China	RCT	Stroke/TIA	Clopidogrel (75 mg) + Aspirin (100 mg)	61.8 ± 8.5; 70.8	365	166 (45.5)	*1	199 (54.5)	*2, *3	Recurrent stroke, MI, death, bleeding events	90 days
Zhu et al., 2016 (PMID: 27137706)	China	Cohort study	IS undergoing stenting	Clopidogrel (75 mg) + Aspirin (100 mg)	64.3 ± 9.3; 90	241	89 (36.9)	*1	152 (63.1)	*2, *3	Recurrent Stroke/TIA, death, bleeding events	12 months

Abbreviations: AMI, acute myocardial infarction; ICAD, intracranial atherosclerotic disease; IS, ischemic stroke; LoF, loss-of-function; MACE, major adverse cardiovascular events; MI, myocardial infarction; NR, not reported; RCT, randomized controlled trial; SD, standard deviation; TIA, transient ischemic attack.

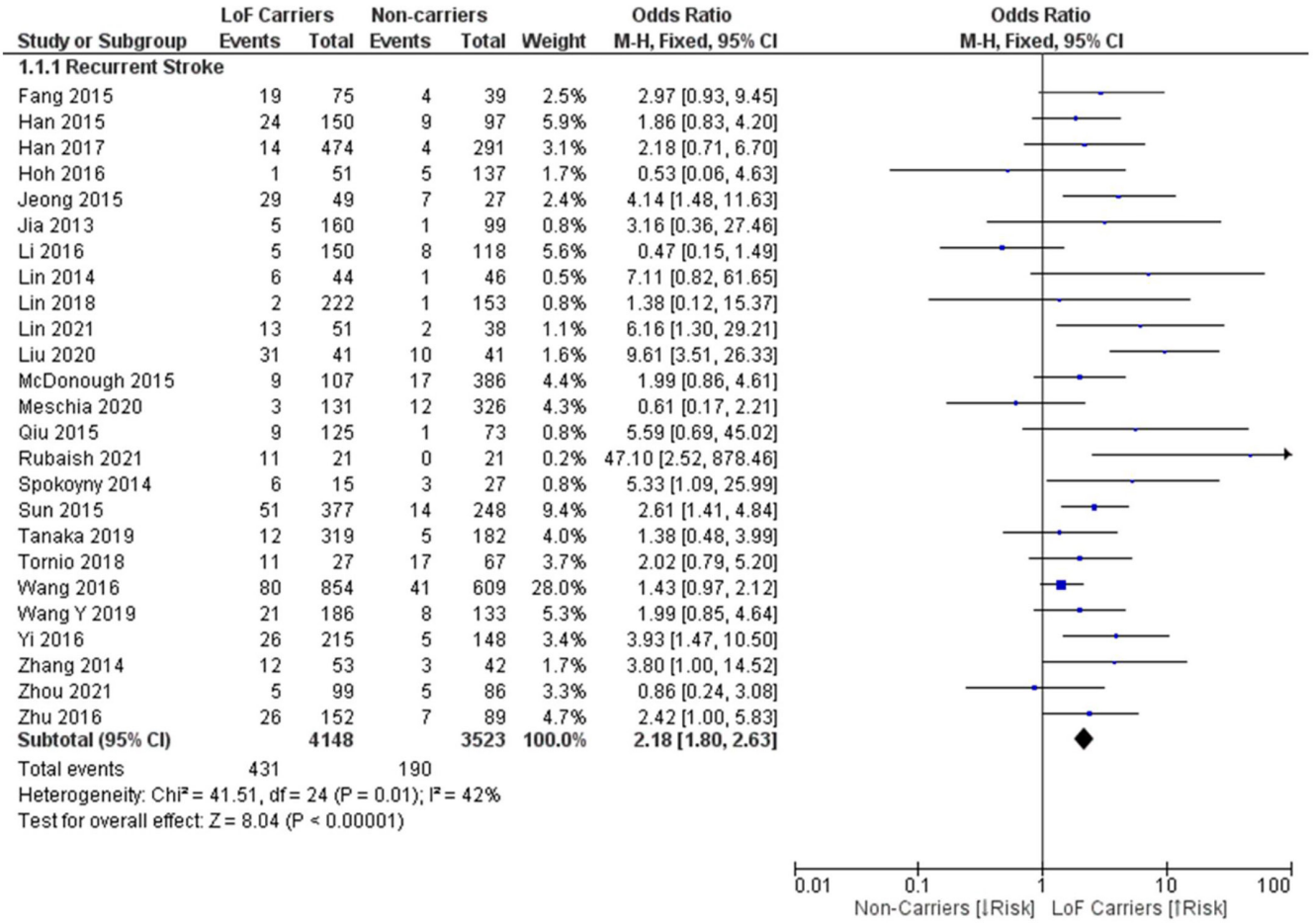

FIGURE 1 Forest plot of the association of *CYP2C19* LoF allele carriers vs. non-carriers on recurrent stroke in stroke/TIA patients taking clopidogrel.

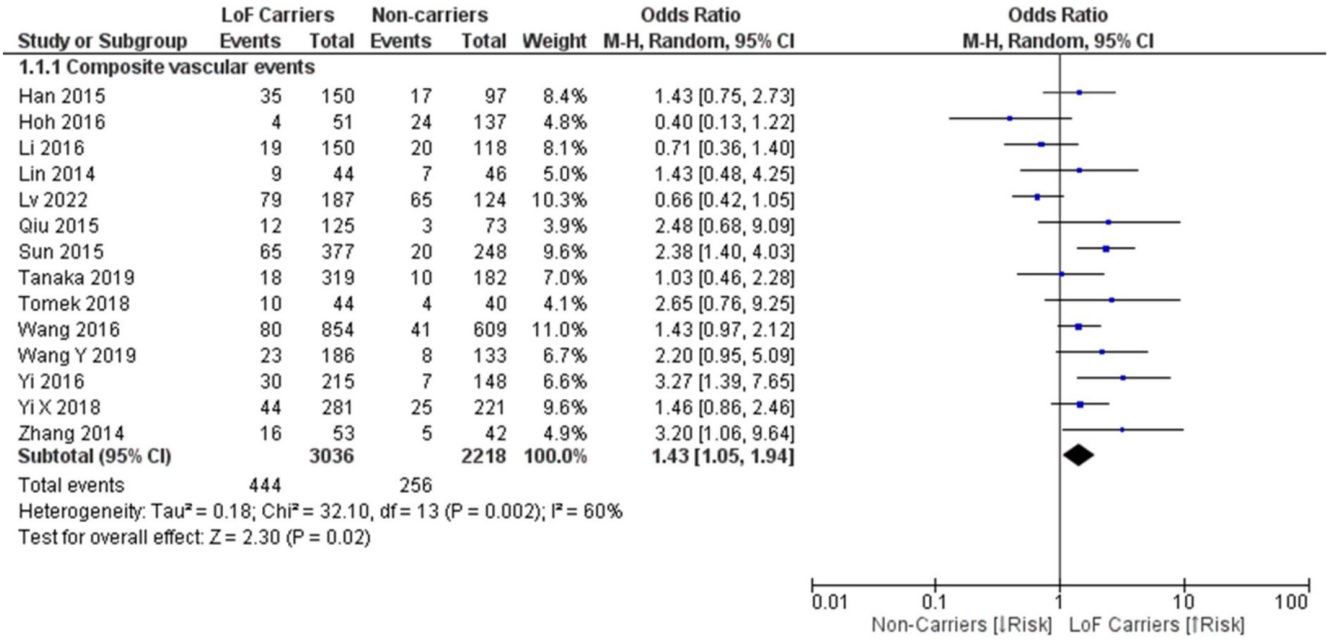

FIGURE 2 Forest plot of the association of *CYP2C19* LoF allele carriers vs. non-carriers on composite vascular events in stroke/TIA patients taking clopidogrel.

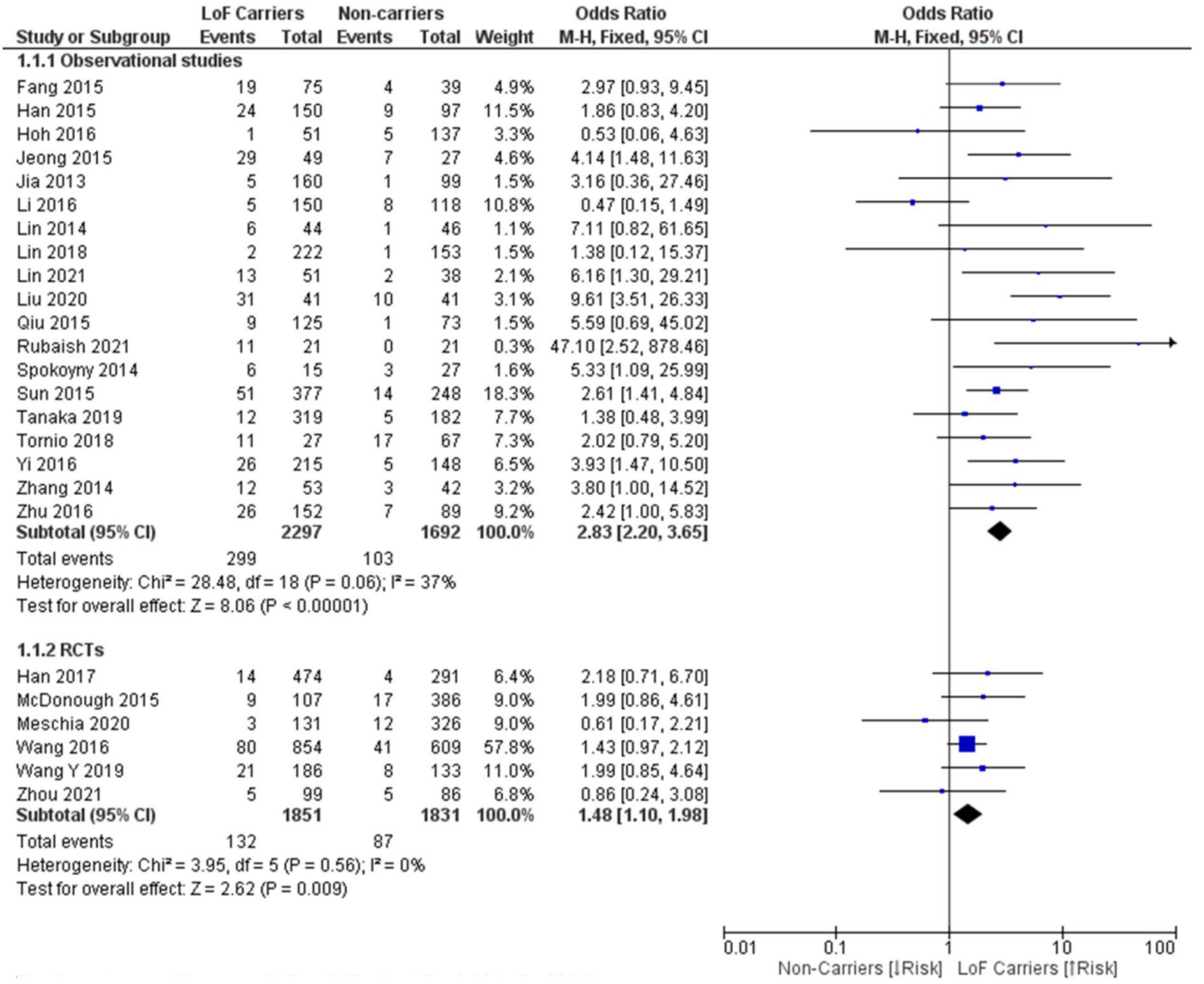

FIGURE 3 Forest plot of the risk of recurrent stroke for *CYP2C19* LoF allele carriers vs. non-carriers on observational studies and RCTs in stroke/TIA patients taking clopidogrel.

the risk ratios, since both study designs (observational vs. RCTs) found associations with a significantly increased risk of recurrent ischemic stroke. From visual inspection of the funnel plot, it was determined that there was no publication bias in the current analysis, as shown in Figure S2.

DISCUSSION

Stroke patients carrying *CYP2C19* LoF alleles treated with clopidogrel had a significantly increased risk of recurrent ischemic stroke and composite vascular events, compared with those who did not carry any *CYP2C19* LoF alleles. The risk of recurrent ischemic stroke was found to be striking in the observational studies and in Asian patients. However, there was no significant difference in the risk of bleeding events between stroke patients with and without *CYP2C19* LoF alleles.

Since the current analysis included 28 studies, comprising 9443 stroke/TIA patients, the findings of this analysis may be considered more encompassing than the previous analysis, which considered only 15 studies with 4762 stroke/TIA patients.[2] The great novelty of the current analysis is that it has investigated and compared the risk of recurrent ischemic stroke identified in both observational studies and RCTs. Since there are methodological differences between observational studies and RCTs, and the findings of RCTs are generally considered more robust, there is an obvious need to assess whether there were any differences in the risk identified between these two types of studies. Although the identified risk of recurrent ischemic stroke was significantly different when comparing observational studies and RCTs for stroke/TIA patients carrying *CYP2C19* LoF alleles and taking clopidogrel compared with the patients without *CYP2C19* LoF alleles, the risk was striking and

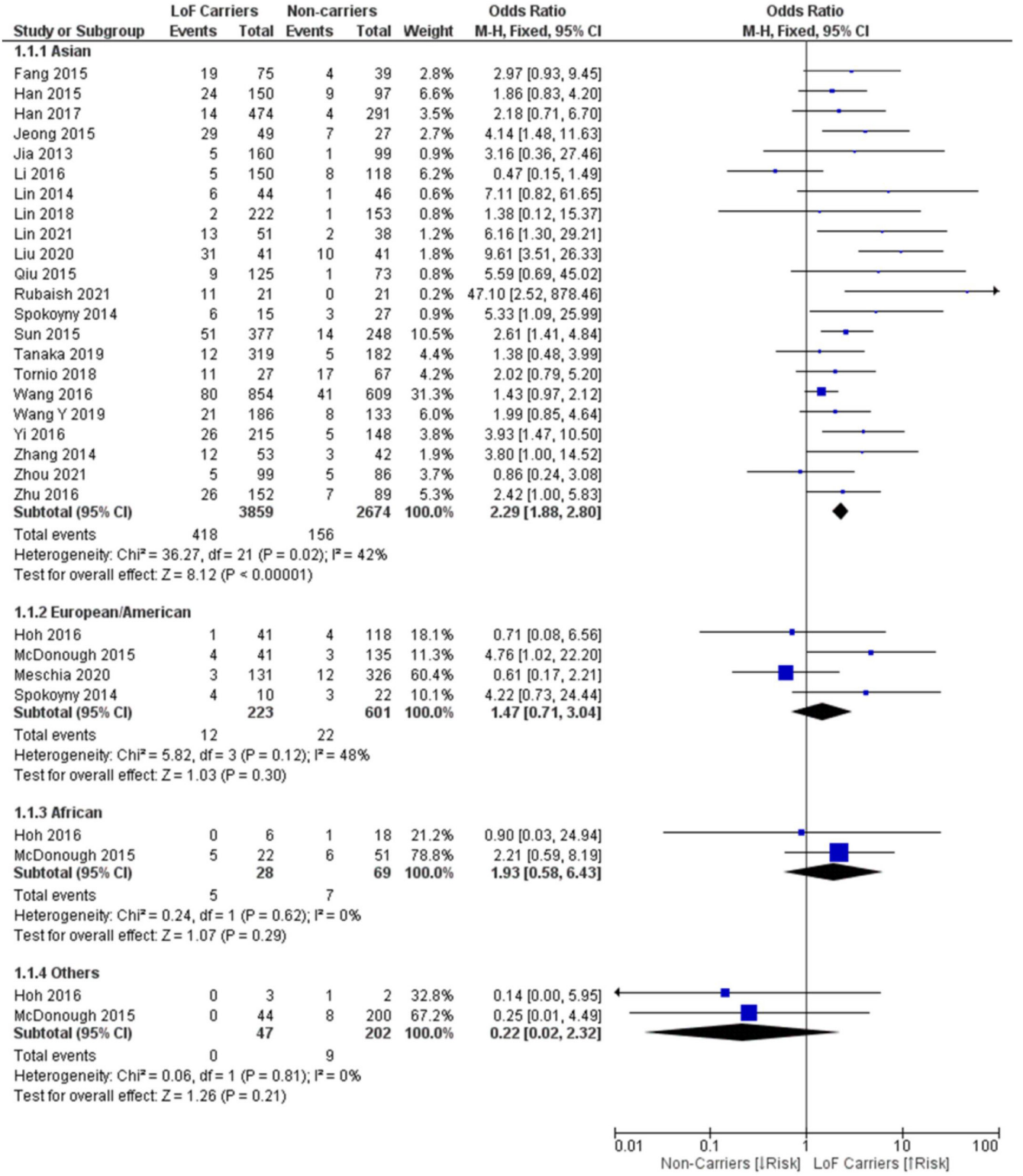

FIGURE 4 Forest plot of the risk of recurrent stroke for *CYP2C19* LoF allele carriers vs. non-carriers on different ethnicities in stroke/TIA patients taking clopidogrel.

profoundly increased in the observational studies compared with the RCTs (OR 2.83 vs. OR 1.48). This might be due to the small number of RCTs (n=6) compared with the large number of observational studies (n=22) included in this comparison analysis. More RCTs are warranted in the future to corroborate the findings of the current analysis.

The current analysis found an increased risk of recurrent ischemic stroke only for Asian stroke/TIA patients and not for other ethnicities. This is not consistent with

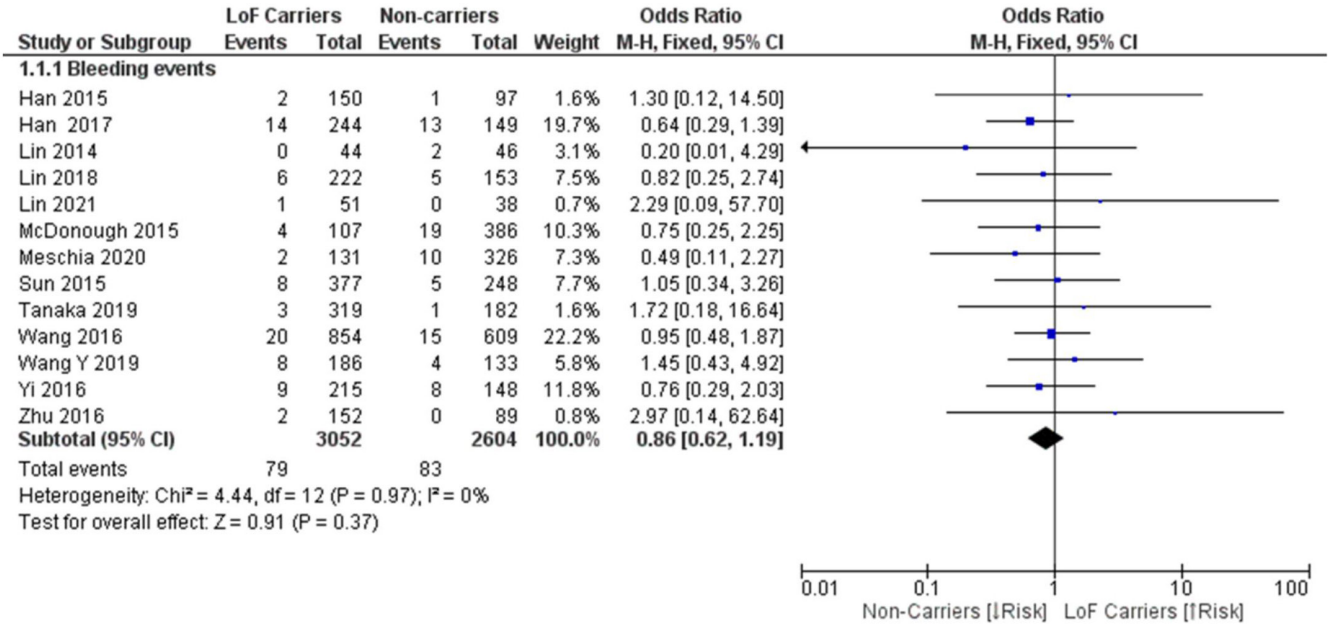

FIGURE 5 Forest plot of the risk of bleeding events for *CYP2C19* LoF allele carriers vs. non-carriers in stroke/TIA patients taking clopidogrel.

the previous analysis, as the authors found a significant difference in European patients.[2] This might be because the previous analysis assessed the effects in only 367 stroke/TIA patients; however, our current analysis included 824 stroke/TIA patients in whom these effects were assessed. Although the risk of adverse cardiovascular events was found to increase significantly in Asian stable CAD or ACS patients carrying *CYP2C19* LoF alleles and taking clopidogrel compared with Caucasian patients,[5,6,47] for stroke patients, such increasing risk trends were most similar for Asian patients only, as found in the current analysis. It is noted that Asian stroke patients carried 58.4% of the identified *CYP2C19* LoF alleles, whereas European stroke patients carried 34.8% of the *CYP2C19* LoF alleles. African and other ethnic patients did not have a significantly increased risk of recurrent ischemic stroke, which might be due to the very small number ($n=2$) of relevant studies included in the analysis. Only a limited number of studies were identified that addressed African patients or those of other ethnicities, which may be considered underpowered to reach statistical significance; therefore, it is assumed that this small number of studies was not associated with any significant difference in increasing the risk of recurrent stroke. It is strongly suggested to conduct future large studies in other ethnic groups relating to the assessment of the impacts of *CYP2C19* LoF alleles on stroke patients.

The overall findings of the current analysis suggest using *CYP2C19* LoF genotype-guided alternative antiplatelets, such as prasugrel or ticagrelor, in stroke patients to optimize the effectiveness of antiplatelet therapy to achieve precision medicine. It has been shown that ticagrelor is not metabolized by the CYP2C19 enzyme; therefore, there is no genetic interference with the effectiveness of this drug.[48] Prasugrel is mainly metabolized by the esterase and subsequently by the CYP enzymes, but the involvement of which specific CYP enzyme is still under investigation.[49] Moreover, *CYP2C19* genotype-guided alternative antiplatelet therapy may reduce adverse cardiovascular events significantly, as found in ACS patients undergoing PCI,[50] and may also be able to reduce the risk of recurrent stroke in stroke/TIA patients, as recently investigated and suggested for stroke/TIA patients.[51] A recent RCT was conducted in China in 2021 consisting of minor ischemic stroke or TIA patients who carried *CYP2C19* LoF alleles. This trial enrolled 6412 patients, where 3205 patients were assigned to the ticagrelor treatment group and 3207 patients were to the clopidogrel treatment group, respectively. The findings of this trial indicated that the risk of stroke at 3 months window was modestly lower with ticagrelor treatment group compared with clopidogrel treatment group. However, the risk of severe or moderate bleeding events did not differ between these two treatment groups (0.3% vs. 0.3%), although total bleeding events were slightly higher in ticagrelor treatment group compared with clopidogrel treatment group (5.3% vs. 2.5%).[52] It is believed that the CPIC guideline should update the prescribing recommendations including stroke patients since the robust evidence established in this analysis, along with other available evidence,[2,16] warrants this.

Given that genetic polymorphisms of *ABCB1*, *CES1*, *Q192R*, *PON1*, and *P2Y12* may affect the pharmacokinetic properties of clopidogrel and may also affect the effectiveness of clopidogrel,[53–55] it is recommended to assess the impacts of such genetic variants in ischemic stroke/TIA patients taking clopidogrel in future studies.

Apart from genetic interferences, some other confounding factors, for example, diet, smoking, and concurrent medications, may also affect the responsiveness of clopidogrel, as well as clinical outcomes.[56] The observed associations between *CYP2C19* LoF allele carriers vs. non-carriers and recurrent ischemic stroke or composite vascular events may be confounded by these factors, especially concomitant medications, such as proton pump inhibitors (PPIs), as observed in a recent analysis for CAD/stroke patients.[57] These confounding factors, especially the impacts of PPIs, were not estimated in the current analysis, due to the limitations of the data, and therefore need to be clarified in future investigations.

Our study had some limitations. First of all, the included studies varied in many aspects, that is, different study populations, doses and regimens of antiplatelet therapy, duration of clopidogrel use, and follow-up duration, which could affect the heterogeneity of this study. Secondly, the results with respect to African/other ethnicity could be underpowered, with only two studies assessing the outcomes for *CYP2C19* LoF interventions in these ethnicities. Thirdly, the existence of the *CYP2C19*17* allele may have an influence on the clinical outcomes, as it may enhance the metabolic conversion of clopidogrel to the active form, which is an entirely opposite function to that of the *CYP2C19* LoF allele. Since the *CYP2C19*17* allele was included in the non-carrier groups, therefore, the net clinical outcome may vary without the *CYP2C19*17* allele, which was not investigated in the current analysis. We were not able to perform analyses accounting for *CYP2C19*17* since the studies did not provide separate outcomes data.

EXPERT OPINION

The findings of the current analysis may have clinical impacts since physicians can determine the *CYP2C19* genetic status if that facility is available before prescribing clopidogrel to ischemic stroke patients to reduce the incidence of recurrent ischemic strokes as well as other associated adverse events. However, in countries where the facility to genotype *CYP2C19* is unavailable, physicians may check other alternative parameters, for example, the magnitude of platelet aggregation and the concentration of clopidogrel's active metabolite as part of the therapeutic drug monitoring process to evaluate the risk of prescribing clopidogrel to these patients. In addition, an alternative strategy such as treating the patients with novel anti-coagulants (NOACs), that is, dabigatran, rivaroxaban, etc., might be beneficial to prevent the incidence of recurrent ischemic stroke, although safety issues, that is, the bleeding risk, must be considered rationally.

CONCLUSIONS

In summary, ischemic stroke/TIA patients who are carriers of LoF alleles and are treated with clopidogrel are at a significantly increased risk of recurrent ischemic stroke and composite vascular events in comparison with *CYP2C19* LoF non-carriers. A significantly increased risk of ischemic stroke was found in both the observational studies and RCTs. The risk of recurrent ischemic stroke was significantly striking only for Asian stroke/TIA patients. The risk of bleeding events was not significantly different between the ischemic stroke/TIA patients with and without *CYP2C19* LoF alleles. The findings of the current analysis may justify *CYP2C19* genetic testing when clopidogrel is clinically indicated, and the use of *CYP2C19* LoF genotype-guided alternative antiplatelets, such as prasugrel or ticagrelor, may be preferred in Asian stroke/TIA patients to optimize the effectiveness of clopidogrel therapy to achieve precision medicine.

AUTHOR CONTRIBUTIONS

C.S. and M.B. designed the research; M.B. and C.S. wrote the manuscript; C.S., M.B., M.S.H., T.A., and S.H. performed the research; M.B. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests in this work.

DATA AVAILABILITY STATEMENT

There were no associated data for this research.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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