Journal of Stroke and Cerebrovascular Diseases

HIGH ON-CLOPIDOGREL PLATELET REACTIVITY IN ISCHAEMIC STROKE OR TRANSIENT ISCHAEMIC ATTACK: SYSTEMATIC REVIEW AND META-ANALYSIS

--Manuscript Draft--

Manuscript Number:	JSCVD-D-19-01620R2
Article Type:	Original Article
Section/Category:	Neurology
Keywords:	High on clopidogrel platelet reactivity; clopidogrel resistance; Ischaemic Stroke; transient ischaemic attack; CYP2C19 polymorphisms
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Abstract:	Objectives To assess the prevalence of high on-clopidogrel platelet reactivity (HCPR) in patients with ischaemic stroke or transient ischaemic attack (IS/TIA), their outcome and genetic basis of on-treatment response variability in IS/TIA patients. Methods We conducted a comprehensive search of PubMed and EMBASE from their inceptions to March 9, 2019. Studies that reported absolute numbers/percentages of HCRP at any time point after IS/TIA onset evaluated with any type of platelet function tests, clinical outcomes and genotyping data were included. Results Among 21 studies of 4312 IS/TIA patients treated with clopidogrel, the pooled prevalence of HCPR was 28% (95%CI: 24-32%; high heterogeneity: I^2 =88.2%, p <0.001). Heterogeneity degree diminished across groups defined by the HCPR testing method. Clopidogrel non-responder IS/TIA patients had poorer outcome compared to responders (RR=2.09, 95%CI: 1.61–2.70; p =0.036; low heterogeneity across studies: I^2 =27.4%, p =0.210). IS/TIA carriers of *CYP2C19*2* or *CYP2C19*3* loss of function alleles had a higher risk of HCPR compared to wild type (RR=1.69, 95%CI: 1.47–1.95; p <0.001; I^2 =0.01%, p =0.475). Conclusions This systematic review shows a high prevalence of clopidogrel resistance in IS/TIA and poor outcome in these patients. *CYP2C19* polymorphisms may potentially influence clopidogrel resistance.

Title Page (with ALL author details included (including names, email addresses, degrees, and phone numbers)

HIGH ON-CLOPIDOGREL PLATELET REACTIVITY IN ISCHAEMIC STROKE OR TRANSIENT ISCHAEMIC ATTACK: SYSTEMATIC REVIEW AND META-ANALYSIS

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Grant support:

None

Keywords:

High on clopidogrel platelet reactivity; clopidogrel resistance; ischaemic stroke; transient ischaemic attack; *CYP2C19* polymorphisms

Abstract

Objectives: To assess the prevalence of high on-clopidogrel platelet reactivity (HCPR) in patients with ischaemic stroke or transient ischaemic attack (IS/TIA), their outcome and genetic basis of on-treatment response variability in IS/TIA patients.

Methods: We conducted a comprehensive search of PubMed and EMBASE from their inceptions to March 9, 2019. Studies that reported absolute numbers/percentages of HCRP at any time point after IS/TIA onset evaluated with any type of platelet function tests, clinical outcomes and genotyping data were included.

Results: Among 21 studies of 4312 IS/TIA patients treated with clopidogrel, the pooled prevalence of HCPR was 28% (95%CI: 24-32%; high heterogeneity: I^2=88.2%, p<0.001). Heterogeneity degree diminished across groups defined by the HCPR testing method. Clopidogrel non-responder IS/TIA patients had poorer outcome compared to responders (RR=2.09, 95%CI: 1.61–2.70; p=0.036; low heterogeneity across studies: I^2=27.4%, p=0.210). IS/TIA carriers of *CYP2C19*2* or *CYP2C19*3* loss of function alleles had a higher risk of HCPR compared to wild type (RR=1.69, 95%CI: 1.47–1.95; p<0.001; I^2=0.01%, p=0.475).

Conclusions: This systematic review shows a high prevalence of clopidogrel resistance in IS/TIA and poor outcome in these patients. *CYP2C19* polymorphisms may potentially influence clopidogrel resistance.

Introduction

Excessive platelet activation plays a major role in the pathophysiology of ischaemic stroke[1-12]. Clopidogrel has been shown to be superior to aspirin in platelet inhibition and reducing the risk of ischaemic stroke[13]. It is metabolized by cytochrome P450 and the active metabolite irreversibly binds to platelet surface receptor P2Y12 inhibiting adenosine diphosphate induced platelet activation[14]. However, the antiplatelet response to clopidogrel is highly variable[15]. The reported prevalence of clopidogrel resistance, also termed "high on-clopidogrel platelet reactivity (HCPR)", ranges from 16% to 65%[16-19]. This wide variation in clopidogrel resistance prevalence is attributed to the profile of the studied population[20] and a lack of consensus on threshold values to define HCPR using different assays which include for example, VerifyNow P2Y12, light transmission aggregometry (LTA), multiple-electrode impedance aggregometry (MEA), vasodilator-stimulated phosphoprotein (VASP), thromboelastography (TEG) and flow cytometry. Causes for decreased platelet inhibition by clopidogrel are multifactorial and include genetic, cellular and co-morbid clinical factors[21-24].

Studies of ischaemic stroke patients with clopidogrel resistance have shown an association with early neurological deterioration and recurrent ischaemic episode with poor recovery[25]. Similarly, patients displaying HCPR have been shown to be at higher risk of thromboembolic events during and after carotid revascularisation[26].

In this article, we undertook a systematic review and meta-analysis of the prevalence of HCPR in patients with ischaemic stroke or transient ischaemic attack (IS/TIA), their outcome and the genetic basis of on-treatment response variability in IS/TIA patients.

Methods

This meta-analysis is presented according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[27] for systematic reviews and meta-analyses. We searched PUBMED and EMBASE for publications from inception up to March 9, 2019, and used the search terms (Clopidogrel*/ resistance*) OR (high*/ OR therapy* OR treatment* OR therapeutics.mp OR therapeutics*/ blood platelets OR blood*/ platelets* OR blood platelets* OR platelet*/ reactivity*) AND (stroke OR stroke*) OR (ischemic attack, transient OR ischemic*/ attack*/ transient* OR transient ischemic attack* OR transient*/ischemic*/attack*). We further performed a search of the Cochrane library, and ClinicalTrials.gov, and a manual search of references from all identified publications.

Two authors (VA, XH) identified studies eligible for further review by performing an initial screen of identified titles or abstracts. We restricted studies to those including patients with ischaemic stroke or TIA on Clopidogrel; those with coronary artery disease were excluded. Studies were considered for inclusion in the meta-analysis if they reported absolute numbers/percentages of HCPR at any time point after ischaemic stroke or TIA onset evaluated with any type of platelet function test, any type of study design with or without reported clinical outcomes or genotyping data. Any disagreement was reviewed by a third reviewer (ACP) and resolved by consensus. Initial screening revealed 33 potential studies and full-text article assessment excluded studies on the same cohort. Twenty-one studies were included for meta-analysis (Figure 1).

*CYP2C19*2* and *CYP2C19*3* alleles that result in impaired metabolism of CYP2C19 substrates were entitled as loss-of-function alleles[28]. Patients with at least 1 loss-of-function alleles

(hetero- or homozygous for *CYP2C19*2* or *CYP2C19*3*) were classified as loss-of-function allele carriers. Of twenty-one studies included for meta-analysis, eight studies provided data on *CYP2C19* loss of function allele carrier status in IS/TIA patients and clinical outcomes (Figure 4). Of eight studies, only four analysed platelet resistance and clinical outcome in *CYP2C19* loss of function allele carriers, and therefore this was not included in Figure 4 (Supplementary Table 1).

The primary end point was HCRP pooled proportion and outcome in clopidogrel-treated IS/TIA. The secondary endpoint was the association between *CYP2C19*2* and *CYP2C19*3* loss of function allele carrier status and HCPR in IS/TIA. Statistical analyses were performed using STATA software (version 15.0, Stata Corporation, College Station, TX). Pooled prevalence of HCPR in IS/TIA cohort across studies was derived. Pooled risk ratios (RR) and 95% confidence intervals (CIs) were calculated as the overall measure of efficacy of clopidogrel response using random-effects models. Two-sided probability values of <0.05 were considered statistically significant. Each analysis was accompanied by the assessment of the corresponding heterogeneity evaluated by the I^2 statistic; the Cochrane Q ($\chi 2$) statistic assessed heterogeneity between studies. Potential publication bias of studies with different sample sizes was examined by visual inspection of funnel plots and trim-and-fill analysis. The guidelines from https://uk.cochrane.org/news/meta-analysis-what-why-and-how were followed.

Results

Our search identified 21 potentially relevant studies with a total of 4312 ischaemic stroke and/or TIA patients on Clopidogrel. Study sizes ranged from 62 to 465 stroke or TIA patients.

Characteristics of the studies are summarised on the Supplementary Table 2. In the overall analysis of all included studies, the pooled prevalence of HCPR was 28% (95%CI: 24–32%). However, the prevalence reported between studies presented great variability as demonstrated by substantial heterogeneity (I^2 = 88.2%, Cochran Q p<0.001) (Figure 2).

The main finding is the significant disparity in many aspects across the studies not only in outcome measure, but also in the patients included, their demographics, the dose of Clopidogrel, the timing of the tests, the laboratory methods used, the definition of HCPR, and so on. In order to explain the heterogeneity, we did several analyses by grouping studies according to factors such as ethnicity (Supplementary Figure 1 and Supplementary Table 2), and laboratory methods assessing HCPR (Supplementary Table 1 and Supplementary Figure 3). Supplementary Figure 2 refers to subgroup analysis on the prevalence of HCPR according to use carotid artery stenting.

Heterogeneity only reduced amongst studies using multiple-electrode impedance aggregometry (MEA), thromboelastography (TEG) and vasodilator-stimulated phosphoprotein (VASP) methods (Table 1); and improved with analysis of studies using light transmission aggregometry (LTA) testing with similar cut-off points defining HCPR (Supplementary Table 1 and Supplementary Figure 4).

In the analysis of eight studies (total of 1887 IS/TIA patients on clopidogrel) providing data on outcome including recurrent stroke or other vascular events, increased modified Rankin Scale (mRS) or National Institutes of Health Stroke Scale (NIHSS) and death, IS/TIA patients with HCPR had poorer outcome compared to clopidogrel responders (RR = 2.09, 1.61–2.70, p=0.036) (Figure 3 and Supplementary Table 3).

From the analysis of eight studies providing data on genotyping, IS/TIA carriers of *CYP2C19* loss of function allele (*2 or *3) had a higher risk for HCPR (RR=1.69, 95%CI: 1.47–1.95; $p<0.001$; $I^2=0.01\%$, $p=0.475$) (Figure 4).

Discussion

The present report is to our knowledge the first meta-analysis that determines the prevalence of HCPR in IS/TIA patients and shows a positive association between the presence of HCPR and poor outcome including recurrent stroke or other vascular events, stroke progression or death. This finding is consistent with previously published systematic reviews and meta-analyses that reported an increased risk of cardiovascular events in patients with HCPR[16]. Meta-analyses in patients with acute coronary syndrome[29] who underwent percutaneous coronary intervention and stenting had a prevalence of HCPR of 21%, with a pooled OR of cardiovascular events of 8.0, which is similar to our finding. However, a peripheral vascular disease[30] meta-analysis reported a prevalence of HCPR of 65%, which is much higher than our result.

There is significant heterogeneity evident across the studies. In particular, the laboratory methods for testing clopidogrel resistance and the definition of HCPR varied from study to study. Currently, multiple laboratory and point of care platelet function testing are used across the world. A recent review[31] comparing existing platelet function tests has emphasised that non-standardised use of these tests and the lack of a proper definition is at least partly responsible for the disparity of the prevalence reported in studies. In one guideline[32] that attempted to standardise the definition of HCPR, the author argued that cut-off values to define HCPR are better determined by the individual laboratory, rather than providing an

arbitrary value generated from previous studies. That report also recommended that multiple assessments of the patients should be done in the same laboratory if possible, to provide meaningful interpretation. The same group[33] suggested additional clinical information and genotyping besides a platelet function test may be a better prediction of the risk of recurrent thromboembolic events.

In all the included studies, there were significant differences in clinical factors such as ethnicity, age, and co-morbidities, which probably have contributed to the heterogeneity of the analysis. In subgroup analysis for Asian/Non-Asian, IS/TIA plus or minus carotid artery stent, this heterogeneity did not dissipate. However, the subgroup analysis of laboratory methods did show much less heterogeneity, but the number of studies in each group was small so the results must be interpreted with caution.

A similar pattern of disparity was observed in analysis of the genetic studies. We nevertheless found that a significant proportion of IS/TIA patients with HCPR were *CYP2C19* loss-function allele carriers. Previous studies showed that among patients with ischemic stroke or TIA treated with clopidogrel, carriers of *CYP2C19* loss-of-function alleles are at increased risk of new stroke and composite vascular events in comparison with noncarriers, whereas bleeding risk is similar[34]. Similarly, the metanalysis[35] of acute coronary syndrome (ACS) patients who were *CYP2C19* loss-of-function carriers, found them to have an increased risk of myocardial infarct (MI), stent occlusion and ischaemic stroke, which supports the conclusion that *CYP2C19* has an important role in clopidogrel metabolism. However, not all patients with HCPR develop recurrent vascular events. The factors relating to this may not rest solely on pharmacokinetic aspects of clopidogrel metabolism but may also involve other genetic variation[36]. On the present evidence, *CYP2C19* genotyping may be a useful addition to the

individualised risk assessment to predict whether patients on clopidogrel are more at risk of recurrent vascular events and merit treatment with an alternative antiplatelet agent. However, further research is needed to assess the applicability of *CYP2C19* genotyping on a routine basis.

Our study has some limitations. First, none of the studies included in the meta-analysis was a randomised study. Second, medications including proton pump inhibitors intake data among studies was scanty and therefore was not included to the meta-analysis. Third, platelet resistance and clinical outcome was not analysed in *CYP2C19* loss of function allele carriers due to limited data among studies.

Clopidogrel resistance has been described for more than a decade, but the quality of published studies is so variable and heterogeneous that firmer conclusions from this meta-analysis cannot be drawn. However, patients with HCRP need evidence based guidance on how to approach their management. In order to determine the true potential benefit of testing for HCPR in the clinical setting, a randomised multicentre study with a single HCPR definition and centralised laboratory testing is warranted.

Abbreviations

ACS	acute coronary syndrome
CR	clopidogrel responders
CI	confidence intervals
ES	effect size
HCPR	high on clopidogrel platelet reactivity
IS	ischaemic stroke

LoF	loss of function
LTA	light transmission aggregometry
MEA	multiple-electrode impedance aggregometry
MI	myocardial infarction
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RR	risk ratios
TEG	thromboelastography
TIA	transient ischaemic attack
VASP	vasodilator-stimulated phosphoprotein

Declarations

1. ETHICS GUIDELINES: not applicable

2. CONSENT FOR PUBLICATION: not applicable

3. AVAILABILITY OF DATA AND MATERIAL: on request

4. COMPETING INTERESTS: none

5. FUNDING: none

6. AUTHORS' CONTRIBUTIONS: ACP and MM conceived the study. ICS oversaw the statistical methodology, analyses plan and crude data interpretation. VA and XH contributed to data acquisition. VA, XH, ICS and SD contributed to data quality assurance and data quality analysis. ACP, VA and XH contributed to data interpretation. VA drafted the initial manuscript and all remaining authors critically revised the manuscript. All authors gave final approval for publication.

7. ACKNOWLEDGEMENTS: We would like to thank Suzanne Drury for her help and comments on this project.

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Figure legends

Figure 1: Flow chart diagram presenting the selection procedure of eligible studies.

Figure 2: Pooled prevalence of all studies: Heterogeneity chi-squared = 169.69 (d.f. = 20), *p*<0.001; I-squared (variation in ES attributable to heterogeneity) = 8788.2%; Estimate of between-study variance Tau-squared = 0.0069; Test of ES=0 : z= 14.22; *p*<0.001. References[25, 37-56]. ID (identification); ES (effect size;) CI, confidence interval.

Figure 3: Overall analysis of all studies providing data on the outcome between non-responders and responders to clopidogrel. References[25, 38, 45-48, 50, 55]. ID, identification; RR (relative risk); CI, confidence interval.

Figure 4**:** HPCR related to *CYP2C19* loss of function**:** Heterogeneity chi-squared = 6.57(d.f. =7) *p* = 0.475; I-squared (variation in ES attributable to heterogeneity) = 0.01%; Estimate of between-study variance Tau-squared = 0.0000; Test of RR=1 : z= 7.32; *p*<0.001. References[39, 45, 47, 50-53, 55]. ID (identification); RR (relative risk); CI (confidence interval).

Table legends

Table 1. Subgroup analyses on the prevalence of HCPR reported in included studies. References[25, 37-56]

Supplementary data

Supplementary Table 1: Laboratory characteristics of the studies included for pooled proportion analysis. References[25, 37-56]

Supplementary Table 2: Clinical characteristics of the studies included for pooled proportion analysis. References[25, 37-56]

Supplementary Table 3: Outcome of the HCPR vs clopidogrel responders. References[39, 45, 47, 50-53, 55]

Supplementary Figure 1: Subgroup analyses on the prevalence of HCPR according to ethnicity. References[25, 37-56]. ID (identification); ES (effect size); CI (confidence interval).

Supplementary Figure 2: Subgroup analyses on the prevalence of HCPR according to carotid artery stenting. References[25, 37-56]. ID (identification); ES (effect size); CI (confidence interval); IS (ischaemic stroke); CAS (carotid artery stenting).

Supplementary Figure 3: Subgroup analyses on the prevalence of HCPR according to test. References[25, 37-56]. ID (identification); ES (effect size); CI (confidence interval); LTA (light transmission aggregometry); VASP (vasodilator-stimulated phosphoprotein); TEG (thromboelastography); MEA (multiple-electrode impedance aggregometry).

Supplementary Figure 4: Subgroup analyses on the prevalence of HCPR according to LTA test different cut-off points. References [25, 41, 43, 44, 55]. ID (identification); ES (effect size); CI (confidence interval); LTA (light transmission aggregometry); platelet aggregation rate <30% or <10% are cut-off points defining HCPR on light transmission aggregation.

Abstract

Objectives: To assess the prevalence of high on-clopidogrel platelet reactivity (HCPR) in patients with ischaemic stroke or transient ischaemic attack (IS/TIA), their outcome and genetic basis of on-treatment response variability in IS/TIA patients.

Methods: We conducted a comprehensive search of PubMed and EMBASE from their inceptions to March 9, 2019. Studies that reported absolute numbers/percentages of HCRP at any time point after IS/TIA onset evaluated with any type of platelet function tests, clinical outcomes and genotyping data were included.

Results: Among 21 studies of 4312 IS/TIA patients treated with clopidogrel, the pooled prevalence of HCPR was 28% (95%CI: 24-32%; high heterogeneity: I^2=88.2%, p<0.001). Heterogeneity degree diminished across groups defined by the HCPR testing method. Clopidogrel non-responder IS/TIA patients had poorer outcome compared to responders (RR=2.09, 95%CI: 1.61–2.70; p=0.036; low heterogeneity across studies: I^2=27.4%, p=0.210). IS/TIA carriers of *CYP2C19*2* or *CYP2C19*3* loss of function alleles had a higher risk of HCPR compared to wild type (RR=1.69, 95%CI: 1.47–1.95; p<0.001; I^2=0.01%, p=0.475).

Conclusions: This systematic review shows a high prevalence of clopidogrel resistance in IS/TIA and poor outcome in these patients. *CYP2C19* polymorphisms may potentially influence clopidogrel resistance.

Introduction

Excessive platelet activation plays a major role in the pathophysiology of ischaemic stroke[1-12]. Clopidogrel has been shown to be superior to aspirin in platelet inhibition and reducing the risk of ischaemic stroke[13]. It is metabolized by cytochrome P450 and the active metabolite irreversibly binds to platelet surface receptor P2Y12 inhibiting adenosine diphosphate induced platelet activation[14]. However, the antiplatelet response to clopidogrel is highly variable[15]. The reported prevalence of clopidogrel resistance, also termed "high on-clopidogrel platelet reactivity (HCPR)", ranges from 16% to 65%[16-19]. This wide variation in clopidogrel resistance prevalence is attributed to the profile of the studied population[20] and a lack of consensus on threshold values to define HCPR using different assays which include for example, VerifyNow P2Y12, light transmission aggregometry (LTA), multiple-electrode impedance aggregometry (MEA), vasodilator-stimulated phosphoprotein (VASP), thromboelastography (TEG) and flow cytometry. Causes for decreased platelet inhibition by clopidogrel are multifactorial and include genetic, cellular and co-morbid clinical factors[21-24].

Studies of ischaemic stroke patients with clopidogrel resistance have shown an association with early neurological deterioration and recurrent ischaemic episode with poor recovery[25]. Similarly, patients displaying HCPR have been shown to be at higher risk of thromboembolic events during and after carotid revascularisation[26].

In this article, we undertook a systematic review and meta-analysis of the prevalence of HCPR in patients with ischaemic stroke or transient ischaemic attack (IS/TIA), their outcome and the genetic basis of on-treatment response variability in IS/TIA patients.

Methods

This meta-analysis is presented according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[27] for systematic reviews and meta-analyses. We searched PUBMED and EMBASE for publications from inception up to March 9, 2019, and used the search terms (Clopidogrel*/ resistance*) OR (high*/ OR therapy* OR treatment* OR therapeutics.mp OR therapeutics*/ blood platelets OR blood*/ platelets* OR blood platelets* OR platelet*/ reactivity*) AND (stroke OR stroke*) OR (ischemic attack, transient OR ischemic*/ attack*/ transient* OR transient ischemic attack* OR transient*/ischemic*/attack*). We further performed a search of the Cochrane library, and ClinicalTrials.gov, and a manual search of references from all identified publications.

Two authors (VA, XH) identified studies eligible for further review by performing an initial screen of identified titles or abstracts. We restricted studies to those including patients with ischaemic stroke or TIA on Clopidogrel; those with coronary artery disease were excluded. Studies were considered for inclusion in the meta-analysis if they reported absolute numbers/percentages of HCPR at any time point after ischaemic stroke or TIA onset evaluated with any type of platelet function test, any type of study design with or without reported clinical outcomes or genotyping data. Any disagreement was reviewed by a third reviewer (ACP) and resolved by consensus. Initial screening revealed 33 potential studies and full-text article assessment excluded studies on the same cohort. Twenty-one studies were included for meta-analysis (Figure 1).

*CYP2C19*2* and *CYP2C19*3* alleles that result in impaired metabolism of CYP2C19 substrates were entitled as loss-of-function alleles[28]. Patients with at least 1 loss-of-function alleles

(hetero- or homozygous for *CYP2C19*2* or *CYP2C19*3*) were classified as loss-of-function allele carriers. Of twenty-one studies included for meta-analysis, eight studies provided data on *CYP2C19* loss of function allele carrier status in IS/TIA patients and clinical outcomes (Figure 4). Of eight studies, only four analysed platelet resistance and clinical outcome in *CYP2C19* loss of function allele carriers, and therefore this was not included in Figure 4 (Supplementary Table 1).

The primary end point was HCRP pooled proportion and outcome in clopidogrel-treated IS/TIA. The secondary endpoint was the association between *CYP2C19*2* and *CYP2C19*3* loss of function allele carrier status and HCPR in IS/TIA. Statistical analyses were performed using STATA software (version 15.0, Stata Corporation, College Station, TX). Pooled prevalence of HCPR in IS/TIA cohort across studies was derived. Pooled risk ratios (RR) and 95% confidence intervals (CIs) were calculated as the overall measure of efficacy of clopidogrel response using random-effects models. Two-sided probability values of <0.05 were considered statistically significant. Each analysis was accompanied by the assessment of the corresponding heterogeneity evaluated by the I^2 statistic; the Cochrane Q ($\chi 2$) statistic assessed heterogeneity between studies. Potential publication bias of studies with different sample sizes was examined by visual inspection of funnel plots and trim-and-fill analysis. The guidelines from https://uk.cochrane.org/news/meta-analysis-what-why-and-how were followed.

Results

Our search identified 21 potentially relevant studies with a total of 4312 ischaemic stroke and/or TIA patients on Clopidogrel. Study sizes ranged from 62 to 465 stroke or TIA patients.

Characteristics of the studies are summarised on the Supplementary Table 2. In the overall analysis of all included studies, the pooled prevalence of HCPR was 28% (95%CI: 24–32%). However, the prevalence reported between studies presented great variability as demonstrated by substantial heterogeneity (I^2 = 88.2%, Cochran Q p<0.001) (Figure 2).

The main finding is the significant disparity in many aspects across the studies not only in outcome measure, but also in the patients included, their demographics, the dose of Clopidogrel, the timing of the tests, the laboratory methods used, the definition of HCPR, and so on. In order to explain the heterogeneity, we did several analyses by grouping studies according to factors such as ethnicity (Supplementary Figure 1 and Supplementary Table 2), and laboratory methods assessing HCPR (Supplementary Table 1 and Supplementary Figure 3). Supplementary Figure 2 refers to subgroup analysis on the prevalence of HCPR according to use carotid artery stenting.

Heterogeneity only reduced amongst studies using multiple-electrode impedance aggregometry (MEA), thromboelastography (TEG) and vasodilator-stimulated phosphoprotein (VASP) methods (Table 1); and improved with analysis of studies using light transmission aggregometry (LTA) testing with similar cut-off points defining HCPR (Supplementary Table 1 and Supplementary Figure 4).

In the analysis of eight studies (total of 1887 IS/TIA patients on clopidogrel) providing data on outcome including recurrent stroke or other vascular events, increased modified Rankin Scale (mRS) or National Institutes of Health Stroke Scale (NIHSS) and death, IS/TIA patients with HCPR had poorer outcome compared to clopidogrel responders (RR = 2.09, 1.61–2.70, p=0.036) (Figure 3 and Supplementary Table 3).

From the analysis of eight studies providing data on genotyping, IS/TIA carriers of *CYP2C19* loss of function allele (*2 or *3) had a higher risk for HCPR (RR=1.69, 95%CI: 1.47–1.95; $p<0.001$; $I^2=0.01\%$, $p=0.475$) (Figure 4).

Discussion

The present report is to our knowledge the first meta-analysis that determines the prevalence of HCPR in IS/TIA patients and shows a positive association between the presence of HCPR and poor outcome including recurrent stroke or other vascular events, stroke progression or death. This finding is consistent with previously published systematic reviews and meta-analyses that reported an increased risk of cardiovascular events in patients with HCPR[16]. Meta-analyses in patients with acute coronary syndrome[29] who underwent percutaneous coronary intervention and stenting had a prevalence of HCPR of 21%, with a pooled OR of cardiovascular events of 8.0, which is similar to our finding. However, a peripheral vascular disease[30] meta-analysis reported a prevalence of HCPR of 65%, which is much higher than our result.

There is significant heterogeneity evident across the studies. In particular, the laboratory methods for testing clopidogrel resistance and the definition of HCPR varied from study to study. Currently, multiple laboratory and point of care platelet function testing are used across the world. A recent review[31] comparing existing platelet function tests has emphasised that non-standardised use of these tests and the lack of a proper definition is at least partly responsible for the disparity of the prevalence reported in studies. In one guideline[32] that attempted to standardise the definition of HCPR, the author argued that cut-off values to define HCPR are better determined by the individual laboratory, rather than providing an

arbitrary value generated from previous studies. That report also recommended that multiple assessments of the patients should be done in the same laboratory if possible, to provide meaningful interpretation. The same group[33] suggested additional clinical information and genotyping besides a platelet function test may be a better prediction of the risk of recurrent thromboembolic events.

In all the included studies, there were significant differences in clinical factors such as ethnicity, age, and co-morbidities, which probably have contributed to the heterogeneity of the analysis. In subgroup analysis for Asian/Non-Asian, IS/TIA plus or minus carotid artery stent, this heterogeneity did not dissipate. However, the subgroup analysis of laboratory methods did show much less heterogeneity, but the number of studies in each group was small so the results must be interpreted with caution.

A similar pattern of disparity was observed in analysis of the genetic studies. We nevertheless found that a significant proportion of IS/TIA patients with HCPR were *CYP2C19* loss-function allele carriers. Previous studies showed that among patients with ischemic stroke or TIA treated with clopidogrel, carriers of *CYP2C19* loss-of-function alleles are at increased risk of new stroke and composite vascular events in comparison with noncarriers, whereas bleeding risk is similar[34]. Similarly, the metanalysis[35] of acute coronary syndrome (ACS) patients who were *CYP2C19* loss-of-function carriers, found them to have an increased risk of myocardial infarct (MI), stent occlusion and ischaemic stroke, which supports the conclusion that *CYP2C19* has an important role in clopidogrel metabolism. However, not all patients with HCPR develop recurrent vascular events. The factors relating to this may not rest solely on pharmacokinetic aspects of clopidogrel metabolism but may also involve other genetic variation[36]. On the present evidence, *CYP2C19* genotyping may be a useful addition to the

individualised risk assessment to predict whether patients on clopidogrel are more at risk of recurrent vascular events and merit treatment with an alternative antiplatelet agent. However, further research is needed to assess the applicability of *CYP2C19* genotyping on a routine basis.

Our study has some limitations. First, none of the studies included in the meta-analysis was a randomised study. Second, medications including proton pump inhibitors intake data among studies was scanty and therefore was not included to the meta-analysis. Third, platelet resistance and clinical outcome was not analysed in *CYP2C19* loss of function allele carriers due to limited data among studies.

Clopidogrel resistance has been described for more than a decade, but the quality of published studies is so variable and heterogeneous that firmer conclusions from this meta-analysis cannot be drawn. However, patients with HCRP need evidence based guidance on how to approach their management. In order to determine the true potential benefit of testing for HCPR in the clinical setting, a randomised multicentre study with a single HCPR definition and centralised laboratory testing is warranted.

Abbreviations

ACS	acute coronary syndrome
CR	clopidogrel responders
CI	confidence intervals
ES	effect size
HCPR	high on clopidogrel platelet reactivity
IS	ischaemic stroke

LoF	loss of function
LTA	light transmission aggregometry
MEA	multiple-electrode impedance aggregometry
MI	myocardial infarction
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RR	risk ratios
TEG	thromboelastography
TIA	transient ischaemic attack
VASP	vasodilator-stimulated phosphoprotein

Declarations

1. ETHICS GUIDELINES: not applicable

2. CONSENT FOR PUBLICATION: not applicable

3. AVAILABILITY OF DATA AND MATERIAL: on request

4. COMPETING INTERESTS: none

5. FUNDING: none

6. AUTHORS' CONTRIBUTIONS: ACP and MM conceived the study. ICS oversaw the statistical methodology, analyses plan and crude data interpretation. VA and XH contributed to data acquisition. VA, XH, ICS and SD contributed to data quality assurance and data quality analysis. ACP, VA and XH contributed to data interpretation. VA drafted the initial manuscript and all remaining authors critically revised the manuscript. All authors gave final approval for publication.

7. ACKNOWLEDGEMENTS: We would like to thank Suzanne Drury for her help and comments on this project.

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Figure legends

Figure 1: Flow chart diagram presenting the selection procedure of eligible studies.

Figure 2: Pooled prevalence of all studies: Heterogeneity chi-squared = 169.69 (d.f. = 20), *p*<0.001; I-squared (variation in ES attributable to heterogeneity) = 8788.2%; Estimate of between-study variance Tau-squared = 0.0069; Test of ES=0 : z= 14.22; *p*<0.001. References[25, 37-56]. ID (identification); ES (effect size;) CI, confidence interval.

Figure 3: Overall analysis of all studies providing data on the outcome between non-responders and responders to clopidogrel. References[25, 38, 45-48, 50, 55]. ID, identification; RR (relative risk); CI, confidence interval.

Figure 4**:** HPCR related to *CYP2C19* loss of function**:** Heterogeneity chi-squared = 6.57(d.f. =7) *p* = 0.475; I-squared (variation in ES attributable to heterogeneity) = 0.01%; Estimate of between-study variance Tau-squared = 0.0000; Test of RR=1 : z= 7.32; *p*<0.001. References[39, 45, 47, 50-53, 55]. ID (identification); RR (relative risk); CI (confidence interval).

Table legends

Table 1. Subgroup analyses on the prevalence of HCPR reported in included studies. References[25, 37-56]

Supplementary data

Supplementary Table 1: Laboratory characteristics of the studies included for pooled proportion analysis. References[25, 37-56]

Supplementary Table 2: Clinical characteristics of the studies included for pooled proportion analysis. References[25, 37-56]

Supplementary Table 3: Outcome of the HCPR vs clopidogrel responders. References[39, 45, 47, 50-53, 55]

Supplementary Figure 1: Subgroup analyses on the prevalence of HCPR according to ethnicity. References[25, 37-56]. ID (identification); ES (effect size); CI (confidence interval).

Supplementary Figure 2: Subgroup analyses on the prevalence of HCPR according to carotid artery stenting. References[25, 37-56]. ID (identification); ES (effect size); CI (confidence interval); IS (ischaemic stroke); CAS (carotid artery stenting).

Supplementary Figure 3: Subgroup analyses on the prevalence of HCPR according to test. References[25, 37-56]. ID (identification); ES (effect size); CI (confidence interval); LTA (light transmission aggregometry); VASP (vasodilator-stimulated phosphoprotein); TEG (thromboelastography); MEA (multiple-electrode impedance aggregometry).

Supplementary Figure 4: Subgroup analyses on the prevalence of HCPR according to LTA test different cut-off points. References [25, 41, 43, 44, 55]. ID (identification); ES (effect size); CI (confidence interval); LTA (light transmission aggregometry); platelet aggregation rate <30% or <10% are cut-off points defining HCPR on light transmission aggregation.

Figure 1

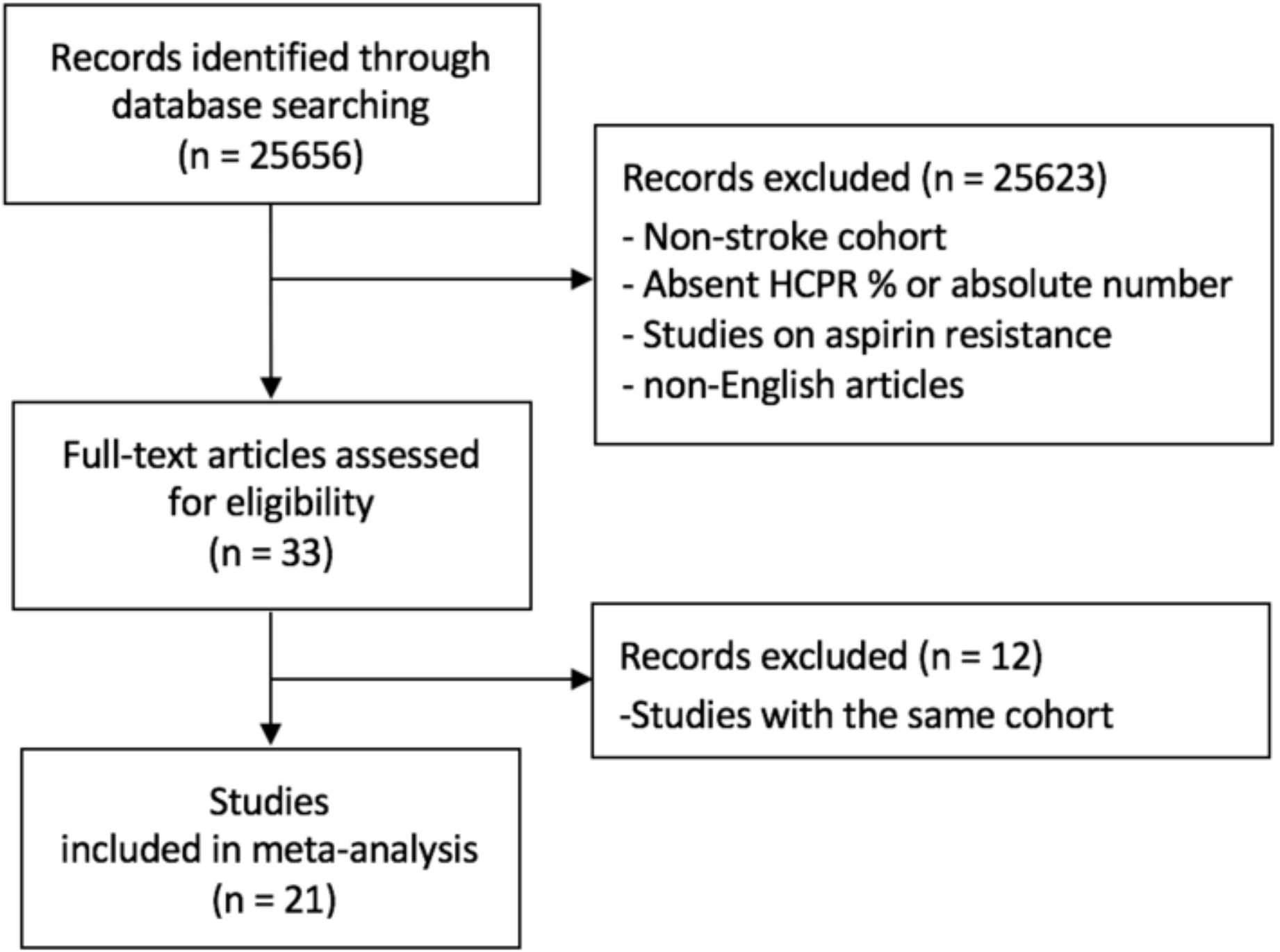

Figure 2

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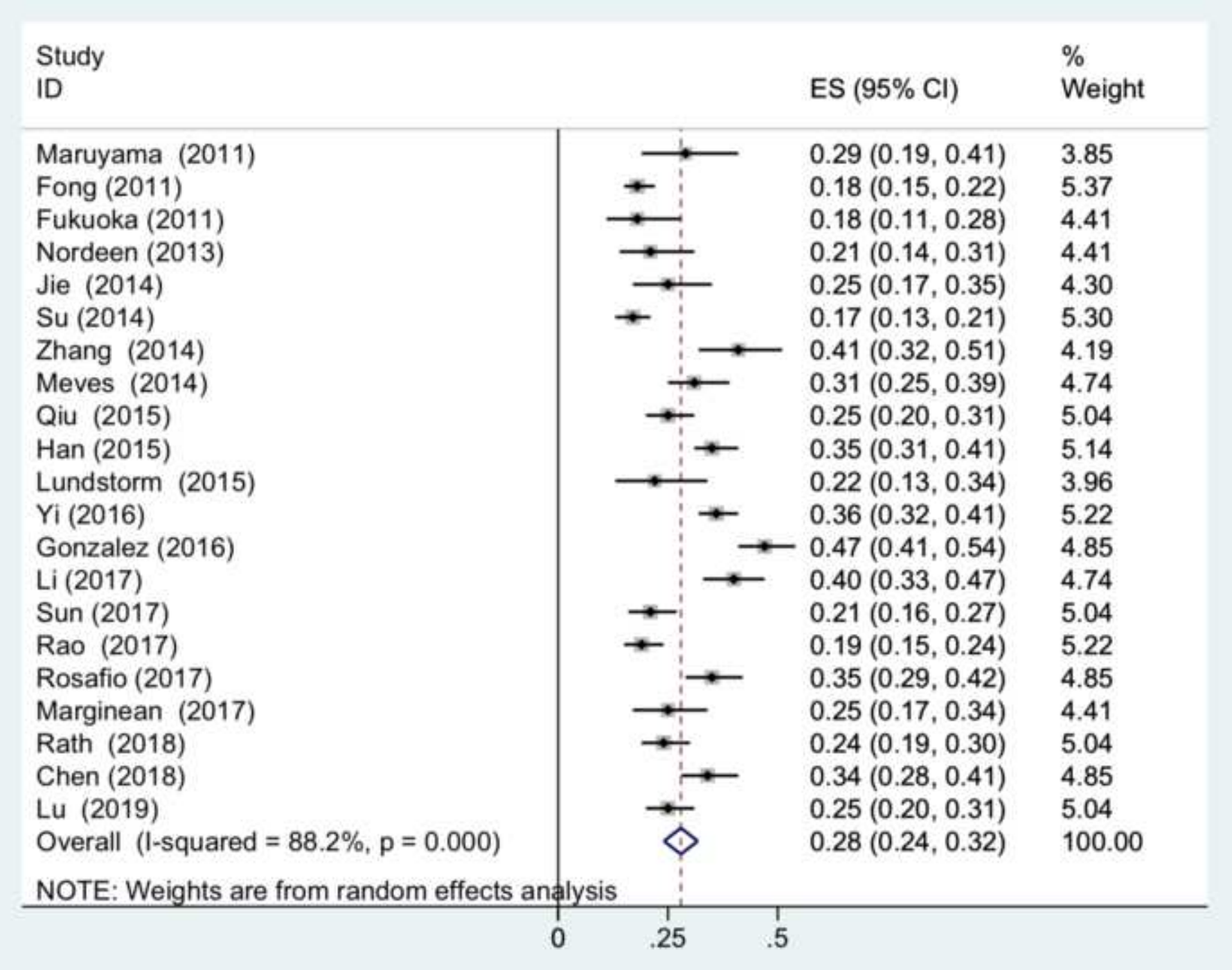

Figure 3

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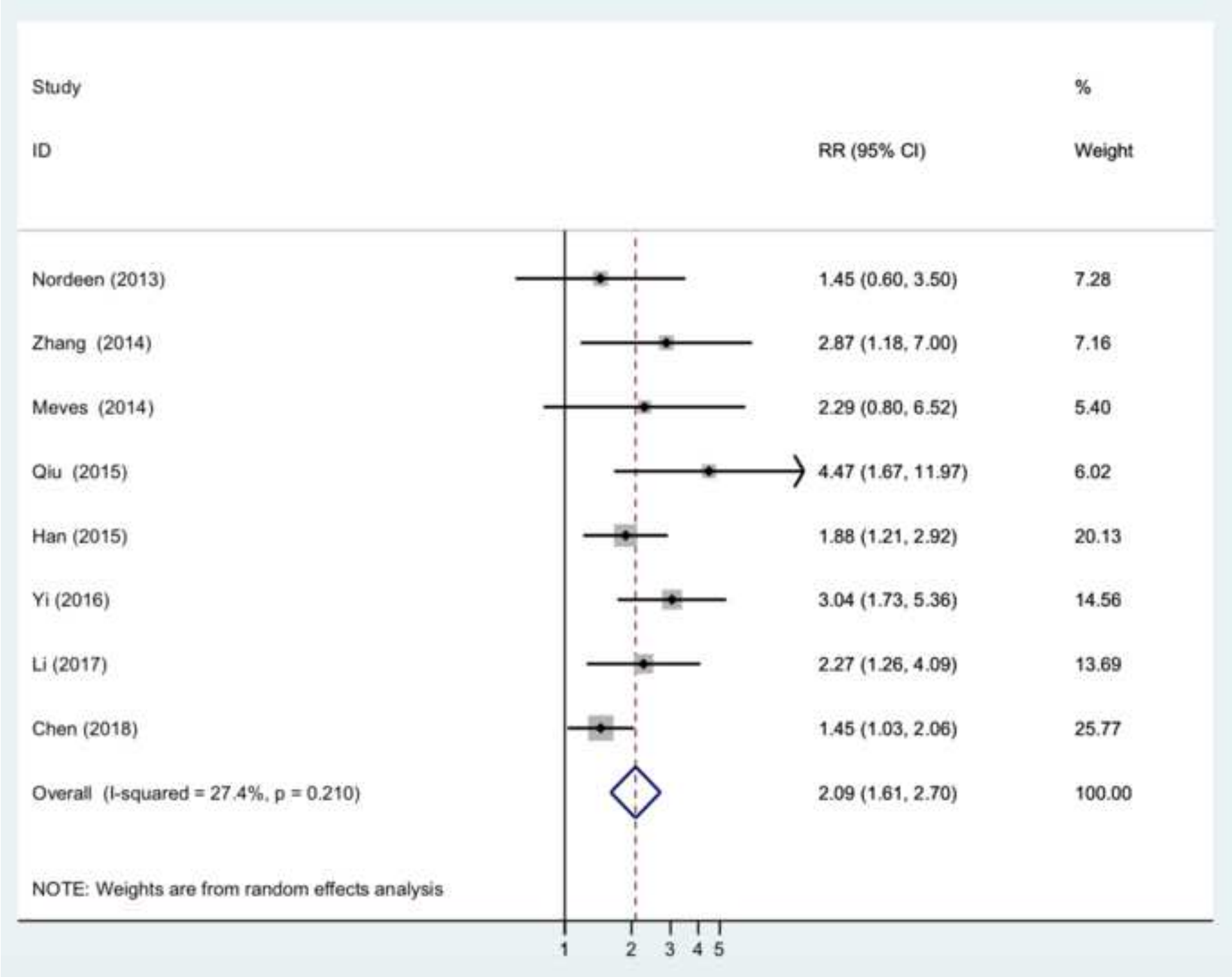

Figure 4

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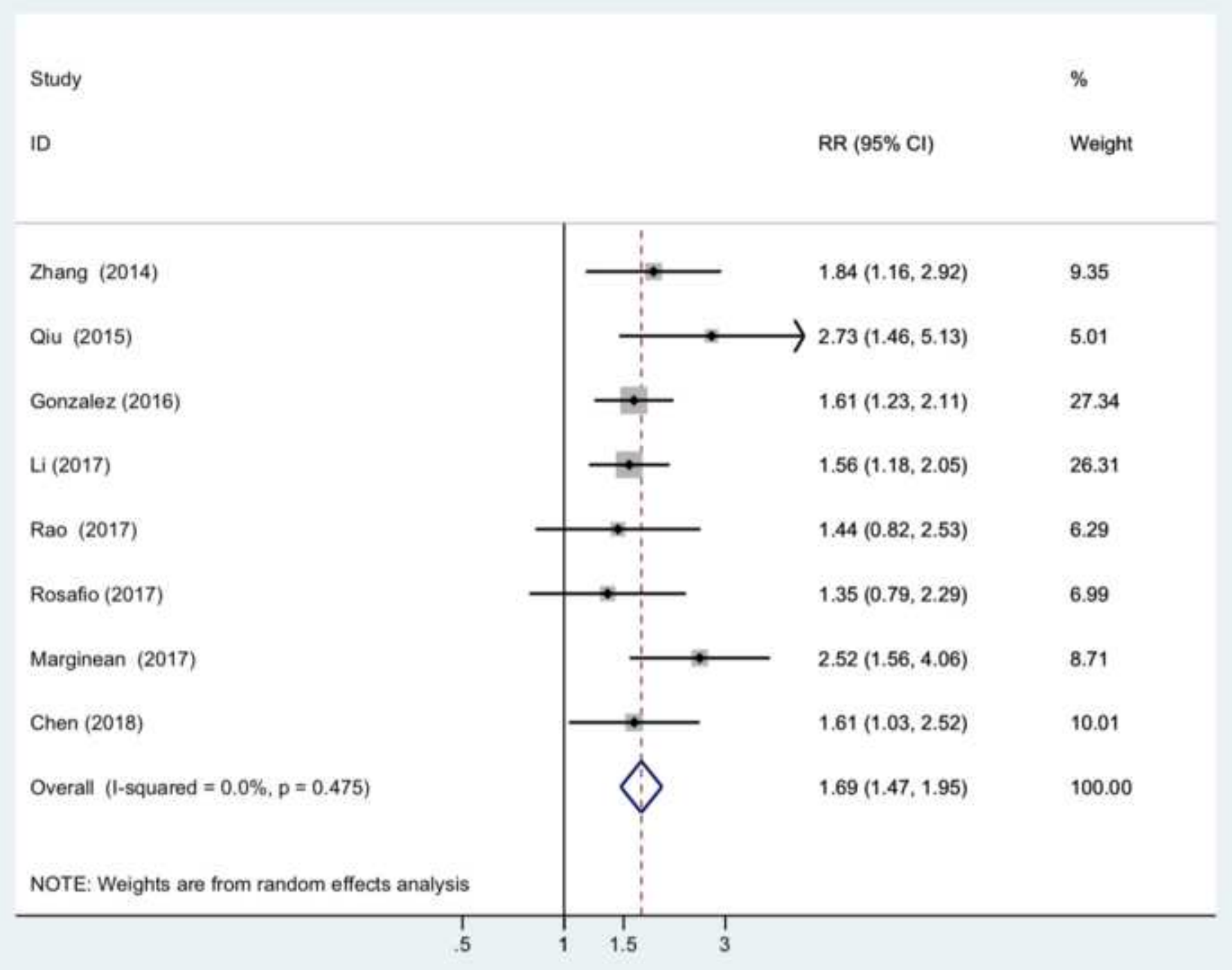

Supplementary Figure 1

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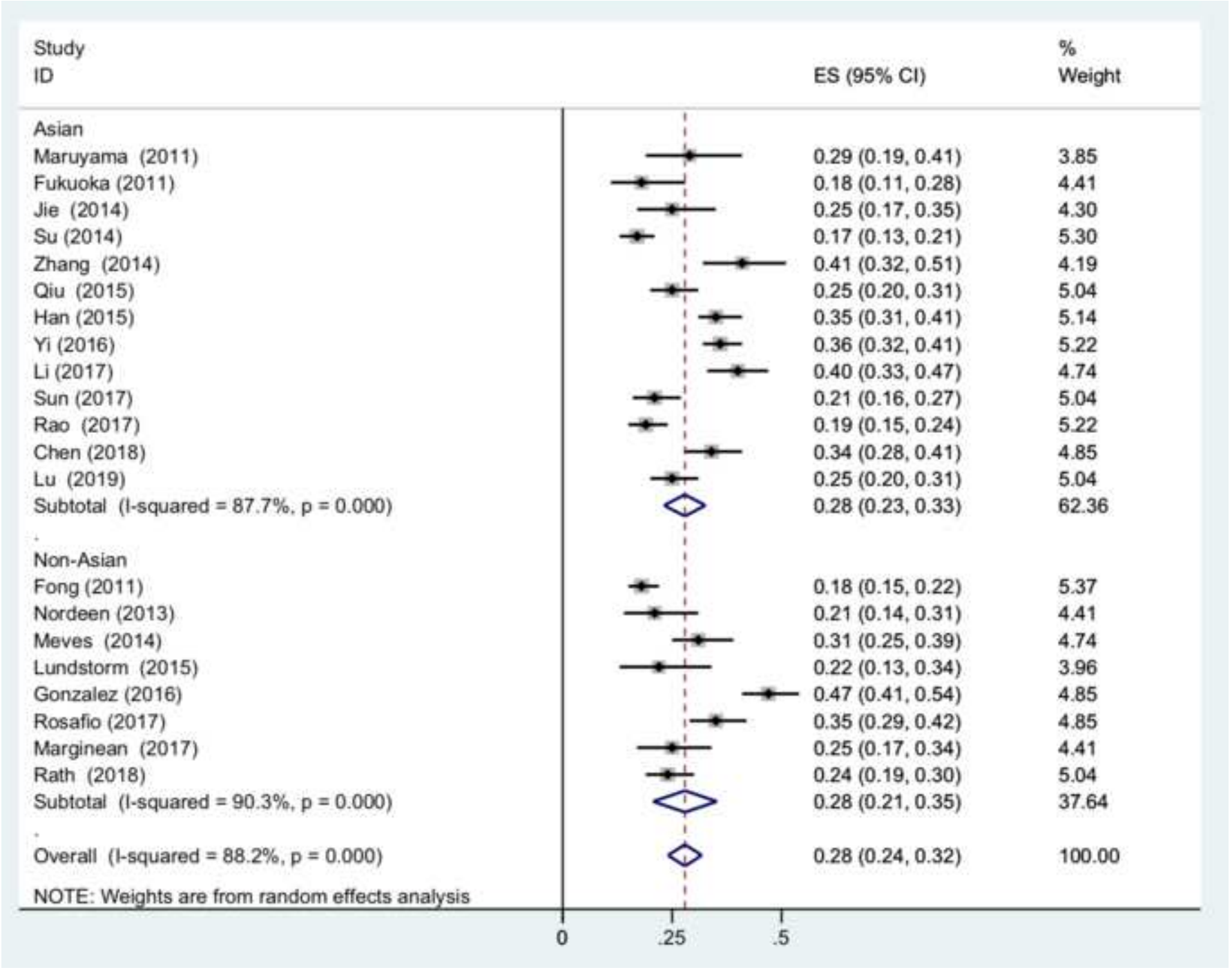

Supplementary Figure 2

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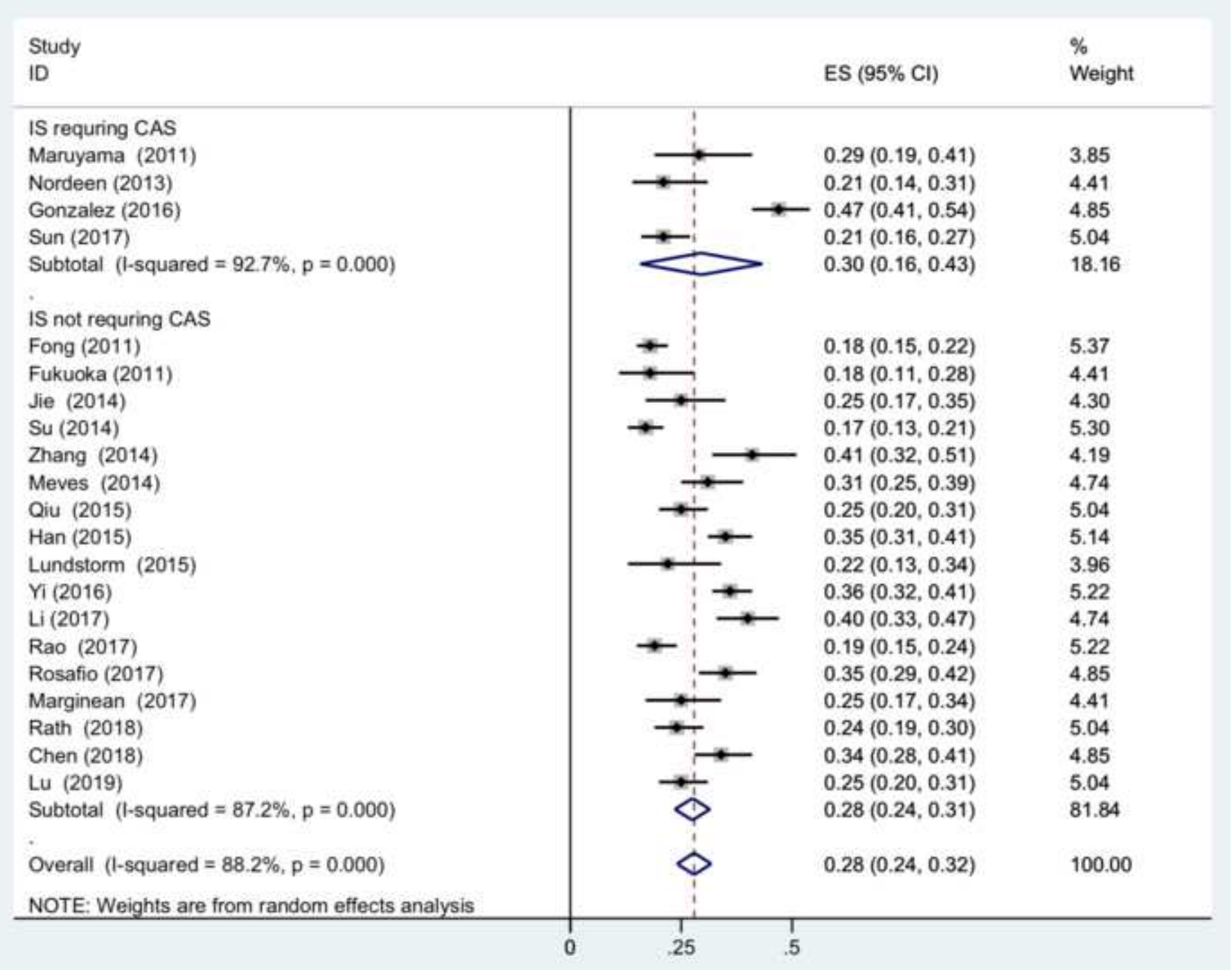

Supplementary Figure 3

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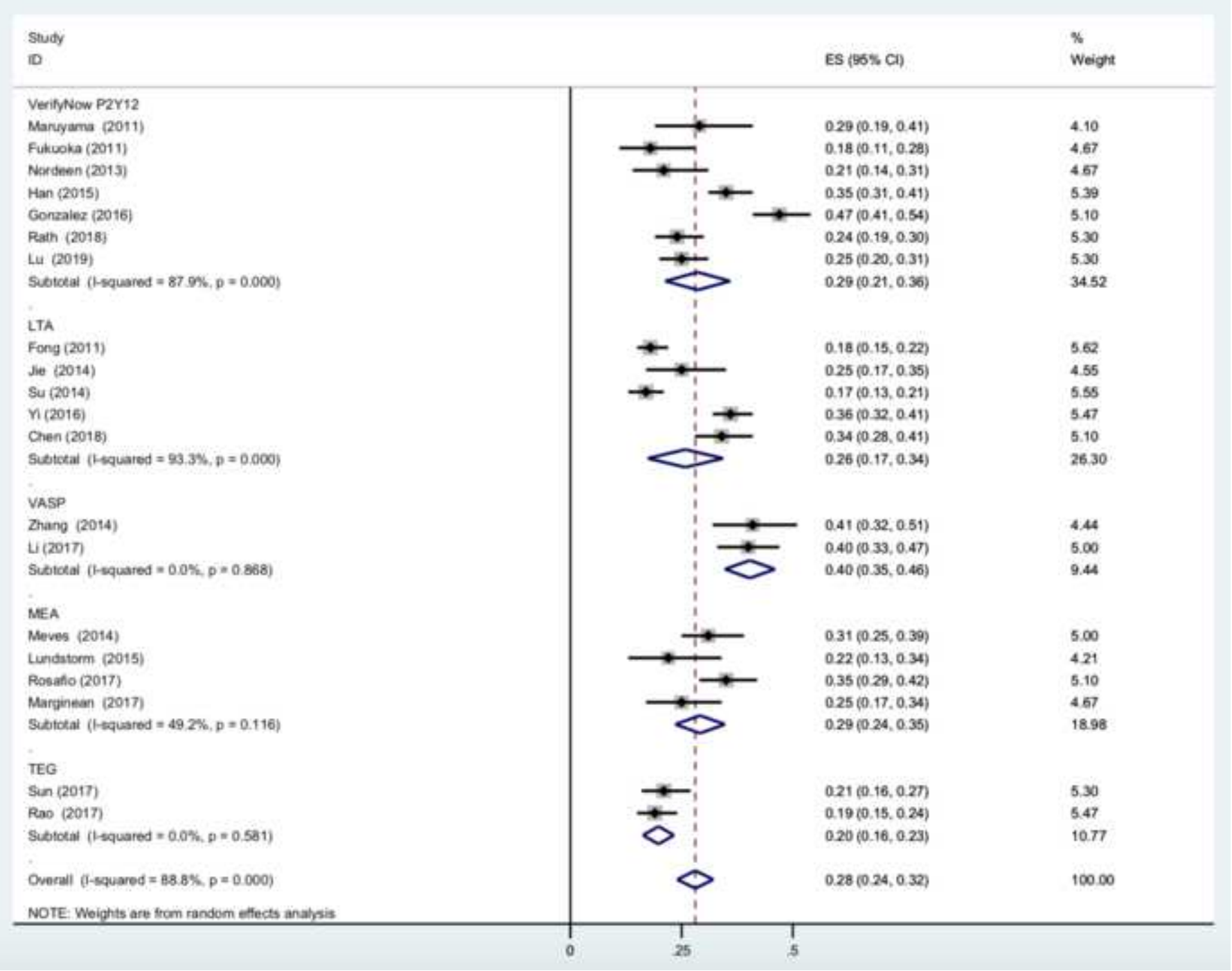

Supplementary Figure 4

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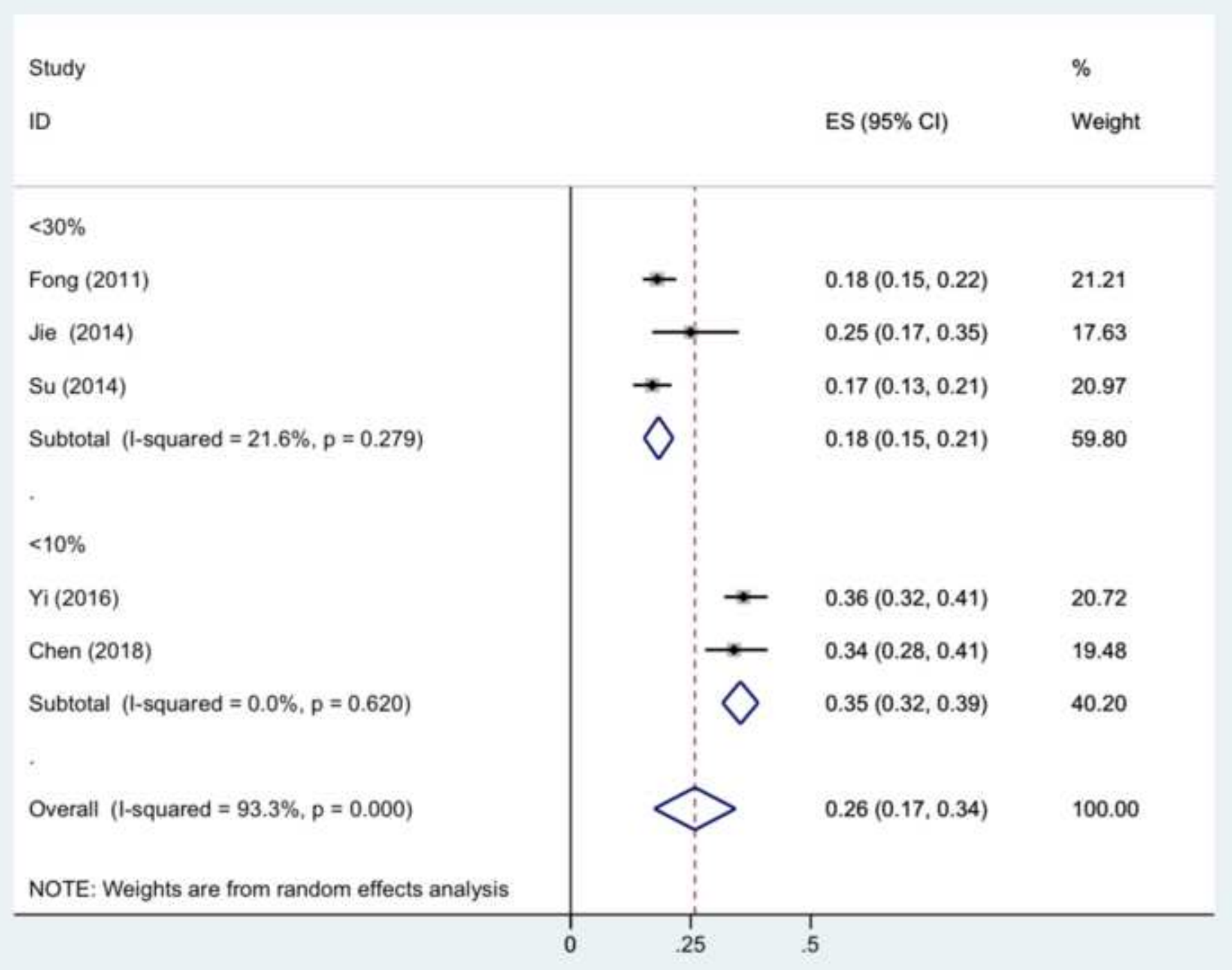

1 Table 1. Subgroup analyses on the prevalence of HCPR reported in included studies.

Subgroup analysis	Prevalence (95%Cl)	I2, Cochran Q
<u>According to ethnicity</u>		
Asian	0.28 (0.23-0.33)	87.7%, p<0.0001
Non-Asian	0.28 (0.21-0.35)	90.3%, p<0.0001
<u>According to stroke type</u>		
IS/TIA with CAS	0.30 (0.16-0.43)	92.7%, *p*<0.0001
IS/TIA without CAS	0.28 (0.24-0.31)	87.2%, *p*<0.0001
<u>According to the method</u>		
VerifyNow System	0.29 (0.21-0.36)	87.9%, *p*<0.0001
LTA	0.26 (0.17-0.34)	93.3%, *p*<0.0001
VASP	0.40 (0.35-0.46)	0.01%, *p*=0.868
MEA	0.29 (0.24-0.35)	49.2%, *p*=0.116
TEG	0.20 (0.16-0.23)	0.01%, *p*=0.581
HCPR, high on clopidogrel platelet reactivity; LTA, light transmission aggregometry; MEA, multiple-electrode impedance aggregometry; TEG, thromboelastography; VASP, vasodilator-stimulated phosphoprotein; IS, ischaemic stroke; TIA, transient ischaemic attack; NICS, non-cardiogenic ischaemic stroke; CAS, carotid artery stenting;		

2

3

Supplementary Table 1. Laboratory characteristics of the studies included for pooled proportion analysis.

Study ID	**HCPR/ cohort N**	**Assay**	**Clopidogrel intake & test interval**	**Cut off**	***CYP2C19* LoF, HCPR/CR, N**	***CYP2C19* HCPR/CR, N**
Maruyama (2011)	18/62	VerifyNow	>7 days	<20%	NA	NA
Fong (2011)	83/465	LTA	NA	<40%	NA	NA
Fukuoka (2011)	13/72	VerifyNow	>7 days	66%	NA	NA
Nordeen (2013)	17/160	VerifyNow	NA	<20%	NA	NA
Jie (2014)	22/87	LTA	>5 days	<35%	NA	NA
Su (2014)	51/303	LTA	>7 days	<30%	NA	NA
Zhang (2014)	39/95	VASP	>7 days	>50%	10/5	29/51
Meves (2014)	50/159	MEA	7 days	> 47	NA	NA
Qiu (2015)	53/211	Flow cytometry	7 days	>28.54	43/86	10/72
Han (2015)	122/345	VerifyNow	5-7 days	≥230	76/124	0/136
Lunsdorm (2015)	16/72	MEA	30 days	<468	NA	NA
Yi (2016)	153/426	LTA	>7 days	<10 %	NA	NA
Gonzalez (2016)	99/209	VerifyNow	>7 days	≥230	35/18	64/92
Li (2017)	78/196	VASP	7 days	< 60%	67/89	65/171
Sun (2017)	46/221	TEG	3-5 days	<30%	NA	NA
Rao (2017)	53/278	TEG	7 days	< 30%	31/115	15/87
Rosafio (2017)	74/209	MEA	7-10 days	<46U	13/21	27/68
Marginean (2017)	25/101	MEA	5 days	>43	7/2	25/56
Rath (2018)	63/219	VerifyNow	8-24 hours	>208	NA	NA
Chen (2018)	65/192	LTA	5-7 days	<10%	43/65	20/61
Lu (2019)	57/230	VerifyNow	7-14 days	>50%	NA	NA

CR, clopidogrel responders; HCPR, high on clopidogrel platelet reactivity; LTA, light transmission aggregometry; MEA, multiple-electrode impedance aggregometry; TEG,

thromboelastography; VASP, vasodilator-stimulated phosphoprotein; LoF, loss of function that is *CYP2C19*2* or **3* alleles.

Supplementary Table 2. Clinical characteristics of the studies included for pooled proportion analysis.

Study ID	Age (SD)	Female%	DM %	Smoking%	Patients	Country
Maruyama (2011)	65.3 (9.9)	32	27	48	IS/CAS	Japan
Fong (2011)	65.6 (13.6)	53	35	NA	IS	US
Fukuoka (2011)	69 (8.0)	28	NA	NA	IS/TIA	Japan
Nordeen (2013)	61 (14.3)	65	41	NA	IS/TIA/NV	US
Jie (2014)	62.9 (8.0)	36	18	18	IS	China
Su (2014)	63.65 (9.6)	23	55	13	IS	China
Zhang (2014)	64.8 (11.3)	40	21	31	NCIS	China
Meves (2014)	72.2 (8.8)	30	40	12	AIS	Germany
Qiu (2015)	66.7 (11.5)	47	36	38	AIS	China
Han (2015)	68.1 (11.5)	32	39	NA	AIS	China
Lunsdorm (2015)	70 (66-77)	56	31	NA	IS/TIA	Sweden
Yi (2016)	69.9 (12.2)	35	52	62	Minor AIS	China
Gonzalez (2016)	67.2 (9.6)	17	47	41	IS/CAS	Spain
Li (2017)	63.67 (11)	29	33	39	NCIS	China
Sun (2017)	59 (8.0)	18	32	62	IS/TIA/CAS	China
Rao (2017)	57.9 (9.5)	26	38	41	Minor IS/TIA	China
Rosafio (2017)	68.6 (13.9)	36	24	31	ASA	Italy
Marginean (2017)	65.6 (11.1)	81	25	28	NCIS	Romania
Rath (2018)	72.8 (10.9)	46	18	16	IS/TIA	Denmark
Chen (2018)	67.0 (13.1)	42	31	26	IS	China
Lu (2019)	68.5 (7.2)	47	58	30	IS	China

IS, ischaemic stroke; TIA, transient ischaemic attack; NICS, non-cardiogenic ischaemic stroke; CAS, carotid artery stenting; NV, neuro-intervention; SD, standard deviation; N, number; NA, no available information; DM, diabetes mellitus; AIS, acute ischaemic stroke.

Supplementary Table 3. Outcome of the HCPR vs clopidogrel responders

Study ID	**HCPR/ clopidogrel responders**	**Poor/good outcome in HCPR**	**Poor/good outcome in clopidogrel responders**	**Clinical outcome measure**	**Follow-up months, drop outs**
Nordeen (2013)	17/64	5/12	13/51	Stroke/ICH recurrence, death	3 months
Zhang (2014)	39/56	12/27	6/50	Increase in NIHSS score ≥2, stroke recurrence or occurrence of other ischaemic vascular events	6 months
Meves (2014)	70/89	9/61	5/84	Stroke/ICH recurrence	Hospital stay
Qiu (2015)	53/158	9/44	6/152	Stroke recurrence, nonfatal MI and CVD death, mRS<2 vs mRS>2	6 months
Han (2015)	90/181	29/61	31/150	Stroke/ICH recurrence	12 months
Yi (2016)	153/273	29/124	17/256	Stroke recurrence, MI, death	3 months
Li (2017)	77/118	22/55	15/104	Increase of NIHSS score ≥ 2, vascular events	6 months
Chen (2018)	65/127	32/33	43/84	>2 mRS, recurrent vascular event, death	12 months

HCPR, high on clopidogrel platelet reactivity; ICH, intracranial hemorrhage; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; MI, myocardial infarction; CVD, cardiovascular disease.