Meta-analyses of the association between cytochrome CYP2C19 loss- and gain-of-function polymorphisms and cardiovascular outcomes in patients with coronary artery disease treated with clopidogrel

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ABSTRACT

Aims To perform a meta-analysis of the association between *CYP2C19* loss- and gain-of-function variants and cardiovascular outcomes and bleeding in patients with coronary artery disease treated with clopidogrel, and to explore the causes of heterogeneity between studies.

Methods A comprehensive literature search was conducted. A random-effects model was used to summarise the results. In the presence of between-study heterogeneity, a meta-regression analysis was performed to identify study characteristics explaining this heterogeneity.

Results Patients who carried a loss-of-function allele, mainly *CYP2C19*2*, did not present an increased risk of a cardiovascular event, HR =1.23 (95% CI 0.97 to 1.55). Substantial heterogeneity was observed between studies (I^2 =35.6), which was partially explained by the study sample size: the pooled HR was higher among studies with a sample size <500 patients (HR =3.55; 95% CI 1.66 to 7.56) and lower among studies with a sample size ≥500 (HR =1.06; 95% CI 0.89 to 1.26). *CYP2C19*2* was associated with an increased risk of a stent thrombosis (HR =2.24; 95% CI 1.52 to 3.30). The gain-of-function allele, mainly *CYP2C19**17, was associated with a lower risk of cardiovascular events (HR =0.75; 95% CI 0.66 to 0.87) and a higher risk of major bleeding (HR =1.26; 95% CI 1.05 to 1.50).

Conclusions Not only *CYP2C19* loss-of-function but also gain-of-function alleles should be considered to define the pharmacogenetic response to clopidogrel. The results question the relevance of the *CYP2C19* loss-of-function alleles in the prediction of major cardiovascular events beyond stent thrombosis in coronary patients treated with clopidogrel. The gain-of-function variant is associated with a lower risk of cardiovascular events but a higher risk of bleeding.

INTRODUCTION

Dual antiplatelet therapy with aspirin and clopidogrel is the standard of care following acute coronary syndrome and percutaneous coronary intervention (PCI) to prevent recurrent ischaemic events and to improve other clinical outcomes.[1–3] However, even with the use of such therapy, a substantial number of subsequent ischaemic events still occur in these groups of patients.[1] A possible explanation is the documented interindividual variability in response to clopidogrel,[4] which results in increased risk of ischaemic events among those patients with attenuated response to this drug.[5]

The mechanisms leading to a poor response to clopidogrel are not clearly understood, although it has been suggested that clinical, cellular or genetic factors may be involved.[6] The prodrug, clopidogrel, is converted into an active metabolite, which selectively and irreversibly binds to the P2Y12 receptor on the platelet membrane.[7–9] Conversion is achieved by the hepatic cytochrome P450 (CYP) system in a two-step oxidative process, and CYP2C19 is involved in both of these steps. In recent years, several studies have reported a series of loss-of-function alleles in *CYP2C19*, in particular the *CYP2C19*2* allele, that result in reduced activation of clopidogrel,[10 11] a lower antiplatelet effect,[12–14] and increased risk of cardiovascular events in patients receiving clopidogrel. Recently, four meta-analyses[15–18] have reported a significant association between the *CYP2C19*2* allele and adverse major cardiovascular clinical outcomes and stent thrombosis in patients with coronary artery disease (CAD) treated with clopidogrel. However, most of these meta-analyses report significant heterogeneity between studies, although the causes of this heterogeneity have not yet been established. Moreover, none of these meta-analyses has explored the role of *CYP2C19* gain-of-function variants in major cardiovascular clinical outcomes and the risk of bleeding.

The aims of this study were to: (i) perform an updated systematic review and a meta-analysis of previous studies that analysed the association between *CYP2C19* loss- and gain-of-function variants and cardiovascular outcomes and major bleeding in patients with CAD treated with clopidogrel; (ii) explore the potential causes of heterogeneity between studies.

MATERIALS AND METHODS

Study selection

A comprehensive search of the PubMed and ISI Web of Science databases from its inception through October 2010 was conducted to identify studies that evaluated the relationship between

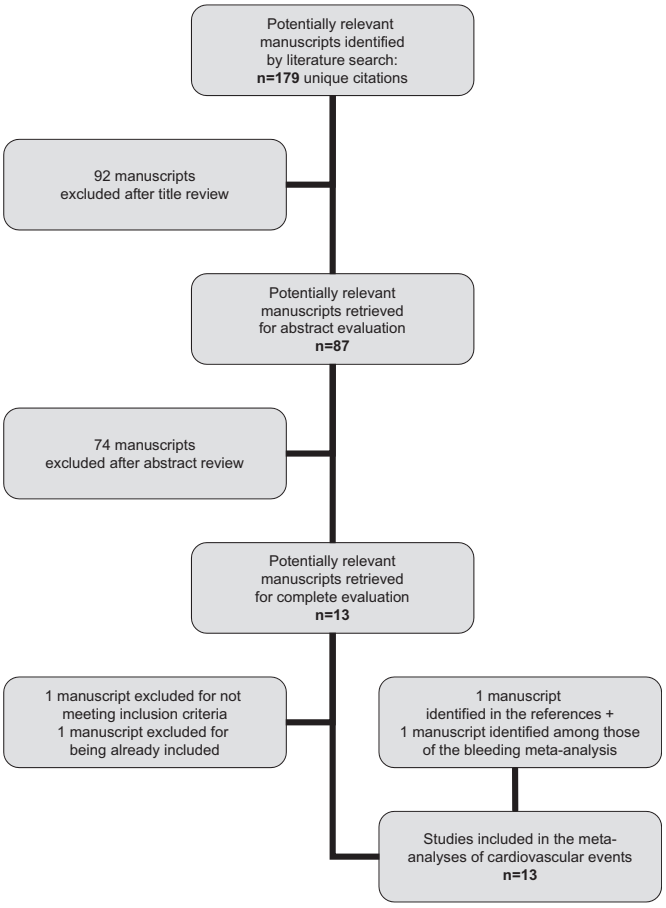

Figure 1 Flow diagram of the systematic review process.

genetic variants in *CYP2C19* and cardiovascular events and bleeding. For cardiovascular events, we queried MeSH terms and the article text for the following search terms: ('clopidogrel') AND ('angioplasty', 'revascularisation', 'outcome', 'cardiovascular events', 'stent thrombosis', 'recurrence', 'acute coronary syndrome', 'percutaneous intervention') AND ('genetics', 'genotype', 'gene', 'snp', 'polymorphism'). For bleeding events, we substituted the list of clinical events for 'bleeding' and we kept the rest of the terms previously mentioned.

The articles returned by this search were manually screened, first on the basis of the title, then the abstract and finally the complete manuscript, to assess their appropriateness for inclusion in the meta-analysis. References cited in these articles were also reviewed to identify additional published articles not identified by the database search.

Case reports, abstracts, editorials and review articles were excluded. The search strategy was limited to articles published in English.

The meta-analysis included genetic association studies that fulfilled the following criteria: (i) the study analysed genotype data for the *CYP2C19* loss- or gain-of-function polymorphisms; (ii) study participants were patients with CAD receiving clopidogrel treatment; (iii) the study used a case–control or prospective design; (iv) the study evaluated clinical outcomes; and (v) the study reported data on the size and variance of the risk effect.

Data extraction

Two investigators (MZ and RE) independently extracted data. The data extracted included information about the study design characteristics, the outcomes assessed, the cohort characteristics, genotype, allele frequencies and genetic model used in the analysis, and the reported results. The following design and cohort characteristics were collected: number of patients, mean age and mean body mass index of the participants, proportion of women, proportion of patients with diabetes, race, clinical inclusion criteria, clopidogrel dosages, primary outcomes of the study, genotype distribution, time of follow-up, estimates of associations, and adjustment for confounding factors. Disagreements were resolved through consensus.

The quality of the reported information included in each article was assessed following the STREGA statement[19] and summarised with the approach used in other previous studies.[20]

Genetic variants

The following *CYP2C19* genetic variants were included in the meta-analysis:

- Loss-of-function: rs4244285 (*2), a 618 G→A substitution; rs4986893 (*3), a 4903T→C substitution; rs28399504 (*4), a 5001A→G substitution; rs56337013 (*5), a 1297C→T substitution
- Gain-of-function: rs12248560 (*17), a 4195C→T/A substitution.

Outcomes of interest

For the current meta-analysis, we defined three outcomes of interest.

(a) Major adverse cardiovascular events. These were defined as any cardiovascular event (fatal and non-fatal myocardial infarction, stroke, unstable angina), recurrent ischaemia (symptoms compatible with ischaemia needing hospital readmission and coronariography), or death from other cardiovascular causes during follow-up.

(b) Definite or probable stent thrombosis. Definite stent thrombosis was defined as acute coronary syndrome and either angiographic or pathological confirmation of thrombosis. Probable stent thrombosis was defined as unexplained death or myocardial infarction in the territory supplied by a stented vessel without angiographic confirmation.

(c) Major bleeding. Defined in most of the studies according to the TIMI criteria (intracranial haemorrhage or clinically significant overt signs of haemorrhage associated with a fall in haemoglobin of >5 g/dl (or, when haemoglobin is not available, an absolute fall in packed cell volume of >15%)).

Statistical analysis

Owing to the low frequency of the minor alleles analysed in this study at the population level, most of the studies have defined a genetic dominant model. We also defined a genetic dominant model for this meta-analysis, in which the carriers of the rare allele were compared with those homozygous for the wild-type allele.

Statistical analysis for the meta-analyses was performed using the *meta. DSL* function from the R package *rmeta*. We calculated the overall HR, 95% CI and two-sided p value under a random-effects model (RE; DerSimonian and Laird).[21] We tested for heterogeneity of effects between studies by computing the I^2 statistic. In the presence of between-study heterogeneity, a meta-regression analysis was performed under a mixed-effects model[22] to establish whether any of the following study characteristics could account for this heterogeneity: study design (observational or clinical trial), clinical outcomes included in the study (acute coronary syndrome or PCI), sample size (<500 patients or ≥500 patients), duration of follow-up (<1 year or ≥1 year), type of patients according to risk (low- and high-risk according to the incidence of cardiovascular outcomes <7% or

Table 1 Characteristics of studies included in the meta-analyses

Author	Year	Country	Sample size	Mean age (years)	Male (%)	Ethnicity	Disease	Genetic variant	Outcome			Follow-up	Covariate adjustment	STREGA	
														n/N	%
Trenk *et al*[23]	2008	Germany	797	66	78	–	Elective PCI	CYPC219*2	MACE: death, MI (n=24)			1 year	Baseline and procedural variables.	26/36	72.2
Malek *et al*[35]	2008	Poland	105	60	70	–	ACS + PCI	CYPC219*2	MACE: death, MI (n=6)			1 year	None	19/38	50.0
Shuldiner *et al*[24]	2009	USA	95	64	60	62% Caucasic; 37% African-American	Elective PCI	CYPC219*2	MACE: MI, revascularisation, death, stroke, hospitalisation for ischaemia (n=20)	Stent thrombosis (n=24)		1 year	Age, gender, race	29/35	82.9
Giusti *et al*[25]	2009	Italy	772	–	75	–	Elective PCI: 34%; ACS+PCI: 66%	CYPC219*2	Stent thrombosis, death (n=29)	Stent thrombosis (n=24)		6 months	Residual platelet reactivity, CV risk factors, procedural variables	25/37	67.6
Sibbing *et al*[26]	2009	Germany	2485	67	78	–	Elective PCI	CYPC219*2	MACE: MI, stroke, death (n=173)	Stent thrombosis (n=17)		30 days	Age, diabetes, type of stent, ACS, use of abciximab	29/33	87.9
Collet *et al*[27]	2009	France	259	40	92	Most white	Acute MI	CYPC219*2	MACE: MI, revascularisation, cardiovascular death (n=26)	Stent thrombosis (n=12)		>4 years	BMI, smoking, diabetes, stent implantation, STEMI, use of proton-pump inhibitors	31/36	86.1
Mega *et al*[11 28]	2009, 2010	USA	1477	60	71	98% Caucasic	ACS+PCI	CYPC219 loss-of-function (*2-5)	MACE: MI, stroke, cardiovascular death (n=129)	Stent thrombosis (n=20)		15 months	–	33/40	82.5
Simon *et al*[29]	2009	France	2208	68	72	–	Acute MI	CYPC219 loss-of-function (*2-5); CYP2C19 gain-of-function (*17)	MACE: MI, stroke, death (n=294)			1 year	Full propensity score	32/40	80.0
Paré *et al*[30]	2010	Canada	2549	64	59	86% Caucasic; 14% Hispanic	ACS	CYP2C19 loss-of-function (*2,*3); CYP2C19 gain-of-function (*17)	MACE: MI, stroke, cardiovascular death (n=230)		Major bleeding (n=135/3119)	1 year	Age, sex, ancestry, use of stent, smoking, waist, diabetes, hypertension, country of origin	30/40	75.0
Wallentin *et al*[31]	2010	Sweden	4904	63	69	98% Caucasic	ACS	CYPC219 loss-of-function (*2-5); CYP2C19 gain-of-function (*17)	MACE: MI, stroke, cardiovascular death (n=481)	Stent thrombosis (n=56/3284)	Major bleeding (n=375/3833)	1 year	Ethnic, sex, use of PPI, aspirin dose, smoking, diabetes	32/38	84.2
Tiroch *et al*[32]	2010	Germany	928	65	75	–	MI + PCI (90%)	Loss-of- function CYP2C19*2; gain-of-function CYP2C19*17	MACE: MI, revascularisation, death (n=239)			1 year	Age, gender, diabetes, extent of coronary artery disease	26/37	70.3

Continued

Table 1 Continued

Author	Year	Country	Sample size	Mean age (years)	Male (%)	Ethnicity	Disease	Genetic variant	Outcome	Follow-up	Covariate adjustment	STREGA n/N	STREGA %
Harmsze *et al*[33]	2010	Netherlands	596	63	78	–	PCI	CYP2C19 loss-of-function (*2, *3)	Stent thrombosis (n=176 cases and 420 controls)	1 year	Age, gender, BMI, smoking, diabetes, prior MI, use of PPIs, ACS as indication of PCI, procedural variables	26/39	66.7
Sibbing *et al*[36]	2010	Germany	1524	67	23	–	PCI	CYP2C19 gain-of-function (*17)	MACE: MI, revascularisation, death (n=56)	Stent thrombosis (n=14)	Major bleeding (n=12)	30 days	Age, sex, BMI, creatinine, use of PPIs or abciximab, clopidogrel loading interval	23/33	78.8

ACS, acute coronary syndrome; BMI, body mass index; CV, cardiovascular; MACE, major adverse cardiovascular event; MI, myocardial infarction; n/N, number of items from the STREGA recommendations that were clearly reported in the manuscript/number of items from the STREGA recommendations that could have been reported in the article; PCI, percutaneous coronary intervention; PPI, proton-pump inhibitor; STEMI, ST elevation myocardial infarction.

≥7% per year, respectively), adjustment for clinical covariates, type of patients according to the mean or proportion of classical risk factors also associated with prognosis in each study such as diabetes, hypertension, proportion of female patients, mean age and mean body mass index, and the study's score on a reporting quality scale based on the STREGA statement (compliance with <70% or ≥70% of items).

To identify studies that individually had strong effects on the pooled OR, a sensitivity analysis was performed by excluding one study at a time and calculating the OR for the remaining studies.

HRs and OR were assumed to represent the same RR. Where more than one result was given in the original report, we used that obtained under the multivariate model with the most complete adjustment for potential confounders. Where ORs or HRs were not reported, effect size estimates were calculated using genotype frequencies stratified according to the presence of the cardiovascular event of interest.

Funnel plots of effect size against SE were examined to detect the presence of publication bias.

RESULTS

Eligible studies

The process of selection of studies for inclusion in the meta-analysis of major adverse cardiovascular events or stent thrombosis is summarised in figure 1. The database search identified 179 unique citations, of which 87 were judged to be of potential interest on the basis of the title. On the basis of the abstract, 13 studies were reviewed in their entirety.[11 23–34] One was excluded because it did not meet the eligibility criteria[34] and a second one was excluded[28] because the data reported were available in a previous study already included in the meta-analysis.[11] One additional study[35] was identified after review of the references cited, and a second one after review of the manuscripts of the meta-analysis of major bleeding[36] (see below). Finally, 13 studies that met the criteria described above were included in the meta-analysis.

For the meta-analysis of major bleeding, we identified 36 unique citations, of which seven were judged to be of potential interest on the basis of the title. On the basis of the abstract, three articles were reviewed in their entirety,[30 31 36] one of them showing results from two different studies: CURE and ACTIVE A.[30] We also reviewed the previously identified 12 studies. Finally, four studies were included in this meta-analysis.

The main characteristics of these studies are shown in table 1, and the quality of the reported information according to the STREGA criteria is presented in online supplementary table 1.

Meta-analysis

Using data from the studies described above, we performed four distinct meta-analyses according to the presence of loss- or gain-of-function alleles and the different clinical outcomes of interest. We could not perform a meta-analysis of the association between the gain-of-function alleles and stent thrombosis because only two studies analysed this association.[31 36] We were also unable to perform a meta-analysis of the association between the loss-of-function alleles and bleeding because only one study analysed this association.[30]

Association between *CYP2C19* loss-of-function alleles and cardiovascular outcomes

Eleven studies incorporating 16 360 individuals were included in this analysis. Patients that carried a loss-of-function allele, mainly *CYP2C19**2, did not have a statistically significant increased risk of a subsequent cardiovascular event (HR =1.23

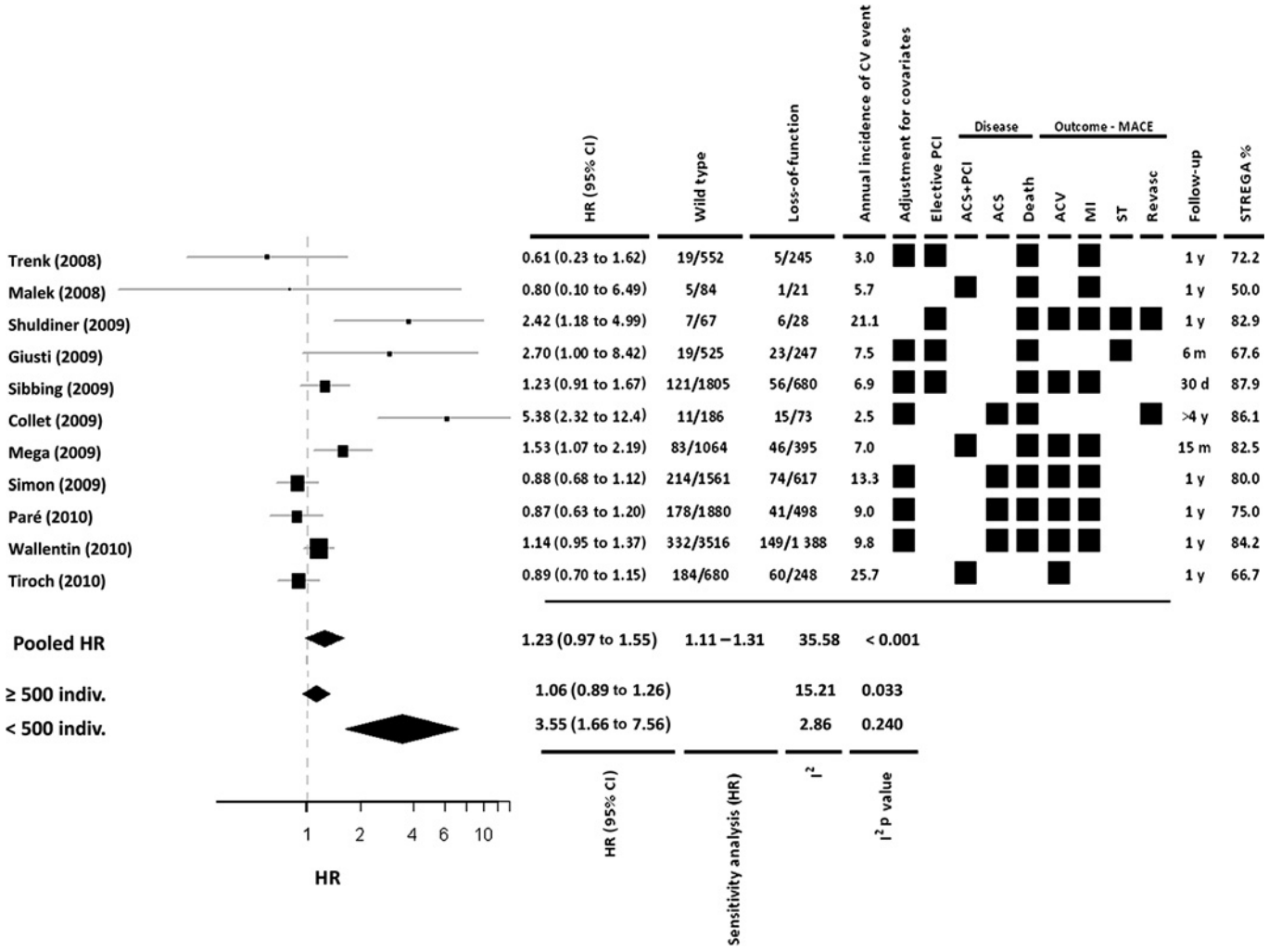

Figure 2 Risk of major cardiovascular adverse events in coronary patients treated with clopidogrel according to the presence of *CYP2C19* loss-of-function alleles and stratified by the sample size of the studies. ACS, acute coronary syndrome; ACV, acute cerebrovascular disease; CV, cardiovascular; MI, myocardial infarction; MACE, major adverse cardiovascular events; PCI, percutaneous coronary intervention; Revasc, revascularisation; ST, stent thrombosis.

(95% CI 0.97 to 1.55)) (figure 2). Sensitivity analysis indicated that no one study significantly affected the results of the meta-analysis (pooled HR ranging from 1.11 to 1.31).

We observed substantial heterogeneity between the effect sizes observed in each study (I^2=35.6; p<0.001). When we analyse the potential causes of this heterogeneity, only study sample size partially explained the observed heterogeneity. Therefore, we stratified the meta-analysis by sample size (N <500 or ≥500), and observed an increase in the pooled HR of a cardiovascular event among studies with a sample size <500 patients (HR =3.55; 95% CI 1.66 to 7.56), and loss of the previously observed association among studies with a sample size ≥500 (HR =1.06; 95% CI 0.89 to 1.26). Significant heterogeneity persisted among the larger studies (I^2=15.2; p=0.033) (figure 2), but not among the smaller studies (I^2=2.9; p=0.240) (figure 2).

The funnel plot of effect size versus SE constructed to investigate possible publication bias was slightly asymmetric, suggesting the presence of some statistical outliers among the studies included (online supplementary figure).

Association between *CYP2C19* loss-of-function alleles and stent thrombosis

Seven studies incorporating 8686 individuals were included in this analysis. Patients who carried a loss-of-function allele, mainly *CYP2C19*2*, had an increased risk of a stent thrombosis (HR =2.24; 95% CI 1.52 to 3.30; p<0.001) (figure 3). We observed no significant heterogeneity between the reported effect sizes (I^2=8.8; p=0.184), and none of the studies had an individually large effect on the result of the meta-analysis (sensitivity analysis pooled HR =1.94−2.49).

Association between *CYP2C19* gain-of-function alleles and cardiovascular outcomes

Four studies incorporating 6584 individuals were included in this analysis. Patients who carried a gain-of-function allele, mainly *CYP2C19*17*, had a lower risk of clinical cardiovascular outcomes (HR =0.75; 95% CI 0.66 to 0.87; p<0.001) (figure 4). We observed no significant heterogeneity between the reported effect sizes (I^2=1.16; p=0.763), and none of the studies had an individually large effect on the result of the meta-analysis (sensitivity analysis pooled HR =0.74−0.78).

Association between *CYP2C19* gain-of-function alleles and major bleeding

Four studies incorporating 7660 patients were included in the analysis. Patients who carried a gain-of-function allele had a higher risk of major bleeding (HR =1.26; 95% CI 1.05 to 1.50; p=0.011) (figure 5). No significant heterogeneity between

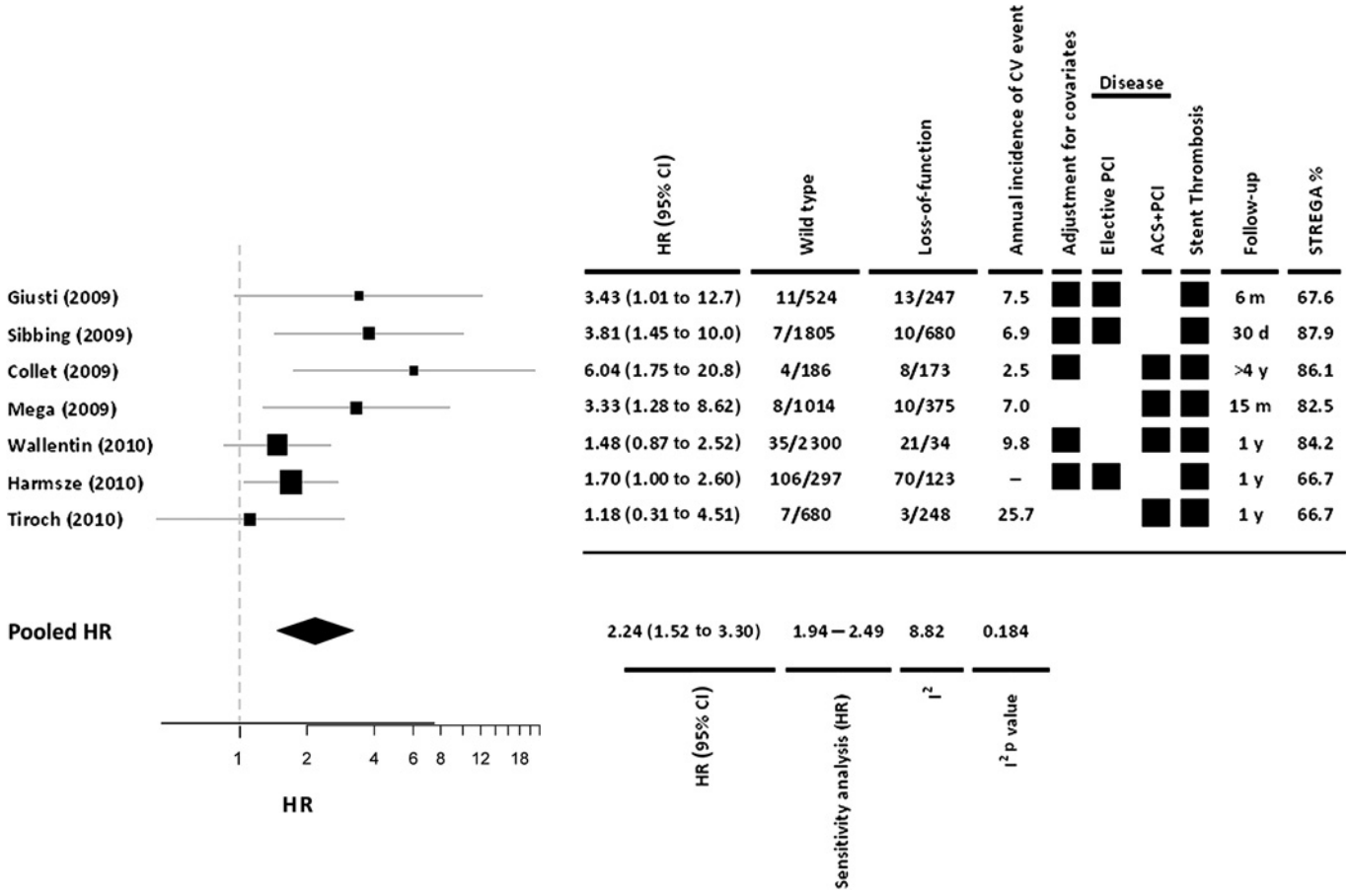

Figure 3 Risk of stent thrombosis in coronary patients treated with clopidogrel according to the presence of *CYP2C19* loss-of-function alleles. ACS, acute coronary syndrome; CV, cardiovascular; PCI, percutaneous coronary intervention.

studies was observed (I^2=0.8; p=0.849), and none of the studies had an individually large effect on the result of the meta-analysis (sensitivity analysis pooled HR ranged from 1.24 to 1.29).

DISCUSSION

In this study, we evaluated previous evidence regarding the association between *CYP2C19* gain- and loss-of-function polymorphisms and cardiovascular outcomes and major bleeding in patients with CAD taking clopidogrel. We observed high heterogeneity between studies analysing the relationship between *CYP2C19* loss-of-function alleles and major cardiovascular outcomes. This heterogeneity was partially related to study sample size: smaller studies reported a significant association between the loss-of-function alleles and a higher risk of cardiovascular outcomes, whereas no significant effect was observed in the pooled analysis of studies with a sample size >500 patients. However, these loss-of-function alleles were associated with a higher risk of stent thrombosis. Moreover, the presence of a *CYP2C19* gain-of-function allele was associated with a lower risk of major cardiovascular recurrences and a higher risk of major bleeding.

In March 2010, the US Food and Drug Administration (FDA) added a 'boxed warning' to the label of clopidogrel including a reference to patients who do not effectively metabolise the drug and therefore may not receive the full benefits on the basis of their genetic characteristics,[37] and more recently the American College of Cardiology Foundation and the American Heart Association have published a consensus document addressing this FDA warning.[38] This guideline states that the role of genetic testing and the clinical implications and consequences of this testing remains to be determined.

In this study and consistent with the previous meta-analyses, we observed an association between the *CYP2C19* loss-of-function alleles and a higher stent thrombosis risk.[15–17] In the previously published meta-analyses, an association between the *CYP2C19* loss-of-function polymorphisms and a higher risk of major cardiovascular events was reported.[15–18] In our analyses we included data from four recently published studies, and when analysing all the available evidence, we did not observe an association between these alleles and cardiovascular recurrences. We conclude that a significant part of the effect size reported in previous studies is driven by the higher effect size observed in small sample size studies. Similar findings were observed in the meta-analysis performed by Hulot *et al*.[17] In their study, the effect size of the association between the loss-of-function alleles and cardiovascular outcomes was significantly higher in studies with a sample size <1000 patients (OR =2.16; 95% CI 1.46 to 3.21) than in the studies with a sample size ≥1000 (OR =1.20; 95% CI 1.03 to 1.40). In our study, we selected a different cut point a priori to analyse the effect of small studies (n <500) to obtain a real estimation of the influence of such studies. We also used the cut point defined by Hulot *et al*[17] and observed similar results to those reported in that study, although the cut point did not explain the heterogeneity observed among studies. The overestimation of the effect size in small genetic association studies is well known and could be related to different factors such as spurious association due to biased publication of positive results in these types of studies.[39]

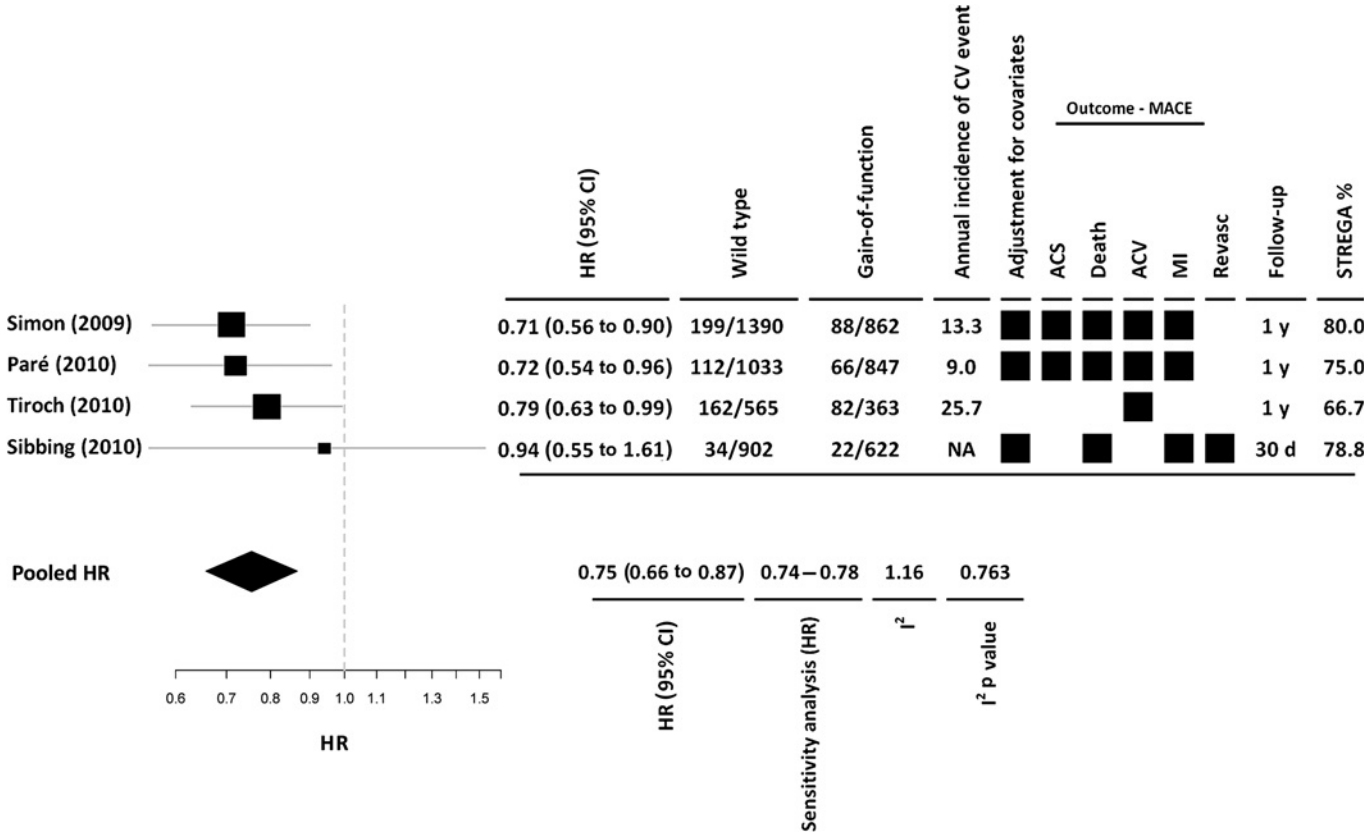

Figure 4 Risk of major cardiovascular adverse events in coronary patients treated with clopidogrel according to the presence of a *CYP2C19* gain-of-function allele. ACS, acute coronary syndrome; ACV, acute cerebrovascular disease; CV, cardiovascular; MACE, major adverse cardiovascular events; MI, myocardial infarction; Revasc, revascularisation.

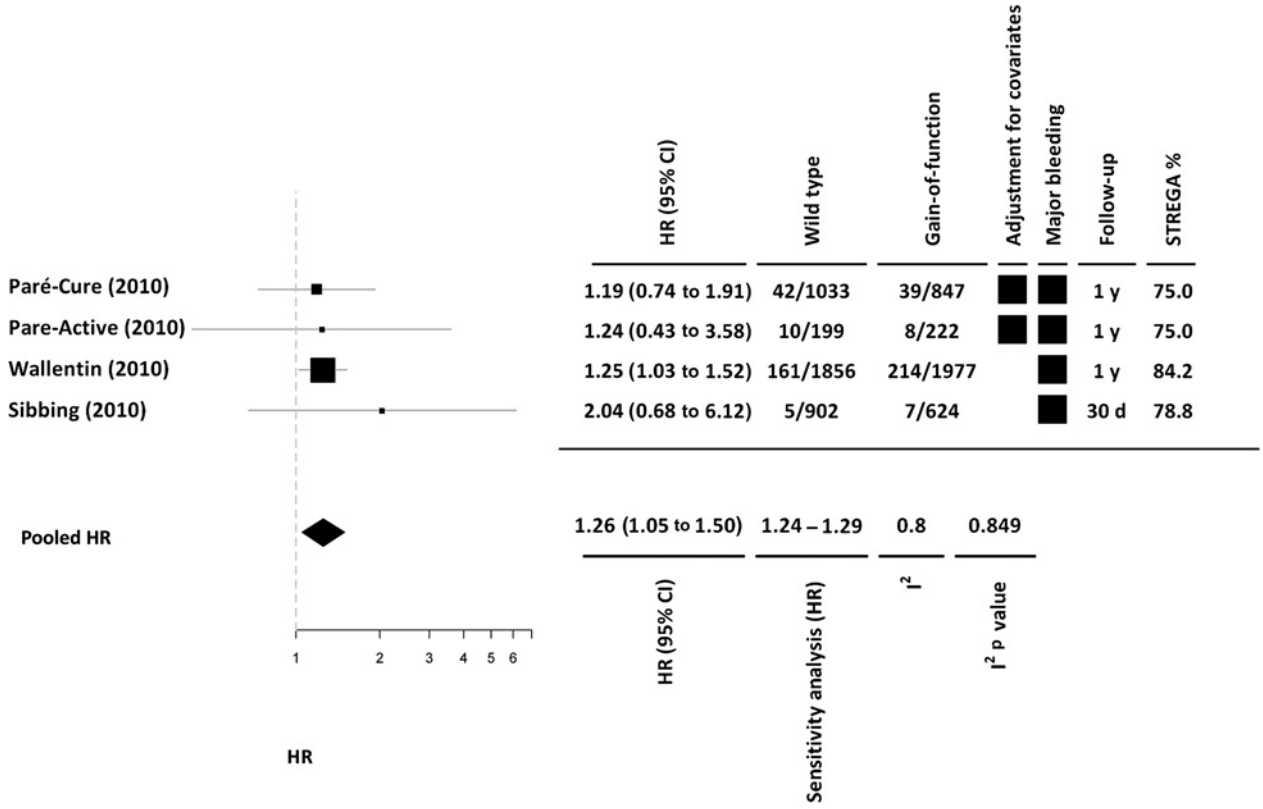

Figure 5 Risk of major bleeding in coronary patients treated with clopidogrel according to the presence of a *CYP2C19* gain-of-function allele.

In some studies the association between the loss-of-function allele and cardiovascular events was higher in those patients treated with PCI and stenting,[29] although not in others.[27] In our meta-analyses, most of the patients with an acute coronary event were also treated with PCI (>70%)[27 29 31] and stenting except in the study of Paré *et al*,[30] in which only 19% were treated with PCI. Therefore, we cannot determine whether the risk of the *CYP2C19* loss-of-function allele is similar in non-stented patients.

To our knowledge, this is the first study that has meta-analysed the effect of a *CYP2C19* gain-of-function allele, *CYP2C19*17*, on cardiovascular recurrence and major bleeding. Although we only included four studies,[29 30 32 36] we observed a clear protective association between this variant and major cardiovascular outcomes. This variant has also been associated with a lower risk of stent thrombosis in one study.[31] However, the results of our meta-analysis indicate that this variant is also associated with a higher risk of major bleeding. Therefore the balance between potential benefits and harms should be considered.

Several clinical trials have evaluated the utility of tailoring antiplatelet therapy according to genotype or platelet reactivity in coronary patients undergoing PCI.[40] The results of the GRAVITAS Study, a clinical trial designed to test if a high-clopidogrel dose strategy for 6 months is superior to the classical dose for the prevention of cardiovascular outcomes after PCI in patients with high residual platelet activity, has shown that the high dose strategy did not reduce the risk of further ischaemic events.[41] In other studies, alternative therapeutic strategies such as triple antiplatelet therapies (adding cilotazol) or other drugs also targeting the P2Y12 receptor such as prasugrel are being tested. However, although the concept of platelet reactivity monitoring of response to thienopyridenes is emerging, a consensus has not yet been reached regarding the threshold of platelet reactivity that should be achieved and the method of assessing platelet reactivity that should be used.[42]

Our study has several limitations, which are also inherent to many meta-analyses. The retrospective nature of our meta-analysis, incorporating data from published studies and not on individual patients, limits the availability of information on some issues, such as different clinical end points, comorbidities or concomitant therapies. We observed substantial heterogeneity between the results reported by different studies, which was partly accounted for by sample size. We attempted to determine if this heterogeneity might also be explained by other variables such as the type of patient (with or without acute coronary syndrome, with or without PCI, etc) included in the different studies, but are unable to provide a reliable answer to this question because we did not have access to individual level data for these variables. Studies including a more homogeneous type of patients are warranted. Finally, in this analysis we were able to consider a dominant genetic model, but could not explore an additive model. However, in the meta-analyses described by Mega *et al*,[18] it was reported that the effect size for major cardiovascular events of the presence of one loss-of-function allele (HR =1.55; 95% CI 1.11 to 1.27) was similar to that observed when two alleles were present (HR =1.76; 95% CI 1.25 to 2.50).

The results of this meta-analysis indicate that not only *CYP2C19* loss-of-function but also gain-of-function alleles should be considered to define the pharmacogenetic response to clopidogrel. The main clinical outcome related to these genetic variants is stent thrombosis. Our results question the relevance of the *CYP2C19* loss-of-function alleles on the prediction of major cardiovascular events and bleeding in coronary patients treated with clopidogrel.

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Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. **Yusuf S,** Zhao F, Mehta SR, *et al*. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001;**345**:494–502. [Erratum published in *N Engl J Med* 2001;**345**:1506].
2. **Sabatine MS,** Cannon CP, Gibson CM, *et al*. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med* 2005;**352**:1179–89.
3. **King SB 3rd,** Smith SC Jr, Hirshfeld JW Jr, *et al*. 2007 Focused Update of the ACC/AHA/SCAI 2005 Guideline Update for Percutaneous Coronary Intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2008;**117**:261–95. [Erratum, *Circulation* 2008;**117**(6):e161].
4. **Wang TH,** Bhatt DL, Topol EJ. Aspirin and clopidogrel resistance: an emerging clinical entity. *Eur Heart J* 2006;**27**:647–54.
5. **Bliden KP,** Dichiara J, Tantry US, *et al*. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J Am Coll Cardiol* 2007;**49**:657–66.
6. **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**:1505–16.
7. **Savi P,** Combalbert J, Gaich C, *et al*. The antiaggregating activity of clopidogrel is due to a metabolic activation by the hepatic cytochrome P450-1A. *Thromb Haemost* 1994;**72**:313–17.
8. **Savi P,** Pereillo JM, Uzabiaga MF, *et al*. Identification and biological activity of the active metabolite of clopidogrel. *Thromb Haemost* 2000;**84**:891–6.
9. **Hollopeter G,** Jantzen HM, Vincent D, *et al*. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* 2001;**409**:202–7.
10. **Kim KA,** Park PW, Hong SJ, *et al*. The effect of CYP2C19 polymorphism on the pharmacokinetics and pharmacodynamics of clopidogrel: a possible mechanism for clopidogrel resistance. *Clin Pharmacol Ther* 2008;**84**:236–42.
11. **Mega JL,** Close SL, Wiviott SD, *et al*. Cytochrome P-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009;**360**:354–62.
12. **Hulot JS,** Bura A, Villard E, *et al*. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006;**108**:2244–7.
13. **Brandt JT,** Close SL, Iturria SJ, *et al*. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J Thromb Haemost* 2007;**5**:2429–36.
14. **Frere C,** Cuisset T, Morange PE, *et al*. Effect of cytochrome p450 polymorphisms on platelet reactivity after treatment with clopidogrel in acute coronary syndrome. *Am J Cardiol* 2008;**101**:1088–93.
15. **Sofi F,** Giusti B, Marcucci R, *et al*. Cytochrome P450 2C19(*)2 polymorphism and cardiovascular recurrences in patients taking clopidogrel: a meta-analysis. *Pharmacogenomics J* 2011;**11**:199–206.
16. **Jin B,** Ni HC, Shen W, *et al*. Cytochorme P450 2C19 polymorphism is associated with poor clinical outcomes in coronary artery disease patients treated with clopidogrel. *Mol Biol Rep* 2011;**38**:1697–702.
17. **Hulot JS,** Collet JP, Silvain J, *et al*. Cardiovascular risk in clopidogrel-treated patients according to cytochrome P450 2C19*2 loss-of-function allele or proton pump inhibitor coadministration: a systematic meta-analysis. *J Am Coll Cardiol* 2010;**56**:134–43.
18. **Mega JL,** Simon T, Collet JP, *et al*. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI. *JAMA* 2010;**304**:1821–30.
19. **Little J,** Higgins JPT, Ionnidis JPA, *et al*. Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE statement. *Ann Intern Med* 2009;**150**:206–15.
20. **Lluís-Ganella C,** Lucas G, Subirana I, *et al*. Qualitative assessment of previous evidence and an updated meta-analysis confirms lack of association between the ESR1 rs2234693 (PvuII) variant and coronary heart disease in men and women. *Atherosclerosis* 2009;**207**:480–6.
21. **DerSimonian R,** Laird N. Meta-analysis in clinical trials. *Controlled Clinical Trials* 1986;**7**:177–88.
22. **Berkey CS,** Hoaglin DC, Mosteller F, *et al*. A random-effects regression model for meta-analysis. *Statistics in Medicine* 1995;**14**:395–411.
23. **Trenk D,** Hochholzer W, Fromm MF, *et al*. Cytochrome P450 2C19 681G_A polymorphism and high on-clopidogrel platelet reactivity associated with adverse

1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or baremetal stents. *J Am Coll Cardiol* 2008;**51**:1925—34.
24. **Shuldiner AR,** O'Connell JR, Bliden KP, *et al*. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA* 2009;**302**:849—57.
25. **Giusti B,** Gori AM, Marcucci R, *et al*. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10 + 12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. *Pharmacogenet Genomics* 2007;**17**:1057—64.
26. **Sibbing D,** Stegherr J, Latz W, *et al*. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J* 2009;**30**:916—22.
27. **Collet JP,** Hulot JS, Pena A, *et al*. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009;**373**:309—17.
28. **Mega JL,** Close SL, Wiviott SD, *et al*. Genetic variants in ABCB1 and CYP2C19 and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON-TIMI 38 trial: a pharmacogenetic analysis. *Lancet* 2010;**376**:1312—19.
29. **Simon T,** Verstuyft C, Mary-Krause M, *et al*. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009;**360**:363—75.
30. **Paré G,** Mehta SR, Yusuf S, *et al*. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. *N Engl J Med* 2010;**363**:1704—14.
31. **Wallentin L,** James S, Storey RF, *et al*. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 2010;**376**:1320—8.
32. **Tiroch KA,** Sibbing D, Koch W, *et al*. Protective effect of the CYPC19 *17 polymorphism with increased activation of clopidogrel on cardiovascular events. *Am Heart J* 2010;**160**:506—12.
33. **Harmsze AM,** van Werkum JW, Ten Berg JM, *et al*. CYP2C19*2 and CYP2C19*3 alleles are associated with stent thrombosis: a case-control study. *Eur Heart J* 2010;**31**:3046—53.
34. **Brackbill ML,** Kidd RS, Abdoo AD, *et al*. Frequency of CYP3A4, CYP3A5, CYP2C9, and CYP2C19 variant alleles in patients receiving clopidogrel that experience repeat acute coronary syndrome. *Heart Vessels* 2009;**24**:73—8.
35. **Malek LA,** Kisiel B, Spiewak M, *et al*. Coexisting polymorphisms of P2Y12 and CYP2C19 genes as a risk factor for persistent platelet activation with clopidogrel. *Circ J* 2008;**72**:1165—9.
36. **Sibbing D,** Koch W, Gebhard D, *et al*. Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation* 2010;**121**:512—18.
37. *FDA Drug Safety Communication: reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug*. http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm190836.htm (accessed 3 Aug 2010).
38. **Holmes DR Jr,** Dehmer GJ, Kauls S, *et al*. ACCF/AHA Clopidogrel Clinical Alert: Approaches to the FDA "Boxed Warning". *Circulation* 2010;**122**:537—57.
39. **Chanock SJ,** Manolio T, Boehnke M, *et al*; NCI-NHGRI Working Group on Replication in Association Studies. Replicating genotype-phenotype associations. *Nature* 2007;**447**:655—60.
40. **Kazui M,** Nishiya Y, Ishizuka T, *et al*. Identification of the human cytochorme P450 enzymes envolved in the two oxidative stops in the bioactivation of clopidogrel to its pharmacologically active metabolite. *Drug Metab Dispos* 2010;**38**:92—9.
41. **Gensch C,** Hoppe U, Böhm M, *et al*. Late-breaking clinical trials presented at the American Heart Association Congress in Chicago 2010. *Clin Res Cardiol* 2011;**100**:1—9.
42. **Bonello L,** de Labriolle A, Scheinowitz M, *et al*. Emergence of the concept of platelet reactivity monitoring of response to thienopyridines. *Heart* 2009;**95**:1214—19.

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