Published in final edited form as:
J Thromb Haemost. 2012 July ; 10(7): 1242–1251. doi:10.1111/j.1538-7836.2012.04756.x.

Influence of the paraoxonase-1 Q192R genetic variant on clopidogrel responsiveness and recurrent cardiovascular events: a systematic review and meta-analysis

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Summary

Background—A poor biological response to clopidogrel is associated with an increased risk of major cardiovascular ischemic events (MACE). Paraoxonase 1 (PON1) enzyme activity is modulated by the PON1-Q192R variant (rs662) and was recently suggested to be strongly involved in clopidogrel bioactivation, but the influence of the PON1-Q192R variant on the risk of MACE in clopidogrel-treated patients is controversial.

Objectives—To determine whether the PON1-Q192R variant influences clopidogrel biological responsiveness and the risk of MACE in patients treated with clopidogrel.

Methods—Systematic review and meta-analysis of studies of the association between the PON1-Q192R polymorphism and the biological response to clopidogrel and/or the risk of MACE during clopidogrel administration.

Results—Seventeen studies were included. In the 12 studies of the biological response to clopidogrel (n = 5302 patients), there was no significant difference between 192QQ and 192QR + 192RR subjects, whatever the laboratory method used (global mean standardized difference = 0.10 [−0.06; 0.25], P = 0.22). Eleven studies assessed the risk of MACE, four using a case–control design (n = 2739 patients) and seven a prospective design (n = 5353 patients). Overall, MACE occurred in 19% of patients in case–control studies and in 6% of patients in prospective cohort studies, with no significant difference between 192QQ and 192QR + 192RR patients (OR = 1.28 [0.97; 1.68], P = 0.08). Similar results were obtained when study design was taken into account. Heterogeneity was mainly driven by one publication.

Conclusions—This meta-analysis suggests that the PON1-Q192R polymorphism has no major impact on the risk of MACE and does not alter the biological response to clopidogrel in clopidogrel-treated patients.

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Disclosure of Conflict of Interests
The authors state that they have no conflict of interest.

Keywords

clopidogrel; ischemic events; paraoxonase-1; platelet function

Antiplatelet drugs are part of the recommended first-line treatment for atherothrombosis [1,2]. Current guidelines recommend a combination of aspirin and clopidogrel to prevent recurrent ischemic events in patients with an acute coronary syndrome (ACS) and for patients undergoing percutaneous coronary interventions (PCI) [3]. However, the biological response to clopidogrel is highly variable [4], and poor responsiveness is associated with a higher risk of recurrent ischemic events in cardiovascular patients [5]. This variability is tightly linked to the efficiency of the biological process through which clopidogrel, a pro-drug, is activated. Indeed, clopidogrel requires enzymatic bioactivation into its active thiol metabolite before interacting with the P2Y12 receptor on blood platelets. Clopidogrel bioactivation is a two-step process in which the cytochrome P450 (CYP) system, especially isoenzyme CYP2C19, plays a major role [6]. Several CYP2C19 gene variants modulate the biological response to clopidogrel [7-9] and the CYP2C19*2 [rs4244285] that leads to a loss-of-function phenotype has been linked to an increased risk of cardiovascular events [10]. Yet this polymorphism explains < 10% of the observed variability of clopidogrel responsiveness in cardiovascular patients [8,11,12], which is at odds with the marked heritability of this phenotype ($h^2 = 0.73$) observed in a familial study [13].

More recently, paraoxonase-1 (PON1), an esterase synthesized in the liver and present in the serum, was reported to be strongly involved in clopidogrel bioactivation. A PON1 genetic variant (Q192R, rs662, A576G) was reported to account for > 70% of the variability of clopidogrel responsiveness, with the PON1-192Q allele being associated with an increased risk of recurrent ischemic events in clopidogrel-treated patients. Of note, the authors found no evidence for the involvement of CYP2C19 in any of the steps of clopidogrel metabolism [14]. However, these associations were recently challenged by the results of several independent studies that showed no influence of the Q192R polymorphism on the biological response to clopidogrel or on the risk of ischemic events. Some studies have shown a trend towards an increased risk of ischemic events [15,16], or significantly increased platelet reactivity, in 192QQ patients [17]. As the failure of most studies to show clinical or biological effect of the PON1-Q192R genotype might have been due to a lack of statistical power, we conducted a systematic review of the literature and meta-analysis of summarized data to determine the influence of the PON1-Q192R polymorphism on clopidogrel biological responsiveness and the risk of recurrent ischemic events in clopidogrel-treated cardiovascular patients.

Methods

We followed published guidelines for meta-analyses of observational studies and their reporting [18].

Search strategy

The search was based on electronic databases (Medline, Embase, Web of Science, and the Cochrane Central Register of Controlled Trials), and abstracts of international meetings held in 2011, with no limitations on the type of publication or language. The electronic database search was last updated on 10 January 2012. A free-text search was conducted will the following key-word combination: (‘PON1’ or ‘PON-1’ or ‘paraoxonase’) and ‘clopidogrel’. Articles were selected on the basis of the abstract, before examining the full text. In addition, the reference lists of selected articles were hand-searched to identify additional

relevant reports. The reviewers were not blinded to the journal, authors or institution of the publications, as this has been shown to be unnecessary [19].

Inclusion criteria

Data extraction—To assess the specificity and characteristics of the identified studies, we abstracted information from each report on the year of publication, the total sample size, the study design, the duration and completeness of follow-up in prospective cohort studies, the type of clinical endpoint, blinding of clinicians to PON1 genotyping results, and study population characteristics (consecutive inclusions, age, sex, vascular risk factors, patients with acute events or stable ischemic disease, and drug compliance monitoring).

Exposed patients were defined as 192QQ. This arbitrary choice was related to the lower frequency of the R allele. Clinical outcome was defined in terms of major adverse cardiovascular events (MACE), including stent thrombosis, acute coronary syndromes, myocardial infarction and revascularization, and was expressed as the number of patients concerned in each genotype group. These numbers were extracted from the articles to prepare 2 × 2 tables. Biological outcome was defined in terms of platelet function test results, expressed as continuous variables. When residual platelet reactivity was evaluated at several time-points in pharmacodynamic studies, we included only the time-point closest to the loading dose in order to minimize heterogeneity. When two or more platelet reactivity tests were used in a given study, we only included the results of the test most specific for the inhibition of the P2Y12 receptor by clopidogrel (VASP > Verify-NowP2Y12 > ADP-induced aggregation > other aggregation-based assays). When different ADP concentrations were used for ADP-induced aggregation, we selected the concentration (20 μ_M) most frequently used in clinical studies, again to minimize heterogeneity. When biological test data were available as the mean and standard deviation (SD) for each of the three PON1 genotypes (192QQ, 192QR and 192RR), we used the Huygens theorem (decomposition of the total variance into an intra-stratum and an extra-stratum variance) to compute the mean and SD for the 192QQ group and the 192QR + 192RR group. When the published data were not sufficient to compute the 2 × 2 table for MACE or when the mean and SD of platelet function test results were not provided, the corresponding author was contacted and kindly requested to provide the required summarized data.

Two authors (PF and JLR) each independently implemented the search strategy, selected the studies and recorded the abstracted data. Disagreements on the study selection or data extraction were resolved by discussion among three authors (CC, PF and JLR).

Assessment of study quality—To estimate the risk of bias, we predefined a customized quality assessment tool based on eight methodological items that were evaluated on a scale from A to C [5]. The mode of patient inclusion was ranked A if consecutive and B if not consecutive or unclear. Quality of follow-up was based on the percentage of patients lost to follow-up (A, < 10%; B, 10–20%; C, more than 20%), identical follow-up and identical clopidogrel treatment for all included patients (A, yes; B, unclear; C, not identical), and evaluation of compliance (A, evaluated or in-hospital study with no clinical outcome; B, unclear; C, not evaluated). Two modes of evaluation were considered: blinding of clinicians to the PON1 genotype (A, blinded; B, unclear or not blinded) and adjudication of clinical events by independent clinicians (A, yes; B, unclear or no independent adjudication).

The overall quality of studies was defined as follows: if a study had one C or more, its overall quality was C. If all items were rated A, then overall quality was A. All other studies were rated B.

Statistical analysis

Descriptive data on the patients were recorded for each study as the mean and standard deviation (SD) or percentage.

The odds ratio (OR) for ischemic events associated with the 192QQ genotype was assessed in each study, along with its 95% confidence interval. The global OR was computed by using the method of the inverse of the variance with random effects [20], and the hypothesis OR = 1 was tested. Heterogeneity was tested with the Cochran Q statistic and measured with the *I*-squared statistic (I^2). The analysis was stratified for the case-control or prospective cohort study design. In case of heterogeneity (Cochran's test with $P < 0.10$ or $I^2 > 50\%$), predefined potential factors of heterogeneity were tested by meta-regression [21] or by comparing subgroups with random effects. For each study the candidate factors included the design, mean age, sex ratio, prevalence of diabetes, mean BMI, length of follow-up in prospective studies, frequency of stent thrombosis, and clinical setting (acute vs. stable patients).

To test for an association between platelet reactivity using different platelet function assays and the PON1 genotype, the standardized mean difference was used for meta-analysis; this was preferred to the mean difference because the test results are from different assays and were originally expressed with different units on different scales. The pooled standardized mean difference was computed for each type of platelet function assay by using the method of the inverse of the variance with random effects. As described above, in case of heterogeneity, predefined potential factors of heterogeneity were tested, including the type of platelet function assay.

Sensitivity analyses were also performed for both the global OR for MACE and the global standardized mean difference for platelet reactivity assays: (i) the pooled results, I^2 and Cochran's test were assessed n times, the studies being removed one by one; (ii) the pooled results were assessed using a dominant model (192QQ + 192QR vs. 192RR) and comparing homozygotes (192QQ vs. 192RR); and (iii) to check the robustness of the overall results of the meta-analysis with respect to studies with missing summarized data (not included in the meta-analysis), two separate meta-analyses were performed with extreme assumptions for the OR or the standardized mean difference.

The potential publication bias was explored for both the OR and the standardized mean difference by visual interpretation of the funnel plot [22], and the asymmetry of the funnel plot was checked with Egger's test [23]. The impact of funnel plot asymmetry on the results of the meta-analyses was evaluated by using the trim-and-fill method, which consists of adding missing studies virtually in order to obtain symmetry, and assessing the pooled results, including those of these missing studies [24].

Data were analyzed by using Comprehensive Meta Analysis Version 2 (BioStat, Englewood, NJ, USA) and Review Manager (RevMan) Version 5.1. (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2011). Significance was assumed at $P < 0.05$ in all analyses.

Results

Selection and characteristics of the studies

The flow of references through the review is shown in Fig. 1. There were 11 449 patients enrolled in the 17 selected studies [12,14-17,25-36], which were all published in 2011 or 2012, most data being derived from existing cohorts. Details of the 17 studies are shown in Table 1. Excepting the few studies of healthy subjects, the studies were quite homogenous

with respect to the patients' mean age (61–68 years in most studies), mean BMI (26–30 kg m^{-2}), prevalence of diabetes (21–30%), length of follow-up (≥ 12 months in all but one of the prospective cohort studies) and the PON1-192Q allelic frequency (0.64–0.73). In contrast, the studies differed markedly with respect to the type of assay used to assess platelet reactivity and the clinical setting (acute coronary syndrome or stable disease).

Regarding methodological quality (Table 2), it was unclear in most studies whether or not compliance was assessed and whether an independent adjudicating committee was used for MACE. Patient follow-up and the clopidogrel regimen were identical within each prospective cohort study but one, and losses to follow-up were lower than 3% in all prospective cohort studies, apart from one study in which follow-up information was unclear.

As shown in the flow chart in Fig. 1, summarized data could not be abstracted from 10 studies, corresponding to five published papers [16,26-28,32] and four conference abstracts [33-36]. All summarized data required to compute a 2 × 2 table for MACE were kindly provided by the corresponding author of the published papers. Similarly, the means and standard deviation of platelet function assay results could not be retrieved from the publications of eight studies [16,27,28,33-36]. In three instances of studies presented at conferences but not yet published, summarized data could not be obtained from the authors (n = 1575) [34-36].

Clinical outcome

Eleven studies assessed the risk of MACE, four in a case–control design (n = 2739) and seven in a prospective design (n = 5353). MACE cases represented 19% of patients in the case–control studies and 6% of patients in the prospective cohorts. There was no significant difference in the incidence of MACE between the 192QQ patients and the 192QR + 192RR patients (OR = 1.28 [0.97; 1.68] P = 0.08) (Fig. 2). Statistical heterogeneity was found (Cochran P = 0.004, I^2 = 62%), but there was no significant subgroup difference in OR between the two types of study design (P = 0.55 and I^2 = 0%).

Platelet reactivity

Four different types of platelet function assay were used (VASP, VerifyNowP2Y12, LTA, and impedance whole-blood aggregometry). For these four assays, the pooled standardized mean difference was never significantly different from zero between 192QQ and 192QR + 192RR subjects (Fig. 3). The pooled standardized mean difference for all platelet function tests combined was close to 0 (−0.02 [−0.10; 0.06], P = 0.68). Statistical heterogeneity was found (Cochran P < 0.00001, I^2 = 86%), but there was no significant difference in the standardized mean difference between PON1 genotypes across the different tests (P = 0.31 and I^2 = 17%).

Publication bias, sensitivity analyses and meta-regression

No publication bias was detected on funnel plots or with Egger's test (P = 0.30 for OR and P = 0.13 for the standardized mean difference). Trim-and-fill analysis showed that the pooled estimates were not sensitive to the missing studies on the left part of the funnel plot (data not shown).

The results of the meta-analyses were similar when patients were grouped in 192QQ + 192QR vs. 192RR or in 192QQ vs. 192RR. The tested potential heterogeneity factors (mean age, sex ratio, prevalence of diabetes, mean BMI, acute or stable patients, length of follow-up and frequency of stent thrombosis in prospective studies) included in meta-regressions were not significantly associated with the OR or the standardized mean difference. In the

five studies [14,16,25,29] reporting analyses adjusted on potential individual confounders for the risk of MACE, the results were only marginally modified. When considering the risk of MACE most of the statistical heterogeneity was explained by two studies reported in one publication [14]. When each of these studies was removed, I^2 fell from 63% to 0% for the group of case–control studies and from 64% to 0% for the group of prospective studies, whereas removal of any of the other studies only marginally affected the level of heterogeneity (from 63% to 80% and from 64% to 70% in case–control and prospective studies, respectively). Removal of both the studies conducted by Bouman *et al.* yielded a global OR of 1.06 ([0.90; 1.24], $P = 0.51$), without statistical heterogeneity. When considering the platelet reactivity test results, most of the statistical heterogeneity was explained by one study [14]. When this study was removed, I^2 fell from 86% to 48% whereas removal of any of the other studies did not affect the level of heterogeneity (I^2 = 86%–87%).

To study the potential impact of platelet reactivity data missing from three studies totalling 1544 patients, a sensitivity analysis was performed with the following extreme hypotheses: the mean difference and standard deviation in platelet assay results between PON1-192QQ and 192QQ + RR subjects in each missing study was set to the highest possible standardized mean difference, knowing that the differences were reported as statistically non-significant in each of the three missing studies. Assuming that all differences were positive, this corresponded to standardized mean differences of 0.42 (0.00; 0.85), 0.32 (0.00; 0.63) and 0.11 (0.00; 0.22), for the studies of 88 [36], 157 [34] and 1299 patients [35], respectively. Following this extreme hypothesis, the pooled standardized mean difference would be 0.12 (−0.01; 0.25) ($P = 0.07$). Assuming all three differences were negative, the pooled standardized mean difference would be 0.03 (−0.10; 0.16) ($P = 0.67$).

Discussion

Inherited factors play a major role in platelet aggregation [37] and familial studies have shown a high degree of heritability of the platelet response to clopidogrel ($h^2 = 0.73$) [13]. This has led to attempts to identify the genetic factors underlying the variable biological response to clopidogrel. Hepatic CYP 2C19 gene variants associated with loss of function have been identified as the main contributors to this variability [6]. However, the proportion of the variability explained by the most frequent loss-of-function allele of CYP 2C19 (2C19*2, rs4244285) is only 12% in healthy volunteers and below 10% in cardiovascular patients [8,11,12] and its role in predicting cardiovascular events is controversial [38]. A new player in clopidogrel pharmacogenetics was recently reported by Bouman *et al.* [14], who identified PON1 as a potential major determinant of the efficiency of clopidogrel bioactivation and this drug's clinical efficacy in patients of European descent.

The results of our meta-analysis of 17 studies (11 449 patients) do not support a major biological or clinical influence of PON1. Indeed, the PON1-Q192R polymorphism was associated neither with platelet reactivity, regardless of the test used, nor with the risk of cardiovascular events in patients treated with clopidogrel, independently of the study design (case–control or prospective cohort). These results are in line with the lack of correlation between PON1 activity and P2Y12 receptor inhibition (evaluated with the specific VASP assay) in 538 stable cardiovascular patients [12] as well as with the lack of correlation between PON1 activity and clopidogrel active metabolite [39] or other *in vitro* assays showing no impact of PON1 inhibition on the production of the clopidogrel active metabolite [40]. The trend towards an association between the PON1-Q192R polymorphism and MACE observed in our meta-analysis was mainly driven by Bouman's study, which accounted for most of the observed heterogeneity. No publication bias was detected by

visual inspection of the funnel plot or by statistical analysis, although the tests used lack power when the number of studies is low.

For meta-analyses, it is strongly recommended to investigate the influence of potential heterogeneity factors in order to avoid simplistic and potentially misleading conclusions [41], but, owing to the danger of over-interpretation, the choice of potential factors should be limited and based on 'reasonable suspicion'. Prior to conducting our meta-analysis, we defined a limited number of potential heterogeneity factors. Overall, the statistical heterogeneity was not explained by any of the factors tested in meta-regressions. These meta-regressions, conducted at the study level, do not exclude a potential interaction of patient-related factors, and further patient-level analysis is needed to reach a firm interpretation. On investigating study-level factors, we found that the heterogeneity was mainly related to two independent studies published in one paper [14]. Indeed, a sensitivity analysis, in which we removed one study at a time, showed that a large part of the statistical heterogeneity was associated with the two studies by Bouman *et al.* [14], whereas heterogeneity was not noticeably affected when any of the other studies were removed.

The reasons for the discrepancy between the original work of Bouman *el al.* [14] and most subsequent studies are unclear. Differences in populations may be an issue; indeed, the frequency of the Q192 allele is somewhat lower in their cohort study (63.9%) than in any of the other studies [12,15-17,25-33,42]. A recent *in vitro* study toy Dansette *et al.* [43] clearly demonstrates that PON1 is involved in clopidogrel metabolism, but the resulting metabolite is a minor isomer by comparison with the active metabolite produced by cytochromes P450. Dansette *et al.* suggested that the analytical method used by Bouman *et al.* was not sufficiently selective to distinguish among the different isomers of the active metabolite, and that it preferentially detected the inactive metabolite produced by PON1. The observed clinical differences between PON1 gene variants may be due to a clopidogrel-unrelated mechanism, as PON1 plays a pivotal role in atherogenesis by protecting low-density lipoprotein from oxidation [44]. This may partly explain the trend towards an association of the PON1-Q192R variant with MACE, best seen in two studies [15,16] in addition to Bouman's studies (Fig. 2).

Differences in clinical endpoints across studies might also be involved. Indeed, Bouman *et al.* [14] found the strongest association with definite stent thrombosis, while the association with all MACE was smaller. The CYP2C19*2 variant also seems to mainly affect the risk of stent thrombosis. In our meta-analysis, most of the studies addressing the association of the PON1-Q192R variant with clinical events involved composite endpoints, with a low incidence of stent thrombosis, possibly leading to an underestimation of the clinical importance of the PON1 genotype (Table 1). Another potential bias is ethnic heterogeneity across the different studies, resulting in genetic differences that might have influenced the response to clopidogrel [45]. Drug–drug interactions, mainly via CYP3A4/5, are another important issue when analyzing the variability of clopidogrel responsiveness [46]. For example, platelet inhibition by clopidogrel is attenuated by co-administration of ketoconazole, a known CYP3A4/5 inhibitor [47], or atorvastatin, a competitive inhibitor [48], though it is debated for this latter drug. Conversely, increased platelet inhibition is observed in hyporesponsive patients when CYP3A4/5 activity is induced by St John's Wort, an herbal remedy used for the treatment of depression [49].

Significant pharmacokinetic interactions also exist with proton pump inhibitors, probably through a CYP2C19 inhibition [50-52]. Hence, an imbalance of co-medication with drugs affecting cytochrome P450 activities across genotype groups might have influenced the findings. Finally, as recently suggested in a study of 74 patients with ACS, the PON1-Q192R variant may modulate the clopidogrel response (and possibly clinical outcome) in a

subset of patients considered to be good responders to this drug [17]. Only a few of the papers describing the studies that we included in our meta-analysis mentioned the prevalence of good responders, meaning that this issue could not be addressed by meta-regression. However, analysis of the ADRIE study data restricted to patients treated with clopidogrel and with a platelet reactivity index below 50% (n = 261) gave results similar to those obtained for the entire population (data not shown) [12].

Study limitations

First, most of the studies included in this meta-analysis were observational, being based on prospective or retrospective registries. However, we applied the MOOSE statement checklist [18], allowing careful selection of studies with systematic use of a quality score, and we provided an indication of statistical uncertainty of findings using sensitivity analyses that are key factors to control for this limitation. Second, although analysis of statistical heterogeneity is recommended, it is well known that meta-regressions may have limited relevance in meta-analyses of summarized data and certainly limited power with the number of studies involved in each meta-regression. However, study-level sub-group analyses yielded similar conclusions irrespective of the biological test used to assess clopidogrel responsiveness and the study design (case–control or prospective cohort). Finally, information on clopidogrel use at the time of the event was incomplete.

Conclusion

The results of this meta-analysis do not support the PON1-Q192R polymorphism as a major determinant of the biological response to clopidogrel or as a risk factor for MACE in clopidogrel-treated patients. As CYP2C19*2 plays only a minor role in the variability of clopidogrel responsiveness, further studies of clopidogrel pharmacogenetics are needed before implementing CYP2C19 genotyping in routine clinical practice [53].

Appendix

The PON1 meta-analysis study group includes, in addition to the authors of the present paper, D. Aradi, J. Delaney, J.-P. Déry, P. Gurbel, J. Lewis, D. Sibbing, D. Taubert, D. Trenk and the Geneva Platelet Group members who contributed to this work: V. Ancrenaz, J. Desmeules, A. Perrier, A. Poncet, J.-C. Sanchez and A. Zufferey.

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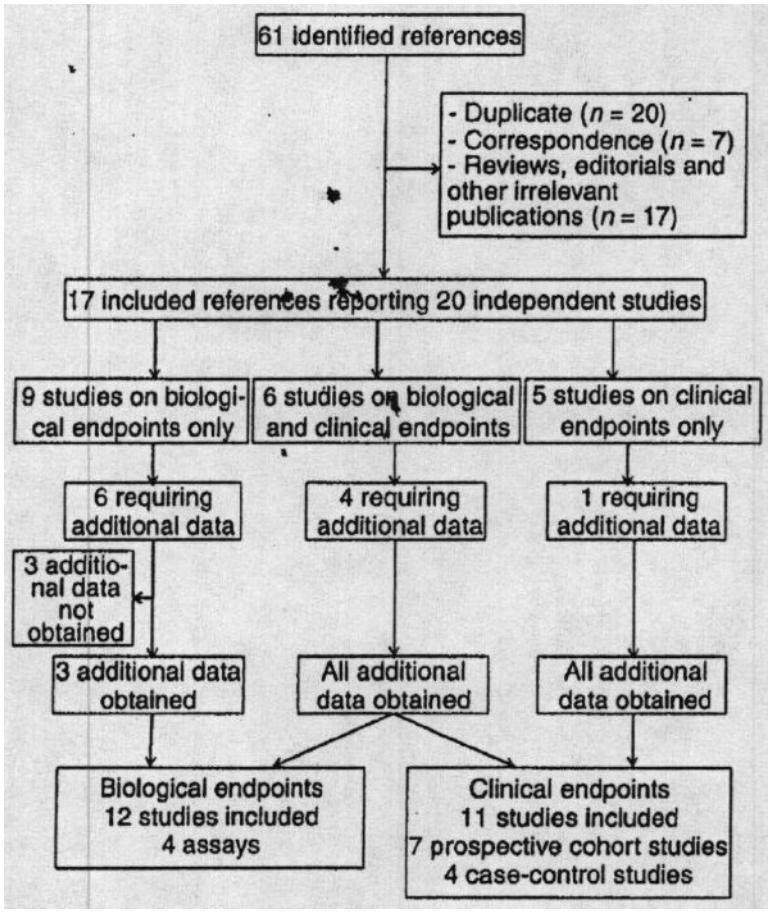

Fig. 1.
Flow chart of the meta-analysis.

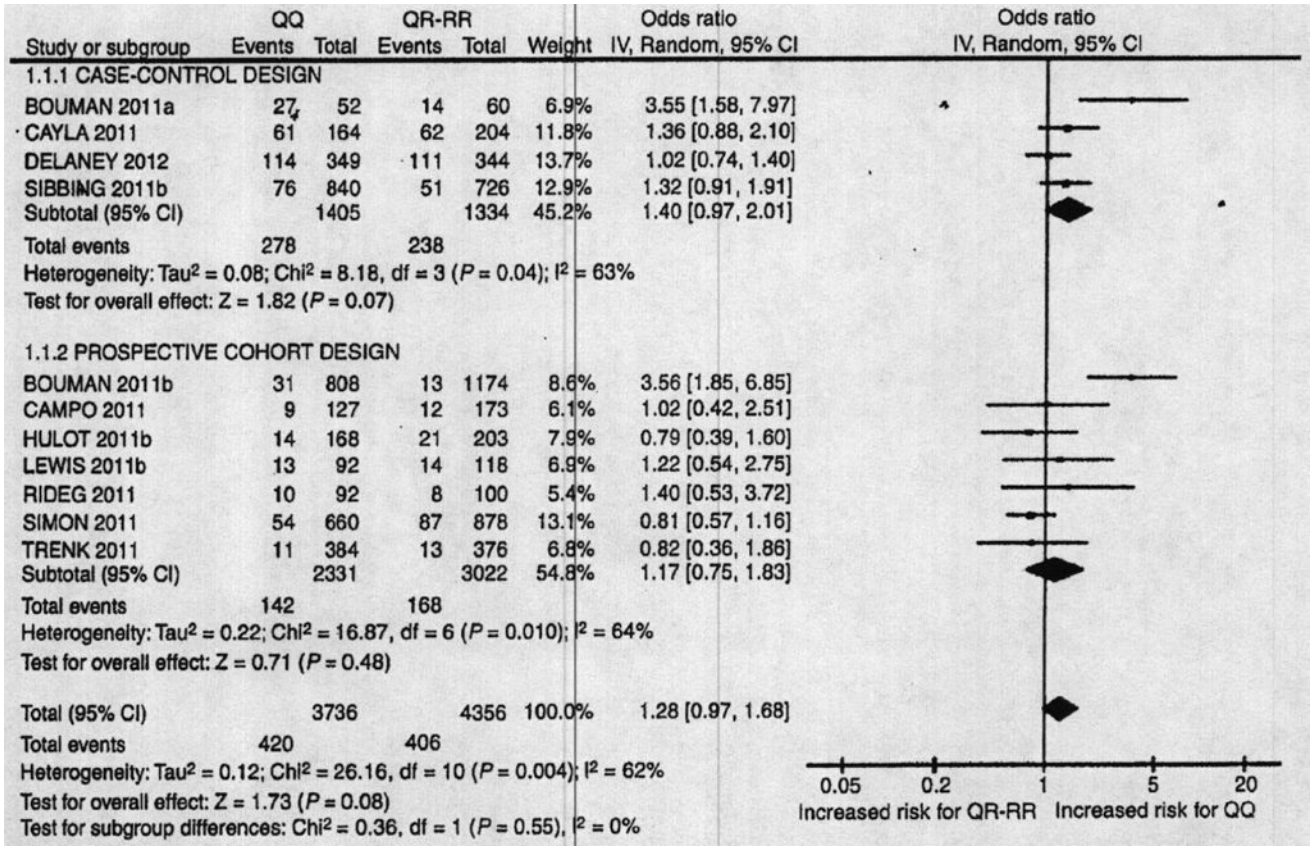

Fig. 2.

PON1-Q192R and risk of MACE. The odds ratios (ORs) for major adverse cardiovascular events (MACE) are presented for PON1 192QQ compared with 192QR + RR patients. The analysis is stratified by study design. The size of the squares corresponds to the weight of the study in the meta-analysis. Horizontal lines represent corresponding 95% confidence intervals (CIs). The global ORs are depicted as black diamonds.

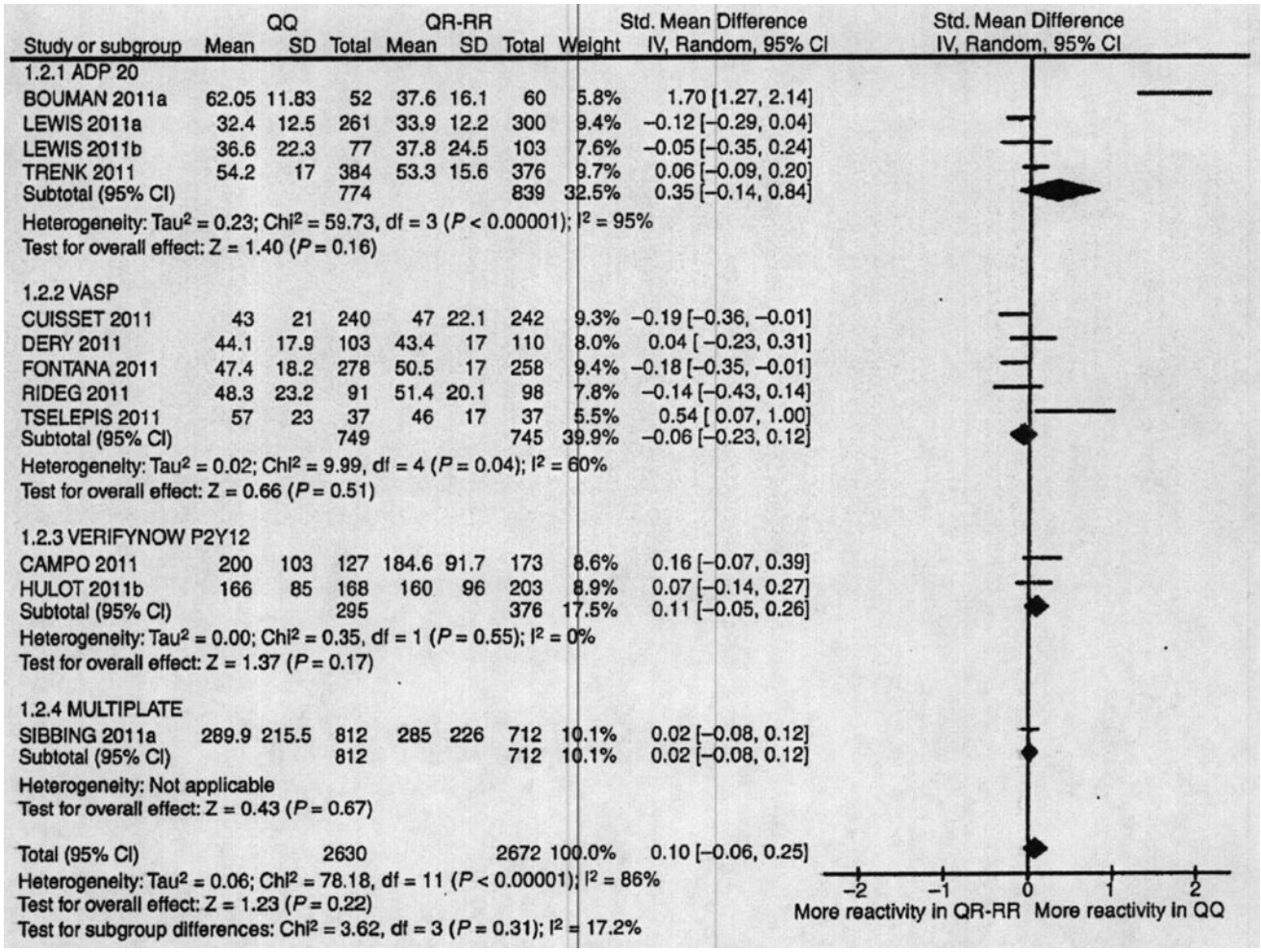

Fig. 3.

PON1-Q192R and platelet reactivity. The standardized mean differences in the platelet reactivity assay results between PON1 192QQ and 192QR + RR patients are shown. The analysis is stratified by the types of assays. The size of the squares corresponds to the weight of the study in the meta-analysis. Horizontal lines represent corresponding 95% confidence intervals (CIs). The global standardized mean differences are depicted as black diamonds.

Table 1

Main characteristics of published studies

Study	*n*	Design	Mean age (y)	Male (%)	Diabetics (%)	BMI (kg m^{-2})	Clinical setting	% of ST	Follow-up (months)	Platelet reactivity assay	% of poor responders	192Q allele frequency (%)
Bouman 2011a	112	Case-cohort	61.2 ± 8.5	79.5	25.9	27.0 + 3.2	41 ST vs. 71 controls	37	18	LTA (ADP 20 μM)	NA	66.9
Bouman 2011b	1982	Cohort	62.2 ± 10.2	71.3	24.3	27.1 + 4.3	ACS	2.2	12	No	NA	63.9
Campo 2011	300/281	Cohort	66 ± 13	77.0	23.7	27.0 ± 4	PCI (61% NSTEMI)	1.3	12	VerifyNow	13	66.7
Cayla 2011	123/246	Case-cohort	62.7 ± 12.2	80.5	25.2	26.6 ± 4.1	ST vs. non-ST	50	–	No	–	67.1
Cuisset 2011	482	Cohort	63.0 ± 10	81.0	28.0	26.3 ± 4.0	ACS	–	No	LTA (ADP 10 μM) and VASP	NA	69.9
Delaney 2012	693	Case-control	68.3 ± 11.7	63.5	34.8	30.3 ± 6.4	MI and PCI patients	1.7	–	–	–	69.0
Dery 2011	213	Cohort	61.1 ± 9.8	83.1	9.8	28 ± 4.2	30 days post-PCI	–	–	LTA (ADP 5 μM), VASP	NA	70.2
Fontana 2011	538	Cohort	62 ± 12.2	81.9	21.2	26.5 ± 3.9	Stable	–	–	LTA (ADP 20 μM), VASP	49.6 (VAS P)	70.8
Hulot 2011	371	Cohort	40.3 ± 5.5	84.6	10.5	25.9 ± 4	Stable young survivors of MI	4.8	96	VerifyNow	NA	65.2
Lewis 2011a	566	Cohort	45.5 ± 13.2	48.7	0.9	27.0 ± 4.7	Healthy subjects	–	–	LTA (ADP 20 μM)	NA	68.0
Lewis 2011b	210	Cohort	64.3 ± 11.4	59.9	36.6	30.4 ± 6.6	Non-emergent PCI	NA	12	LTA (ADP 20 μM) in 180 patients	NA	NA
Rideg 2011	189	Cohort	61.8 ± 8.4	61.4	37.6	29.4 ± 5.2	Non-emergent PCI	0.5	12	LTA (ADP 5 μM) and VASP	NA	70.9
Sibbing 2011a*	1524	Cohort	67.5 ± 10.5	77.4	28.2	27.5 ± 4.4	PCI (67% stable)	–	–	MEA	20	72.7
Sibbing 2011b	127/1439	Case-control	67.6 ± 10.5	77.5	29.0	NA	Cases with ST	8.1	–	No	–	NA
Simon 2011	1538	Cohort	64.4 ± 13.1	74.7	29.7	27.4 ± 4.7	ACS with PCI only	NA	12	–	–	66.7
Trenk 2011	760	Cohort	66.3 ± 9.2	78.3	24.3	27.7 ± 3.9	Elective PCI	2.1	12	LTA (ADP 5 and 20 μM), FACS	NA	70.5
Tselepis 2011	74	Cohort	63.3 ± 8.6	70.3	NA	NA	ACS	–	–	LTA (ADP 5 and 10 μM), VASP, FACS	23 (at 5 days with VASP)	70.3

ACS, acute coronary syndrome; ADP, adenosine diphosphate; LTA, light transmission aggregometry; MI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; PCI, percutaneous coronary intervention; ST, stent thrombosis.

*Used for platelet reactivity data only.

Table 2

Methodological quality assessment and risk of bias

Study	Consecutive patients	Losses to follow-up (%)	Identical follow-up and clopidogrel treatment	Assessment of compliance	Clinician blinding to platelet reactivity	Independent adjudicating committee	Quality score
Bouman 2011a	Unclear	–	–	Unclear	–	Unclear	B
Bouman 2011b	Unclear	2.8	Yes	Unclear	–	Unclear	B
Campo 2011	Unclear	0	Yes	Unclear	Unclear	Unclear	B
Cayla 2011	Unclear	–	–	Yes	–	No	B
Cuisset 2011	Unclear	–	–	Yes	–	–	B
Delaney 2012	Yes	–	–	Unclear	–	Yes	B
Dery 2011	No	–	–	Unclear	–	–	B
Fontana 2011	Yes	–	–	Yes	–	–	A
Hulot 2011	Yes	0	Yes	Yes	–	Yes	A
Lewis 201la	–	–	–	Unclear	–	–	B
Lewis 2011b	Unclear	Unclear	Yes	Yes	Yes	Unclear	B
Rideg 2011	Unclear	Unclear	No	Yes	Yes	Yes	C
Sibbing 2011a	Yes	1.4	Yes	Yes	Yes	Yes	A
Sibbing 2011b	Unclear	–	–	Unclear	–	Unclear	B
Simon 2011	Yes	1%	Yes	Unclear	–	Unclear	B
Trenk 2011	Yes	< 1%	Yes	Unclear	Unclear	Yes	B
Tselepis 2011	Yes	–	–	Unclear	–	–	B