Original Article
Associations between four types of single-nucleotide polymorphisms in PLA2G7 gene and clinical atherosclerosis: a meta-analysis

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Abstract: Background: Previous studies suggested that some types of single nucleotide polymorphisms (SNPs) in PLA2G7 genes, encoding Lp-PLA2 have been reported to yield an antiatherogenic effect, but other studies mentioned otherwise. Thus, a comprehensive study to explore the effect of SNPs in PLA2G7 genes (V279F, A379V, R92H, I198T) toward clinical atherosclerosis is needed. Methods: We searched eligible studies from PubMed, EBSCO, ProQuest, Science Direct, Springer, and Cochrane databases for case-control studies to assess the between four types of SNPs in PLA2G7 gene with risk of clinical atherosclerosis (CVD = cardiovascular disease, CAD = coronary artery disease, PAD = peripheral artery disease, ischemic stroke). All studies were assessed under Hardy-Weinberg Equilibrium, an additive model. This meta-analysis was performed by RevMan 5.3 to provide pooled estimate for odds ratio (ORs) with 95% confidence intervals (95% CIs). Results: Fourteen clinical studies met our inclusion criteria. Those included 12,432 patients with clinical atherosclerosis and 10,171 were controls. We found that ORs of two variants SNPs (V279F, R92H) were associated with clinical atherosclerosis {V279F, OR = 0.88 (95% CI, 0.81-0.95); p = 0.0007, I^2 = 40%}, {R92H, OR = 1.29 (95% CI, 1.09-1.53); p = 0.003, I^2 = 73%}. Meanwhile, there was no significant associations between the other two, A379V {OR = 1.08 (95% CI, 0.93-1.26); p = 0.31, I^2 = 78%} and I198T {OR = 1.12 (95% CI, 0.79-1.59); p = 0.53, I^2 = 81%}. Conclusions: These results suggested that V279F polymorphism in PLA2G7 gene has a protective effect for clinical atherosclerosis, whereas R92H polymorphism might contribute toward increased risk of clinical atherosclerosis.

Keywords: Lipoprotein-associated phospolipase A2 (Lp-PLA2), single-nucleotide polymorphisms (SNPs), atherosclerosis, PLA2G7

Introduction

Oxidative stress and inflammation contribute to atherosclerosis. Lipoprotein-associated phospholipase A2 (Lp-PLA2) and other human phospholipases A2 (such as secretory phospholipase A2) [1, 2] propagate inflammation by producing precursors of arachidonic acid from membrane glycerophospholipids [3]. Lp-PLA2 (also known as platelet-activating factor acetylhydrolase or PAF-AH) hydrolyzes oxidized phospholipids to produce pro-inflammatory products that are implicated in endothelial dysfunction, plaque inflammation, and formation of a necrotic core in atherosclerotic plaque. Therefore, circulating Lp-PLA2 has been studied rigorously as a marker of cardiovascular disease (CVD) risk because the enzyme exhibits pro-inflammatory and oxidative stress properties [2]. Clinical and epidemiological studies demonstrate an association with an incident and recurrent coronary artery disease events [4,5]. Beside the traditional CVD risk factors, higher plasma Lp-PLA2 is associated with adverse long-term CVD outcomes [6, 7].

Heritability studies revealed that approximately 62% of the variation in Lp-PLA2 activity was

deemed to genetic factors [8]. Consequently, the endeavor of proving the association between single nucleotide polymorphisms (SNP) on PLA2G7 function and CVD outcome had been conducted by recent studies. In Caucasian population, some studies had revealed that non-synonymous coding polymorphism (A379V) associated with plasma Lp-PLA2 levels [9, 11]. However, the association between the A379V polymorphism and the enzymatic levels and atherosclerotic outcome had been diverse among studies, with some studies showing V379 allele to be associated with increased enzyme levels [9, 11], whereas others exhibiting lower levels [10]. There was a significant association between the A379V polymorphism and premature myocardial infarction, but V379 allele was paradoxically associated with decreased risk of myocardial infarction [10].

Additionally, another allele such as R92H in exon 4 that is associated with coronary artery disease in the USA [12]. Similar to both A379V and R92H, missense mutation of V279F in exon 9 of PAF-AH gene leads to a complete loss of catalytic activity and might be a risk factor for stroke in Japanese [13]. This loss-of-function mutation has been reported to be associated with an increased CAD risk [14, 15], supporting the anti-atherogenic role of PAF-AH. On the other hand, the other studies in European population have proven otherwise [6]. Furthermore, in Korean men, there was a lack of function in Lp-PLA2 activity due to a variant of PLA2G7 279 allele, thus protecting from CAD [16]. Similar to three alleles mentioned above, amino acid alterations caused by a mutation in Ile198Thr may lead to lower PAF-AH expression. Nevertheless, all these findings are still considered to be inconclusive [17].

Thus, it is still unknown whether Lp-PLA2 levels result in a pro- or anti-atherogenic effects in human. Therefore, studies on the association of Lp-PLA2 genetic variations may reveal a support for a causal role of Lp-PLA2 in clinical atherosclerosis. Unfortunately, several studies have shown polymorphisms in this gene, but none to our knowledge has really proven the causal relationship with atherosclerosis.

Therefore, it is justified to investigate the associations of SNPs in PLA2G7 genes (V279F, A379V, R92H, I198T) with clinical atherosclerosis by conducting a meta-analysis. It is needed to estimate the strength of the associations (eg, effect size, heterogeneity, publication bias and cumulative evidence).

Methods

Literature search

We carried out a comprehensive electronic search to identify all published prospective cohort, and case-control studies that investigate the relationship between four types gene polymorphisms (V279F, A379V, R92H, I198T) in Lp-PLA2 gene and risk of clinical atherosclerosis. This study population is clinical atherosclerosis, *namely*: coronary artery disease (CAD), cerebrovascular disease (CVD), ischemic stroke, TIA and peripheral arterial disease (PAD) presumed to be of atherosclerotic origin. PubMed, Science Direct, ProQuest, EBSCO, Springer, and Cochrane databases were searched from March 2016 through October 2016, by using the following search terms: "Lipoprotein-associated phospholipase A2" ("Lp-PLA2" or "platelet-activating factor acetylhydrolase" or "PAF-AH") and "gene polymorphisms" ("gene variants" or "SNP" or "single nucleotide polymorphisms") and "atherosclerosis" ("CHD" or "CAD" or "coronary heart disease" or "coronary artery disease" or "acute coronary syndromes" or "ischemic-stroke" or "cerebrovascular disease" or CVD or "peripheral artery disease"). A manual search was also carried out to retrieve potential articles cited in the previous meta-analysis, systematic review, and those considered to be pertinent by the reviewers.

Inclusion criteria

All studies were considered potentially eligible if they aimed to investigate the relationship between four types gene polymorphisms in PLA2G7 gene and risk of clinical atherosclerosis. We had included a total of 14 studies that met the following specified criteria: (1) each clinical atherosclerosis terms (defined based on the American College of Cardiology/American Heart Association) [18] considered as studied population; (2) prospective cohort, and case-control studies as appropriate study design; (3) risk of clinical atherosclerosis reported as RR (relative risk) and/or OR (odds ratio) with the 95% confidence interval; (4) each genotype and allele frequency (V279F, A379V, I198T, R92H) reported as independent

variables; (5) includes human subjects; (6) published in English since 2001 and beyond.

Exclusion criteria

The excluded literatures were as follows: (1) cross-sectional study, case report or series, editorial and narrative review; (2) duplicate publication; (3) flawed study design, namely: having indefinite population recruited and unqualified data; (4) non-English articles; (5) studies assessing surrogate outcome; (6) published prior to 2000. We excluded those study designs because both selected case-control and prospective cohort were much better on defining causal association between PLA2G7 gene polymorphisms and risk of clinical atherosclerosis.

Study selection

Four investigators (R.M., F.A., I.M., and A.D.P.) independently conducted the literature search and retrieved the titles, abstracts, and full texts of articles that matched with our searched terms. The remaining investigators (A.S and T.H) read full selected articles that met the requirements and provided final suggestions. These articles were fully read, and those that fulfilled our criteria were included. Final inclusion of studies was merely based on the agreements of all investigators; the disagreement was resolved by consensus.

Data extraction

Data were extracted by using a standardized reporting form. The eligible articles included the first author's name, country of origin, year of publication, study design, gender composition, mean age or age range, major CVD end point, selection of controls (population-based or hospital-based), allele frequencies of PLA2G7 gene, genotype distribution in case patients and controls, confounding factors by matching, and study size, ethnicity, genotyping method.

Quality assessment

The quality of the studies had been evaluated using the guidelines for reporting observational studies, developed by the STROBE initiative and STREGA [19, 20]. The STROBE initiative developed recommendations on what should be included in an accurate and complete report of an observational study. The scope of this recommendation is to cover three main study designs: cohort, case-control and cross-sectional studies. The STREGA recommendations further seek to enhance the transparency of genetic study's reporting, so it would assure the quality of the genetic study [20].

Statistical analysis

Hardy-Weinberg Equilibrium (HWE) in the control group was examined by Chi Square test. Review Manager 5.0 software (The Cochrane Collaboration, Oxford, UK) and Comprehensive Meta-Analysis (version 3.3.070, 2014) software was used for meta-analysis. The contrast frequencies of genotype F-allele versus V-allele; V-allele versus A-allele; H-allele versus R-allele and T-allele versus I-allele (additive model) of the Lp-PLA2 (V279F, A379V, R92H, I198T) polymorphisms were evaluated.

Heterogeneity was assessed with the Q-statistic test and I^2 test. The I^2 statistic measured the percentage of total variation across the studies due to clinical or methodological heterogeneity rather than chance. The pool estimated ORs was measured with models based on fixed effects or random effects assumptions. If the significant Q statistic ($P < 0.05$) indicated heterogeneity across the studies, a random effects model was used for meta-analysis. Otherwise, a fixed effect model should be chosen. The 95% confidence interval (CI) of pool estimated OR was also calculated.

Publication bias

Inverted funnel plots of the four SNPs of PLA2G7 gene and Egger's test [21] were performed to look for evidence of publication bias. The inverted funnel plot should be asymmetric and Egger's test result was significant ($P < 0.05$) once there is a publication bias and otherwise in the case of no publication bias.

Results

Study characteristics

Initially, 151,269 studies abstract was primarily identified from the database. Potential relevant articles (n = 495) were initially selected, moreover four hundred thirty-three of them had
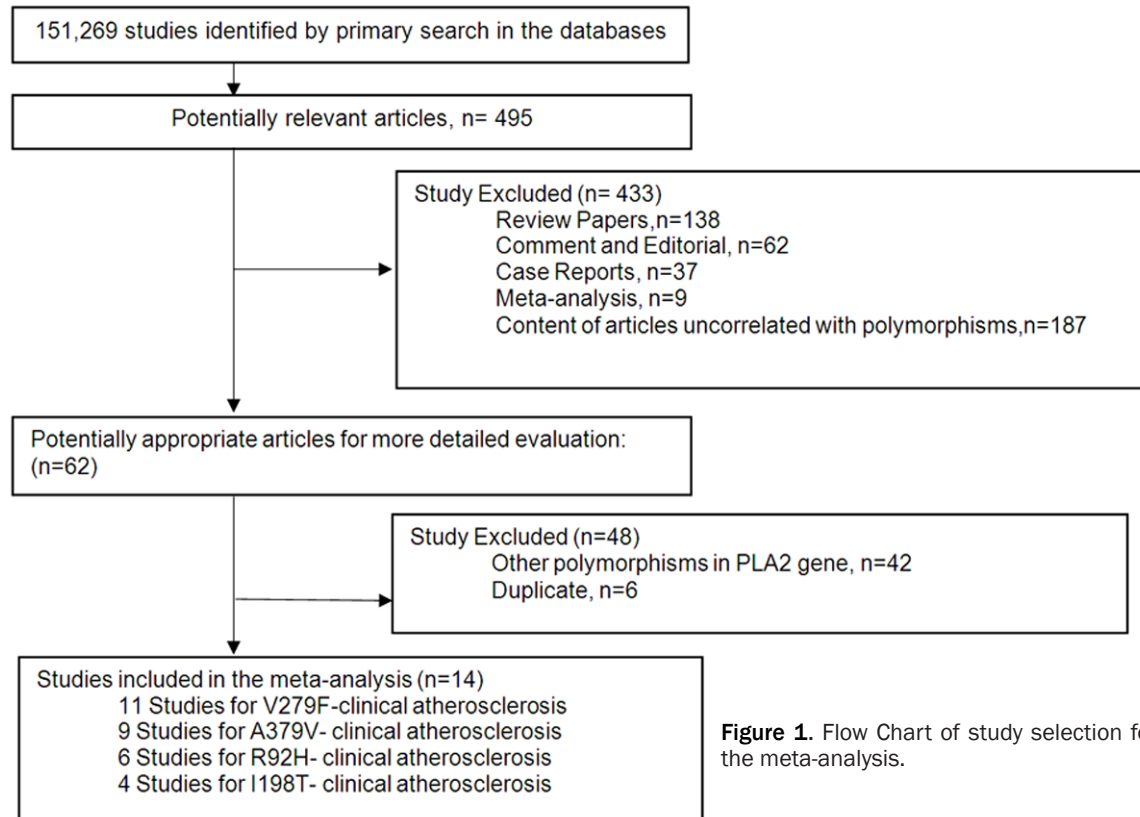
Figure 1. Flow Chart of study selection for the meta-analysis.

been excluded since they were considered as review articles (n = 138); commentaries and editorials (n = 62); case report (n = 37); meta-analysis (n = 9); and article unrelated with polymorphisms (n = 187). Eventually, a total of 62 potentially articles were screened and met the STROBE and STREGA recommendations. Nonetheless, after thoroughly scrutinizing them, 42 studies should be excluded from the analysis due to studying other polymorphisms in the genes. Furthermore, 6 studies were also excluded due to duplicate and overlapping publications. Finally, simply 14 studies [9, 10, 16, 22-32] were included in this meta-analysis and all together recruited 22,603 individuals (**Figure 1**), and they consisted of 12,432 cases and 10,171 controls.

We had examined four PLA2G7 gene polymorphisms, namely V279F, A379V, R92H, and I198T because other SNPs were less reported in the previous studies. The majority of the clinical atherosclerosis was represented by coronary heart disease, except 3 studies recruited subjects with peripheral arterial occlusive disease [25] and ischemic stroke [29, 31] and mainly the study design was employing case-

control study, only one study was nested-case control design [9]. The recruited samples consisted of males in 4 studies [16, 22, 23, 29] and the remaining consisted of both gender in 10 studies [9, 10, 24-28, 30-32]. Most studies matched controls to cases by age, gender and ethnicity, in order to avoid selection bias. All ethnicity largely consisted of Oriental and Asian subjects, except 3 studies [9, 22, 26] comprised Caucasian subjects and 1 study assessed Turkish population [24]. So, Caucasian subjects were only represented by a small proportion (5.25%) in the present meta-analysis. The controls from eight studies were hospital-based patient, whilst those from the rest of the studies were healthy subjects. The characteristics of our final studies were summarized in **Tables 1** and **2**.

Association of V279F polymorphism with clinical atherosclerosis

Eleven eligible studies with 8,050 cases and 8,427 controls were examined for the association of V279F variants and clinical atherosclerosis. Majority studies had Asian descent, except 1 study had Turkish subjects. There was

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

Study	Study design, Publication year	Sample size Case (N = 12,432)	Control (N = 10,171)	Ethnicity	Matching Variable	Genotyping method	End point assessment
Abuzeid AM	Case control (2003)	527	566	Caucasian	Age	PCR	CHD, Post MI
Ninio E	Nested-case control (2004)	1314	485	Caucasian	None	PCR-SSCP	CAD
Jang Y	Case control (2006)	532	670	Korean	None	SnaPShot	CVD
Sekuri C	Case control (2006)	115	128	Turkish	None	PCR-RFLP	Premature CVD
Liu PY	Case control (2006)	200	200	China	Sex, Age	AFLP-PCR	Premature MI
Unno N	Case control (2006)	150	158	Japan	Sex, Age	AS-PCR	PAOD Patients
Huo L	Case control (2009)	827	947	China	None	PCR-RFLP	CHD
Hoffmann MM	Case control (2009)	2541	693	Caucasian	None	PCR–RFLP	CAD Patients
Jang Y	Case control (2011)	3767	4358	Korean	None	AS-PCR	CAD
Li L	Case control (2011)	806	482	China	Age	PCR	CAD
Zheng GH	Case control (2014)	570	317	China	None	SNaPshot	CHD & Blood Stasis Syndrome
Liu X	Case control (2014)	386	386	China	Sex, Age	PCR-LDR	Ischemic Stroke
Ma Y	Case control (2015)	375	370	China	None	AFLP-PCR	Ischemic Stroke
Hong M	Case control (2015)	322	411	Asian	None	PCR-RFLP	CHD

AS-PCR the allele specific polymerase chain reaction; AFLP-PCR amplified fragment length polymorphism- polymerase chain reaction; PCR-SSCP polymerase chain reaction-single-strand conformation polymorphism; PCR-RFLP polymerase chain reaction-restriction fragment length polymorphism; PCR-LDR polymerase chain reaction-ligation detection reaction method; CHD coronary heart disease; MI myocardial infarction; CAD coronary artery disease; CVD cardiovascular disease; PAOD peripheral artery occlusive disease; CHD coronary heart disease.

Table 2. The ORs and 95% CI in PLA2 gene polymorphism and risk of clinical atherosclerosis under additive genetic model

Allele contrast in additive model	OR (95% CI)	P value for Z test	I² for heterogeneity (%)	P value for heterogeneity	P value for Egger's test
V279F					
F allele vs V allele	0.88 (0.81, 0.95)	0.0007*	40	0.08	0.741
A379V					
V allele vs A allele	1.08 (0.93, 1.26)	0.31	78	< 0.0001*	0.038*
R92H					
H allele vs R allele	1.29 (1.09, 1.53)	0.003*	73	0.002*	0.007*
I198T					
T allele vs I allele	1.12 (0.79, 1.59)	0.53	81	0.001*	0.431
Egger's test for all SNPs					0.094

*P < 0.05.

a significantly inverse association of V279F polymorphism with clinical atherosclerosis with pool estimated OR of 0.88 (95% CI: 0.81-0.95; P = 0.0007), and with a statistically insignificant heterogeneity across the studies (I^2 = 40%; P = 0.08) (**Figure 3** and **Table 2**). Among controls, the prevalence of F-allele was obtained in 10.39% in Asian but none in Turkish.

Interestingly, Turkish population was particularly younger (46.2 ± 6.1 years old) than the other Asian/Oriental subjects. Genotype frequency among control group had not deviated from Hardy-Weinberg Equilibrium (P = 0.08) and all populations were in Hardy-Weinberg Equilibrium except three studies [24, 28, 30]

(**Table 2**). When we did a sub-group analysis of Asian/Oriental population there was still consistently an inverse association of V279F polymorphism with clinical atherosclerosis with pool OR of 0.88 (95% CI: 0.81-0.94; P = 0.0005) (**Figure 2**).

Association of A379V polymorphism with clinical atherosclerosis

Nine studies with 7,703 cases and 4,746 controls were recruited in the A379V-clinical atherosclerosis analysis. Only 3 studies examined Caucasian descent, and the remaining studies recruited Asian. Thus, the Caucasian subjects comprised 50.24% of the population in this
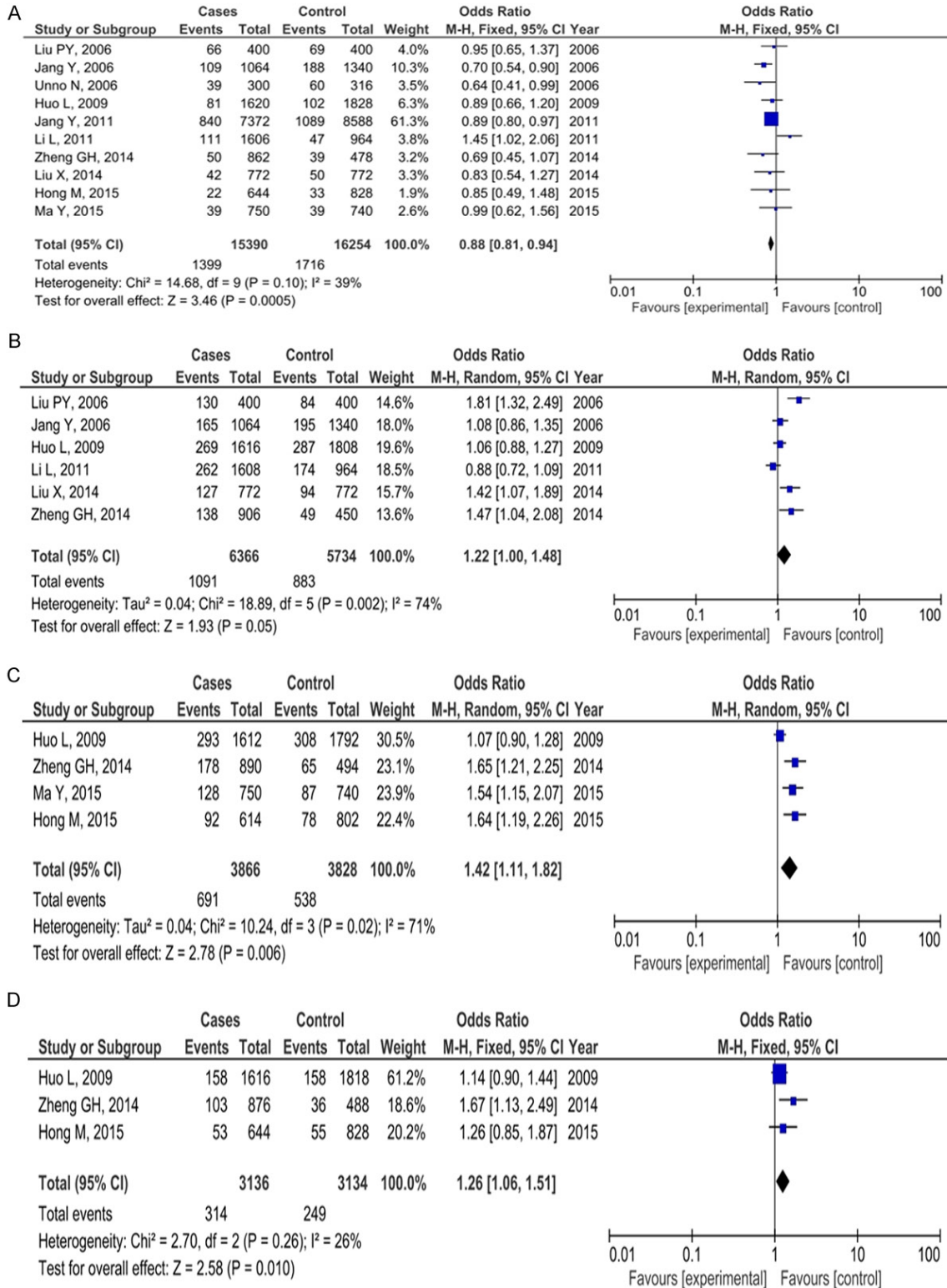
Figure 2. (A) V279F polymorphism of PLA2G7 gene in Asian population (Chinese, Japanese, and Koreans). (B) A379V polymorphism of PLA2G7 gene in Asian population (Chinese, Japanese, and Koreans). (C) R92H polymorphism of PLA2G7 gene in Asian population (Chinese, Japanese, and Koreans). (D) I198T polymorphism of PLA2G7 gene in Asian population (Chinese, Japanese, and Koreans). Forest plot of association between single nucleotide polymorphisms of PLA2G7 gene in Asian population, (A) V279F, (B) A379V, (C) R92H, (D) I198T and clinical atherosclerosis (additive model). The *p* values for the association between V279F, A379V, R92H, I198T and clinical atherosclerosis in Asian population were 0.0005, 0.05, 0.006, and 0.010, respectively.
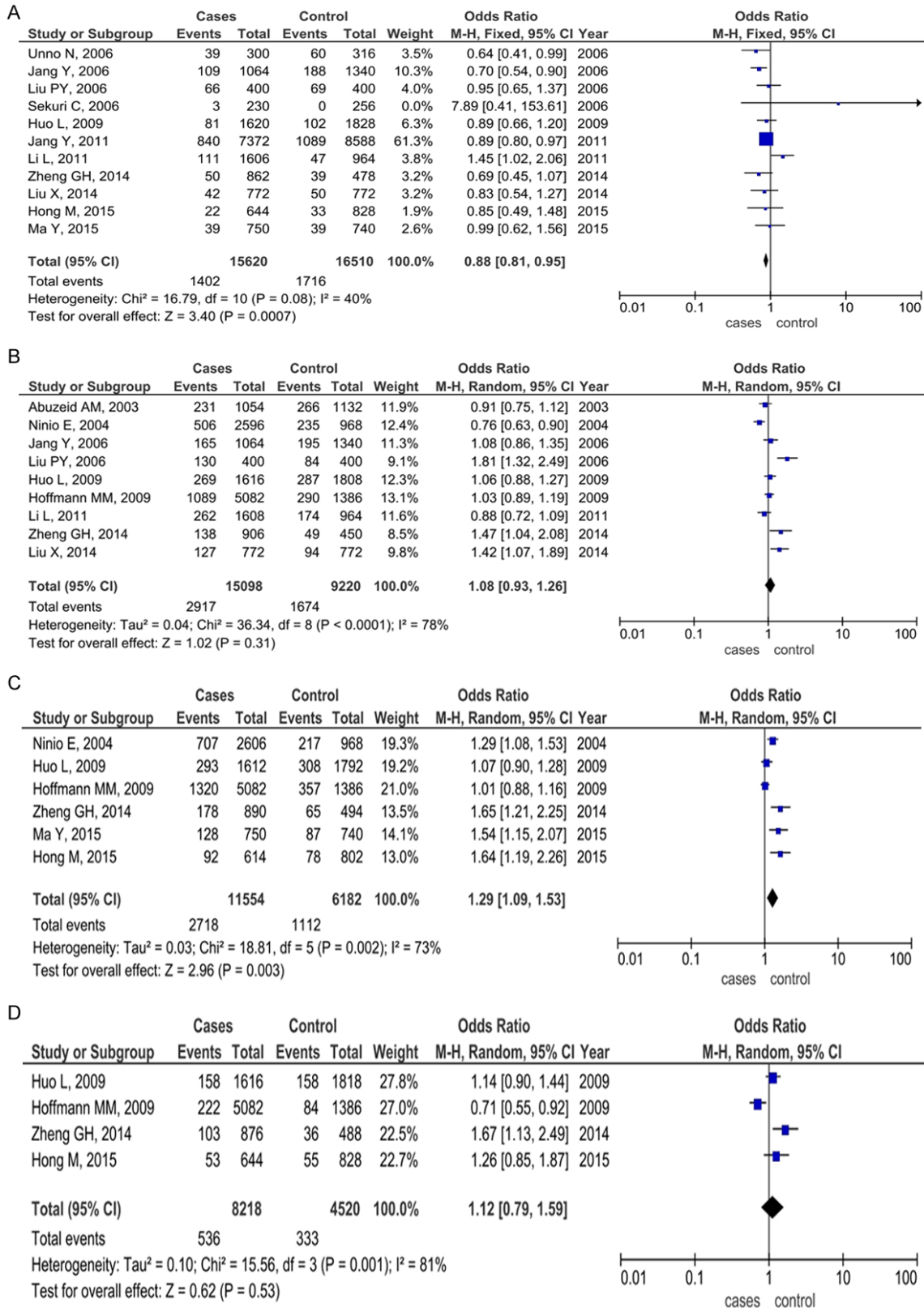
Figure 3. (A) V279F polymorphism of PLA2G7 gene in Asian and Caucasian population. (B) A379V polymorphism of PLA2G7 gene in Asian and Caucasian population. (C) R92H polymorphism of PLA2G7 gene in Asian and Caucasian population. (D) I198T polymorphism of PLA2G7 gene in Asian and Caucasian population. Forest plot of association for single nucleotide polymorphisms of PLA2G7 gene in Asian and Caucasian population, (A) V279F, (B) A379V,

(C) R92H, (D) I198T and clinical atherosclerosis (additive model). The *p* values for the association between V279F, A379V, R92H, I198T and clinical atherosclerosis in all population were 0.0007, 0.31, 0.003, and 0.53, respectively.

sub-analysis. The prevalence of V-allele among genotyped controls was 18.15%. We found no significant association between V-allele compared with A-allele (OR = 1.08; 95% CI = 0.93-1.26; *P* = 0.31) with clinical atherosclerosis (**Figure 3**), and with a statistically significant heterogeneity across the studies (I^2 = 78%; *P* < 0.0001). Observed genotype frequency among control group was significantly different from the expected frequency by HWE (*P* < 0.0001) (**Table 2**). On sub-group analysis applied only in Asian/Oriental population, it consistently presented no significant association between V-allele to A-allele (OR = 1.22; 95% CI = 1.00-1.48) to atherosclerosis (**Figure 2**).

Association of R92H polymorphism with clinical atherosclerosis

There were only 6 studies with 5,949 cases and 3,223 controls available. Only 2 studies had recruited Caucasian subjects, consisted of 56.6% of the all subjects. There was a significantly positive association of R92H polymorphism with clinical atherosclerosis (OR = 1.29; 95% CI: 1.09-1.53; *P* = 0.003) (**Figure 3**); however, we found that the 92 H allele was weakly associated with increased risk of clinical atherosclerosis. A significant heterogeneity across the studies was noted with I^2 = 73%; *P* = 0.002 (**Table 2**). Among controls, the observed frequency of the H-allele was more prevalent in Caucasian (9.3%) compared with Asian (8.7%). There was a more reliable significant association between H-allele toward clinical atherosclerosis with OR of 1.42 (95% CI; 1.11-1.82; *P* = 0.006) in sub-group analysis carried out in Asian/Oriental population (**Figure 2**).

Association of I198T polymorphism with clinical atherosclerosis

Only 4 studies with 4,260 cases and 2,368 controls were available. Although the Caucasian subjects were only assembled in 1 study, but their proportion reached 50.7% compared to Asian. However, we obtained no significant association between T-allele compared with I-allele (OR = 1.12; 95% CI = 0.79-1.59; *P* = 0.53) to atherosclerosis (**Figure 3**). And a significant heterogeneity across the studies was noted as well, with I^2 = 81%; *P* = 0.001 (**Table 2**). When the sub-group analysis was done in Asian/Oriental population, interestingly there was a weak positive association between T-allele and clinical atherosclerosis (OR = 1.26; 95% CI = 1.06-1.51; *P* = 0.01) (**Figure 2**).

In term of publication bias estimating, we did not observe any visual asymmetry, and according to Egger's test results was insignificant (*P* = 0.741 and *P* = 0.43 successively) particularly in V279 polymorphism and I198T polymorphism groups. But in the remaining groups, there were still evidences of publication bias (**Table 2**). Overall for the whole SNP's we did not find any publication bias (*P* = 0.094 in Egger's test).

Discussion

In the absence of very large individual studies and limited ethnicity involved, however we had carried out this updated meta-analysis for clarifying the associations between PLA2G7 polymorphism and clinical atherosclerosis. Diagnostic criteria of clinical atherosclerosis are based on 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults [18]. Clinical atherosclerosis or clinical ASCVD (atherosclerotic cardiovascular disease) is defined by using the inclusion criteria for the secondary prevention statin RCTs (acute coronary syndromes, or a history of myocardial infarction, stable or unstable angina, coronary or other arterial revascularization, stroke, TIA, or peripheral arterial disease presumed to be of atherosclerotic origin). This guideline highly recommended the clinical treatment of blood cholesterol levels to reduce ASCVD risk using the highest levels of evidence, namely: randomized controlled trials (RCTs) and systematic reviews and meta-analysis of RCTs. Accordingly the definition of clinical outcomes and recommendations are very robust [18].

The largest sample size available in this individual study was only 8,125 subjects. And the only available ethnicity recruited within the present study was as the following: Asian/Oriental, Caucasian and Turkish population. The studies about PLA2G7 polymorphism had been emerging since year 2000. To date, only very

few studies have investigated the relationship between PLA2G7 polymorphism and clinical atherosclerosis. Thus, we chose a wider time frame than the previous meta-analysis, with a range of 16 years, in order to have more eligible studies included in the present study.

In this study, R92H polymorphism yielded a significant positive association with clinical atherosclerosis, and with a strong evidence of heterogeneity across the available studies. Interestingly, the positive association of R92H polymorphism occurred only in the Chinese population, not in the Caucasian. This association had been translated into increased levels of plasma Lp-PLA2 (gain of function). Likewise, R92H variants in PCOS (polycystic ovary syndrome) subjects in China were related to increased plasma Lp-PLA2 and changes in plasma lipoprotein levels, insulin resistance, aging and weight gain, possibly increased the future risk of CVD [33]. Accordingly, the activity and mass of plasma Lp-PLA2 were heritable traits. For Lp-PLA2 mass, there were 12 SNPs achieved genome-wide significance. All was clustering in a region on chromosome 6p12.3 closed to PLA2G7 gene [34]. Additionally, Lp-PLA2 activity and mass presented the continuous association with risk of coronary heart disease, in parallel with the magnitude to that with plasma non-HDL cholesterol and systolic blood pressure. Anyway, an association of Lp-PLA2 mass and activity was not exclusive to vascular outcomes, this association depends partly on lipids role [35]. It is corroborating the joint effects of both predictors.

Similarly, there was a significant negative association between V279F polymorphism with clinical atherosclerosis, without any heterogeneity across the studies. A rare non-synonymous polymorphism (V279F) was only encountered in Japanese, Turk, Kyrgyz and Azerbaijan population [36, 37]. These were associated with a decreased enzymatic level of Lp-PLA2 in heterozygous and complete loss of enzymatic levels in homozygous subjects [37, 38]. Loss-of-function mutations in the PLA2G7 gene, which are common in this East Asian population, consequently this effectively eliminates Lp-PLA2 activity [37]. Consequently, lifelong lower Lp-PLA2 activity in this population had been proven not associated with major risk of clinical atherosclerosis [38]. This considerable evidence had emphasized that V279F po-

lymorphism in Asian subjects acting as a protective factor for clinical atherosclerosis.

Since known Lp-PLA2 genotype that is also fairly common in European subjects have quite weak effects on Lp-PLA2 activity; their study really would need very large number of patients recruited to prove the association [26]. These above associations were undoubtedly considered to be robust, because when we did the sensitivity analysis, by analyzing only Asian/Oriental population and then the results were still consistent. Let alone, the OR of the Asian/Oriental subjects increased comparing to the whole group (ORs = 1.29 → ORs = 1.42).

On the other hand, in Indonesian V279F genetic variants associated with acute myocardial infarction (MI) risk, shown by the GG genotype of 2.9 times greater risk of acute MI compared to GT/TT genotype (279Phe) [40]. Given the small sample size, this study could not be representing the population. This could be inferred that R92H polymorphism is considered as an emerging risk predictor for clinical atherosclerosis, and V279F variants as a protective factor.

Respectively, the previous meta-analysis [41] revealed that the same result for the R92H variant with CHD, they indicated R92H allele had probably increased the risk of CHD, while the hypothesized effects of A379V and V279F polymorphisms on CHD could not be confirmed in that previous study. Our study suggested that there was no association between A379V and I198T polymorphisms with clinical atherosclerosis as well. Otherwise, there was an inverse significant association between V279F variants with clinical atherosclerosis and regarded as a protective factor in our study with OR of 0.88 (95% CI: 0.81-0.95). The more subjects employed in our meta-analysis compared to the previous one likely explain the significant association of V279F with clinical atherosclerosis.

Thus this present study was affirming the dual effect of Lp-PLA2 levels on atherosclerosis compared to the previous studies. Though we believe that the results of this meta-analysis supposed to be robust, but we could not comprehensively measure the combined effects of genetics and CVD risk factors. There were still evidences of significant heterogeneity

among the studies, additionally publication bias might slightly disturb this meta-analysis. Nevertheless, our study had a greater external validity than the previous meta-analysis because of larger sample size and wider time frame, and the internal validity was appropriately achieved basically after accomplishing the sensitivity analysis.

The limitations of this study were as the following; *first*, this meta-analysis only included a limited number of the eligible studies, particularly available ethnicity. Second, we were not able to get the raw data from each individual study; consequently, there is no meta-regression analysis done.

In conclusion, this study revealed that R92H polymorphism in PLA2G7 gene associated with increased risk of clinical atherosclerosis and also suggested that V279F polymorphism has a protective risk of clinical atherosclerosis.

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Disclosure of conflict of interest

None.

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