PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Two polymorphisms in the fracalkine receptor CX3CR1 gene influence the development of atherosclerosis: a meta-analysis	Title
ABSTRACT			
Structured summary	2	Background: The associations between the Fractalkine receptor (CX3CR1) gene T280M (rs3732378) and V249I (rs3732379) polymorphisms and atherosclerosis (AS) risk are conflicting. The aim of this meta-analysis was undertaken to assess their associations. Methods: PubMed, Embase, Web of Science, Medline, Cochrane database and CNKI were searched to get the genetic association studies. All statistical analyses were done with Stata 11.0. Results: Twenty-five articles involving 49 studies were included in the final meta-analysis. The analysis showed that the 280M allele carriers of the CX3CR1 T280M polymorphism decreased the risk of AS and coronary artery disease (CAD) in the heterozygous state, but increased the risk of ischemic cerebrovascular disease (ICVD) in the homozygote state. The 249I allele carriers of the CX3CR1 V249I polymorphism decreased the risk of AS and CAD in the heterozygous state. The V249I-T280M combined genotype VITM and IITM also decreased the risk of AS. Conclusions: The present meta-analysis suggests that the CX3CR1 T280M and V249I polymorphisms are associated with the susceptibility to AS. However, the results should be interpreted with caution because of the high heterogeneity in the meta-analysis.	Abstract
INTRODUCTION			
Rationale	3	It has recently been accepted that AS is predominantly an inflammatory process [32, 33], and begins with a fatty streak, which is made up almost entirely of monocyte-derived macrophages [33, 34]. During the process of vascular inflammation, chemokines and adhesive molecules exert a vital role via mediating the activation of inflammatory cells and their aggregation or adhesion to vascular walls [35, 36]. FKN is a special chemotactic factor existing in both membrane-bound and soluble form [37], the expression of FKN and its receptor CX3CR1 is upregulated in AS lesions [38-40], and the severity of AS is greatly improved by inhibiting their expression [40-42], suggesting that the FKN/CX3CR1 is closely correlated to AS. Given the crucial role of CX3CR1 in the inflammatory process, the mutations in the CX3CR1 may also play a significant role in the development of atherosclerotic diseases. Recently, a number of molecular epidemiological studies have been done to evaluate the associations between the CX3CR1 gene polymorphisms (T280M and V249I) and the risk of atherosclerotic diseases [6-31]. However, the results of different studies are inconsistent, possibly due to small sample sizes in the individual studies.	Introduction
Objectives	4	In 2009, Apostolakis et al. [43] performed a meta-analysis to evaluate the association between the CX3CR1T280M and V249I polymorphisms and CAD, and demonstrated that the CX3CR1280M allele was associated with a reduced risk of CAD in the heterozygous state and 249I-280M haplotype was atheroprotective effect on CAD. However, they just studied the 280M allele and 249I-280M haplotype of CX3CR1in Caucasians. Considering the meta-analysis only focused on the association of the CX3CR1 polymorphism with the single atherosclerotic disease, we therefore performed this meta-analysis of all the studies available now to get a more precise estimation of the associations between the CX3CR1 T280M (rs3732378) and V249I (rs3732379) polymorphisms and overall AS risk.	Introduction
METHODS			

PRISMA 2009 Checklist

Protocol and registration	5	No protocol and registration.	
Eligibility criteria	6	To be included in the present meta-analysis, the studies had to comply with the following major criteria: (1) case-control or cohort studies evaluating the associations between the CX3CR1 T280M and V249I polymorphisms and AS risk; (2) published studies with full text articles; (3) sufficient published data for calculating odds ratios (ORs) with their 95% confidence intervals (CIs); (4) study population were adults; (5) The diagnosis of ischemic heart disease (CAD and AMI) is accorded with the result of coronary angiography, criteria of World Health Organization (WHO), criteria of European Society of Cardiology (ESC), or criteria of American College of Cardiology/American Heart Association (ACC/AHA); the diagnosis of ICVD is accorded with result of computed tomography (CT) or magnetic resonance imaging (MRI); the diagnosis of carotid atherosclerosis (CAA) was assessed by ultrasound color Doppler (USCD), and peripheral arterial disease (PAD) was diagnosed by the following criteria: clinical symptoms of PAD (intermittent claudication, rest pain, or gangrene) accompanied by an ankle-brachial-index and significant stenosis of leg arteries confirmed by FCDS and/or angiography. Studies were excluded if they were: (1) Review or meta-analysis; (2) Not conducted in humans; (3) Duplicate studies.	Inclusion and exclusion criteria
Information sources	7	Eligible literatures published before the end of March 2014 were identified by the search of PubMed, Embase, Web of Science, Cochrane database and CNKI. In addition, all references cited were reviewed to identify additional studies.	Literature search and selection
Search	8	Library using combinations of the following keywords: ("chemokine receptor 1" OR "FKN" OR "CX3CR1" OR "fractalkine") AND ("polymorphism" OR "mutation" OR "variant" OR "variation" OR "genotype") AND ("coronary artery disease" OR "CAD" OR "coronary heart disease" OR "CHD" OR "myocardial infarction" OR "MI" OR "ischemic cardiovascular disease" OR "ischemic cardiovascular events" OR "ischemic stroke" OR "IS" OR "cerebrovascular disease" OR "ischemic cerebrovascular events" OR "cerebral infarction" OR "cerebral ischemia" OR "brain infarction" OR "carotid artery stenosis" OR "CAAD" OR "transient ischemic attack" OR "TIA" OR "peripheral Arterial Disease" OR "PAD" OR "peripheral artery occlusive disease" OR "PAOD" OR "renal artery stenosis" OR "RAS" OR "retinal artery occlusion" OR "RAO" OR vaortic aneurysm" OR "atherosclerosis"). In addition, all references mentioned in the identified original articles were reviewed by hand-searching in order to investigate additional literature that was not indexed.	Literature search and selection
Study selection	9	The present study met the PRISMA statements and PRISMA flow chart (Checklist S1 and Figure 1). A total of 667 articles were identified after searching. After careful review, 26 articles involving 49 studies (24 studies for T280M and 25 studies for V249I polymorphisms) met the inclusion criteria and were selected in this meta-analysis [6-31], one duplicate study was cut [18]. For the CX3CR1 T280M polymorphism, 7732 AS cases and 5905 controls were included to assess the association between the variant and AS risk [6-12, 15-19, 21-28, 30, 31]. For the CX3CR1 V249I polymorphism, 7952 AS cases and 6035 controls were included to assess the association between the variant and AS risk [6-12, 15-24, 26-31]. Main characteristics of the included studies were listed in Tables 1 and 2. The most commonly atherosclerotic disease included in the present meta-analysis was CAD in 21 studies. In addition, there were 8 studies involving carotid atherosclerosis, 8 studies involving cerebral infarction, and 6 study involving AMI, 2 studies involving ICVD, 2 studies involving ischemic stroke (IS) and 2 studies involving PAD. There were 24 studies of Asians, 25 studies of Caucasians. Three studies did not follow the HWE [8, 23, 26]. The results of GRADE were shown in Table S1.	Study characteristics
Data collection process	10	Data were independently extracted from original publications by two reviewers (J Wu and QZ Lin) according to the inclusion criteria listed above. Discrepancy between the reviewers was resolved by consensus or a third reviewer (RX Yin).	Data extraction

Data items	11	Data, including name of the first author, year of publication, study population (country, ethnicity), type of atherosclerotic disease, source of controls (population-based studies and hospital-based studies), sample size (total numbers of cases and controls), and number of genotypes in cases and controls, were extracted from each study.	Data extraction
Risk of bias in individual studies	12	The quality of the individual studies was evaluated and scored by two reviewers independently based on the Newcastle-Ottawa Scale (NOS). Each study was assessed based on three broad perspectives: selection, comparability, and exposure), and each satisfactory answer received one point. The NOS ranges between zero (none of the quality criterion was met) up to nine stars (all the quality criteria were met), and the high-quality study was considered as the one with a score equal to or higher than seven. The third reviewer (RX Yin) examined the results, and a consensus was reached.	Quality assessment for individual studies
Summary measures	13	The principal summary measures are odds ratios(ORs) and 95% confidence intervals (CIs).	Statistical analysis
Synthesis of results	14	For the controls of each study, Hardy-Weinberg equilibrium (HWE) was assessed using the chi-square test ($P < 0.05$ was considered significant deviation from HWE). We performed a haplotype analysis based on the genotype data, the haplotype frequencies were calculated by CubeX analysis software for each study separately and for the whole sample [47]. The strength of associations between the CX3CR1T280M and V249I polymorphisms and AS risk was assessed by ORs with 95% CIs. The pooled ORs were performed for dominant model (T/M+M/M vs. T/T for T280M; V/I+I/I vs. V/V for V249I), recessive model (M/M vs. T/T+T/M for T280M; I/I vs. V/V+V/I for V249I), co-dominant model (T/M vs. T/T + M/M for T280M; V/I vs. V/V+I/I for V249I), additive model (M/M vs. T/T for T280M; I/I vs. V/V for V249I), and allelic model (M allele vs. T allele for T280M; I allele vs. V allele for V249I). Bonferroni correction was used to control for the multiple testing in view of five genetic models under investigation (significance was set at 0.05/5 = 0.01), then other statistical significance was set as $P < 0.05$.	Statistical analysis

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Heterogeneity of the included studies is the most important drawback when analyzing genotyping data, either for the CX3CR1 T280M polymorphism or for the CX3CR1 V249I polymorphism.	Discussion
Additional analyses	16	Subgroup analyses were performed based on ethnicity (Caucasians and Asians), atherosclerotic disease (CAD, CAA, PAD and ICVD) and source of controls (population-based studies and hospital-based studies). Sensitivity analyses were performed by limiting the meta-analysis to studies conforming to HWE and the high quality studies (according to the NOS score).	Statistical analysis
RESULTS			
Study selection	17	The flow diagram of the study selection for this meta-analysis was shown in the Figure 1. A total of 667 articles were identified after searching. After careful review, 26 articles involving 49 studies (24 studies for T280M and 25 studies for V249I polymorphisms) met the inclusion criteria and were selected in this meta-analysis [6-31], one duplicate study was cut [18]. For the CX3CR1 T280M polymorphism, 7732 AS cases and 5905 controls were included to assess the association between the variant and AS risk [6-12, 15-19, 21-28, 30, 31]. For the CX3CR1 V249I polymorphism, 7952 AS cases and 6035 controls were included to assess the association between the variant and AS risk [6-12, 15-24, 26-31]	Study characteristics

Study characteristics	18	Table 1 shows the studies included in the meta-analysis and their main characteristics.	Study characteristics
Risk of bias within studies	19	The NOS results were shown in Table 1.	Study characteristics
Results of individual studies	20	The main results of individual studies were shown in Table 2.	Genotype–phenotype association
Synthesis of results	21	As shown in Table 2, the overall results showed no significant association between the CX3CR1 T280M polymorphism and the susceptibility to AS in five genetic models (P > 0.01 for all). When we performed a subgroup analysis, there was significant association between T280M polymorphism and the susceptibility to AS in the CAD group in dominant model (P < 0.01), co-dominant model (P < 0.01), additive model (P < 0.01) and allelic model (P < 0.01), but not in recessive model (P > 0.01), suggesting that the 280M allele carriers decreased the risk of CAD in the heterozygous state. The significant association between T280M polymorphism and the susceptibility to AS was also found in the ICVD group in recessive model (P < 0.01) and additive model (P < 0.01), but not in dominant model (P > 0.01), co-dominant model (P > 0.01) or allelic model (P > 0.01), suggesting that the 280M allele carriers increased the risk of ICVD in homozygote state. Then we considered the TT genotype as the baseline risk, we found TM genotype was a protective role for AS (OR = 0.81, 95% CI = 0.66-0.99, P = 0.04; Table 3 and Figure 2), subgroup analysis showed that the TM genotype was a protective role for CAD (OR = 0.67, 95% CI = 0.52-0.87, P < 0.01; Table 3 and Figure S1) and MM genotype was a risk factor for ICVD (OR = 2.88, 95% CI = 1.64-5.04, P < 0.001; Table 3 and Figure S2). There was no association between the T280M polymorphism and the susceptibility to AS in the other groups (Tables 2 and 3). The overall results showed no significant association between the CX3CR1 V249I polymorphism and the susceptibility to AS in five genetic models (P > 0.01 for all, Table 2). Subgroup analysis showed significant association between V249I polymorphism and the susceptibility to AS in CAD group in dominant model (P < 0.01), co-dominant model (P < 0.01), additive model (P < 0.01) and allelic model (P < 0.01), but not in recessive model (P > 0.01), suggesting that the 249I allele decreased the risk of CAD in heterozygote state. Significant associations were also found between this variant and the susceptibility to AS in the population-based (PB) group in recessive and additive models (P < 0.01 for each). Then we considered the VV genotype as the baseline risk, we found VI genotype was a protective role for AS (OR = 0.84, 95% CI = 0.72 - 0.98, P = 0.02; Table 3 and Figure 3), subgroup analysis showed that the VI genotype was a protective role for CAD (OR = 0.72, 95% CI = 0.59-0.90, P < 0.01; Table 3 and Figure S3). There was no association between V249I polymorphism and the susceptibility to AS in the other groups (Tables 2 and 3).	Genotype–phenotype association
Risk of bias across studies	22	Begg's funnel plot and Egger's regression test were performed to assess potential publication bias. For the CX3CR1 T280M polymorphism, visual inspection of the funnel plot (Figure 4A) displays symmetrical distribution of OR estimations, suggesting no publication bias. In addition, the results of Egger's regression test also provided evidence for no publication bias (TM vs. TT, P > 0.05 for all genetic models). For the CX3CR1 V249I polymorphism, no obvious asymmetry was observed in any genetic model according to the visual assessment of funnel plot (Figure 4B). The results of Egger's regression test did not provide any statistical evidence for publication bias (VI vs. VV, P > 0.05 for all genetic models).	Publication bias
Additional analysis	23	**Subgroup analyses:** When we performed a subgroup analysis, there was significant association between T280M polymorphism and the susceptibility to AS in the CAD group in dominant model (P < 0.01), co-dominant model (P < 0.01), additive model (P < 0.01) and allelic model (P < 0.01), but not in recessive model (P > 0.01), suggesting that the 280M allele carriers decreased the risk of CAD in the heterozygous	Genotype–phenotype association, Sensitivity analysis, and Heterogeneity

		state. The significant association between T280M polymorphism and the susceptibility to AS was also found in the ICVD group in recessive model (P < 0.01) and additive model (P < 0.01), but not in dominant model (P > 0.01), co-dominant model (P > 0.01) or allelic model (P > 0.01), suggesting that the 280M allele carriers increased the risk of ICVD in homozygote state. Then we considered the TT genotype as the baseline risk, we found TM genotype was a protective role for AS (OR = 0.81, 95% CI = 0.66-0.99, P = 0.04; Table 3 and Figure 2), subgroup analysis showed that the TM genotype was a protective role for CAD (OR = 0.67, 95% CI = 0.52-0.87, P < 0.01; Table 3 and Figure S1) and MM genotype was a risk factor for ICVD (OR = 2.88, 95% CI = 1.64-5.04, P < 0.001; Table 3 and Figure S2). There was no association between the T280M polymorphism and the susceptibility to AS in the other groups (Tables 2 and 3). Subgroup analysis showed significant association between V249I polymorphism and the susceptibility to AS in CAD group in dominant model (P < 0.01), co-dominant model (P < 0.01), additive model (P < 0.01) and allelic model (P < 0.01), but not in recessive model (P > 0.01), suggesting that the 249I allele decreased the risk of CAD in heterozygote state. Significant associations were also found between this variant and the susceptibility to AS in the population-based (PB) group in recessive and additive models (P < 0.01 for each). Then we considered the VV genotype as the baseline risk, we found VI genotype was a protective role for AS (OR = 0.84, 95% CI = 0.72 - 0.98, P = 0.02; Table 3 and Figure 3), subgroup analysis showed that the VI genotype was a protective role for CAD (OR = 0.72, 95% CI = 0.59-0.90, P < 0.01; Table 3 and Figure S3). There was no association between V249I polymorphism and the susceptibility to AS in the other groups (Tables 2 and 3). **Sensitivity analyses:** Sensitivity analyses were conducted to determine whether modification of the inclusion criteria of the meta-analysis affected the final results. The included studies were limited to those conforming to HWE or the high quality studies (NOS score ⩾7), the corresponding pooled ORs were not materially altered, either for the CX3CR1 T280M polymorphism or for V249I polymorphism. This suggested that the overall results of this meta-analysis were statistically robust. The main results of sensitivity analyses are shown in Table 2. **Heterogeneity analysis:** For the both CX3CR1 T280M and V249I polymorphisms, significant heterogeneity existed in the overall comparisons in five genetic models. We performed subgroup analyses based on the ethnic (Caucasians and Asian), atherosclerotic diseases (CAD, PAD, CAA and ICVD) and source of control (hospital-based or population-based), heterogeneity was distinctly reduced, although it was still significant, we could not point out other possible sources of heterogeneity. The data were shown in Table S3. For the V249I-T280M combined polymorphism, when the VVTT genotype was taken as the baseline risk, we found significant heterogeneity in the VITM genotype (PQ < 0.1, I2 = 69.2%). When the 249V-280T haplotype was taken as the baseline risk, we showed obvious heterogeneity in the 249I-280M haplotype (PQ < 0.1, I2 = 82.2%; Table 3).	analysis
DISCUSSION			
Summary of evidence	24	The overall findings showed that there was no association between the CX3CR1 polymorphisms and the risk of AS in five genetic models. To make a more comprehensive analysis, subgroup analyses were performed based on ethnicity, atherosclerotic disease and source of controls. For the CX3CR1 T280M polymorphism, Apostolakis et al. [43] showed that the 280M allele carriers reduced the risk of CAD in heterozygote state. In the present study, we also found atheroprotective effect to AS in the CAD group. In addition, we found that the 280M allele carriers increased the risk of ICVD in homozygote state. For the CX3CR1 V249I polymorphism, significant associations were found between this variant and the atheroprotective effect on AS in CAD and PB groups. These results suggested that the 249I allele carriers reduced the risk of CAD in heterozygote state. The above statistical results were based on Bonferroni correction to control for the	Discussion

		multiple testing in view of under investigation. For the combined genotype, the VITM and IITM genotypes played an atheroprotective effect on AS. The combined VVTM genotype was more common in the cases than in the controls, however, the rarity of the VVTM genotype makes any conclusion rather unsafe. LD analysis indicated a strong association between T280M and V249I, and a protective role of the 249I-280M haplotype was also observed in the control population compared to case subjects. No association was observed between the 249I-280T haplotype and the susceptibility to AS. These results were consistent with those of a previous study [43].	
Limitations	25	**Limitations:** However, some limitations of this meta-analysis should be acknowledged. Firstly, there was significant heterogeneity in this meta-analysis. Heterogeneity may affect the precision of overall results, despite the use of appropriate meta-analytic techniques with random-effects model. Secondly, in the subgroup analyses, the sample sizes in some subgroup, such as the PAD and CAA groups of the CX3CR1 T280M and V249I polymorphisms, were relatively small, not having enough statistical power to explore the real association. Thirdly, AS is a complex disease and involves potential interactions of gene-environment. However, many eligible studies included in this meta-analysis did not consider the environmental factors. Therefore, studies with larger sample sizes and better design are needed.	Discussion
Conclusions	26	**Conclusion：** The present meta-analysis suggested that the CX3CR1 280M and 249I allele carriers had atheroprotective roles on AS in heterozygote state, and the 280M allele carriers were associated with the susceptibility to AS in homozygote state. The combined genotypes of VITM and IITM also had atheroprotective roles on AS. Consequently, this effect may be attributed to the haplotype of 249I-280M. However, the results should be interpreted with caution because of its limitations. Further studies with large sample size, especially with the consideration of gene-gene and gene-environment interactions, should be needed to confirm our findings.	Discussion
FUNDING			
Funding	27	This study was supported by the National Natural Science Foundation of China (No: 30960130)	Acknowledgements

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Table S1 GRADE profile evidence of the included studies.

Position	Category	No. of studies	Quality assessment					Quality	Importance
			Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias		
T280M	Dominant model	24	Serious[1]	Very serious[2]	No	No	Undetected	⊕○○○ (Very low)	Critical
	Recessive model	22	Serious[1]	No	No	No	Undetected	⊕⊕⊕○ (Moderate)	Critical
	Co-dominant model	24	Serious[1]	Serious[2]	No	No	Undetected	⊕⊕○○ (Low)	Critical
	Additive model	22	Serious[1]	No	No	No	Undetected	⊕⊕⊕○ (Moderate)	Critical
	Allelic model	24	Serious[1]	Very serious[2]	No	No	Undetected	⊕○○○ (Very low)	Critical
	TM vs. TT	24	Serious[1]	Serious[2]	No	No	Undetected	⊕⊕○○ (Low)	Critical
V249I	Dominant model	25	Serious[1]	Serious[2]	No	No	Undetected	⊕⊕○○ (Low)	Critical
	Recessive model	24	Serious[1]	No	No	No	Undetected	⊕⊕⊕○ (Moderate)	Critical
	Co-dominant model	25	Serious[1]	No	No	No	Undetected	⊕⊕⊕○ (Moderate)	Critical
	Additive model	24	Serious[1]	No	No	No	Undetected	⊕⊕⊕○ (Moderate)	Critical
	Allelic model	25	Serious[1]	Very serious[2]	No	No	Undetected	⊕○○○ (Very low)	Critical
	VI vs. VV	25	Serious[1]	Serious[2]	No	No	Undetected	⊕⊕○○ (Low)	Critical

[1] Evidence limited by study design and implementation (observational study).

[2] High heterogeneity existed in the comparison.

Table S2 Genotype distribution in cases and controls.

First author	SS(case /control)	Cases (n)									Controls (n)								
		VVTT	VVTM	VVMM	VITT	VITM	VIMM	IITT	IITM	IIMM	VVTT	VVTM	VVMM	VITT	VITM	VIMM	IITT	IITM	IIMM
McDermott	197/142	122	0	0	28	36	0	1	3	7	70	0	0	22	38	0	1	4	7
Moatti	151/249	97	0	0	24	23	0	2	2	3	126	0	0	51	53	0	2	12	5
Gugl	492/503	261	0	0	69	129	0	3	14	16	268	0	0	56	154	0	2	11	12
McDermott	204/1655	105	0	0	44	44	0	3	4	4	794	0	0	285	450	0	23	52	51
Ghilardi	108/204	63	0	0	20	14	0	4	4	3	104	0	0	36	52	0	4	6	2
Hattori	235/306	207	10	0	3	11	2	0	0	2	277	3	1	0	21	0	0	2	2
Niessner	720/432	390	2	0	114	160	0	9	28	17	245	0	0	73	89	0	1	11	12
Apostolakis	210/165	111	0	0	42	39	0	6	9	3	86	0	0	22	37	0	1	16	3
An	108/80	72	0	0	18	16	0	0	0	2	41	0	0	16	17	0	0	4	2
Nassar-a	149/149	69	4	0	25	38	0	3	7	3	63	0	0	25	44	0	6	4	7
Nassar-b	150/149	53	5	0	37	38	2	2	6	7	63	0	0	25	44	0	6	4	7
Zhao	318/292	194	0	0	29	37	0	23	19	16	164	0	0	25	40	0	12	30	21
Singh-a	152/300	93	18	0	31	6	0	2	0	2	150	0	0	32	86	0	9	13	10
Singh-b	156/300	86	10	0	32	14	2	6	4	2	150	0	0	32	86	0	9	13	10
total	3350/4925	1923	49	0	516	605	6	64	100	87	2601	3	1	700	1211	0	76	182	151

Table S3 Heterogeneity test of *CX3CR1* T280M and V249I polymorphisms and risk of AS in each subgroup.

	Dominant model		**Recessive model**		**Co-dominant model**		**Additive model**		**Allelic model**	
Position	I^2 **(%)**	P_Q	I^2 **(%)**	P_Q	I^2 **(%)**	P_Q	I^2 **(%)**	P_Q	I^2 **(%)**	P_Q
Overall analysis										
T280M	78.8	< 0.1	21.5	0.18	75.2	< 0.1	31.9	< 0.1	79.2	< 0.1
V249I	74.1	< 0.1	53.3	< 0.1	58.0	< 0.1	61.6	< 0.1	78.3	< 0.1
Subgroup analysis based on ethnicity										
T280M (C)	56.9	< 0.1	18.3	0.26	56.4	< 0.1	20.0	0.25	54.1	< 0.1
T280M (A)	86.5	< 0.1	31.1	0.16	82.9	< 0.1	47.0	< 0.1	87.1	< 0.1
V249I (C)	33.9	0.11	55.6	< 0.1	0	0.52	60.0	< 0.1	53.9	< 0.1
V249I (A)	83.9	< 0.1	51.0	< 0.1	74.2	< 0.1	62.0	< 0.1	85.5	< 0.1
Subgroup analysis based on type of diseases										
T280M (CAD)	71.8	< 0.1	0	0.97	71.8	< 0.1	0	0.92	67.2	< 0.1
T280M (PAD)	-	-	-	-			-	-	-	-
T280M (CAA)	77.5	< 0.1	0	0.42	73.9	< 0.1	14.1	0.32	76.2	< 0.1
T280M (ICVD)	77.0	< 0.1	0	0.99	76.1	< 0.1	0	0.98	77.2	< 0.1
V249I (CAD)	75.4	< 0.1	41.4	< 0.1	58.1	< 0.1	59.3	< 0.1	78.6	< 0.1
V249I (PAD)	-	-	-	-			-	-	-	-
V249I (CAA)	0	0.97	38.3	0.18	0	0.74	24.8	0.26	0	0.66
V249I (ICVD)	79.9	< 0.1	37.2	0.17	76.3	< 0.1	51.9	< 0.1	81.0	< 0.1
Subgroup analysis based on source of controls										

T280M (HB)	68.9	< 0.1	23.4	0.21	58.1	< 0.1	32.1	0.13	72.7	< 0.1
T280M (PB)	83.2	< 0.1	0	0.67	82.5	< 0.1	0	0.58	81.5	< 0.1
V249I (HB)	46.3	< 0.1	43.2	< 0.1	0	0.46	52.4	< 0.1	60.1	< 0.1
V249I (PB)	83.1	< 0.1	14.5	0.31	75.7	< 0.1	41.3	< 0.1	83.9	< 0.1

$P_{Q,}$ *P* value for heterogeneity test. A: Asians; C: Caucasians; CAD: coronary artery disease; CAA: carotid artery atherosclerosis; ICVD: Ischemic cerebrovascular disease; PAD: peripheral arterial disease; PB: population-based; HB: hospital-based.