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## The 9p21 Myocardial Infarction risk allele increases risk of Peripheral Artery Disease in older people:

Cluett – 9p21 alleles and Peripheral Artery Disease

Christie Cluett, MSc, Mary McGrae McDermott, MD, Jack Guralnik, MD, PHD, Luigi Ferrucci, MD, PHD, Stefania Bandinelli, MD, Iva Miljkovic, MD, PHD, Joseph M Zmuda, PHD, Rongling Li, MD, PHD, Greg Tranah, PHD, Tamara Harris, MD, MS, Neil Rice, MSc, William Henley, PHD, Timothy M Frayling, PHD, Anna Murray, PHD, and David Melzer, PHD

From Peninsula Medical School, Exeter, UK (CC, NR, WH, TMF, AM, DM); Department of Medicine, Northwestern University's Feinberg School of Medicine, Chicago, USA (MMM); National Institute on Aging/NIH, Baltimore, USA (DM); Longitudinal Studies Section, Clinical Research Branch, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, USA (LF); Geriatric Unit, Azienda Sanitaria di Firenze, Florence, Italy (SB); Department of Epidemiology, Graduate School of Public Health University of Pittsburgh, Pittsburgh, USA (IM, JMZ); Department of Preventive Medicine and Center for Genomics and Bioinformatics, College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, USA (RL); San Francisco Coordinating Center, California Pacific Medical Center Research Institute, San Francisco, California, USA (GT); Laboratory of Epidemiology, Demography and Biometry, National Institute on Aging, Bethesda, Maryland, USA (JG, TH).

### Abstract

**Background**—A common variant at chromosome 9p21 (tagged by the rs1333049 or rs10757278 SNP) is strongly associated with Myocardial Infarction (MI) and major arterial aneurysms. An association with Peripheral Arterial Disease (PAD) was also reported in a sample aged <75 years, but this disappeared on removal of respondents with a MI history, resulting in an odds ratio for PAD of 1.09 ( $p=0.075$ ). We aimed to estimate the association of this variant with Ankle Brachial Index (ABI) and PAD in three older populations.

**Methods and Results**—We used data from the InCHIANTI, Baltimore Longitudinal Study of Aging and Health, Aging and Body Composition studies. In 2,630 Caucasian individuals (mean age 76.4 years) the C allele at rs1333049 was associated with lower mean ABI measures and with increased prevalence of PAD. These associations remained after removal of baseline and incident MI cases over a 6 year follow-up for both ABI ( $-0.017$  ABI units, 95% CI:  $-0.03$ – $-0.01$ ,  $p=1.3 \times 10^{-4}$ ) and PAD (per allele OR: 1.29, 95% CI: 1.06–1.56,  $p=0.012$ ). These associations also remained after adjustment for known atherosclerosis risk factors including Diabetes Mellitus, smoking, hypercholesterolemia and hypertension.

**Conclusions**—The C allele at rs1333049 is associated with an increased prevalence of Peripheral Arterial Disease and lower mean Ankle Brachial Index. This association was independent of the presence of diagnosed MI and atherosclerotic risk factors in 3 older Caucasian populations.

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**Address for Correspondence:** Prof David Melzer, Epidemiology and Public Health Group, Peninsula Medical School, RD&E Wonford Site, Barrack Road, Exeter, Devon. EX2 5DW, United Kingdom. Tel (Sec) (44) 1392 406753. Fax (44) 1392 406767, david.melzer@pms.ac.uk.

**Conflict of Interest Disclosures**

None

## Keywords

Genetics; Myocardial Infarction; Peripheral Vascular Disease; 9p21; CDKN2a/2b

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## INTRODUCTION

Recent genome wide association studies and subsequent replication studies have found consistent associations between a common variant on chromosome 9p21 and Myocardial Infarction (MI)<sup>1</sup> or Coronary Artery Disease (CAD)<sup>2–5</sup>. The nearest genes to this marker are *CDKN2b* and *CDKN2a*, which are key regulators of the cell cycle. The possible involvement of these cell cycle control genes in heart disease may be mediated via reduced re-growth of arterial intimal cells<sup>6</sup>, a phenomenon implicated in the development of atherosclerosis. Three independent genome wide scans have demonstrated that the variant associated with MI is best captured by the SNP rs1333049<sup>2, 4</sup> or its proxy rs10757278<sup>1</sup> (which are in perfect linkage disequilibrium,  $r^2=1$  in HapmapII). Rs10757278 has also been shown to be associated with abdominal aortic aneurysms and intracranial aneurysms, independent of the validated effect on MI<sup>6</sup>. However, similar associations with Peripheral Artery Disease (PAD) and cardiogenic stroke were no longer significant when subjects with known CAD were removed, in a study population with age at onset before 70 for males and before 75 for females.

Although a number of atherosclerotic risk factors contribute to PAD and a lower Ankle Brachial Index (ABI) measure, genetic factors contribute 21–48% of the variability of both the continuous ABI level and presence of PAD, defined using an ABI threshold<sup>7, 8</sup>. Low ABI (the ratio of systolic blood pressure at the ankle divided by that at the brachial artery) is a marker of peripheral arterial narrowing and is used in the definition of PAD. Decreased ABI is a well-characterized predictor of increased cardiovascular events and all-cause mortality<sup>9–11</sup>. In addition, ABI values across the entire range<sup>10, 12</sup> including those above the typical PAD threshold of 0.90<sup>13</sup> are implicated in a graded inverse correlation with risk of coronary heart disease, stroke and pre-clinical atherosclerosis, suggesting that inclusion of both measures is relevant for the examination of lower extremity peripheral arterial disease<sup>14</sup>.

In this study, we aimed to further examine the association of the 9p21 MI SNP (rs1333049) with peripheral arterial disease in older adults using three independent community-dwelling study populations, while accounting for the association of this SNP with MI.

## METHODS

### Study Populations

The InCHIANTI study is a population based longitudinal study designed to investigate the causes of decline in mobility in older subjects<sup>15</sup>. The sample is representative of the population of two small towns from the Chianti region in Tuscany, Italy. The study includes 1,453 individuals of white European descent ranging from 20–102 years of age at baseline, when blood samples were taken. Follow-up measures were collected during two waves at three year intervals from baseline interview. For our investigation of associations with ABI and PAD, only subjects 65 years or older at baseline were included in analyses. Of the 1,453 samples in the cohort, 1,155 were  $\geq 65$  years and of these 804 have genotype and phenotype information available.

The Health, Aging and Body Composition study (Health ABC) is a longitudinal cohort study designed to investigate factors contributing to changes in body composition in old age which contribute to disease and disability. 3,075 black and white US subjects, aged 70–79 at baseline, who reported no difficulty in daily activity related mobility at baseline were sampled. Follow-

up measures were collected annually for seven years though ABI measurements were taken at year one and year four only. The sample for Health ABC was selected to have a substantial number of blacks (42%). Large differences between the allele frequencies at rs1333049 have been reported for different ethnic populations (<http://www.HapMap.org/>). Within Health ABC those reporting their ethnicity as ‘white’ or ‘black’ had a frequency for the C allele at the variant of 0.49 and 0.26 respectively. Therefore the sample contributing to the meta-analysis was restricted to those 1,780 of European decent, of which 1,569 participants have phenotypic and genotypic information available, while data for those 1,266 of ‘black’ ethnicity, of which 1,070 have genotypes and phenotypes, were analysed separately.

The Baltimore Longitudinal Study of Aging (BLSA) is a longitudinal study designed as a scientific study of aging. Of the more than 1,400 participants, genotype information is available for 1,230 participants, aged 17–92. The ethnicity of each sample was assessed against the Hapmap populations, using the first two principle components from an EIGENSTRAT<sup>16</sup> analysis. A strong CEU (CEPH – Utah residents with ancestry from northern and western Europe <http://www.HapMap.org>) subset of the population was selected and following a sex-check using the X-chromosome homozygous data to validate the reported gender, 858 samples were available for use within the analysis. 372 of these remaining participants had ABI measured, of which 257 were 65 years or older.

### Phenotypic Measures

The ABI was measured to investigate an association with arterial disease in the lower extremities. ABI was defined as the ratio of systolic blood pressure in the ankle to systolic blood pressure in the arm. In InCHIANTI systolic blood pressure was measured with a hand-held Doppler stethoscope (Parks Electronics model 41-A, Aloha, OR) in both posterior tibial arteries and the right brachial artery. The highest pressure at each set was used to calculate the index and the final ABI was calculated using the lower of the right and left posterior tibial pressures divided by the brachial artery pressure<sup>17</sup>. Two follow-up measurements were also taken at three and six years after the baseline interview and these were used in assessment of incident PAD.

In Health ABC ABI was defined as the ratio of blood pressure in the right ankle to the right upper-arm and similarly for the left. Two measurements were taken and a mean calculated for each leg<sup>18</sup>. One follow-up measurement was taken during the fourth year interview and was used to compile incident PAD cases. In BLSA blood pressure was taken at both right and left arms and ankles using an automated testing device (Colin VP2000/1000). The minimum value between the left and right legs was used in the analysis. ABI measures were also taken at the fourth year follow-up interview and were used to define incident cases.

Those with an ABI measure greater than 1.40 were removed as this is indicative of non-compressible, calcified arteries<sup>14</sup> and although this also carries risk of mortality<sup>19</sup> inclusion of these individuals could have led to a misclassification of arterial extremity disease. An ABI measure less than 0.90 in either leg was used to define the presence of PAD, as this has been reported to be highly sensitive and specific for defining angiographically documented PAD<sup>20</sup>. PAD cases at baseline across all three studies were analysed within the main analysis. Incident PAD was calculated for both studies as those individuals without PAD at baseline who had developed PAD by the follow-up(s) interview, as defined by an ABI measure  $\leq 0.9$ .

### Covariates

A MI event was recorded using evidence from questionnaires with the question ‘Have you ever had a MI?’ at baseline for BLSA and InCHIANTI and also ‘Have you had a MI event since last interview?’ at follow-up interviews for InCHIANTI only. In Health ABC, subjects are

asked if a health professional had ever told them that they had had a heart attack and their current medications are checked to assess for those compatible with a history of heart disease. At follow-up interviews, participants are asked if they have been told of a heart attack or hospitalized and events are adjudicated according to specific algorithms that involve data from hospitalization such as electrocardiographic findings and enzyme results. Information was combined to record individuals who had had a MI event at baseline or would go on to experience an event in the follow-up period. Smoking exposure and status at baseline in each study was included as two covariates; the first recorded as a categorical variable where 0 was never smoked, 1 was former smoker and 2 was current smoker and the second as number of pack-years exposure, where one pack-year is calculated as one pack of cigarettes per day for one year from individual self-report data. Diabetes Mellitus and hypertension were recorded using self-report questionnaires in InCHIANTI and BLSA, whilst hypercholesterolemia was adjusted for using baseline LDL-cholesterol levels which were calculated using the Friedewald formula.

In Health ABC, those with diabetes at baseline were identified first by report of being told by a health professional that they had diabetes or by the identification of medications specific for diabetes treatment. For hypertension, those who reported hypertension or who were on medications consistent with treatment were considered hypertensive. In addition to total cholesterol  $\geq 240$ mg/dl participants taking statins were considered to be hypercholesterolemic even if their cholesterol did not exceed this limit.

### Genotyping

Genotyping of rs1333049 in InCHIANTI and Health ABC was performed in-house using conventional Taqman probes (Applied Biosystems, Foster City, CA, USA). Genotyping of its proxy rs4977574 ( $r^2$  0.89) in BLSA was performed using the Illumina Infinium HumanHap550 genotyping chip (ver1 and ver3 chips were used)<sup>21</sup>. Samples were assessed for MAF ( $>1\%$ ), genotype success rate ( $>99\%$ ) and HWE ( $p > 0.0001$ ). There were no duplicate errors and the SNPs were in Hardy-Weinberg equilibrium ( $p > 0.05$ ) within all included studies.

### Statistical Analysis

Logistic regression was used to test for an association of the three genotypes in an additive model with PAD. Linear regression was performed to test for a similar association with log-transformed ABI. Meta-analysis was performed using an inverse variance weighting method as implemented using the Stata 'metan' command, and heterogeneity was tested using the Q statistic and  $I^2$  metric.

ABI measures were log-transformed to correct for a modest non-normal distribution. MI was classified in a binary variable as the presence of a definite or possible MI event. Pack-years smoking exposure was grouped into a dichotomized variable at 20 pack-years due to its highly skewed distribution.

Associations with phenotypes indicative of PAD were adjusted in a three-tier design. Firstly, we adjusted our analyses for age and sex. Associations were then estimated after participants with definite or possible MI at baseline or within the 6 year follow-up period were removed from the analysis. Finally, we additionally adjusted for known atherosclerotic risk factors: Diabetes Mellitus, smoking status, hypercholesterolemia and hypertension<sup>22, 23</sup>. All analyses were conducted using StataSE 9.0.

## RESULTS

Summary statistics of the individual studies are detailed in Table 1. The mean age of genotyped participants ranged from 73.7 to 77.6 years and an average 49.2% were female across the three studies. Prevalence of PAD was 13.3%, 9.4% and 7.0% for the InCHIANTI, Health ABC and BLSA studies respectively with an average 9.2% of participants with a reported history of MI across the three populations.

For the C allele at rs1333049, across the three studies, we found a per-allele difference of  $-0.020$  ABI units (95% CI:  $-0.03 - -0.01$ ,  $p=1.1 \times 10^{-5}$ ) (Table 2). Similarly, combining estimates across the three studies we found that each additional copy of the C allele at rs1333049 increased the risk of PAD (OR: 1.34, 95% CI: 1.11 – 1.62,  $p=0.002$ ) (Table 2) when compared to the G allele homozygotes.

We next investigated whether the association with PAD was independent of the expected association with history of MI. After removing baseline and incident MI cases (Table 2), rs1333049 remained significantly associated with both a lower ABI ( $\beta$ :  $-0.017$ , 95% CI:  $-0.03 - -0.01$ ,  $p=1.3 \times 10^{-4}$ ) and PAD (OR: 1.29, 95% CI: 1.06–1.57,  $p=0.012$ ) across the three studies.

These associations remained after adjusting for known atherosclerotic risk factors (Table 2), including the presence of Diabetes Mellitus, hypercholesterolemia and hypertension and smoking status.

It has been suggested that as ABI is a blood pressure ratio designed to detect obstruction to flow and that blood flow doesn't start to drop until an ABI of 0.9 that individuals in the range of 1.0–1.3 may not be informative. In order to assess the relationship of the variant with the lower ranges of continuous ABI a sensitivity analysis was performed on those individuals with an ABI  $\leq 1.0$  (Table 3). The variant was associated with a per C allele change in ABI of  $-0.028$  (95% CI:  $-0.05 - -0.01$ ,  $p=0.014$ ) following adjustment for diagnosed MI and known atherosclerotic risk factors.

In addition we investigated whether the relationship between PAD and rs1333049 was similar in other ethnic groups using the black population of Health ABC. Within 1,070 subjects the associations between rs1333049 and both ABI and PAD were non-significant ( $\beta$ :  $-0.0006$ , 95% CI:  $-0.02 - 0.02$ ,  $p=0.95$ ; OR: 1.06, 95% CI: 0.83–1.33,  $p=0.653$  respectively). The association remained non-significant when removing MI cases, adjusting for atherosclerotic risk factors and when restricting analysis to those individuals with ABI  $\leq 1.0$ .

Finally the contribution of the variant to incident PAD in InCHIANTI and Health ABC was assessed on 99 and 74 incident cases respectively. These incident cases had a MAF of 0.51 which was slightly lower than the baseline PAD cases. The association of the C allele at rs1333049 with an increased risk of incident PAD across the two Caucasian populations did not reach significance (OR: 1.09, 95% CI: 0.87, 1.38,  $p=0.460$ ) in this small sub-sample. Similarly the association was non-significant in Health ABC's black population (OR: 0.95, 95% CI: 0.65, 1.38,  $p=0.787$ ).

### Sensitivity Analysis

The associations between ABI and PAD do appear weaker in BLSA than in the other two larger studies. However the 95% confidence intervals around the estimated BLSA effect sizes encompasses that of the InCHIANTI and Health ABC estimates and formal testing for heterogeneity in the meta-analysis showed no evidence for heterogeneity between the samples for ABI or PAD ( $p=0.287$ ,  $I^2=20\%$  and  $p=0.278$ ,  $I^2=22\%$  respectively), leading to the selection of a fixed effects model (which makes the assumption that the true effect of the risk allele is



the same in each study). Huedo-Medina et al<sup>24</sup> reported that heterogeneity statistics, both  $I^2$  and the Q statistic, suffer from low power when the number of studies included is small. To address the possibility of some heterogeneity between studies, we carried out a sensitivity analysis on both ABI and PAD meta-analyses using random effects models which resulted in only the ABI estimates altered to a small degree ( $\beta$ : -0.019, 95% CI: -0.03, -0.008,  $p=2.3 \times 10^{-4}$ ). Finally we estimated the effect of rs1333049 on ABI and PAD following exclusion of the BLSA study, which resulted in very similar effect sizes due to BLSA's small population size and thus its relatively small contribution to the meta-analysis. Following removal of MI cases from the InCHIANTI and Health ABC samples and adjustment for atherosclerotic risk factors the rs1333049 C allele remains significantly associated with lower ABI and increased risk of PAD ( $\beta$ : -0.018, 95% CI: -0.03- -0.01,  $p=1.5 \times 10^{-4}$ ; OR: 1.35, 95% CI: 1.09, 1.67,  $p=0.007$  respectively)

Alternative approaches to testing whether the reported associations with ABI and PAD were independent of MI diagnosis were considered. These included adding both MI history as a covariate and an interaction term between MI history and genotype into the regression models. Neither adjustment altered the reported estimates (Data not shown).

We performed sensitivity tests to check the validity and suitability of the additive model of inheritance. Firstly we performed a likelihood ratio test of the 1 degree-of-freedom(df) additive model against the genotypic 2df model and found no deviation from additivity in the three populations. In addition we tested both dominant and recessive models of inheritance for the variant against both ABI and PAD and found that neither provided a better model of fit to that of the additive model across the three datasets.

## DISCUSSION

The 9p21 SNP rs1333049 has been shown to be associated with MI and major arterial aneurysms. In a previous study, the relationship between this marker and PAD was also explored resulting in an association with an odds ratio of 1.14<sup>6</sup>, which disappeared when removing those with histories of MI, suggesting a lack of independent effect on the peripheral arterial system. In this study we examined the association of the rs1333049 polymorphism with peripheral arterial function in older populations. We found that the C allele, present in 49–54% of the participants in our meta-analysis, was associated with both a lower ABI and increased prevalence of PAD. Removing baseline and incident MI cases had very little effect on the association, indicating that the association with PAD is statistically independent of clinical MI in older subjects. In addition the effect of the C allele of the variant increased when the analysis was restricted to those participants with an ABI  $\leq 1.0$ .

PAD is a common disease with an estimated 27 million individuals affected in North America and Europe in 2007<sup>25</sup>. Up to 50% of patients experience both CAD and PAD concomitantly<sup>26, 27</sup> as atherosclerosis is involved in both conditions. A genome-wide association study of sub-clinical atherosclerotic measures had previously found no evidence for an association with continuous ABI and the *CDKN2a/2b* locus, but their analysis was based on 984 young- to middle-aged individuals<sup>28</sup>, suggesting a lack of power to detect the small effect size expected. Using a sample of 2,630 older individuals, we have shown that the rs1333049 variant, known to be associated with MI and major vessel aneurysms, is also associated with a higher prevalence of PAD, independently of diagnosed MI. This is also true for the association with lower ABI values, which suggests an effect on the full range of PAD, including the milder forms which may later progress to more serious disease<sup>29</sup>. Unfortunately the available data does not allow a detailed analysis of the time sequence of onsets of MI and PAD: future work could focus on this, in sufficiently large cohorts with multiple waves of data.

Our analysis also suggests that rs1333049 can be considered a risk factor for PAD independent of common atherosclerotic risks such as Diabetes Mellitus, smoking, hypercholesterolemia and hypertension. Additionally the C allele increased the risk of PAD in the small number of incident cases from InCHIANTI and Health ABC's follow-up ABI measurements, though this failed to reach significance.

The rs1333049 SNP is distal to the cyclin-dependent kinase inhibitor genes *CDKN2a* and *CDKN2b* locus, which code for p16 and p15 respectively, closely related proteins involved in cell cycle control and cellular senescence. In a range of mice and human tissues, p16 expression increases with age<sup>30, 31</sup>. Experimental up-regulation of p16 in mice promotes aging related changes in a range of cell types, resulting in cell senescence and decreased tissue regeneration<sup>32-35</sup>. Although evidence for the mechanism of action of the 9p21 polymorphism in human MI is limited, it has been suggested that cellular senescence plays a critical role in vascular pathophysiology and atherosclerosis (Reviewed in 36). Accumulation of vascular senescent cells could lead to a reduction in regeneration and repair capabilities leading to atherosclerotic damage. Lower levels and poorer quality of circulating endothelial progenitor cells have been reported in preliminary data on PAD patients<sup>37, 38</sup>, which are implicated as determinants of subclinical atherosclerosis<sup>39</sup>. Although it should be noted that recent work by Samani et al<sup>40</sup> has found no association between early atherosclerotic markers in 1,295 middle-aged to elderly individuals there is evidence that the downstream ANRIL locus, which is partially overlapped by the CAD risk haplotype, is expressed in a number of cells and tissues affected by atherosclerosis<sup>41</sup> suggesting a potential role in heart disease despite the unknown function of its non-coding RNA transcript.

Also it is interesting to note that other common (but independent) genetic variants near the p16/p15 locus have been associated with other aging phenotypes including Type II Diabetes<sup>2, 42, 43</sup> and impaired physical functioning in elderly individuals<sup>44</sup>.

In our analysis, we also examined SNP-phenotype associations in 'black' origin respondents from the Health ABC study. In this population rs1333049 SNP was found not to be associated with ABI or PAD in any of the analysis models performed. This is not surprising, as the rs1333049 SNP is not associated with MI in this population or in African Americans from the ADVANCE study<sup>45</sup>. This lack of association may be due to a different Linkage Disequilibrium (LD) structure in the locus of interest in non-European individuals, making this SNP a poor marker of the biologically relevant locus. Additionally it is possible that the effect size is much smaller in subjects of African descent than that seen in populations of European descent resulting in a lack of power to detect the effect in 1,194 individuals.

In conclusion, the common variant near the *CDKN2a/2b* locus on chromosome 9p21 is associated with a lower Ankle Brachial Index and increased risk of Peripheral Arterial Disease in three studies of elderly Caucasians. This association was independent of MI diagnosis and known atherosclerotic risk factors. Further work is needed to understand the mechanism of effect of this variant.

A genetic variant at chromosome 9p21 (present in 46% of people of Caucasian origin) is strongly associated with Myocardial Infarction (MI): heterozygote carriers are at 28% increased risk

As peripheral arterial disease (PAD) often co-occurs with MI, we examined associations between the risk allele and both ankle brachial index (ABI) and PAD in 2,630 Caucasian individuals (mean age 76.4 years) from three cohorts. The relevant risk allele (C allele at rs1333049) was associated with lower mean ABI measures and with increased prevalence of PAD – OR for PAD per risk allele 1.34 (1.12, 1.62)  $p=0.002$ : ie CC homozygotes may be at 68% increased risk of PAD compared to TT homozygotes.

The biological mechanisms involved are believed to relate to endothelial cell repair, and in our analyses were unrelated to known atherosclerosis risk factors: adjustment for diabetes mellitus, smoking, hypercholesterolemia and hypertension did not attenuate the association of the risk allele with ABI or prevalence of PAD. Our finding adds to the body of evidence implicating the chromosome 9p21 variant in vascular disease.

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Table 1

Summary characteristics of baseline PAD\* cases and controls by study.

Measure	InCHIANTI <sup>†</sup>		Health ABC Caucasian		BLSA		Health ABC 'Blacks' <sup>‡</sup>	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Sample N	107	697	148	1421	18	239	229	841
rs1333049 C allele frequency	0.61	0.52	0.56	0.47	0.53 <sup>‡</sup>	0.53 <sup>‡</sup>	0.27	0.26
rs1333049 genotype GG frequencies	14 (13.1)	162 (23.2)	31 (21.0)	385 (27.1)	6 (33.3) <sup>‡</sup>	55 (23.0) <sup>‡</sup>	125 (54.6)	456 (54.2)
	55 (51.4)	340 (48.8)	69 (46.6)	724 (51.0)	5 (27.8) <sup>‡</sup>	114 (47.7) <sup>‡</sup>	83 (36.2)	332 (39.5)
	38 (35.5)	195 (28.0)	48 (32.4)	312 (22.0)	7 (38.9) <sup>‡</sup>	70 (29.3) <sup>‡</sup>	21 (9.2)	53 (6.3)
Age (years)	77.7 (7.1)	73.8 (6.7)	74.5 (2.8)	73.6 (2.8)	85.8 (6.7)	77.0 (7.6)	74.1 (2.9)	73.2 (2.9)
Number Female	39 (36.5)	411 (59.0)	68 (46.0)	679 (47.8)	13 (72.2)	144 (60.3)	131 (57.2)	479 (57.0)
Ankle Brachial Index (ABI Units)	0.70 (0.14)	1.08 (0.10)	0.74 (0.16)	1.13 (0.10)	0.75 (0.15)	1.16 (0.07)	0.73 (0.15)	1.09 (0.10)
Myocardial Infarction history reported	19 (17.8)	98 (9.8)	17 (11.5)	102 (7.2)	2 (11.1)	20 (8.4)	26 (11.4)	44 (5.2)
Diabetes history reported	11 (10.7)	69 (10.0)	27 (18.4)	135 (9.5)	5 (27.8)	43 (18.0)	68 (30.0)	152 (18.2)
Hypertension history reported	49 (47.6)	308 (44.8)	93 (63.7)	631 (44.6)	14 (77.8)	143 (59.8)	159 (69.7)	537 (64.5)
LDL Cholesterol (mg/dl)	142.4 (33.5)	135.7 (33.0)	120.0 (33.6)	119.7 (33.5)	97.3 (24.5)	107.5 (31.7)	127.0 (36.2)	123.5 (36.6)
Smoking Status <i>Never</i>	37 (34.6)	437 (62.7)	40 (27.0)	620 (43.7)	7 (38.9)	106 (44.4)	71 (31.1)	408 (48.5)
<i>Former</i>	38 (35.5)	178 (25.5)	87 (58.8)	716 (50.5)	10 (55.6)	126 (52.7)	97 (42.5)	325 (38.6)
<i>Current</i>	32 (29.9)	82 (11.8)	21 (14.2)	83 (5.9)	1 (5.6)	7 (2.9)	60 (26.3)	108 (12.8)
Pack-year Exposure ≥ 20	54 (50.5)	152 (21.8)	81 (55.9)	496 (35.5)	8 (44.4)	51 (21.5)	103 (45.8)	223 (26.8)

\* PAD defined as ABI less than or equal to 0.90.

<sup>†</sup> InCHIANTI dataset was restricted to those subjects 65 years and over.<sup>‡</sup> A allele of rs4977574 used as proxy for C allele of rs1333049 in BLSA.

§ Health ABC 'Blacks' have been analysed separately due to differences in allele frequency between ethnicities and thus are not included in the meta-analysis.



Table 2

Per risk allele ('C') linear regression based associations between rs1333049 status and ankle-brachial index, plus logistic regression based associations with peripheral arterial disease.

Measure	Study	Sample n	Model A Age and sex adjusted		Model B* Plus MI history excluded		Model C† Plus adjustment for vascular risk factors	
			Beta (95% CI)	p- value	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Ankle-Brachial Index (ABI Units)	InChianti	804	-0.024 (-0.04, -0.005)	0.012	-0.020 (-0.04, -0.00)	0.036	-0.019 (-0.04, -0.001)	0.038
	Health ABC	1569	-0.022 (-0.03, -0.01)	1.6×10 <sup>-4</sup>	-0.020 (-0.03, -0.01)	8.1×10 <sup>-4</sup>	-0.017 (-0.03, -0.006)	0.002
	BLSA‡	257	-0.002 (-0.03, 0.02)	0.867	0.003 (-0.02, 0.03)	0.842	-0.0007 (-0.03, 0.03)	0.958
	<b>Meta-Analysis</b>	<b>2630</b>	<b>-0.020 (-0.03, -0.01)</b>	<b>1.1×10<sup>-5</sup></b>	<b>-0.017 (-0.03, -0.01)</b>	<b>1.3×10<sup>-4</sup></b>	<b>-0.016 (-0.03, -0.01)</b>	<b>1.7×10<sup>-4</sup></b>
Measure	Study	n cases/ controls	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value	Odds Ratio (95% CI)	p-value
Peripheral Artery Disease‡	InChianti	107/697	1.35 (0.99, 1.83)	0.054	1.33 (0.96, 1.85)	0.087	1.37 (0.97, 1.94)	0.077
	Health ABC	148/1421	1.42 (1.11, 1.82)	0.005	1.36 (1.05, 1.76)	0.019	1.33 (1.01, 1.75)	0.042
	BLSA‡	18/239	0.83 (0.41, 1.69)	0.613	0.66 (0.31, 1.42)	0.292	0.58 (0.26, 1.30)	0.185
	<b>Meta-analysis</b>	<b>273/2357</b>	<b>1.34 (1.12, 1.62)</b>	<b>0.002</b>	<b>1.29 (1.06, 1.57)</b>	<b>0.012</b>	<b>1.27 (1.03, 1.57)</b>	<b>0.024</b>

\* Estimates adjusted for age and sex, following removal of participants with definite or possible MI at baseline and/or within 6 year follow-up period.

† Estimates adjusted for age, sex, current or former smokers, Diabetes Mellitus, hypercholesterolemia and hypertension following removal of participants with MI at baseline and within 6 year follow-up period.

‡ PAD defined as ABI less than or equal to 0.90.

§ rs4977574 used in analysis as a proxy for rs1333049 r<sup>2</sup>=0.9

**Table 3**  
Per risk allele ('C') linear regression based associations between rs1333049 status and ankle-brachial index  $\leq 1.0$

Measure	Study	Sample n	Model A Age and sex adjusted		Model B* Plus MI history excluded		Model C† Plus adjustment for vascular risk factors	
			Beta (95% CI)	p-value	Beta (95% CI)	p-value	Beta (95% CI)	p-value
Ankle-Brachial Index $\leq 1.0$ (ABI Units)	InChianti	342	-0.023 (-0.05, -0.008)	0.151	-0.017 (-0.05, 0.02)	0.297	-0.019 (-0.05, 0.01)	0.248
	Health ABC	306	-0.051 (-0.08, -0.02)	0.002	-0.048 (-0.08, -0.02)	0.003	-0.037 (-0.07, -0.01)	0.019
	BLSA‡	25	-0.041 (-0.18, 0.10)	0.540	-0.042 (-0.19, 0.11)	0.576	-0.007 (-0.19, 0.17)	0.931
	<b>Meta-analysis</b>	<b>673</b>	<b>-0.037 (-0.06, -0.01)</b>	<b>4.3 * 10<sup>-4</sup></b>	<b>-0.033 (-0.06, -0.01)</b>	<b>0.003</b>	<b>-0.028 (-0.05, -0.01)</b>	<b>0.014</b>

\* Estimates adjusted for age and sex, following removal of participants with definite or possible MI at baseline and/or within 6 year follow-up period.

† Estimates adjusted for age, sex, current or former smokers, Diabetes Mellitus, hypercholesterolemia and hypertension following removal of participants with MI at baseline and within 6 year follow-up period.

‡ rs4977574 used in analysis as a proxy for rs1333049  $r^2=0.9$