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Association Between Chromosome 9p21 Variants and the Ankle-Brachial Index Identified by a Meta-Analysis of 21 Genome-Wide Association Studies

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Abstract

Background—Genetic determinants of peripheral arterial disease (PAD) remain largely unknown. To identify genetic variants associated with the ankle-brachial index (ABI), a noninvasive measure of PAD, we conducted a meta-analysis of genome-wide association study data from 21 population-based cohorts.

Methods and Results—Continuous ABI and PAD (ABI≤0.9) phenotypes adjusted for age and sex were examined. Each study conducted genotyping and imputed data to the ~2.5 million SNPs in HapMap. Linear and logistic regression models were used to test each SNP for association with ABI and PAD using additive genetic models. Study-specific data were combined using fixed-effects inverse variance weighted meta-analyses. There were a total of 41,692 participants of European ancestry (~60% women, mean ABI 1.02 to 1.19), including 3,409 participants with PAD and with GWAS data available. In the discovery meta-analysis, rs10757269 on chromosome 9 near *CDKN2B* had the strongest association with ABI ($\beta = -0.006$, $p=2.46\times10^{-8}$). We sought replication of the 6 strongest SNP associations in 5 population-based studies and 3 clinical samples (n=16,717). The association for rs10757269 strengthened in the combined discovery and replication analysis ($p=2.65\times10^{-9}$). No other SNP associations for ABI or PAD achieved genome-wide significance. However, two previously reported candidate genes for PAD and one SNP associated with coronary artery disease (CAD) were associated with ABI : *DAB21P* (rs13290547, $p=3.6\times10^{-5}$); *CYBA* (rs3794624, $p=6.3\times10^{-5}$); and rs1122608 (*LDLR*, p=0.0026).

Conclusions—GWAS in more than 40,000 individuals identified one genome-wide significant association on chromosome 9p21 with ABI. Two candidate genes for PAD and 1 SNP for CAD are associated with ABI.

Keywords

cohort study; genetic association; genome-wide association study; meta-analysis; peripheral vascular disease

Peripheral arterial disease (PAD) affects approximately 27 million people in Europe and North America (1) and is associated with increased risk for myocardial infarction, stroke, and mortality.(2–6) Measurement of ankle and arm blood pressures with a Doppler device and calculation of the ankle-brachial index (ABI) is a simple and reliable method to detect PAD. An ABI≤0.90 is indicative of definite PAD.(7) In previous work, the Ankle Brachial Index Collaboration demonstrated a reverse J shaped relationship of ABI with mortality and coronary events with a low risk ABI ranging from 1.11 to 1.40.(8)

Little is known about genetic susceptibility to PAD but familial aggregation and heritability estimates suggest a significant genetic component.(9–13) A study of 112 biological candidate genes identified only two single nucleotide polymorphisms (SNPs) in *NOS3* significantly associated with ABI.(14) The candidate gene approach to identify novel genetic variants for PAD has been limited by modest study sample size, relatively small number of genes examined, and lack of replication in independent samples.(13)

Genome-wide association studies (GWAS) have successfully led to the discovery of novel genetic variants for several common diseases including coronary artery disease (CAD).(15) The association between genetic variants on chromosome 9p21 and CAD has demonstrated replication (16;17), persistent association across race/ethnicity (18), and association with other vascular diseases.(19–21) Notably, GWAS of subclinical atherosclerosis phenotypes such as intima-medial thickness or ABI are sparse. Therefore, we conducted a meta-analysis of GWAS findings for ABI within an international consortium of 21 population-based cohort studies that included 41,692 participants of European ancestry among whom 3,409 participants had PAD (ABI ≤0.90). We conducted replication analyses of our strongest findings in over 16,000 individuals from population-based cohort studies and clinically based samples of PAD. We hypothesized that this approach would lead to the unbiased identification of genetic variants associated with ABI. Further, we hypothesized that some genetic variants for ABI would be identical to those reported to be associated with CAD and/or its risk factors given shared underlying biologic pathways, while some genetic variants would be uniquely associated with PAD.

Methods

Discovery Studies

Our analyses were conducted within the international Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (22) and included four of the five original CHARGE cohorts: Atherosclerosis Risk in Communities Study (ARIC, n=7,630), the Cardiovascular Health Study (CHS, n=3,193), the Framingham Heart Study (FHS, n=3,572) and the Rotterdam Study (RS-I, n=5,169 and RS-II n=1,642). Ten additional population-based cohorts joined the collaboration for analysis of ABI phenotypes: the Family Heart Study (FamHS, n=1,736), Genetic Epidemiology Network of Arteriopathy Study (GENOA, n=991), Gutenberg Heart Study (GHS, n=3,122), Health, Aging, and Body Composition (Health ABC, n=1,564), the Invecchiare in Chianti Study (InCHIANTI, n=1,130), Cooperative Health Research in the Region of Augsburg (KORA F3, n=1,581 and KORA F4, n=1,407), Netherlands Study of Anxiety and Depression (NESDA, n=1,612), Nijmegen Biomedical Study (NBS, n=544), Study of Health in Pomerania (SHIP, n=543). A further 6 studies derived from population isolates were also available for the analyses:

Amish Study (Amish, n=1,183), Croatia-Vis (n=897), Croatia-Korcula (n=851), Croatia-Split (n=499), Erasmus Rucphen Family Study (ERF, n=2,133), and the Orkney Complex Disease Study (ORCADES, n=693). For all studies participating in the meta-analyses, each participant self-identified as European or European American and provided written informed consent and the Institutional Review Board at the parent institution for each respective cohort approved the study protocols. More detailed study-specific information is provided in the Supplementary Methods.

Ankle-brachial index Phenotypes—Ankle and brachial blood pressure measurements for each participating study were obtained from the baseline examination or the first examination the measurement was obtained. Details on the ABI protocol used and the calculation performed in each study are provided in Supplementary Method Table 1. To calculate the ABI for each leg, the systolic blood pressure at each ankle was divided by the systolic blood pressure in the arm. If the systolic blood pressure was measured in both arms, the higher arm reading was used in the ABI calculation. If replicate readings were obtained, the mean of the two measurements for each limb was used to calculate the ABI with the exception of InCHIANTI which used the higher of the two readings of each measurement set to calculate the ABI. The lower of the ABIs from the two legs was used for analysis. In ARIC and FamHS, the ABI was measured in only one leg chosen at random. Participants with an ABI >1.40 were excluded since this high ABI may represent medial sclerosis, fibrocalcific disease secondary to diabetes mellitus, or other causes of non-compressible vessels.

To maximize the sample size and the power to detect genetic variants with modest effects and to examine the entire range of ABI values given the recent evidence of increased CVD risk associated with ABI values up to 1.1(8), we examined the continuous range of ABI <1.40. As a secondary analysis to provide a clinical phenotype, we defined PAD as ABI ≤0.90 and conducted a case (ABI≤0.9)/control (ABI >0.90 and < 1.40) comparison analysis.

Genotyping and Imputation

Different genotyping platforms were used by the 21 studies (Supplementary Methods Table 2). Each study imputed the genotype “dosage” (0–2) for the expected number of alleles for ~2.5 million Phase II HapMap CEU SNPs for each participant using currently available imputation methods. (23) CHS used BIMBAM (available at http://stephenslab.uchicago.edu/software.html) (24), GHS, InCHIANTI, NESDA and SHIP used IMPUTE (25) and all other cohorts used MACH (http://www.sph.umich.edu/csg/abecasis/MaCH/).

Statistical Analysis

We devised a GWAS analysis plan for the ABI and PAD phenotypes that each study independently implemented. Sex-specific and age-adjusted residuals of ABI were created from linear regression models and used as phenotypes in the analysis. No transformation of the ABI measure was performed prior to analysis. In FHS, residuals were also obtained separately in the original and offspring cohorts. Multi-site studies (ARIC, CHS, FamHS) additionally adjusted for field study site. Each SNP was tested for association with ABI in additive genetic models using linear regression. The Amish Study, FamHS, FHS, and GENOA cohorts used linear mixed effects (LME) models to account for familial correlations. CROATIA-Vis, CROATIA-Korcula, CROATIA-Split, ERF, and ORCADES used the “mmscore” function of the GenABEL package for R statistical software for the association test under an additive model. This score test for family-based association takes into account pedigree structure and allows unbiased estimations of SNP allelic effect when relatedness is present between examinees. Logistic regression adjusting for age and sex was

used to test each SNP for association with the PAD phenotype. The FamHS, FHS, and GENOA cohorts used generalized estimating equations (GEE) clustering on family to account for family correlations.

A genome-wide meta-analysis using a fixed effects approach with inverse-variance weighting, was then conducted in METAL(26) [www.sph.umich.edu/csg/abecasis/metal] for 2,669,158 SNPs in the meta-analysis excluding the population isolates (2,670,732 SNPs including the population isolates) that met imputation and quality control criteria (Supplementary Methods Table 2). Prior to meta-analysis genomic control was applied to each study. The association of ABI per each additional risk allele was quantified by the regression slope (β), its standard error [SE(β)], and the corresponding p-value. We calculated a meta-analysis odds ratio (OR) for each of the most significant SNP associations for PAD. The meta-analysis OR estimates the increase in odds of PAD for each additional copy of the risk allele of the SNP. SNP associations were considered to be significant on a genome-wide level at $p<5 \times 10^{8}$.(27;28) Standardized gene and SNP annotations were created using a PERL script.(29) We also tested for heterogeneity of study-specific regression parameters using Cochran's Q statistic. Due to concerns about heterogeneity, we conducted analyses of non-isolate studies and of the full group of studies. We selected SNPs for replication using results from the meta-analysis excluding the population isolates because the available replication samples did not include isolates. We excluded SNP association results if the total meta-analysis sample was less than 20,000 and if the average minor allele frequency of the SNP was <5%.

Replication

We sought to replicate independent SNP associations for ABI that attained genome-wide significance (1 region) and SNPs with suggestive associations (5 regions, $p<10^{-5}$) and bioinformatics data supporting the signal. The bioinformatic analyses are described in detail in the Supplementary Material. In addition, we sought to replicate one SNP associated with both ABI and PAD at $p<10^{-4}$. The replication studies included 5 population-based studies and 3 clinically-based studies including a total of over 16,000 participants: the Bruneck Study (n=786), the Copenhagen City Heart Study (CCHS, n=5,330), the Multi-Ethnic Study of Atherosclerosis (MESA, n=2,611), the National Health and Nutrition Examination Surveys (NHANES 1999–2002, n=2,335), Prevention of Renal and Vascular End-stage disease (PREVEND, n=3,691) cohort, Cardiovascular Disease in Intermittent Claudication (CAVASIC, n=443) Study, Genetic Determinants of Peripheral Arterial Disease (GenePAD, n=850), and the Linz Peripheral Arterial Disease (LIPAD, n=671) Study. Each collaborating study was provided with a SNP list and a detailed analysis plan. MESA and PREVEND used *in silico* genotyping (Supplementary Methods Table 2) and the remaining studies genotyped the SNPs using Taqman assays or Sequenom. Relative excess heterozygosity (REH) analysis demonstrated that all genotyped SNPs were compatible with Hardy-Weinberg equilibrium at the nominal 5% test-level (Supplementary Methods Table 3).(30)

Examination of candidate genes associated with peripheral artery disease and coronary artery disease/myocardial infarction

We selected candidate genes for ABI and/or PAD from the published literature using PubMed search terms "((ankle-brachial index) OR (peripheral arterial disease)) AND polymorphism". Association studies with at least 100 cases and 100 controls were included regardless of whether the original study results were positive or negative. Using the discovery meta-analysis results for ABI, we then identified the most strongly associated SNP based on p-values within the gene region ±100 kb upstream or downstream of the candidate gene. Due to the high correlation of imputed genotypes, the effective number of loci were calculated for each gene region (31) using the genotype scores from the KORA F4

Study (see Supplementary Methods). Bonferroni correction of p-values was then applied in each region using the effective number of loci. Subsequently, false discovery rates (FDR) were calculated using these corrected p-values, accounting for the number of gene regions examined (see Supplementary Methods). Lastly, we examined the association with ABI of 30 SNPs strongly associated with CAD in recent GWAS.(32–34) Our ABI discovery meta-analysis did not include 2 of the 30 SNPs (rs17465637 and rs3798220) and we were unable to identify proxy SNPs available in our data. Using the p-values for the 28 SNPs in our discovery meta-analysis, we then calculated the FDR for each CAD SNP accounting for the 28 regions examined.

Results

Study Sample

The study sample included 41,692 participants of European ancestry (56% women, 6,256 from population isolates) with ABI data and genome-wide genotyping. Participant characteristics at the time of ABI measurement for each cohort are provided in Supplementary Table 4. Across the studies the mean age ranged from 41.8 years to 73.8 years, the mean ABI ranged from 1.02 to 1.19, and 8.2% (n=3,409) had PAD (ABI<0.9). Characteristics of the replication samples were similar to the discovery set (Supplementary Table 5).

ABI-SNP associations

We conducted a meta-analysis with (n=41,692) and without (n=35,434) the population isolates (Supplementary Figures 1 and 2, QQ-plots and Manhattan plots, study specific lambdas ranged from 0.997 to 1.044). Our primary meta-analysis excluded studies from population isolates because of concern for study heterogeneity and the lack of availability of replication samples from isolates. The strongest SNP association for ABI was rs10757269 on chromosome 9 near *CDKN2B* ($\beta = -0.006$, $p = 2.46 \times 10^{-8}$, p for heterogeneity=0.23, Table 1; meta-analysis results including the population isolates, Supplementary Table 7). Among the 96 SNP associations for ABI with $p<10^{-5}$, 79 were located in the chromosome 9p21 region (Supplementary Table 6). The ABI SNP rs10757269 is in strong LD with several SNPs in the region previously reported to be associated with CAD or myocardial infarction ($r^2>0.8$) but this ABI SNP is not in LD with SNPs previously associated with the type 2 diabetes mellitus (Figure 1). We repeated the meta-analysis to examine the association between ABI and rs10757269 first adjusting for CAD and then excluding individuals with CAD among the non-isolate studies. The association remained but was no longer genome-wide significant (adjusting for CAD: $p = 5.56 \times 10^{-6}$; excluding CAD: $p = 3.79 \times 10^{-5}$). Next, we sought to replicate the association between rs10757269 and ABI in both population-based and clinically-based samples (n=16,717). The magnitude and direction of the association in the replication studies was similar to the discovery set ($\beta = -0.0035$, p=0.0176) providing evidence of replication. In the combined stage 2 discovery plus replication meta-analysis the ABI-rs10757269 association became stronger ($p = 2.65 \times 10^{-9}$). The study-specific estimates of effect for the discovery studies, population isolates, replication studies and overall discovery plus replication meta-analyses are presented in Figure 2. Two studies among the population isolates (the Amish Study and Croatia-Split) had effect estimates in the opposite direction to the other studies. None of the other SNP associations for ABI achieved genome-wide significance. The significance of the associations for the additional SNPs chosen for replication diminished in the discovery plus replication meta-analysis (Table 1, Supplementary Table 7).

PAD-SNP Associations

None of the SNP associations for the PAD phenotype (defined by an ABI≤0.9) achieved genome-wide significance (Table 2, for meta-analysis results including population isolates Supplementary Table 8). The strongest association was found for rs6584389 on chromosome 10 near the *PAX2* gene (odds ratio 1.17, 95% confidence interval 1.10, 1.25, $p=2.34 \times 10^{-6}$). Of note, the chromosome 9 SNP rs10757269 association with PAD was in a direction consistent with the ABI association but did not achieve statistical significance (Table 1, β=0.0849, p=0.004, increasing the odds of PAD).

Overlap in SNP Associations for ABI and PAD

While the directions of effect for the ABI SNPs in Table 1 were consistent with the PAD association result (lower ABI, increased odds of PAD), there was little overlap in the top associations for the two phenotypes. Only three regions marked by SNPs in/near *IDE* (10q23-q25), *DAB21P* (9q33.2), and *GRAMD1C* (3q13.31) in addition to the chromosome 9p21 region showed association with both ABI and PAD at the $p<10^{-4}$ level (Supplementary Table 9). SNP rs7100623 in *IDE* demonstrated the strongest novel association with both ABI (β= −0.005, $p=1.89 \times 10^{-5}$) and PAD (β= 0.139, $p=8.39 \times 10^{-5}$) at $p<10^{-4}$; however the association p-value was not significant in the replication stage and diminished in the combined discovery plus replication meta-analysis.

Examination of PAD Candidate Genes

Among the 55 candidate genes or regions previously tested for association with ABI and/or PAD, eight regions showed nominally significant p-values (p<0.05) after correction for the number of effective loci for each gene region. After accounting for the number of regions examined using a false discovery rate (FDR<0.10), we found evidence of association between ABI and *CYBA* (rs3794624, uncorrected $p=6.3 \times 10^{-5}$, corrected p=0.0036, FDR=0.0665) and *DAB21P* (rs13290547, uncorrected $p=3.6 \times 10^{-5}$, corrected p=0.0035, FDR=0.0665) in addition to the chromosome 9p21 locus (rs1333049) reported to be associated with ABI (Table 3) (35). We found no evidence of association between ABI and any of the other candidate genes previously tested for association with ABI or PAD (Supplementary Table 10).

Examination of Coronary Artery Disease/Myocardial Infarction Candidate Genes

Among the 30 SNPs previously reported by GWAS to be associated with CAD or myocardial infarction, 28 SNPs were available in our discovery meta-analysis of ABI and 2 of these SNPs demonstrated an association (FDR <0.10) with ABI including rs4977574 near *CDKN2B* ($p=2.33 \times 10^{-6}$) and rs1122608 in *LDLR* (p=0.0026) (Table 3, Supplementary Table 11).

Discussion

Our GWAS meta-analysis for ABI conducted in more than 40,000 adults of European ancestry has several notable findings. First, we identified and replicated one genome-wide significant association between a SNP in the chromosome 9p21 region and ABI. No other ABI-SNP associations achieved genome-wide significance. Second, in our discovery sample over 3000 adults had PAD (ABI≤0.9); however, none of the SNP associations were significant. Third, the directions of effect were consistent across the two phenotypes for the most significant ABI SNPs (lower ABI, increased odds of PAD): however, we observed minimal overlap in the top SNP associations for ABI and PAD. Finally the effect size for the 9p21 SNP was modest. The association itself is, however, intriguing and may provide insights into the biologic mechanisms contributing to generalized atherosclerosis.

Chromosome 9p21 locus and atherosclerosis susceptibility

Common genetic variants in the 9p21 locus are strongly associated with myocardial infarction and CAD (17;33;36) and confer risk for other atherosclerotic diseases including stroke (19), cerebral and abdominal aortic aneurysm (20;21), and clinically diagnosed PAD; however, the relation with PAD was diminished when coronary artery disease cases were excluded.(20) SNP associations at the 9p21 locus with subclinical measures of atherosclerosis have been conflicting. Initially no association was observed with carotid intima-medial thickness or flow mediated dilation in young or older adults (37;38); however more recent reports demonstrate an association with the development and progression of carotid atherosclerosis (39) and with the suggestion of a stronger effect in men.(40) To further investigate the ABI-9p21 SNP association noted in this study, we conducted the meta-analysis after adjusting for CAD and after exclusion of individuals with CAD. Not surprisingly, the association persisted but was no longer genome-wide significant. Both CAD and PAD are manifestations of underlying atherosclerosis and nearly two-thirds of individuals with PAD have coexisting coronary or cerebrovascular disease.(41) One previous report conducted in three studies of older adults identified an association between a variant at 9p21 and lower ABI as well as an increased risk for PAD.(35) The primary affect of the chromosome 9p21 region may be on the atherosclerotic process itself, and there are likely to be many other factors both genetic and environmental that determine whether it manifests as CAD, PAD, or another clinical atherosclerotic phenotype. The primary biologic mechanism underlying the association with ABI is unknown but appears to be independent of two major PAD risk factors, diabetes and smoking, as the ABI SNP in the 9p21 region we identified is not in linkage disequilibrium with the SNPs in the region associated with diabetes risk (42;43) or smoking related behaviors.(44) The mechanism may be related to modulation of platelet reactivity (45), atheroma formation, plaque instability, thrombosis, or biologic processes not yet identified.(46) The SNP associated with ABI is nearest to *CDKN2B*, a well recognized tumor-suppressor gene that encodes a cyclin-dependent kinase inhibitor and is involved in regulation of the cell cycle. *CDKN2B* is abundantly expressed in human atherosclerotic lesions (47) and animal models suggest that altered *CDKN2A/B* expression results in abnormal regulation of vascular cell proliferation.(48) Functional studies reveal a long non-coding RNA at this locus named ANRIL, and a mouse model has confirmed the essential role of ANRIL in regulation of *CDKN2B* expression through a cis-acting mechanism.(49;50) ANRIL is implicated in proliferation and senescence.

PAD Candidate Genes

We performed a literature search to identify all candidate gene regions previously investigated for association with PAD and/or ABI, irrespective of whether the association was reported to be positive or negative. This approach revealed two further associated gene regions: *DAB2IP and CYBA. DAB2IP* rs13290547 was not only associated with ABI but also with PAD ($p=3.62 \times 10^{-5}$ and 2.2×10^{-5}, respectively) (Supplementary Table 10). The *DAB2IP* gene encodes an inhibitor that is involved in the regulation of cell survival and proliferation. One variant in the *DAB2IP* gene (rs70254486) has recently been detected in a GWAS of abdominal aortic aneurysm.(51) That study also detected an association with PAD as a secondary endpoint in 3,690 cases versus 12,271 controls ($p=3.9 \times 10^{-5}$). The same SNP showed an association with CVD within a meta-analysis of case-control studies.(52) The *CYBA* gene is involved in NADPH oxidase regulation, which contributes to oxidative stress and plays a key role in the pathophysiology of coronary disease. Only one report investigated a SNP (rs4673) in this gene for association with PAD among 324 cases and 295 controls, but did not find an association.(53) Our study found an association of rs3794624 ($r^2=0.5$ with rs4673), with continuous ABI, which may indicate that the earlier study likely lacked power to find this association. None of the other gene regions had sufficient evidence for association with continuous ABI in our meta-analysis. Another very wide-reaching

approach designed to systematically examine a large number of genes related to intermediate phenotypes of atherosclerosis such as blood pressure regulation, lipoprotein metabolism, inflammation, oxidative stress, vascular wall biology, obesity and diabetes found only eNOS to be significantly associated with ABI.(14) This gene could not be confirmed by our candidate gene examination.

Coronary candidate genes

Besides the chromosome 9 locus, one other SNP reported to be associated with coronary disease in recent GWAS, also showed an association with ABI in our study; rs1122608 in *LDLR*. The *LDLR* gene plays an important role in cholesterol homeostasis and mutations at this gene have been shown to influence LDL cholesterol levels and the subsequent risk for coronary disease.(54) The association of *LDLR* gene with ABI in our study is a confirmation of the shared biologic pathways underlying both subclinical and clinically apparent disease.

Strengths/limitations

Our meta-analysis represents the largest collaborative effort to date to identify genome-wide SNP associations for variation in ABI and PAD (ABI ≤0.90) and our findings suggest the absence of common variants with large effects on ABI. Use of ABI as our primary phenotype has major advantages of detecting asymptomatic PAD as the ABI is an objective measurement whereas clinical PAD requires subjective symptoms of exertional leg discomfort and mobility of the individual. However, several limitations of our meta-analysis merit comment. The blood pressure measurement protocol and ABI calculation was heterogeneous across participating studies. While protocols were standardized within each study, the studies were not designed to be fully standardized and comparable across studies (Supplementary Methods Table 1). This phenotype heterogeneity may have impacted our ability to detect associations. Furthermore, for many studies information about a previous revascularization intervention was not available. This lack of data may have resulted in the misclassification of some of the most affected persons by placing them into an ABI range of unaffected individuals and consequently reducing our power to detect true associations. Our sample was restricted to individuals of European ancestry and thus our findings cannot yet be generalized to individuals of other race/ethnic groups. Furthermore, some PAD susceptibility variants may be race/ethnic specific and can only be uncovered through the study of non-Europeans. For example, African Americans have a higher prevalence of PAD that cannot be attributed to traditional or novel risk factors.(55) This observation raises the hypothesis that polymorphisms unique to African Americans may partially be responsible for the higher prevalence of PAD.(55) We did not evaluate gene by environment interactions which may be especially relevant for cigarette smoking, a strong risk factor for PAD (56) and a factor known to interact with other genes to modulate atherosclerosis. (57)

Conclusions

In conclusion, a common variant near the *CDKN2B* gene in the chromosome 9p21 locus is associated with a lower ABI. PAD represents a diffuse form of atherosclerosis associated with increased risk for death and incident CVD events. Thus, the identification of genetic variants associated with ABI may provide an important opportunity not only to unravel the biologic basis of PAD but also to improve our understanding of the causes of the variation in degree of atherosclerosis from one arterial bed to another. Additional studies are warranted to identify the causal variants in the 9p21 locus and to characterize their functional significance. The search for genes influencing predilection to PAD remains elusive and alternative approaches are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

see Supplementary Material

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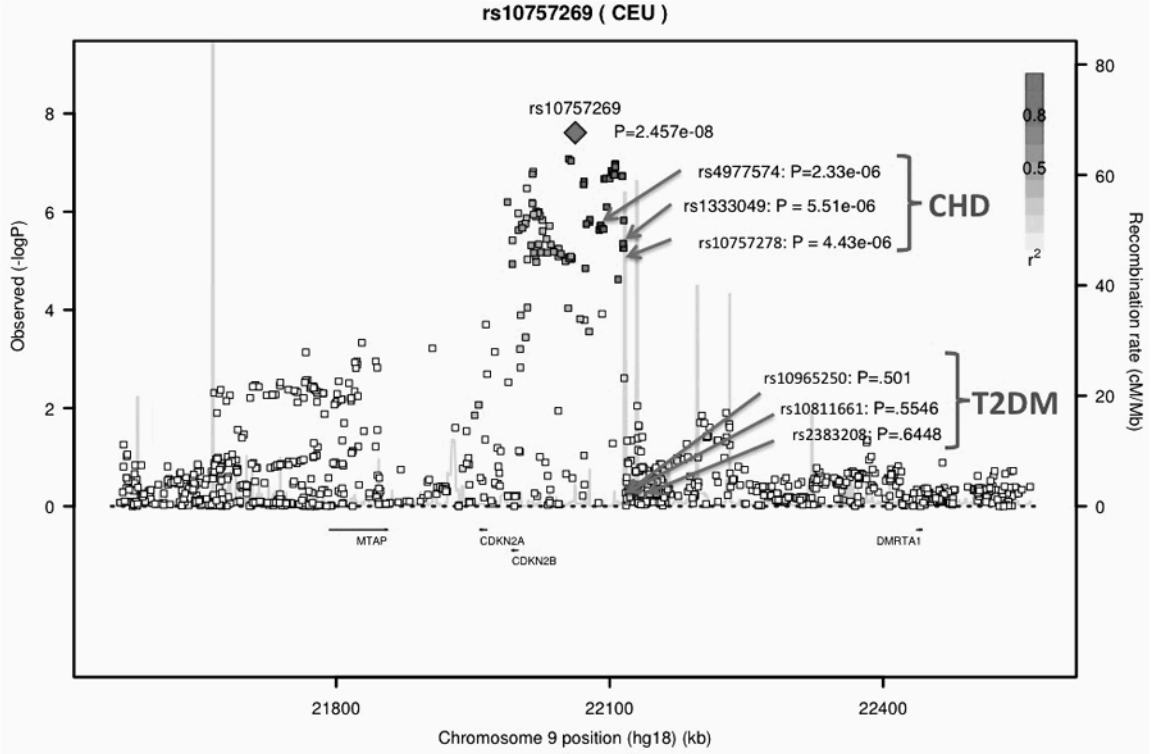

Figure 1.

Genomic context of the genome-wide significant signal at chromosome 9p21 plotted against the $-\log_{10}$ p values. r^2 between the top signal (rs10757269) and each SNP shown in red. SNPs previously reported from GWAS to be associated with coronary artery disease (CAD, green arrows) and type 2 diabetes (T2DM, orange arrows) and p-value for association with ankle-brachial index shown. Chromosome positions are based on build hg18.

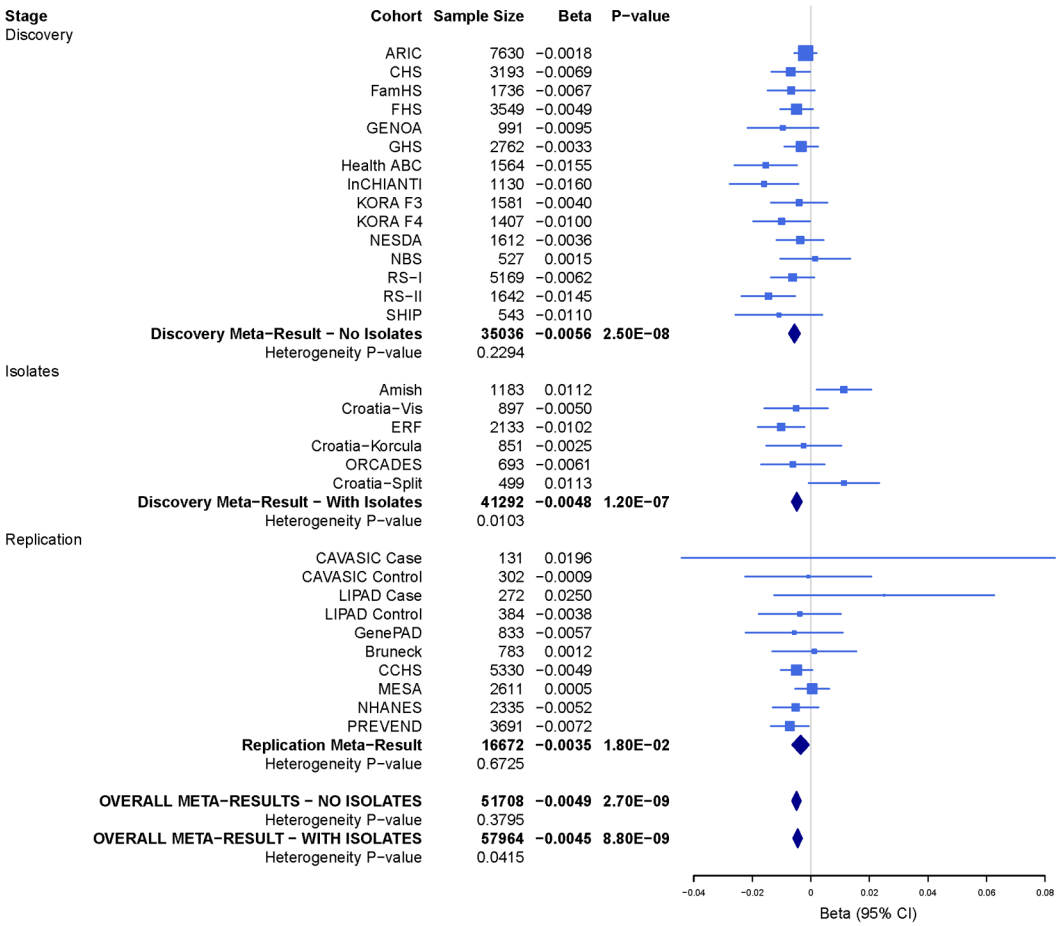

Figure 2.

Ankle-brachial index-chromosome 9p21 (rs10757269) association: study-specific estimates of effect for the discovery studies, population isolates, replication studies and overall discovery and replication meta-analyses.

Table 1

Meta-analysis results: ABI-SNP associations with $p<10^{-5}$ in the primary discovery analysis with population isolates excluded.

SNP	Chr	Physical Position	Closest Gene	Risk/Non-risk Allele	Risk Allele frequency	Meta-analysis	N	Beta	SE	P value	P_{het}
rs10757269	9	22062264	*CDKN2B*	G/A	0.49	ABI Discovery	35036	−0.0056	0.001	2.46E-08	0.23
						ABI Replication	16672	−0.0035	0.0015	1.76E-02	0.67
						ABI Combined	**51708**	**−0.0049**	**0.0008**	**2.65E-09**	**0.38**
						PAD† Discovery	34555	0.0849	0.0296	4.15E-03	0.32
rs4659996	1	238912747	*GREM2*	A/G	0.48	ABI Discovery	28087	−0.006	0.0012	4.44E-07	0.34
						ABI Replication	16658	−0.0018	0.0016	2.67E-01	0.65
						ABI Combined	**44745**	**−0.0045**	**0.001**	**2.12E-06**	**0.32**
						PAD Discovery	27574	0.0725	0.0319	2.31E-02	0.52
rs7003385	8	41705907	*ANK1*‡	T/C	0.67	ABI Discovery	35375	−0.0053	0.0011	5.24E-07	0.49
						ABI Replication	16690	−0.002	0.0016	2.20E-01	0.52
						ABI Combined	**52065**	**−0.0043**	**0.0009**	**1.11E-06**	**0.43**
						PAD Discovery	34903	0.0838	0.0314	7.57E-03	0.24
rs819750	1	99469651	*LPPR4*‡	G/T	0.12	ABI Discovery	35278	−0.007	0.0015	3.64E-06	0.51
						ABI Replication	16660	0.0022	0.0023	3.22E-01	0.99
						ABI Combined	**51938**	**−0.0041**	**0.0013**	**1.01E-03**	**0.31**
						PAD Discovery	34780	0.1068	0.0437	1.45E-02	0.06
rs9485528	6	102221473	*GRIK2*‡	A/G	0.17	ABI Discovery	35339	−0.0061	0.0013	4.63E-06	0.78
						ABI Replication	16679	0.0002	0.002	9.24E-01	0.63
						ABI Combined	**52018**	**−0.0041**	**0.0011**	**1.77E-04**	**0.48**
						PAD Discovery	34850	0.1172	0.0380	2.02E-03	0.80
rs722453	7	84037497	*SEMA3A*	G/A	0.42	**ABI Discovery**	**26200**	**−0.0054**	**0.0012**	**6.43E-06**	**0.69**
						ABI Replication	6300	−0.0046	0.0025	5.74E-02	0.08
						ABI Combined	**32500**	**−0.0052**	**0.0011**	**1.02E-06**	**0.59**
						PAD Discovery	25706	0.0575	0.0318	7.05E-02	0.63
rs16824978	2	211380306	*CPS1*	T/C	0.25	ABI Discovery	34950	−0.0054	0.0012	7.77E-06	0.37
						ABI Replication	14340	0.0000	0.0019	9.94E-01	0.22
						ABI Combined	**49290**	**−0.0039**	**0.001**	**1.48E-04**	**0.11**
						PAD Discovery	34518	0.0760	0.0343	2.65E-02	0.39

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P_{het}= p value for heterogeneity

‡SNP is located within the gene; rs819750 is within 60kb of the gene

†PAD discovery: ABI≤0.9 vs. ABI>0.9

Table 2

Meta-analysis results: SNP associations for PAD (ABI ≤0.9 vs ABI >0.9) with $p<10^{-5}$ with population isolates excluded.

SNP	Chr	Physical Position	Closest Gene	Risk/Non- risk Allele	Risk Allele frequency	N	OR	95% Confidence Interval	P value	P_{het}
rs6584389	10	102459392	*PAX2*	C/A	0.50	24474	1.17	(1.10, 1.25)	2.34E-06	0.37
rs9998941	4	162544312	*FSTL5**	A/G	0.23	34670	1.18	(1.10, 1.27)	2.34E-06	0.61
rs11751656	6	42751046	*UBR2**	G/A	0.07	27470	1.61	(1.32, 1.96)	2.46E-06	0.75
rs4535726	8	68938371	*DEPDC2*	T/C	0.20	34915	1.18	(1.10, 1.26)	3.79E-06	0.01
rs2090205	17	73897869	*PGS1**	A/C	0.24	34912	1.16	(1.09, 1.24)	5.01E-06	0.17
rs11933540	4	25729099	*RBPJ*	C/T	0.30	34830	1.15	(1.08, 1.23)	9.86E-06	0.08

P_{het}= p value for heterogeneity

* SNP is located within the gene

Table 3

Literature-reported candidate genes for peripheral artery disease (PAD) and coronary artery disease (CAD) and their association with ankle-brachial index (ABI) in the CHARGE GWAS discovery sample (population isolates excluded) with FDR <0.10[†].

SNP	Chr	Physical Position	Closest Gene	Risk/Non-risk Allele	Risk Allele frequency	N	Beta	SE	P value[*]	# of effective loci [‡]	P value corrected[‡]	False discovery rate[‡]
PAD genes												
rs10757269	9	22,062,264	*CDKN2B*	G/A	0.51	35036	−0.006	0.001	2.50E-08	69	1.70E-06	9.32E-05
rs3794624	16	87,244,575	*CYBA*	G/A	0.34	31035	−0.005	0.001	6.30E-05	58	3.60E-03	0.0665
rs13290547	9	123,527,316	*DAB2IP*	T/C	0.06	32135	−0.009	0.002	3.60E-05	97	3.50E-03	0.0665
CAD genes												
rs4977574	9	22,088,574	*CDKN2B*	G/A	0.49	35411	−0.0047	0.001	2.33E-06	-	-	6.52E-05
rs1122608	19	11,024,601	*LDLR*	G/T	0.74	35384	−0.0035	0.001	2.56E-03	-	-	0.036

[*] P value from Discovery GWAS of ABI

[†] Candidate genes for PAD were selected for testing with ABI if an association study with at least 100 cases and 100 controls was available in the literature independent of whether the study was positive or negative. Genes for CAD were only considered for testing with ABI if they were identified by recent GWAS to be genome-wide significantly associated with CAD. The table shows only the genes which showed an experiment-wise significant association with ABI after correction for multiple testing. The entire list of genes can be seen in Supplementary tables 10 and 11 for PAD and CAD genes, respectively.

[‡] Due to the high correlation of imputed genotype scores, the effective number of loci was calculated for each PAD gene region (31) using the genotype scores from the KORA F4 Study. Bonferroni correction of p-values was then applied in each region using this number. Furthermore, the corrected P value thresholds of significance for 28 CAD loci (tested in Suppl Table 11, α=0.05/28, 1.85×10^{-3}) and 55 PAD loci (tested in Suppl Table 10, α=0.05/effective number of loci) were calculated. We also calculated a false discovery rate (FDR) using the corrected p-values accounting for the number of gene regions examined. **An FDR <0.10 defined evidence of a significant association.**