Genetic Loci Associated With Nonobstructive Coronary Artery Disease in Caucasian Women

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Abstract (242 words)

Nonobstructive coronary artery disease (CAD) in women is associated with adverse cardiovascular (CV) outcomes, however information regarding genetic variants that predispose women to nonobstructive CAD is lacking. Women from the Women's Ischemia Syndrome Evaluation Study (WISE) and the St. James Women Take Heart Study (WTH) were genotyped using the Cardio-MetaboChip. WISE enrolled women with symptoms and signs of ischemia referred for coronary angiography; WTH enrolled asymptomatic, community-based women without heart disease. Analyses were conducted using a case (WISE) – control (WTH) design and multivariate logistic regression models to investigate genetic variation associated with likelihood of nonobstructive CAD. One genetic marker, SNP rs2301753 on chromosome 6 in RNF39 achieved chip-wide significance for nonobstructive CAD ($p < 9.5 \times 10^{-7}$). After adjusting for baseline characteristics, no variants achieved chip-wide significance. However, SNP rs2301753 on chromosome 6 in RNF39 was associated with reduced likelihood of nonobstructive CAD (odds ratio (OR) 0.42 and 95% confidence interval (CI) of 0.29 to 0.68), at a nominal level of $p = 5.6 \times 10^{-6}$, while SNP rs12818945 in the ATP2B1 locus on chromosome 12 was associated with increased odds for nonobstructive CAD, (OR 2.38 and 95% CI of 1.63 to 3.45), nominal $p=5.8\times10^{-6}$. The functions of RNF39 and ATP2B1 raise the possibility that genes involved in cardio-dysfunction may contribute to nonobstructive CAD in Caucasian women and may provide insights into novel approaches for therapy and prevention. If replicated, incorporation of these genetic variants into diagnostic evaluation may identify women at high risk for nonobstructive CAD.
Introduction

Metabolic conditions such as pre-diabetes, diabetes, prehypertension, hypertension, and dyslipidemia predispose individuals to ischemic heart disease, which is the leading cause of death in both men and women in United States (14). While ischemic heart disease due to obstructive coronary artery disease (CAD) is well studied (29), nonobstructive CAD also contributes to cardiovascular (CV) morbidity and mortality (1, 12, 16), but is less well characterized particularly in women, as nonobstructive CAD risk is often underdiagnosed and underestimated (18, 19). In women compared with men, there is more remodeling of the coronary arteries and more endothelial dysfunction, increasing their risk for developing nonobstructive CAD (2, 35). Nonobstructive CAD is a prevalent condition in women and is associated with an increase in risk for ischemia-related adverse outcomes including mortality (15). Furthermore, findings from the WISE have previously shown that myocardial ischemia is linked with metabolic traits and endothelial dysfunction (18, 20, 30).

Whether there are genetic factors that predispose women to pathological metabolic conditions that are mechanistically-related to nonobstructive CAD is not known. This study was designed to investigate genetic variants associated with nonobstructive CAD in Caucasian women by utilizing clinical data and biological material collected from two large and complementary cohorts, the Women’s Ischemia Syndrome Evaluation Study (WISE) (25), which enrolled women with chest pain and/or suspected myocardial ischemia who were ultimately diagnosed with nonobstructive CAD, and the St. James Women Take Heart Study (WTH) (11), which enrolled asymptomatic women without known heart disease. These phenotypically defined
cohorts represent a unique opportunity to investigate specific associations between confirmed metabolic cardiovascular genetic variants and clinically defined nonobstructive CAD, that would not be possible in more diverse or clinically less well characterized populations.

Given the high prevalence of adverse metabolic conditions that associate with nonobstructive CAD in women, we used the custom-designed Cardio-MetaboChip, which includes SNPs related to metabolic traits as well as CV disease, to examine potential genetic variants that might associate with nonobstructive CAD phenotype in women.

**Method and Materials**

**General design**

The general design is a case controlled study where cases were women enrolled in the the WISE study who presented with chest pain and/or suspected myocardial ischemia and were ultimately diagnosed with nonobstructive CAD, and controls were women enrolled in the Women Take Heart study, who were asymptomatic without known heart disease. Demographic and clinical measurements from these cohorts were available for use in the current analysis.

**Women’s Ischemia Syndrome Evaluation (WISE)**

The WISE is a prospective study of women who underwent clinically indicated coronary angiography for signs and symptoms of myocardial ischemia, with the objective of improving strategies for diagnosing CAD in women. Of the 935 women originally
enrolled in WISE between 1997 and 2000, 512 Caucasians were genotyped with the Cardio-MetaboChip. At the baseline visit, demographic information was collected, the women underwent a physical examination, and a medical history which included information regarding CV symptoms was obtained. Coronary angiography was quantitatively and qualitatively evaluated for the presence and extent of CAD by the WISE angiographic core laboratory (masked to historical data) as previously described (33), to classify CAD status (25). Women with obstructive CAD defined as ≥50% stenosis in any epicardial coronary artery were excluded. Caucasian women with signs and symptoms of ischemia and with nonobstructive CAD (defined as <50% stenosis in any coronary artery) were included in this analysis (10).

St. James Women Take Heart (WTH)

WTH is a prospective study dedicated to evaluating risk factors for heart disease in asymptomatic women volunteers from the greater Chicago metropolitan area. A total of 5932 participants were enrolled from 1992 to 2000 (11) and 1036 Caucasian women were genotyped on the Cardio-MetaboChip. All women were free of diagnosis of any CV disease, and were able to walk on a treadmill at a moderate pace. Women were excluded if they were pregnant, had experienced typical anginal symptoms or myocardial infarction within the previous 3 months, weighed more than 147 kg, or had a BP ≥170/110 mm Hg. Women from WTH with signs/symptoms of CAD based on exercise test were excluded from this study. All participants underwent a physical examination and resting electrocardiogram, and supine BP measurements.

Genotyping
All women enrolled in the WISE and WTH provided voluntary informed consent for participation and for collection of a DNA sample. All DNA samples were genotyped using the Cardio-MetaboChip, which is a custom Illumina genotyping array with ~200,000 selected SNPs of interest from loci identified in GWAS for metabolic and atherosclerotic/CV disease traits (37). Content on the chip was selected on the basis of large scale meta-analysis of relevant traits (including up to 100,000 individuals) and of HapMap and 1000 Genomes Project SNP content. The chip was designed by collaborating representatives of the CARDIoGRAM (coronary artery disease), DIAGRAM (type 2 diabetes), GIANT (height and weight), MAGIC (glycemic traits), Lipids (lipids), ICBP-GWAS (blood pressure), and QT-IGC (QT interval) GWAS meta-analysis consortia (37). After removing SNPs with call rate <0.05, monomorphic SNPs with MAF<0.0001 and SNPs not in Hardy-Weinberg equilibrium (p<0.0001), a total of 121,313 SNPs were available. Linkage disequilibrium (LD) pruning with cutoff $r^2=0.5$ yielded 64,687 unlinked SNPs. Because our goal was to identify more common genetic variants associated with nonobstructive CAD, we removed the rare genetic variants, defined as a minor allele frequency of less than 3%, which further reduced the total number of SNPs included in the association analyses to 52,371, and yielded a suggested p-value of $9.5 \times 10^{-7}$ ($0.05/52,371$) for chip-wide significance. A p-value of $1 \times 10^{-4}$ was considered suggestive of significance.

**Statistical analysis**

Although all participants included were Caucasian based on self-report, principal component analysis was performed with EIGENSOFT to analyze genetically determined ancestry information. Outliers were excluded based on this ancestral analysis. Logistic
regression with an additive model was conducted between the 332 WISE cases and the 1003 WTH controls with and without adjustment for covariates, in PLINK version 1.07. Genomic inflation factor (\(\lambda\)) was calculated with the adjust function in plink. Covariates in the logistic regression model included age, BMI, history of diabetes and principal components for population stratification. Because the top identified signals, loci in ATP2B1, a gene involved with intracellular calcium homeostasis, and RNF39, a gene thought to contribute in an early phase of synaptic plasticity, were previously identified as markers for diastolic blood pressure and waist hip ratio, respectively (23, 37), and the loci were identified in our analysis as associated with nonobstructive CAD, we conducted separate sensitivity analyses in additional logistic regression models that included waist hip ratio and diastolic blood pressure. For the sensitivity analysis of the ATP2B1 signal and diastolic blood pressure, we included use of antihypertensive medications as a covariate in the model, and we also tested the association in a smaller cohort that excluded women who reported using antihypertensive medications. Lastly, we performed univariate analyses of our top genetic signals and other characteristics (history of hypertension, family history of ischemic heart disease and smoking status) that differed significantly at baseline to confirm an association with nonobstructive CAD. Haploview 4.2 was employed to generate Manhattan plots and run haplotype analysis. Regional plots were generated by LocusZoom (https://statgen.sph.umich.edu/locuszoom). The Student T-test was used to examine the differences for continuous characteristics, while Chi-square tests were employed to estimate the differences for dichotomous characteristics, comparing the groups. A power calculation was performed with Quanto using log-additive model. Power
estimation with a 2-sided alpha level of $9.5 \times 10^{-7}$ in our datasets suggest that, we have
80% power to detect genetic variants with odds ratio (OR) of 2.6 for MAF = 0.05, 2.1 for MAF = 0.10 and 1.8 for MAF = 0.20.
Results

Clinical characteristics of the cohorts

WISE: Of the 512 Caucasian women with available genotype data, 346 met the definition of nonobstructive CAD (≤50% stenosis) and were included in the genetic analysis. After quality control procedures, 14 women were excluded: 8 due to likely relatedness and 6 whose ancestry identifiers did not cluster with CEU samples (Centre d’Etude du Polymorphisme Humain people from Utah, USA) in HapMap 3, leaving 332 women included in the final WISE dataset for genetic analysis (Figure 1).

WTH: There were 1,036 Caucasian women with available genotype data. After quality control procedures, 33 women were excluded: 28 women due to likely relatedness and 5 women whose ancestry identifiers did not cluster with CEU samples in HapMap 3, yielding 1003 women included in the final WTH dataset for analysis (Figure 1).

Pertinent baseline characteristics of the women included in this genotyping study are summarized in Table 1. As expected, since they were without known CVD, the WTH participants were younger and leaner than those from the WISE cohort. Also as expected the prevalence of diabetes, hypertension and smoking were significantly greater in the WISE participants than those in the WTH (p<0.0001 for all). Neither HDL cholesterol nor LDL cholesterol differed between the cohorts, which may be due to the use of lipid-lowering drugs in both cohorts.

Unadjusted association analysis
Using the SNP genotype characterized by the Cardio-MetaboChip, a logistic regression was first performed between the 332 WISE cases and 1003 WTH controls, without adjusting for any covariates. As shown in the Manhattan plot (Figure 2), 3 SNP clusters (on chromosome 6, 12 and 15) are noteworthy. One SNP, rs2301753 in RNF39 on chromosome 6, reached the specified level of statistical significance for the association with nonobstructive CAD in Caucasian women, with a p-value of $7.3 \times 10^{-7}$ (Table 2). A functional search revealed that rs2301753 represents a missense variant (C>A), altering the RNF39 coding sequence at position #304 from alanine to glutamic acid. The top SNP cluster on chromosome 12 was found to be in the ATP2B1 locus, while the top SNP cluster on chromosome 15 is in an intergenic region.

Adjusted Association Analysis

After adjusting for age, body mass index, diabetes and ancestry-informative principal components, logistic regression analysis was conducted again in the two cohorts. The genomic inflation factor ($\lambda$) is 1, suggesting the populations were similar in the two cohorts. After adjustment, as shown in Figure 3, no signals achieved chip-wide significance, although SNP rs2301753 in RNF39 on chromosome 6 remained the top signal. The regional plot of the RNF39 locus suggests that rs2301753 is in high linkage disequilibrium with several SNPs in RNF39 and downstream genes coding for human leukocyte antigens (HLAs) (Figure 4A). Top signals from the adjusted analysis were also found on chromosome 12 in the ATP2B1 locus, either in an intron or near the gene, and were in high linkage disequilibrium (Figure 4B). Table 3 summarizes the top signals from the adjusted analysis.
Because *ATP2B1* has been identified in GWAS as a hypertension marker (associated with diastolic blood pressure) (23, 36), we also tested the association of the 5 SNPs in *ATP2B1* (chr12:88382885, rs34205054, rs10506975, rs12818945, rs73198547) and diastolic blood pressure in a linear regression of the combined WISE and WTH participants. We observed a consistent and similar trend for association of these *ATP2B1* markers and diastolic blood pressure (Table 4). Therefore, diastolic blood pressure was added as a covariate in the logistic model, and SNPs rs2301753 and rs12818945 remained at the top of the list of associated signals (Table 5). Association with this ATP2B1 marker remained after including use of antihypertensive medications in the model or excluding women who reported use of antihypertensive medications (data not shown). In addition, rs2301753 was identified in GWAS as a marker for waist-hip ratio (37), and we tested this association in our dataset with a univariate model and found that rs2301753 significantly correlated with waist-hip ratio, p=0.002. When waist-hip ratio was included in the regression model, rs2301753 remained significantly associated with reduced odd of nonobstructive CAD, with p=4x10^-4. Finally, when the top signals were tested in univariate regression models for other characteristics that differed at baseline among the WISE and WTH cohorts, rs2301753 and rs12818945 were consistent and remained at the level of suggestive associations for nonobstructive CAD.

Discussion

This study using the Cardio-MetaboChip identified two suggestive genetic loci associated with nonobstructive CAD in well characterized cohorts of women, one on chromosome 6 and one on chromosome 12. These genetic signals were previously
identified and replicated in CAD GWAS analyses, and now we have extended the
association of these signals to the nonobstructive CAD phenotype. The SNPs on
chromosome 6 are located in the major histocompatibility complex, a region in which the
majority of genes are involved in immune response and inflammation. In contrast, the
SNPs on chromosome 12 are involved in blood pressure susceptibility.

The top signal, rs2301753 in RNF39, achieved chip-wide significance in the unadjusted
analysis. This SNP causes a missense change in the RNF39 protein, from a non-polar
to a polar amino acid, indicating the potential for a substantial change in protein
function, although loss of function was not confirmed using Polyphen-2 (that rated the
variant as benign with a score of 0) (http://genetics.bwh.harvard.edu/pph2/). RNF39 is a
protein with RING finger domain, a Cys$_3$HisCys$_4$ zinc finger which binds two zinc
cations. Although the exact role of RNF39 in vivo remains to be established in humans,
its chromosomal position, encompassed by HLA genes, indicates its potential role in
immune response. In a Japanese cohort, RNF39 was associated with Behcet’s disease,
an autoimmune disorder causing inflammation in blood vessels (22). All of these data
suggest the SNP rs2301753 could substantially affect function of RNF39 in immune
response and inflammation, possibly in the vessel wall. Therefore, we hypothesize that
loss of function of RNF39 caused by the rs2301753 minor allele, the allele associated in
WISE, compromises or disables the buildup of immune reactions. However, this down-side may have some interesting “beneficial” effect in ischemic heart disease in women.

As growing evidence has pointed out, an inflammatory milieu may well be the
underlying cause of the pathophysiology of nonobstructive CAD (4, 7), which in women
is thought to encompass the smaller coronary arteries/arterioles, and has been implicated to cause myocardial ischemia (4).

The loss of function of RNF39 may lower the risk for formation of endothelial plaque in coronary microvessels. This is consistent with our study that the rs2301753 minor allele was associated with reduced odds of nonobstructive CAD, suggesting a relative protective effect. Previous investigations from WISE and others have characterized C-reactive protein, IL-6 and serum amyloid A as inflammation risk factors contributing to nonobstructive CAD in women (17, 31, 34, 38). Furthermore, development of global measures of inflammation, and simply counting the number of inflammatory markers with high levels, improve CVD risk stratification (21). Recently, an improved algorithm to estimate CV risk in women, the Reynolds Risk Score, was developed by incorporating the level of C-reactive protein into the traditional Framingham Risk Score, which may improve the prediction of the 10-year risk of cardiac death or myocardial infarction (26, 32). Additionally, the Cardiovascular Inflammation Reduction Trial (CIRT) is currently testing the inflammation hypothesis of atherothrombosis by evaluating whether or not low-dose methotrexate will reduce rates of adverse CV outcomes among a cohort of stable CAD patients with diabetes or metabolic syndrome, conditions associated with an enhanced pro-inflammatory response (8). Our discovery that variation in rs2301753 in RNF39 is associated with risk for nonobstructive CAD is consistent with previous findings in WISE that inflammatory processes play a key role and further highlight the critical role of inflammatory factors in the development of nonobstructive CAD in women.
However, inflammation is not the only factor contributing to nonobstructive CAD. Other cardiac risk factors, such as hypertension, diabetes and high levels of low density lipoprotein cholesterol, contribute to nonobstructive CAD, dependently or independently. *ATP2B1* codes for an ATP-driven calcium channel on the cell membrane, and SNPs in *ATP2B1* have been associated with hypertension and CAD in previous GWA studies (23, 36). This calcium channel functions to pump Ca$^{2+}$ out of the cell and maintain cellular Ca$^{2+}$ homeostasis. Down-regulation of *ATP2B1* abundance causes increased Ca$^{2+}$ level in vascular smooth muscle cells, resulting in vasoconstriction and elevated blood pressure (28). Previous findings from WISE indicate that an intronic variant in *ATP2B1* (rs12817819) is associated with increased risk for resistant hypertension (9). Even though the top 5 SNPs in *ATP2B1* locus (Table 2) are either in introns or intergenic regions, a query with RegulomeDB (http://regulomedb.org/) indicates that rs10506975, which is in high linkage disequilibrium with our lead signal rs12818945, is predicted to be highly functional (RegulomeDB score 1f). A meta-analysis of two GWAS studies of CAD in a sample including ~33,000 Chinese with Han ancestry identified the *ATP2B1* locus to be associated with CAD with genome-wide significance(24), although the GWA studies did not evaluate the effect of sex.

It is reasonable to hypothesize a synergistic mechanism between inflammation and blood pressure, with increasing levels of both contributing to nonobstructive CAD. In the setting of hypertension, the altered mechanical force across the arterial walls likely impairs the endothelium. However, the causal relationship between calcium regulation at the level of the microvasculature and immunological processes is not well described.
When diastolic blood pressure and waist-hip ratio were respectively included in the final regression models, we found that, while the further adjustment with diastolic blood pressure didn’t affect much of the significance of the top signals (e.g. rs2301753 and rs12818945), the additional adjustment of waist-hip ratio attenuated the contribution of rs2301753 toward the risk of non-obstructive CAD, with the level of significance decreasing from 8.9x10^-6 to 4.0x10^-4. This attenuated significance suggests that the metabolic milieu identified by the waist-hip ratio contributes to the development of nonobstructive CAD in this cohort of women. Previous studies have described an association of waist-hip ratio and endothelial function, CAD and CV death. (3, 13, 27, 39).

Several of the SNPs identified among the top signals (Table 2) in the present study have been implicated previously to be associated with CAD or other CV diseases. Duan et al. in 2013 published their identification of AGPAT4, a gene encoding a member of the 1-acylglycerol-3-phosphate O-acyltransferase family and involved in de novo phospholipid biosynthesis with CAD susceptibility via integrative network analysis (5). Westaway et al. in 2011 reported that common variants in NOS1AP, a gene encoding a cytosolic protein that binds to the signaling molecule, neuronal nitric oxide synthase (nNOS), were significantly associated with the risk of sudden death in CAD patients (40). Ellinor et al. in 2012 demonstrated that genetic variants in SYNE2, a gene encoding a nuclear outer membrane protein that binds cytoplasmic F-actin and involved in the maintenance of the structural integrity of the nucleus, were significantly associated with atrial fibrillation in European descendants through meta-analysis (6). The association of these loci with CAD or CV diseases in the literature were not studied
specifically related to men or women. However, they greatly decrease the likelihood that
the observed signals in the studied cohort of women are spurious. Further investigation
is warranted to assess the potential for these genetic markers to aid in the prediction of
increased or decreased risk for nonobstructive CAD in other groups of women.

There are several limitations to the present study. The sample size is relatively small
(332 cases and 1003 controls) with limited power to detect significant associations,
particularly for rare genetic variants. Additionally, this study was conducted in
Caucasian women from two cohorts with some differing baseline characteristics which
may introduce some confounding with regard to risk factor associations. Results from
our study cannot be generalized to other race groups or populations. Lastly, use of the
Cardio-MetaboChip which was developed in 2012, only includes GWAS signals related
to metabolic and CV diseases identified prior to that time. Newer genetic variants
associated with metabolic or CV diseases were not included in this study. Despite these
limitations, we did identify biologically plausible genetic markers for nonobstructive CAD
that warrant further investigation and confirmation in an independent population.

In conclusion, this study identified suggestive evidence that genetic variants in $RNF39$
and $ATP2B1$ are associated with nonobstructive CAD in women. These findings further
underscore the importance of inflammation and blood pressure variation and reactivity
in the development of nonobstructive CAD and warrant further investigation and
replication in other cohorts. If replicated, incorporation of these genetic variants into
predictive models may improve the prediction of nonobstructive CAD in Caucasian
women.
References:


20. Kip KE, Marroquin OC, Kelley DE, Johnson BD, Kelsey SF, Shaw LJ, Rogers WJ, and Reis SE. Clinical importance of obesity versus the metabolic


from the Asymptomatic Cardiac Ischemia Pilot (ACIP) study angiographic core laboratory. *J Am Coll Cardiol* 29: 78-84, 1997.


Figure Legends

Figure 1. Flow diagram of WISE and WTH participants enrolled for the analysis.
The numbers of participants excluded and used in our analysis from the two cohorts are indicated. WISE, Women’s Ischemia Syndrome Evaluation; WTH, St. James Women Take Heart; NCAD, nonobstructive coronary artery disease.

Figure 2. Manhattan plot of associations with nonobstructive CAD in selected WISE and WTH participants without adjustment. X-axis, the chromosome position of all SNPs; Y-axis, -log10 transformed p-values for the associations. Chr, chromosome.

Figure 3. Manhattan plot of associations with nonobstructive CAD in selected WISE and WTH participants. The covariates for the logistic model include age, body mass index, history of diabetes and principal components for population correction. X-axis, the chromosome position of all SNPs; Y-axis, -log10 transformed p-values for the associations. Chr, chromosome.

Figure 4. Regional plot of SNPs in RNF39 and ATP2B1. The -log10 transformed p-values for each association are shown on the Y-axis, with X-axis showing physical position on chromosome 12. SNPs are colored based on their r² with the labeled top SNP which has the smallest p-value in the region, indicated in purple diamond. Recombination rates estimated from individuals in the HapMap population are indicated by horizontal blue lines. Genes within the recombination region of the top SNPs are labeled in the bottom. A, rs2301753 in RNF39 locus. B, rs12818945 in ATP2B1 locus.
<table>
<thead>
<tr>
<th>Demographic baselines</th>
<th>WISE (n=332)</th>
<th>WTH (n=1003)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age±SD (years)</td>
<td>55.8±10.3</td>
<td>53.3±10.9</td>
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</tr>
<tr>
<td>Mean BMI±SD (kg/m^2)</td>
<td>29.4±6.7</td>
<td>27.2±6.6</td>
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<td>Diabetes (%)</td>
<td>12.4%</td>
<td>3.5%</td>
<td>&lt;0.0001</td>
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<tr>
<td>Hypertension (%)</td>
<td>49.1%</td>
<td>16.1%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Family history of IHD (n, %)</td>
<td>228, 68.8%</td>
<td>464, 46.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean HDL-C±SD (mg/dL)</td>
<td>53.6±15.4</td>
<td>51.7±14.9</td>
<td>0.10</td>
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<tr>
<td>Mean LDL-C±SD (mg/dL)</td>
<td>126.1±45.3</td>
<td>124.0±32.8</td>
<td>0.47</td>
</tr>
<tr>
<td>Mean SBP±SD (mm Hg)</td>
<td>134.1±20.2</td>
<td>129.3±19.7</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mean DBP±SD (mm Hg)</td>
<td>76.3±10.4</td>
<td>81.4±11.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean WHR ± SD</td>
<td>0.78±0.08</td>
<td>0.84±0.11</td>
<td>&lt;0.0001</td>
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<tr>
<td>Smoking (%)</td>
<td>30.1%</td>
<td>13.6%</td>
<td>&lt;0.0001</td>
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<td>Nonobstructive CAD</td>
<td>60.2% (&lt;20%</td>
<td>NA</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>stenosis); 39.8% (20-49% stenosis)</td>
<td>NA</td>
<td></td>
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Note: 
*: Continuous characteristics are presented as mean ± standard deviation; Categorical characteristics are presented as percentage. BMI, body mass index; IHD, ischemic heart diseases; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; WHR, waist hip ratio; CAD, coronary artery disease. NA, not available.
Table 2. Top signals of logistic regression ($p<10^{-4}$) without adjustment.

<table>
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<tr>
<th>SNP</th>
<th>CHR</th>
<th>Base Pair Position</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>Selection Category†</th>
<th>Host gene</th>
<th>OR (95% CI)</th>
<th>P Value</th>
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<tr>
<td>rs2301753</td>
<td>6</td>
<td>30147219</td>
<td>A</td>
<td>0.117</td>
<td>WHR</td>
<td>RNF39</td>
<td>0.40 (0.28, 0.57)</td>
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<td>2.41 (1.68, 3.47)</td>
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<td>A</td>
<td>0.040</td>
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<td>rs8095193</td>
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<td>A</td>
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<td>QT</td>
<td>intergenic</td>
<td>1.60 (1.30, 1.96)</td>
<td>7.33E-06</td>
</tr>
<tr>
<td>rs550338</td>
<td>12</td>
<td>24403304</td>
<td>A</td>
<td>0.238</td>
<td>T2D</td>
<td>SOX5</td>
<td>0.61 (0.48, 0.76)</td>
<td>1.16E-05</td>
</tr>
<tr>
<td>rs11657937</td>
<td>17</td>
<td>56508571</td>
<td>A</td>
<td>0.063</td>
<td>SBP</td>
<td>BCAS3</td>
<td>2.01 (1.47, 2.75)</td>
<td>1.18E-05</td>
</tr>
<tr>
<td>rs12818945</td>
<td>12</td>
<td>88542365</td>
<td>A</td>
<td>0.047</td>
<td>DBP</td>
<td>ATP2B1</td>
<td>2.19 (1.53, 3.13)</td>
<td>1.84E-05</td>
</tr>
<tr>
<td>rs9379976</td>
<td>6</td>
<td>27406179</td>
<td>G</td>
<td>0.236</td>
<td>MHC</td>
<td>intergenic</td>
<td>1.53 (1.25, 1.87)</td>
<td>3.18E-05</td>
</tr>
<tr>
<td>rs17532886</td>
<td>16</td>
<td>50026847</td>
<td>G</td>
<td>0.226</td>
<td>FG</td>
<td>intergenic</td>
<td>0.62 (0.50, 0.78)</td>
<td>3.19E-05</td>
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<tr>
<td>rs2445887</td>
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<td>78345800</td>
<td>A</td>
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<td>FG</td>
<td>DMGDH</td>
<td>0.69 (0.58, 0.83)</td>
<td>4.78E-05</td>
</tr>
<tr>
<td>chr1:160598245</td>
<td>1</td>
<td>160598245</td>
<td>G</td>
<td>0.371</td>
<td>QT</td>
<td>NOS1AP</td>
<td>1.45 (1.21, 1.74)</td>
<td>5.22E-05</td>
</tr>
<tr>
<td>rs9266772</td>
<td>6</td>
<td>31460092</td>
<td>A</td>
<td>0.185</td>
<td>SBP</td>
<td>intergenic</td>
<td>1.56 (1.26, 1.93)</td>
<td>5.59E-05</td>
</tr>
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<td>228915982</td>
<td>G</td>
<td>0.436</td>
<td>MICAD</td>
<td>AGT</td>
<td>0.70 (0.58, 0.83)</td>
<td>7.91E-05</td>
</tr>
<tr>
<td>rs2024366</td>
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<td>153663907</td>
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<td>SBP</td>
<td>DPP6</td>
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<td>8.03E-05</td>
</tr>
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<td>QT</td>
<td>C13orf33</td>
<td>1.48 (1.22, 1.81)</td>
<td>8.08E-05</td>
</tr>
</tbody>
</table>

Note: SNP, SNP identifier; CHR, chromosome number; MAF, minor allele frequency; Host gene, gene locus of SNP; OR, odds ratio; 95% CI, 95% confidence interval; P, asymptotic p-value for t-statistic.

†: Selection category is the functional annotation of the selected SNP on the Cardio-MetaboChip. WHR, waist-hip ratio; QT, QT interval; HDL, high-density lipoprotein; T2D, type 2 diabetes; SBP, systolic blood pressure; DBP, diastolic blood pressure; MHC, major histocompatibility complex; FG, fasting glucose level; MICAD, myocardial infarction and coronary artery diseases.
Table 3 Top signals of logistic regression (p<10^{-4}) in WISE and WTH participants adjusted for age, BMI, diabetes, and principal components for ancestral correction

<table>
<thead>
<tr>
<th>SNP</th>
<th>CHR</th>
<th>Base Pair Position</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>Selection Category†</th>
<th>Host gene</th>
<th>OR (95% CI)</th>
<th>P Value^</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12818945</td>
<td>12</td>
<td>88542365</td>
<td>A</td>
<td>0.047</td>
<td>DBP</td>
<td>ATP2B1</td>
<td>2.38 (1.63, 3.45)</td>
<td>5.82E-06</td>
</tr>
<tr>
<td>rs2301753</td>
<td>6</td>
<td>30147219</td>
<td>A</td>
<td>0.117</td>
<td>WHR</td>
<td>RNF39</td>
<td>0.42 (0.29, 0.62)</td>
<td>8.91E-06</td>
</tr>
<tr>
<td>chr17:30331990</td>
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<td>30331990</td>
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<td>QT</td>
<td>LIG3</td>
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<td>1.20E-05</td>
</tr>
<tr>
<td>rs17532886</td>
<td>16</td>
<td>50026847</td>
<td>G</td>
<td>0.226</td>
<td>fastGlu</td>
<td>intergenic</td>
<td>0.61 (0.48, 0.77)</td>
<td>3.02E-05</td>
</tr>
<tr>
<td>rs3852892</td>
<td>19</td>
<td>59414407</td>
<td>A</td>
<td>0.113</td>
<td>N/A</td>
<td>intergenic</td>
<td>1.76 (1.35, 2.31)</td>
<td>3.85E-05</td>
</tr>
<tr>
<td>rs6906489</td>
<td>6</td>
<td>161573429</td>
<td>G</td>
<td>0.047</td>
<td>DBP</td>
<td>AGPAT4</td>
<td>0.27 (0.15, 0.51)</td>
<td>3.90E-05</td>
</tr>
<tr>
<td>chr15:61195932</td>
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<td>61195932</td>
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<td>HDL</td>
<td>intergenic</td>
<td>2.41 (1.58, 3.70)</td>
<td>5.19E-05</td>
</tr>
<tr>
<td>rs550338</td>
<td>12</td>
<td>24403304</td>
<td>A</td>
<td>0.238</td>
<td>T2D</td>
<td>SOX5</td>
<td>0.62 (0.49, 0.78)</td>
<td>5.25E-05</td>
</tr>
<tr>
<td>chr1:160598245</td>
<td>1</td>
<td>160598245</td>
<td>G</td>
<td>0.371</td>
<td>QT</td>
<td>NOS1AP</td>
<td>1.48 (1.22, 1.78)</td>
<td>5.56E-05</td>
</tr>
<tr>
<td>rs9379976</td>
<td>6</td>
<td>27406179</td>
<td>G</td>
<td>0.236</td>
<td>MHC</td>
<td>intergenic</td>
<td>1.54 (1.25, 1.91)</td>
<td>5.71E-05</td>
</tr>
<tr>
<td>rs10503586</td>
<td>8</td>
<td>16792998</td>
<td>G</td>
<td>0.074</td>
<td>T2D</td>
<td>intergenic</td>
<td>1.92 (1.40, 2.63)</td>
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</tr>
<tr>
<td>chr12:88382885</td>
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<td>88382885</td>
<td>A</td>
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<td>SBP</td>
<td>TUWD12</td>
<td>2.53 (1.60, 4.00)</td>
<td>7.22E-05</td>
</tr>
<tr>
<td>rs9266772</td>
<td>6</td>
<td>31460092</td>
<td>G</td>
<td>0.185</td>
<td>SBP</td>
<td>intergenic</td>
<td>1.57 (1.25, 1.96)</td>
<td>8.00E-05</td>
</tr>
<tr>
<td>rs12025601</td>
<td>1</td>
<td>96796873</td>
<td>A</td>
<td>0.249</td>
<td>BMI</td>
<td>intergenic</td>
<td>1.52 (1.23, 1.87)</td>
<td>8.36E-05</td>
</tr>
<tr>
<td>rs9379977</td>
<td>6</td>
<td>27406258</td>
<td>G</td>
<td>0.235</td>
<td>MHC</td>
<td>intergenic</td>
<td>1.53 (1.24, 1.89)</td>
<td>8.37E-05</td>
</tr>
<tr>
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<td>G</td>
<td>0.156</td>
<td>HbA1C</td>
<td>SYNE2</td>
<td>1.63 (1.28, 2.09)</td>
<td>9.17E-05</td>
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</tbody>
</table>

CHR, chromosome number; SNP, single nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; 95% CI, confidence interval; ^asymptotic p-value for t-statistic.

†: Selection category is the functional annotation of the selected SNP on the Cardio-MetaboChip. DBP, diastolic blood pressure; WHR, waist-hip ratio; QT, QT interval; fastGlu, fasting glucose level; HDL, high-density lipoprotein; T2D, type 2 diabetes; MHC, major histocompatibility complex; SBP, systolic blood pressure; BMI, body mass index; HbA1C, hemoglobin A1c; N/A, not available.
Table 4. Associations of *ATP2B1* SNPs with diastolic blood pressure in univariate model in WISE/WTH cohorts.

<table>
<thead>
<tr>
<th>SNP</th>
<th>CHR</th>
<th>Base Pair Position</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>BETA</th>
<th>P</th>
</tr>
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<tbody>
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<td>88382885</td>
<td>A</td>
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</tr>
<tr>
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<td>88471152</td>
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<td>0.046</td>
<td>-1.72</td>
<td>0.074</td>
</tr>
<tr>
<td>rs10506975</td>
<td>12</td>
<td>88510654</td>
<td>G</td>
<td>0.047</td>
<td>-1.85</td>
<td>0.058</td>
</tr>
<tr>
<td>rs12818945</td>
<td>12</td>
<td>88542365</td>
<td>A</td>
<td>0.047</td>
<td>-1.78</td>
<td>0.068</td>
</tr>
<tr>
<td>rs73198547</td>
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<td>88579287</td>
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<td>0.047</td>
<td>-1.86</td>
<td>0.057</td>
</tr>
</tbody>
</table>

Note: SNP, SNP identifier; CHR, chromosome number; MAF, minor allele frequency; BETA, regression coefficient; P, asymptotic p-value for t-statistic.
Table 5. Top signals of logistic regression (p<10^{-4}) adjusted for age, body mass index, diabetes, principal components for ancestral correction, and diastolic blood pressure.

<table>
<thead>
<tr>
<th>SNP</th>
<th>CHR</th>
<th>Base Pair Position</th>
<th>Minor Allele</th>
<th>MAF</th>
<th>Selection Category†</th>
<th>Host gene</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>rs9266772</td>
<td>6</td>
<td>31460092</td>
<td>G</td>
<td>0.185</td>
<td>SBP</td>
<td>intergenic</td>
<td>1.67 (1.32, 2.10)</td>
<td>1.50E-05</td>
</tr>
<tr>
<td>rs6906489</td>
<td>6</td>
<td>161573429</td>
<td>G</td>
<td>0.047</td>
<td>DBP</td>
<td>AGPAT4</td>
<td>0.25 (0.13, 0.47)</td>
<td>1.85E-05</td>
</tr>
<tr>
<td>rs3852892</td>
<td>19</td>
<td>59414407</td>
<td>A</td>
<td>0.113</td>
<td>N/A</td>
<td>intergenic</td>
<td>1.78 (1.35, 2.35)</td>
<td>5.18E-05</td>
</tr>
<tr>
<td>rs10503586</td>
<td>8</td>
<td>16792998</td>
<td>G</td>
<td>0.074</td>
<td>T2D</td>
<td>intergenic</td>
<td>1.99 (1.42, 2.77)</td>
<td>5.82E-05</td>
</tr>
<tr>
<td>rs2301753</td>
<td>6</td>
<td>30147219</td>
<td>A</td>
<td>0.117</td>
<td>WHR</td>
<td>RNF39</td>
<td>0.45 (0.30, 0.67)</td>
<td>5.95E-05</td>
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<tr>
<td>chr1:160598245</td>
<td>1</td>
<td>160598245</td>
<td>G</td>
<td>0.371</td>
<td>QT</td>
<td>NOS1AP</td>
<td>1.49 (1.22, 1.82)</td>
<td>6.69E-05</td>
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<tr>
<td>rs12818945</td>
<td>12</td>
<td>88542365</td>
<td>A</td>
<td>0.047</td>
<td>DBP</td>
<td>ATP2B1</td>
<td>2.23 (1.50, 3.31)</td>
<td>6.97E-05</td>
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<tr>
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<td>A</td>
<td>0.249</td>
<td>BMI</td>
<td>intergenic</td>
<td>1.54 (1.24, 1.92)</td>
<td>9.10E-05</td>
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</table>

Note: SNP, SNP identifier; CHR, chromosome number; MAF, minor allele frequency; Host gene, gene locus of SNP; OR, odds ratio; 95% CI, 95% confidence interval; P, asymptotic p-value for t-statistic.

†: Selection category is the functional annotation of the selected SNP on Cardio-MetaboChip. SBP, systolic blood pressure; DBP, diastolic blood pressure; N/A, not available; T2D, type 2 diabetes; WHR, waist-hip ratio; QT, QT interval; BMI, body mass index.
WISE study

936 patients recruited in WISE
Genotyped with Cardio-MetaboChip

512 Caucasian patients genotyped
Exclude 166 patients with obstructive IHD

346 NCAD patients
Remove 8 patients for processing errors

338 NCAD patients with credible genotypes
Remove 6 patients for ancestral discrepancy

332 NCAD patients with correct ethnic identity

Case group

WTH study

5932 women included
Genotyped with Cardio-MetaboChip

1036 Caucasian participants genotyped
Remove 28 patients for processing errors

1008 participants with credible genotypes
Remove 5 patients for ancestral discrepancy

1003 participants with correct ethnic identity

Control group