Sex-Specific Genetic Variants are Associated With Coronary Endothelial Dysfunction

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Background—Endothelial dysfunction is an early stage of atherosclerosis. Single-nucleotide polymorphisms (SNPs) have been associated with vascular dysfunction, cardiac events, and coronary artery remodeling. We aimed to detect SNPs associated with endothelial dysfunction and determine whether these associations are sex specific.

Methods and Results—Six hundred forty-three subjects without significant obstructive coronary artery disease underwent invasive coronary endothelial function assessment. We collected data from 1536 SNPs that had previously been associated with vasoreactivity, angiogenesis, inflammation, artery calcification, atherosclerotic risk factors, insulin resistance, hormone levels, blood coagulability, or with coronary heart disease. Coronary vascular reactivity was assessed by the percent change in coronary artery diameter $\leq -20\%$ after an intracoronary bolus injection of acetylcholine on invasive coronary physiology study. SNPs significantly associated with coronary epicardial endothelial dysfunction were *ADORA1*, *KCNQ1*, and *DNAJC4* in the whole cohort, *LPA*, *MYBPH*, *ADORA3*, and *PON1* in women and *KIF6* and *NFKB1* in men ($P<0.01$).

Conclusions—We have identified several significant SNPs that are associated with an increased risk of coronary endothelial dysfunction. These associations appear to be sex specific and may explain gender-related differences in development of atherosclerosis. (*J Am Heart Assoc.* 2016;5:e002544 doi: 10.1161/JAHA.115.002544)

Key Words: acetylcholine • coronary disease • endothelium • genetics

Coronary endothelial dysfunction (CED) is an early stage in the development of atherosclerosis and is an independent predictor of adverse cardiovascular outcomes.[1–5] Sex has been identified as an independent factor contributing to cardiovascular disease morbidity and mortality and endothelial dysfunction, but the role of CED has not been fully explored.[2,6]

Sex plays a role in the development of atherosclerosis, but sex-specific genetic associations and the relationship with endothelial dysfunction have not been identified.[7] Healthy women have been shown to have higher endothelium-dependent dilation when compared to their male counterparts, and the presence of cardiovascular risk factors has been associated with sex-specific effects on endothelium-dependent dilation.[8] The Women's Ischemic Syndrome Evaluation study also suggested that sex plays a role in development of atherosclerosis, reporting increased likelihood of diffuse plaque formation in women when compared to men who developed discrete coronary lesions.[9] Moreover, men have been shown to have a greater atheroma burden with more eccentric atheroma and diffuse epicardial endothelial dysfunction than women, suggesting increased structural and functional epicardial abnormalities when compared to their female counterparts.[10] The reason for this difference is not entirely understood, and may be affected by genetic variability among single-nucleotide polymorphisms (SNPs) and their association with early atherosclerosis and CED.[10] Genetic variations have been thought to play a role in the development of cardiovascular disease, and recent studies have focused on defining the genes that are responsible.[11–14] New loci associated with cardiovascular risk factors, subclinical indexes, and disease end points have provided important

insights that shed light on biologic pathways that may be involved in the development of cardiovascular disease. These can plausibly be targeted for prevention and treatment in the future.[6] Identification of genotypic predictors of disease may enhance our understanding of molecular mechanisms underlying CED and the development of atherosclerosis. While several studies have focused on determination of genetic associations with cardiovascular disease, few have focused on genetic and sex-specific associations with endothelial dysfunction, a precursor of cardiovascular disease. We aimed to evaluate this genetic variability and determine the association of sex-specific SNPs with epicardial CED.

Methods

Patient Population

The study was performed at Mayo Clinic in Rochester, Minnesota. The study protocol was approved by the Mayo Clinic Institutional Review Board. Informed consent was obtained from each patient.

As described previously,[15] cardiac catheterization and coronary microvascular function testing was performed on 643 patients enrolled between January 1993 and December 2010. The decision to pursue cardiac catheterization and invasive coronary physiology testing was made by the referring cardiologist. Patients presented with chest pain and had clinical indication for routine coronary angiography to rule out underlying coronary artery disease. All patients enrolled in the study had no evidence of obstruction of coronary arteries on coronary angiography. They then underwent a coronary physiology study to evaluate for coronary microvascular dysfunction.

All patients who met criteria to undergo a coronary physiology study were included in the study. Exclusion criteria included unstable angina pectoris, history of uncontrolled systemic hypertension that requires long-term therapy, valvular heart disease, left ventricular ejection fraction <40%, and/or significant endocrine disorders including diabetes, hepatic, renal, or inflammatory disease.

Study Protocol

Vasoactive medications were discontinued for at least 36 hours prior to catheterization. Eating, drinking, or tobacco use was discontinued for at least 12 hours prior to the procedure.[15–22] Diagnostic coronary angiography was performed with a 6F or 7F guiding catheter using a standard femoral percutaneous approach. Nonionic contrast material was used. No nitroglycerin was given prior to the diagnostic procedure. Unfractionated intravenous heparin was administered for an activated clotting time of ≈250 s.

Coronary vascular reactivity was studied using a 0.014-inch Doppler tipped guidewire (FloWire: Volcano Corp, CA) in the left anterior descending coronary artery.[15–22] Intracoronary infusion of incremental doses of acetylcholine to a maximum tolerable dose (10^{-6}, 10^{-5}, and 10^{-4} mol/L at 1 mL/min at 3-minute intervals) was given. Epicardial coronary artery endothelium-dependent function was calculated as the percent increase in coronary artery diameter, (CAd) in response to acetylcholine. The cut-off points were derived from the presence or absence of impaired epicardial endothelial function, defined as percent change in CAd (% CAd) to acetylcholine less than −20%.[16,20]

Genomic Data and Blood Collection

As previously described,[15,23] DNA was extracted from blood using the Mayo Clinic Biospecimens Accessioning and Processing facility. Pico Green analysis was run on all samples to assess quality. Samples were genotyped at the Mayo Genotyping Core facility using an Illumina custom GoldenGate panel (San Diego, CA).[24] Per 96-well plate, there were 85 unique samples, 5 duplicate DNA samples, and 6 quality control CEPH samples. The 1536 tag SNPs represented genes with previously identified associations with coronary vasoreactivity, angiogenesis, inflammation, artery calcification, atherosclerosis risk factors, insulin resistance, female hormones, blood coagulation system, or prevalence of coronary heart disease (CHD). Of these, 242 SNPs were excluded from the analysis due to minor allele frequencies <5%, Hardy Weinberg Equilibrium, *P*-values <0.001, or SNP call rates <95% (ie, missing values for at least 5% of the subjects). The majority of SNPs failed because they were monomorphic or had a very low minor allele frequency. Genetic positions were listed in Build 36.

Statistic Methodology

All statistical analysis was performed using PLINK and SAS version 9.3 (SAS Institute Inc., Cary, NC).[25] Categorical data were analyzed using the χ^2 test and continuous variables were analyzed using two-sample *t* test and summarized using mean±SD. Logistic regression was run using the end point of %CAd response to acetylcholine < −20 to test for genetic differences after adjusting for age, sex, diabetes, smoking status, and body mass index, assuming a log-additive genetic model. Models were run testing for an interaction between sex and each SNP to determine sex-specific associations.

Results

Baseline Characteristics

Overall, median age was 49.7±11.4 years. Median age of women was 51.4±10.9 years, while it was 46.6 years for

men. The majority of patients were of European ancestry (93% white+5.6% unknown and presumed white). Baseline characteristics are summarized in Table 1. Of women enrolled, 58% were postmenopausal. While only 8% of the population had diabetes, hypertension was present in 41%, dyslipidemia in 55%, and 13% were current smokers. Aspirin was used by 50%, 37% used calcium channel blockers, and 39% used lipid lowering drugs. Of women, 27% were using hormone replacement therapy.

Coronary Epicardial Endothelial Dysfunction

The %CAd induced by acetylcholine was lower in men than in women (Table 1). Diabetes and smoking were significantly higher in patients with abnormal endothelial function when compared to patients with normal endothelial function. Medication was similar between men and women.

Coronary Epicardial Endothelial Dysfunction and SNP Analysis

We identified several SNPs associated with epicardial endothelial dysfunction (Figure). In women (Table 2), rs12038000 was associated with the adenosine A3 receptor gene (*ADORA3*), rs 16851008 with *ADORA1*, and k1_201395343 and rs16851020 with both *ADORA1* and with myosin binding protein H gene (*MYBPH*). Moreover, rs7767084, rs9365171, rs3798221, rs 9364564, rs7453899, rs35600881, rs13202636, and rs 1321195 were all associated with *LPA*, and rs2237583 is associated with paraoxonase 1 gene (*PON1*). These SNPs were

Table 1. Patient Characteristics

	All	Women	Men	
	n=643	n=426	n=217	P Value
Age, y	49.7 (11.4)	51.4 (10.9)	46.6 (11.7)	<0.001
Body mass index, kg/m^2	29.1 (6.2)	29.2 (6.8)	28.9 (4.7)	0.63
Postmenopausal	243 (58%)	243 (58%)	— (—)	
% change CAd (Ach)	−15.1 (21.2)	−13.6 (20.3)	−17.9 (22.7)	0.015
Risk factor				
Diabetes mellitus	52 (8%)	28 (7%)	24 (11%)	0.049
Hypertension	264 (41%)	166 (39%)	98 (45%)	0.13
Dyslipidemia	353 (55%)	218 (52%)	135 (62%)	0.010
Family history	409 (65%)	271 (65%)	138 (66%)	0.77
Lipoprotein A	24.0 (31.0)	23.7 (30.5)	24.6 (32.3)	0.76
hsCRP, mg/dL	2.7 (24.0)	3.4 (29.5)	1.4 (3.5)	0.37
Homocysteine, μmol/L	8.0 (4.4)	7.8 (5.0)	8.4 (2.9)	0.12
Smoking				<0.001
Never	326 (51%)	242 (57%)	84 (39%)	
Former	232 (36%)	146 (34%)	86 (40%)	
Current	83 (13%)	37 (9%)	46 (21%)	
Drugs				
Aspirin	326 (51%)	206 (48%)	120 (55%)	0.010
Calcium channel blockers	239 (37%)	150 (35%)	89 (41%)	0.12
Angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker	104 (16%)	62 (15%)	42 (19%)	0.12
β-Blocker	186 (29%)	128 (30%)	58 (27%)	0.38
Diuretics	106 (16%)	85 (20%)	21 (10%)	<0.001
Lipid-lowering drugs	248 (39%)	151 (35%)	97 (45%)	0.020
Estrogen replacement therapy	117 (27%)	117 (27%)	— (—)	

Values are given as n (%) or mean (SD). *P*-value shows women vs men. Ach, acetylcholine; CAd, coronary artery diameter; hsCRP, high-sensitivity C-reactive protein.
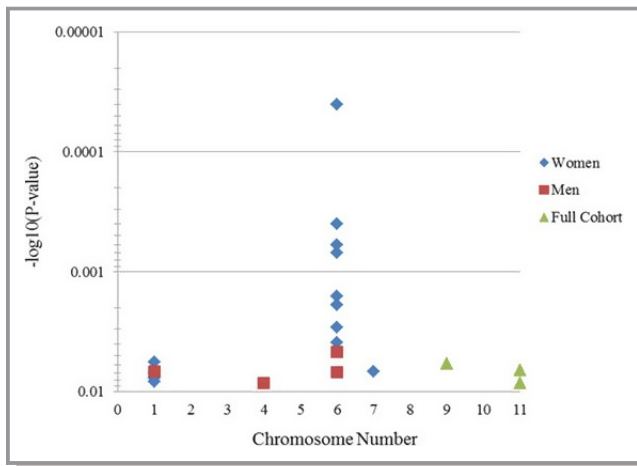
Figure. Macrovascular significant SNPs: *P* values (minus log-transformed) are shown in a signal intensity (Manhattan) plot relative to their genomic position in macrovascular endothelial function. Each SNP is plotted with respect to its chromosomal location (*x* axis) and its *P* value (*y* axis on the left). The minimum *y* axis marks the threshold for significance (*P*=0.01). SNP indicates single-nucleotide polymorphism.

associated with an increased risk of abnormal %CAd induced by acetylcholine (*P*<0.01).

In men (Table 3), we found that rs17511046 was associated with *ADORA1*, rs3774933 and rs1599961 were associated with nuclear factor of κ light polypeptide gene enhancer in B-cell 1 gene (*NF-κB1*), and rs20456 is associated with

both *LOC100124373* and *KIF6.* Also, k6_39474093 is associated with KIF6. These SNPs were associated with increased risk of abnormal coronary artery dilation indicating epicardial endothelial dysfunction (*P*<0.01). Only *ADORA1* and *NFKB1* SNPs showed significant differences between men and women. The strongest evidence for differences between men and women was seen in SNPs on *LPA* and *PON1* (interaction *P*<0.01) (Table 3).

Comparison of SNPs was also made by stratifying patients into 3 groups: men, premenopausal women, and postmenopausal women, in order to assess the effect of menopause on these findings. We find that certain SNPs remain significantly associated with macrovascular endothelial dysfunction in premenopausal women, with others found to be significant in postmenopausal women (Tables 4 and 5). For example, rs12038000 was associated with the adenosine A3 receptor gene (*ADORA3*), rs16851008 with *ADORA1,* and k1_201395343 and rs16851020 with both *ADORA1* and with myosin binding protein H gene (*MYBPH*) in postmenopausal women but not in premenopausal women (Tables S2 and S3). rs2237583 was significantly associated with *PON1* in postmenopausal women but not premenopausal women, and rs7767084 was associated with LPA in premenopausal women but not in postmenopausal women. rs9365171, rs3798221, rs9364564, and rs7453899 were associated with *LPA* in both premenopausal women and postmenopausal women.

Table 2. SNPs Associated With Macrovascular/Epicardial Endothelial Dysfunction Significant Only Among Females

Chr.	Significant SNP in Females	Position	Gene Region	Risk Allele X/Y	Overall Allele X Frequency	OR Overall	*P* Value Overall	OR Men	*P* Value Men	OR Women	*P* Value Women	*P* Value SNP×Sex
1	rs12038000	111859362	*ADORA3*	A/G	0.61	1.30	0.0357	0.93	0.7307	1.54	0.0057*	0.0708
1	rs16851008	201387919	*ADORA1*	G/A	0.87	1.33	0.1066	0.79	0.3910	1.93	0.0077*	0.0354
1	k1_201395343	201395343	*ADORA1/ MYBPH*	A/G	0.87	1.28	0.1549	0.73	0.2538	1.94	0.0083*	0.0254
1	rs16851020	201400275	*MYBPH/ ADORA1*	C/A	0.87	1.29	0.1502	0.70	0.2132	1.96	0.0070*	0.0182
6	rs7767084*	160882493*	*LPA**	G/A	0.16	1.32	0.0780	0.71	0.2483	1.73	0.0039*	0.0075*
6	rs9365171	160901726	*LPA*	A/C	0.35	1.37	0.0118	0.99	0.9691	1.65	0.0016*	0.0571
6	rs3798221*	160918138*	*LPA**	T/G	0.19	1.58	0.0023	0.80	0.4244	2.08	0.00004*	0.0061*
6	rs9364564*	160919030*	*LPA**	A/G	0.18	1.50	0.0134	0.77	0.3854	2.00	0.0004*	0.0097*
6	rs7453899	160930756	*LPA*	T/A	0.35	1.35	0.0167	1.00	1.0000	1.60	0.0029*	0.0848
6	rs35600881	160946754	*LPA*	A/G	0.23	1.50	0.0046	0.95	0.8481	1.79	0.0006*	0.0487
6	rs13202636	160949718	*LPA*	G/A	0.23	1.49	0.0053	0.95	0.8481	1.78	0.0007*	0.0520
6	rs1321195	161004146	*LPA*	T/C	0.13	1.48	0.0256	0.80	0.4877	1.90	0.0019*	0.0267
7	rs2237583*	94788113*	*PON1**	G/A	0.70	1.15	0.3002	0.70	0.0898	1.63	0.0068*	0.0030*

OR indicates odds ratio; SNPs, single-nucleotide polymorphisms.
*SNPs both significant in sex and SNP×Sex.

Table 3. SNPs Associated With Macrovascular/Epicardial Endothelial Dysfunction Significant Only Among Males

Chr.	Significant SNP in Males	Position	Gene Region	Risk Allele X/Y	Overall Allele X Frequency	OR Overall	P Value Overall	OR Men	P Value Men	OR Women	P Value Women	P Value SNP×Sex
1	rs17511046*	201376668*	*ADORA1*	G/A	0.93	1.17	0.5066	3.88	0.0068*	0.68	0.1609	0.0034*
4	rs3774933*	103645369*	*NFKB1*	G/A	0.39	1.06	0.6362	1.72	0.0085*	0.80	0.1472	0.0068*
4	rs1599961*	103662599*	*NFKB1*	A/G	0.39	1.06	0.6362	1.72	0.0085*	0.80	0.1472	0.0068*
6	rs20456	39432901	*LOC100124373/KIF6*	C/T	0.43	1.31	0.0283	1.70	0.0069*	1.10	0.5601	0.0622
6	k6_39474093	39474093	*KIF6*	T/A	0.06	1.95	0.0060	4.00	0.0047*	1.44	0.2188	0.0582

OR indicates, odds ratio; SNPs, single-nucleotide polymorphisms.
*SNPs both significant in sex and SNP×Sex.

Discussion

The current study demonstrates sex-specific differences in SNPs and overlap region in some genes associated with epicardial CED in humans. These observations may explain differences in the propensity for development of early atherosclerosis between men and women and may have potential sex-specific therapeutic implications.

Genetic Associations in Women

In women, variations within *ADORA3*, *ADORA1*, *MYBPH*, *LPA*, and *PON1* genes were associated with epicardial CED. This is a plausible association that plays a crucial role in vascular homeostasis, which may be regulated by sex hormones, especially estrogen.[26] Estrogen contributes to regulation of vascular tone, modulates recruitment of circulating cells, effects platelet function, and plays a role in processes responsible for vascular repair.[26] Gene variants in women modulate the function of estrogen, its receptors, and are implicated in aspects of cardiovascular inflammation, platelet function, and vascular repair.[26,27]

Table 4. Genes With Significant SNPs in Men for Macrovascular Endothelial Dysfunction

Gene With Associated Significant SNPs in Men	Significant SNP	SNP×Sex Significant P Values
ADORA1	rs17511046*	0.0034*
KIF6	rs20456	
	k6_39474093	
NFKB1	rs3774933*	0.0068*
	rs1599961*	0.0068*
LOC100124373	rs20456	
ADORA1	rs17511046*	0.0034*

SNPs indicates single-nucleotide polymorphisms.
*SNPs both significant in sex and SNP×Sex.

Moreover, risk associated with *LPA*, *PON1*, and *KCNQ1* variants affecting CED may be mediated through dysfunction in lipid metabolism. *LPA* risk alleles correlate with high plasma lipoprotein (a), which is associated with atherosclerotic vascular disease leading to CHD and may be thrombogenic.[28] It inhibits fibrinolysis, accumulates in the vascular wall in atherosclerotic lesions, and may proliferate in human smooth muscle cells.[28,29] *PON1* exerts anti-atherogenic effects by protecting low-density lipoproteins against oxidation, which has been implicated in oxidative stress and coronary spasm.[29] Thus, *PON1* is associated with ox-low-density lipoprotein, which is associated with CED.[30] *KCNQ1* SNPs are associated with type 2 diabetes mellitus via a reduction in insulin secretion and higher fasting glucose. This may affect lipid metabolism in patients with type 2 diabetes and CHD.[31]

Adenosine plays an important role in cardiac reperfusion response to ischemia.[32] Variants in adenosine receptor genes including *ADORA3* and *ADORA1* may predict cardiac response to ischemia or injury.[32] Upregulation of adenosine A3 receptors mRNA upon preconditioning is sex specific and depends on a woman's menstrual cycle.[33] In this study, the gene region of A1 receptors was alike in both women and men. Our study suggests that sex-specific SNPs on *ADORA3* relate to epicardial CED and that *ADORA1* polymorphisms play a sex-specific role in endothelial function, depending on the variation of the SNP.

Myosin binding protein H (*MYPH*) was also associated with epicardial CED in women. It is known that *MYPH* is expressed in ventricular Purkinje cells,[34] but how *MYBPH* gene mediates coronary epicardial endothelial function has yet to be elucidated. While little is known about *MYPH* and its association with coronary epicardial endothelial function, awareness of various genetic polymorphisms associated with epicardial CED is important since further studies on these genes may implicate a mechanism explaining this association.

Estrogen likely plays a significant role in explaining these findings and may partially explain the sex-specific differences.

Table 5. Genes With Significant SNPs in Women for Macrovascular Endothelial Dysfunction

Gene With Associated Significant SNPs in Women	Significant SNP	SNP×Sex Significant *P* Values
ADORA1	rs16851008	
	k1_201395343	
	rs16851020	
LPA	rs7767084*	0.0075*
	rs9365171	
	rs3798221*	0.0061*
	rs9364564*	0.0097*
	rs7453899	
	rs36500881	
	rs13202636	
	rs1321195	
MYBPH	k1_201395343	
	rs16851020	
ADORA3	rs12038000	

SNPs indicates single-nucleotide polymorphisms.
*SNPs both significant in sex and SNP×Sex.

Several genetic variations were noted in postmenopausal women but not premenopausal women. On the other hand, several genetic variations do not appear to be associated with menopausal status, and rather simply female sex. Additional investigation to further define the association with menopause is necessary.

Genetic Associations in Men

In men, genetic variations within *ADORA1*, *NFKB1*, *LOC 100124373*, and *KIF 6* genes are associated with epicardial CED. Gene variants related with the activity of nitric oxide synthase itself, angiogenesis, and inflammation modify CED in men in our study. These SNPs in men may mediate testosterone or its receptors and have adverse effects on cardiovascular morbidity and mortality.[35]

Nuclear factor-κB denotes a family of transcription factors involved in both pro-inflammatory and anti-inflammatory processes in atherogenesis.[36] Polymorphisms in *NFKB1* promoter are associated with an increased risk of CHD and heart failure.[36] Inflammatory mechanisms affect all phases of coronary artery disease. Errors in genes encoding inflammatory or anti-inflammatory molecules are candidates for increasing risk of developing complications from coronary artery disease.[36] There are sex-specific variations in NF-κB activity, which may play a role in development of CED and atherosclerosis.[37,38]

It is important to note the overlap in gene regions between women and men in our study. We found overlap of gene region in *ADORA1* and *KIF6* without duplication of SNPs. The kinesin family member 6 gene encodes an intracellular protein, which transports cellular cargo and is expressed in coronary endothelial cells.[39] The association between *KIF6* polymorphism and increased risk of CHD in male patients has been previously described.[40] Arg^{719} allele of *KIF6* was associated with increased risk of CHD and myocardial infarction in populations of healthy women with low prevalence of CHD.[40] *KIF6* polymorphisms may be involved in interrupting intracellular transport in endothelial cells in women or men depending on the SNPs predisposing to development of CED and CHD.

Limitations

Our study has several inherent limitations. First, this is a cross-sectional study in a unique patient population with early coronary atherosclerosis as defined by endothelial dysfunction. Further studies are needed to determine why a significant variant might have an effect in males and not in females with similar cardiovascular risk factors.

While most SNPs that we identified as significantly associated with early coronary atherosclerosis characterized by CED lie within currently presumed noncoding intronic sequences, there are several potential mechanisms that could explain their association. These SNPs may be in linkage disequilibrium with promoter SNPs that have not yet been identified or that were not genotyped in this study.[41] Furthermore, these intronic SNPs may have promoter functions that have not yet been identified, and intronic variants may potentially affect receptor function through alternative splicing mechanisms[41] SNPs in the 5′ upstream region could play a significant role affecting gene transcription.

Additionally, the number of tests performed is another limitation of the study. We have performed a large number of tests and it is possible that these results are significant purely by chance. Further studies are necessary to confirm these findings.

Conclusions

The current study reports for the first time the association between sex-specific gene variants and physiological functional abnormalities related to early coronary atherosclerosis in humans characterized by epicardial endothelial dysfunction in coronary arteries. The study may help explain sex-specific differences in development of coronary endothelial function and atherosclerosis.

Disclosures

None.

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ORIGINAL RESEARCH

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Table S1: Patient characteristics

	All n=643		Premenopausal n=178		Postmenopausal n=426		Men n=217		P-value
Age, years	49.7	(11.4)	43.8	(9.1)	56.9	(8.6)	46.6	(11.7)	<0.001
Body Mass Index, kg/m^2	29.1	(6.2)	29.1	(7.0)	29.1	(6.6)	28.9	(4.7)	0.91
% change CAd (Ach)	-15.1	(21.2)	-12.0	(20.4)	-14.9	(20.1)	-17.9	(22.7)	0.022
Risk Factor									
Diabetes Mellitus	52	(8%)	12	(7%)	16	(7%)	24	(11%)	0.16
Hypertension	264	(41%)	52	(29%)	112	(46%)	98	(45%)	<0.001
Dyslipidemia	353	(55%)	72	(41%)	145	(60%)	135	(62%)	<0.001
Family History	409	(65%)	113	(65%)	156	(66%)	138	(66%)	0.96
LipoProtein A	24.0	(31.0)	22.2	(28.2)	25.1	(32.7)	24.6	(32.3)	0.68
hsCRP, mg/dL	2.7	(24.0)	5.3	(43.5)	1.9	(2.8)	1.4	(3.5)	0.30
Homocysteine, umol/L	8.0	(4.4)	7.5	(4.7)	8.0	(5.3)	8.4	(2.9)	0.19
Smoking									<0.001
Never	326	(51%)	98	(55%)	142	(59%)	84	(39%)	
Former	232	(36%)	60	(34%)	84	(35%)	86	(40%)	
Current	83	(13%)	20	(11%)	16	(7%)	46	(21%)	
Drugs									
Aspirin	326	(51%)	82	(46%)	123	(51%)	120	(55%)	0.19
Calcium Channel Blockers	239	(37%)	60	(34%)	88	(36%)	89	(41%)	0.30

Angiotensin-converting enzyme – inhibitor/ Angiotensin II receptor blocker	104	(16%)	28	(16%)	34	(14%)	42	(19%)	0.29
Beta-blocker	186	(29%)	55	(31%)	71	(29%)	58	(27%)	0.65
Diuretics	106	(16%)	29	(16%)	56	(23%)	21	(10%)	<0.001
Lipid-lowering drugs	248	(39%)	53	(30%)	96	(40%)	97	(45%)	0.008
Estrogen Replacement Therapy	117	(27%)	9	(5%)	108	(44%)	-	(-)	<0.001

Values are given as n (%) or mean (standard deviation). P-value shows women vs. men.

Table S2: SNPs associated with Macrovascular/ Epicardial Endothelial dysfunction significant only among Females

Chr.	Significant SNP in females	Position	Gene region	Risk Allele X/Y	Overall allele X frequency	OR Overall	P-value Overall	OR Men	P-value Men	OR Premeno	P-value Premeno	OR Postmeno	P-value Postmeno
1	rs12038000	111859362	*ADORA3*	A/G	0.61	1.30	0.0357	0.93	0.7307	1.37	0.1832	1.73	**0.0097**
1	rs16851008	201387919	*ADORA1*	G/A	0.87	1.33	0.1066	0.79	0.3910	2.48	0.0792	1.77	0.0424
1	k1_201395343	201395343	*ADORA1/ MYBPH*	A/G	0.87	1.28	0.1549	0.73	0.2538	2.88	0.0619	1.74	0.05
1	rs16851020	201400275	*MYBPH/ ADORA1*	C/A	0.87	1.29	0.1502	0.70	0.2132	2.48	0.0792	1.77	0.0424
6	**rs7767084**	**160882493**	***LPA***	G/A	0.16	1.32	0.0780	0.71	0.2483	2.35	**0.0058**	1.48	0.1085
6	rs9365171	160901726	*LPA*	A/C	0.35	1.37	0.0118	0.99	0.9691	1.79	0.0213	1.61	0.0224
6	rs3798221	160918138	*LPA*	T/G	0.19	1.58	0.0023	0.80	0.4244	1.94	0.0115	2.21	**0.0017**
6	rs9364564	160919030	*LPA*	A/G	0.18	1.50	0.0134	0.77	0.3854	1.94	0.0206	2.09	**0.0077**
6	rs7453899	160930756	*LPA*	T/A	0.35	1.35	0.0167	1.00	1.0000	1.68	0.0357	1.58	0.0269
6	rs35600881	160946754	*LPA*	A/G	0.23	1.50	0.0046	0.95	0.8481	1.63	0.0521	2	**0.0029**
6	rs13202636	160949718	*LPA*	G/A	0.23	1.49	0.0053	0.95	0.8481	1.62	0.057	1.99	**0.0032**
6	rs1321195	161004146	*LPA*	T/C	0.13	1.48	0.0256	0.80	0.4877	1.82	0.0475	2.03	0.0148
7	rs2237583	94788113	*PON1*	G/A	0.70	1.15	0.3002	0.70	0.0898	1.33	0.3269	2.02	**0.0034**
11	rs757092	2455754	KCNQ1	A/G	0.64	1.2	0.1337	1.05	0.8214	2.05	**0.0058**	1.02	0.9126
11	rs11022996	2458902	KCNQ1	A/G	0.61	1.15	0.2288	1.17	0.3798	1.93	**0.0068**	1.08	0.7061
11	rs1080017	2468376	KCNQ1	G/A	0.64	1.23	0.0935	1.03	0.8661	2.1	**0.0046**	1.06	0.7654
11	rs594942	63762868	FKBP2	T/C	0.35	1.18	0.1757	1.02	0.9067	1.9	**0.0093**	1.02	0.9413

Table S3: SNPs associated with Macrovascular/ Epicardial Endothelial dysfunction significant only among Males

Chr.	Significant SNP in males	Position	Gene region	Risk Allele X/Y	Overall allele X frequency	OR Overall	P-value Overall	OR Men	P-value Men	OR Premeno	P-value Premeno	OR Postmeno	P-value Postmeno
1	**rs17511046**	**201376668**	*ADORA1*	G/A	0.93	1.17	0.5066	3.88	**0.0068**	0.79	0.5397	0.55	0.132
4	**rs3774933**	**103645369**	*NFKB1*	G/A	0.39	1.06	0.6362	1.72	**0.0085**	0.81	0.4177	0.78	0.2167
4	**rs1599961**	**103662599**	*NFKB1*	A/G	0.39	1.06	0.6362	1.72	**0.0085**	0.81	0.4177	0.78	0.2167
6	rs20456	39432901	*LOC100124373/ KIF6*	C/T	0.43	1.31	0.0283	1.70	**0.0069**	1.35	0.2205	0.98	0.7137
6	k6_39474093	39474093	*KIF6*	T/A	0.06	1.95	0.0060	4.00	**0.0047**	1.35	0.5081	1.55	0.2736