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Single Nucleotide Polymorphisms Associated with Abnormal Coronary Microvascular Function

Satoshi Yoshino, MD[1], **Rebecca Cilluffo, MD**[2], **Patricia J.M. Best, MD**[2], **Elizabeth J. Atkinson, MS**[3], **Tatsuo Aoki, MD, PhD**[2], **Julie M. Cunningham, PhD**[4], **Mariza de Andrade, PhD**[3], **Byoung-Joo Choi, MD, PhD**[5], **Lilach O. Lerman, MD, PhD**[6], and **Amir Lerman, MD**[2]

[1]Department of Cardiovascular Medicine and Hypertension, Kagoshima University Hospital, Sakuragaoka, Kagoshima, JAPAN

[2]Division of Cardiovascular Diseases and Internal Medicine, Mayo Clinic and College of Medicine, Rochester, Minnesota, USA

[3]Division of Biomedical Statistics and Informatics, Mayo Clinic and College of Medicine, Rochester, Minnesota, USA

[4]Division of Genomics Shared Resource, Mayo Clinic and College of Medicine, Rochester, Minnesota, USA

[5]Department of Cardiology, Ajou University Medical Center, Suwon, KOREA

[6]Division of Nephrology and Internal Medicine, Mayo Clinic and College of Medicine, Rochester, Minnesota, USA

Abstract

Background—Single nucleotide polymorphisms (SNPs) are the most common source of genetic variation. Although microvascular pathology is associated with cardiovascular events, genetic phenotypes causing microvascular disease remain largely unknown. This study identifies gender specific SNPs associated with coronary microvascular dysfunction.

Methods and Results—Six-hundred and forty-three patients without significant obstructive coronary heart disease (CHD) were enrolled, referred for cardiac catheterization, and underwent invasive coronary microcirculatory assessment. Patient data was collected from 1529 autosomal SNPs and 7 X chromosome SNPs which were selected to represent the variability from 76 candidate genes having published associations with coronary vasoreactivity, angiogenesis, inflammation, vascular calcification, atherosclerosis risk factors, female hormones, blood coagulation, or CHD. Coronary flow reserve (CFR) was assessed by intracoronary injection of adenosine. Patients were categorized according to a CFR above or below 2.5 and were stratified by sex.

After adjusting for age, sex, and BMI, this study demonstrates that SNPs within *VEGFA* and *CDKN2B-AS1* are associated with abnormal CFR (P<0.005). SNPs within *MYH15*, *VEGFA* and

Correspondence: Amir Lerman, MD, Division of Cardiovascular Diseases and Internal Medicine, Mayo Clinic, 200 1st Street SW Rochester, MN 55905, USA, Tel: +1-507-255-4152 Fax: +1-507-255-2550, lerman.amir@mayo.edu.

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NT5E are associated with abnormal CFR in men. No SNPs were associated with abnormal CFR in women.

Conclusions—Genetic variation within defined regions of *VEGFA* and *CDKN2B-AS1* genes are associated with coronary microvascular dysfunction. Furthermore, sex-specific allelic variants within MYH15, *VEGFA* and *NT5E* are associated with an increased risk of coronary microvascular dysfunction in men.

Keywords

Single nucleotide polymorphisms; coronary microvascular dysfunction; coronary flow reserve

INTRODUCTION

Many genomic variants underlie variation causing cardiovascular disease. The large number of new loci associated with cardiovascular risk factors, subclinical indexes, and disease end points have provided insights into the biologic pathways that underlie disease (1). Although conventional risk factors are important, both rare and common genetic variants account for more than 50% of a person's susceptibility to coronary heart disease (CHD)(2). Single nucleotide polymorphisms (SNPs) are the most common sources of genetic variations (2).

The development of coronary heart disease depends on a person's genetic predisposition and accumulation of risk factors that lead to a wide array of molecular and cellular abnormalities within vessel wall, such as those related to sustenance and regeneration of microvessels(3). These abnormalities can contribute to the development of coronary microvascular dysfunction and atherosclerosis(3).

Coronary microvascular dysfunction, defined as abnormal coronary flow reserve in response to adenosine, is associated with an increased annual major adverse cardiovascular event rate which includes death, nonfatal myocardial infarction, nonfatal stroke, and congestive heart failure(4). Previous studies on the genetic predictors of cardiovascular disease have primarily focused on macrovascular disease traits and genetic analyses of microvascular disease phenotypes which are unavailable, although both macrovascular and microvascular pathology are associated with cardiovascular disease(5).

Several studies demonstrate gender-specific variations in coronary microcirculation (6–8) however; there is a lack of understanding as to how gender-related pathobiologic and genetic differences influence the development of vascular abnormalities that underlie CHD. Knowledge of such genotypic predictors may enhance our understanding of the molecular mechanisms causing coronary microvascular dysfunction. Thus, the current study was designed to assess the association between SNPs and invasive assessment of coronary microvascular dysfunction in humans and to investigate the sex-specific SNPs related to coronary microvascular dysfunction.

METHODS

Study population

This study includes 643 subjects (426 women and 217 men) who underwent invasive coronary microcirculatory assessment from 1993 to 2010. The referral for cardiac catheterization and coronary microvascular function testing was made at the discretion of the referring cardiologist and operating interventionalist. Patients did not have significant fixed coronary artery stenosis, meaning that they had less than 30% obstruction in their coronary arteries. The majority of subjects were of European ancestry (93% white + 5.6% unknown and presumed white). The median age was 51 for women (range 20 to 75) and 46 for men (range 18 to 78).

Pre-defined exclusion criteria were unstable angina pectoris, uncontrolled systemic hypertension, valvular heart disease, left ventricular ejection fraction<40%, and/or significant endocrine, hepatic, renal, or inflammatory disease. Systemic hypertension was defined as a history of elevated blood pressure requiring long-term therapy. Hypercholesterolemia was defined as either a total cholesterol serum concentration of ≥240 mg/dL or intake of lipid-lowering therapy. Diabetes mellitus diagnosis was based on the patient's clinical record.

The study was approved by the Mayo Clinic Institutional Review Board, and informed consent was obtained from every patient. This study complies with the Helsinki Declaration of 1975, as revised in 2008.

Study protocol

As previously described(9–14), all patients refrained from taking any vasoactive medication for at least 36 hours prior to catheterization. Patients refrained from any food, drinks, and tobacco for at least 12 hour prior to catheterization. Diagnostic coronary angiography was performed using a 6F or 7F guiding catheter with a standard femoral percutaneous approach. Unfractionated intravenous heparin was administered to achieve an activated clotting time of approximately 250 seconds. Non-ionic contrast material was used for all patients. No nitroglycerin was given prior to the diagnostic procedure.

Coronary vascular reactivity responses were studied, as previously reported (9–12). In brief, a 0.014-inch Doppler tipped guidewire (FloWire: Volcano Corp, CA, USA) was introduced into the left anterior descending coronary artery (LAD). Coronary flow reserve (CFR) was assessed by escalating doses of intracoronary bolus injection of adenosine (36–60 μg) until maximal CFR was obtained. Doppler flow velocity spectra were analyzed on-line to determine averaged peak velocity (APV). CFR was calculated as the ratio of maximal CBF induced by adenosine to basal CBF.

Patients were divided into two groups according a CFR greater than, less than or equal to 2.5. These cut-off points were derived from previous reports (9–12). Impaired coronary microvascular function was defined as CFR less than 2.5, and favorable coronary microvascular function was defined as CFR more than or equal to 2.5. Furthermore, the patients were sub-classified into two groups stratified by gender.

Genomic data and blood collection

DNA was extracted from all samples at the same time, after storing them all at −80 degrees C, by the Biospecimens Accessioning and Processing (BAP) facility. Picogreen analysis was run on all the samples to assess quality. The data was genotyped at the Mayo Genotyping Core facility using an Illumina custom GoldenGate panel (15). Per 96 well plate, there were 85 unique samples, 5 duplicate DNA samples, and 6 quality control samples. A total of 1529 autosomal tag SNPs and 7 SNPs on the X chromosome were originally chosen to represent 76 genes with known associations to coronary vasoreactivity, angiogenesis, inflammation, artery calcification, atherosclerosis risk factors, female hormone, blood coagulation system, or prevalence of CHD. Of these, 351 of these were eliminated from the analysis due to minor allele frequencies less than 5%, Hardy Weinberg Equilibrium (HWE) p-values less than 0.001, or SNP call rates less than 95% (i.e. missing values for at least 5% of the subjects). The majority of SNPs failed because they were monomorphic (had the same value for all subjects) or had a very low minor allele frequency (an alternate SNP value was seen in only a few subjects). Genetic positions were listed in Build 36. Expanded methods are included in the supplementary materials.

Ethical Considerations

This study was approved by the Mayo Clinic Institutional Review Board. This study also complies with principles stated in the Declaration of Helsinki. All patients enrolled in the study signed consent forms after reviewing the protocol for the study which included the risks, burdens and benefits of the study. Precautions were taken to maintain confidentiality of all identifying patient information used in this study using secure firewalled, pass-word-secured databases and special consideration was taken to prevent patients undergoing cardiac catheterization from enduring any unnecessary punctures.

Statistical analysis

Categorical data were analyzed using the chi-square test; continuous variables were analyzed using the two sample t-test and summarized using mean ±SD. Logistic regression was run using the endpoint of CFR<2.5 to determine if genetic differences existed after adjusting for age, sex, and body mass index (BMI), assuming a log-additive genetic model. Models were also run testing for a sex-SNP interaction. A p-value of less than 0.005 was considered statistically significant (Table 1). Statistical analysis was performed using Plink 1.07 (16).

RESULTS

Patient characteristics

There were 643 subjects who had physiologic coronary testing in the cardiac catheterization laboratory (426 women and 217 [34%] men). Patient characteristics are summarized in Table 1. The median age was 51 for women (range 20 to 75) and 46 for men (range 18 to 78). Women had a significantly lower CFR than men (2.8±0.6, 3.2±0.8, p<0.001).

Comparisons between abnormal and normal CFR group are shown in Table 2. Age was significantly higher in the abnormal CFR group than for those in the normal group

(53.4±11.0 yrs vs. 48.2±11.2 yrs, p<0.001). BMI and proportion of men was significantly lower in the abnormal CFR group than that in the normal CFR group (27.8±5.5 vs. 29.5±6.3, p<0.001; 2.2 vs. 3.3, p<0.001, respectively). There were no differences in prevalence of diabetes, hypertension, dyslipidemia, family history, and smoking between the two groups. Based on this analysis, age, sex and BMI were used as adjustors prior to investigation of the SNPs.

Relationship of SNPs with abnormal CFR

Figure 1 displays Manhattan plot showing the minus log-transformed p-values for the individual 1529 SNPs against their genomic position. The solid horizontal line marks the threshold for significance (P=0.005). The top SNPs which are associated with increased risk of abnormal CFR are shown in Table 3. This includes one signal within the gene *VEGFA* (vascular endothelial growth factor A) represented by one SNP (odd's ratio = 1.68, p=0.004) and one signal within the gene *CDKN2B-AS1* (CDKN2B antisense RNA1) represented by five correlated SNPs (top SNP's odd's ratio = 1.5, p=0.003).

Sex-specific differences seen in SNPs related to abnormal CFR

We tested for differential effects of SNPs concerning abnormal CFR for males and females by testing for SNP-sex interactions (Table 4). Figures 2 and 3 display Manhattan plots showing minus log-transformed p-values for the individual SNPs against their genomic position in women and men, respectively. The solid horizontal line marks the threshold for significance (P=0.005). Genes with at least one SNP that had different effects between males and females include *MYH15*, *VEGFA*, and *NT5E*. Among these four SNPs (Table 4) associated with those genes, the odd's ratio for the SNPs ranged from 2.27 to 2.85 for males (p-values from 0.0006–0.0029) and 1.06–1.25 for females (no p-values < 0.005).

DISCUSSION

In this current study, we have indicated novel regions of genetic variation within *VEGFA* and *CDKN2B-AS1* genes that are associated with coronary microvascular dysfunction. Furthermore, there were sex-specific differences in SNPs which are associated with microvascular dysfunction. This study may support a role for genetic variation in the heterogeneity of coronary blood flow reserve that can lead to myocardial ischemia.

SNPs related to the increased risk of abnormal CFR

Our study demonstrated that the risk allele of *VEGFA* and *CDKN2B-AS1* gene SNPs reside at introns which are associated with the increased risk of abnormal CFR.

Coronary morphogenesis constitutes the proliferation and migration of angioblasts, tube formation, and further assembly of the vascular wall (17). Regulators of this process are multiple growth factors including vascular endothelial growth factors (VEGF). During the early stage in myocardial vascularization, VEGF expression is closely related to the sites of vascular tube formation and microvascular permeability (17). The role of VEGF and its receptors is not only to generate new vessels but has a control the function of the vascular system in its on-going process. Recent evidences provided the potential effects of

polymorphism in *VEGFA* genes upon the development of CHD (3, 17, 18). The decrease in VEGFA function induced by gene variants is correlated with vascular dysfunction, including microvascular cell damage, impaired microvascular cell survival, decreased anti-apoptotic effect of VEGF, and abnormal vascular repair (19). All of these VEGF functions can lead to the development of coronary atherosclerosis. The current study suggests that polymorphisms in the *VEGF* gene are candidate contributors to the pathogenesis of coronary microvascular dysfunction.

Recently, a major genetic susceptibility locus for CHD was identified. This locus is located within 9p21.3, mapping to the large non-coding antisense RNA transcript *CDKN2BAS*, formerly called *ANRIL* (20). ANRIL is expressed in vascular endothelial cells and coronary smooth muscle cells (21). Genetic variants of *CDKNBAS* are associated with angiogenesis and atherosclerosis pathogenesis in vascular cells by mediating the response to inflammatory signaling (22). Modulations of the expression levels of CDKN2BAS may affect vascular cell proliferation and senescence. Its deficiency may cause vascular injury in patients with microvascular dysfunction.

Sex differences of SNPs related to abnormal CFR

Cardiovascular structural and functional adaptation to aging and disease differs substantially between women and men(23). Women with symptomatic ischemic heart disease undergoing coronary angiography have less extensive or obstructive coronary lesions than men (23, 24). Despite this, women have a poorer prognosis compared with men, suggesting the existence of female-specific pattern of coronary artery disease with a high frequency of coronary microvascular dysfunction (23, 24).

In the current study, we observed sex-specific differences in SNPs, and some duplication in the gene regions of vasculature and *MYH15* without overlap of SNP between women and men. Sex-specific differences in microvascular blood flow and vasodilatory capacity are observed very early in development. In a study on skin microcirculation in newborn preterm (24–28 weeks) infants, female infants had a lower baseline flow than males (25) This suggests that the mechanism of myocardial ischemia in women may be localized to the microvascular coronary arteries, and that abnormal microvascular function may have prognostic implications (26).

Polymorphisms in *MYH15* are associated with MI and an increased risk of CHD (27). *MYH15* encodes myosin heavy polypeptide 15 (27). Further studies are needed to clarify how MYH15 might be involved in vascular biology or how this polymorphism in the downstream region of *MYH15* affects the risk of vascular disease between women and men.

In men, the gene variant of *NT5E* is associated with abnormal CFR. Mutations in *NT5E* are associated with arterial calcification (28). This gene encodes CD73, which converts adenosine monophosphate (AMP) to adenosine, supporting a role for this metabolic pathway in inhibiting vascular calcification (28, 29). CD73 deficiency leads to reduction in extracellular adenosine levels, causing vascular calcification. Double knockout mice for the *NT5E* gene have reduced but not absent levels of adenosine (30) indicating *NT5E* SNPs role in microvascular dysfunction.

Han et al (31) described sex differences in atheroma burden and CFR in patients with early coronary atherosclerosis, demonstrating that men have greater atheroma burden and more eccentric atheroma than women and that CFR was significantly lower in women than in men. Gene variant in *NT5E* may play a role in vascular calcification and function of adenosine, and its role may depend on sex-specific differences. *NT5E* SNP rs6922 effect on risk is independent of all known risk factors, including dyslipidemia, hypertension, diabetes, obesity, and markers of inflammation; this implies a new biologic pathway that is relevant to CHD in that its effects on adenosine working normally as a vasodilator might possibly be lacking and subsequently lead to earlier development of coronary microvascular dysfunction in patients with this SNP.

The aspects that may account for differences in outcomes between women and men are related to vascular genetic and biological factors such as a smaller atheroma burden and slower progression in women, lower CFR, more vascular stiffness, differences in remodeling, and functional differences of smooth muscle cells in the wall.

Clinical implications

The vast majority of cardiovascular diseases are polygenic, with both heritable and environmental contributions. Familial segregation of cardiovascular disease suggests that these diseases share a common genetic predisposition that interact with the environment and may predispose individuals to vascular disorders, which manifest at different time points throughout life. This study suggests that the mechanism of the development of microvascular dysfunction seen in men may be different than that in women and may suggest that treatment options will need to be tailored in specific ways.

While most SNPs that we identified as significantly associated with abnormal CFR lie within currently presumed non-coding 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. 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. SNPs in 5’ upstream region could play a significant role in affecting gene transcription, and those in 3’ untranslated region (3’ UTR) can change mRNA stability and participate in the development of human disease.

Limitations

This study has several limitations. First, this is a cross-sectional study and we have not studied follow-up of cardiovascular events in these patients. Second, the sample size is relatively limited and therefore significance level used (0.005) does not fully account for the multiple testing issues with the SNPs. Given 1185 SNPs and the correlation between the SNPs, the significance level should theoretically be set at 7.1e-5 in order to retain a type I level of 0.05. However, in order to detect odd’s ratios that are clinically reasonable in the size of 1.5–2, this study would need to be triple the sample size. Therefore, we have chosen a lower significance cut-off, recognizing that there are false positive results that need to be validated n additional cohorts. Third, we limited analysis to Caucasian subjects, as we had

insufficient number of non-Caucasian subjects to date to allow for statistical accommodation of gene admixture. Our findings were not definitive and should be viewed as exploratory until further validation studies are conducted in other Caucasian populations with microvascular dysfunction. While we have indicated regions that contain genetic variants independently associated with coronary microvascular dysfunction, there is considerable linkage disequilibrium within these regions and the SNPs identified are only indicators that there may be associations in these regions. We do not have a validation set. Because of the challenge of obtaining such a physiologically quantified group and because the assessment of CFR with adenosine in patients without CAD is performed in very few centers, we do not have a validation set. To our knowledge, we are the only center performing CFR assessment with adenosine as well as collecting blood samples on patients without CAD. Finally, our findings do not identify mechanistic pathways that link identified SNP associations to development of coronary microvascular dysfunction. Further investigation is required to determine how these SNPs associations identified in this study relate to development of coronary microvascular dysfunction.

CONCLUSIONS

This clinical study suggests that genetic variation within defined regions of *VEGFA* and *CDKN2B-AS1* genes are associated with coronary microvascular dysfunction. Furthermore, there are sex-specific differences in genetic variation in alleles MYH15, *VEGFA*, and *NT5E* which are associated with an increased risk of coronary microvascular dysfunction in men. Finally, there is no overlap of SNPs related to microvascular dysfunction when the analysis is run separately for women and men. Our findings may help focus research on novel genes and pathways involving the microvasculature and its role in the pathogenesis and development of CHD, and lead to potential future directed therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

none

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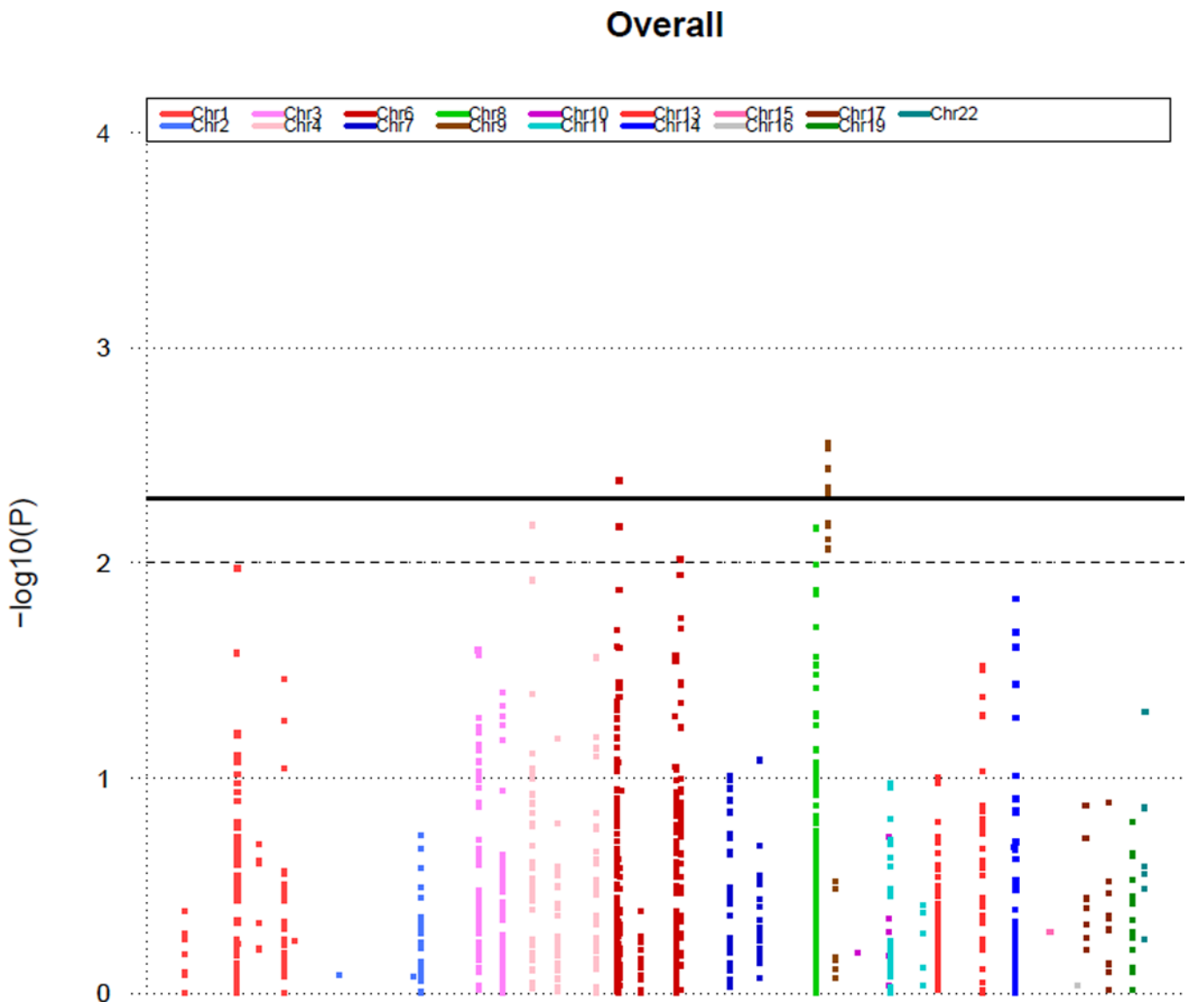

Figure 1.

P values (minus log-transformed) of whole population are shown in a signal intensity (Manhattan) plot relative to their genomic position. Each SNP is plotted with respect to its chromosomal location (*x* axis) and its *P* value (*y* axis on the left). The solid horizontal line marks the threshold for significance in our study (P=0.005). The dashed line represents a standard *P* value (P=0.01).

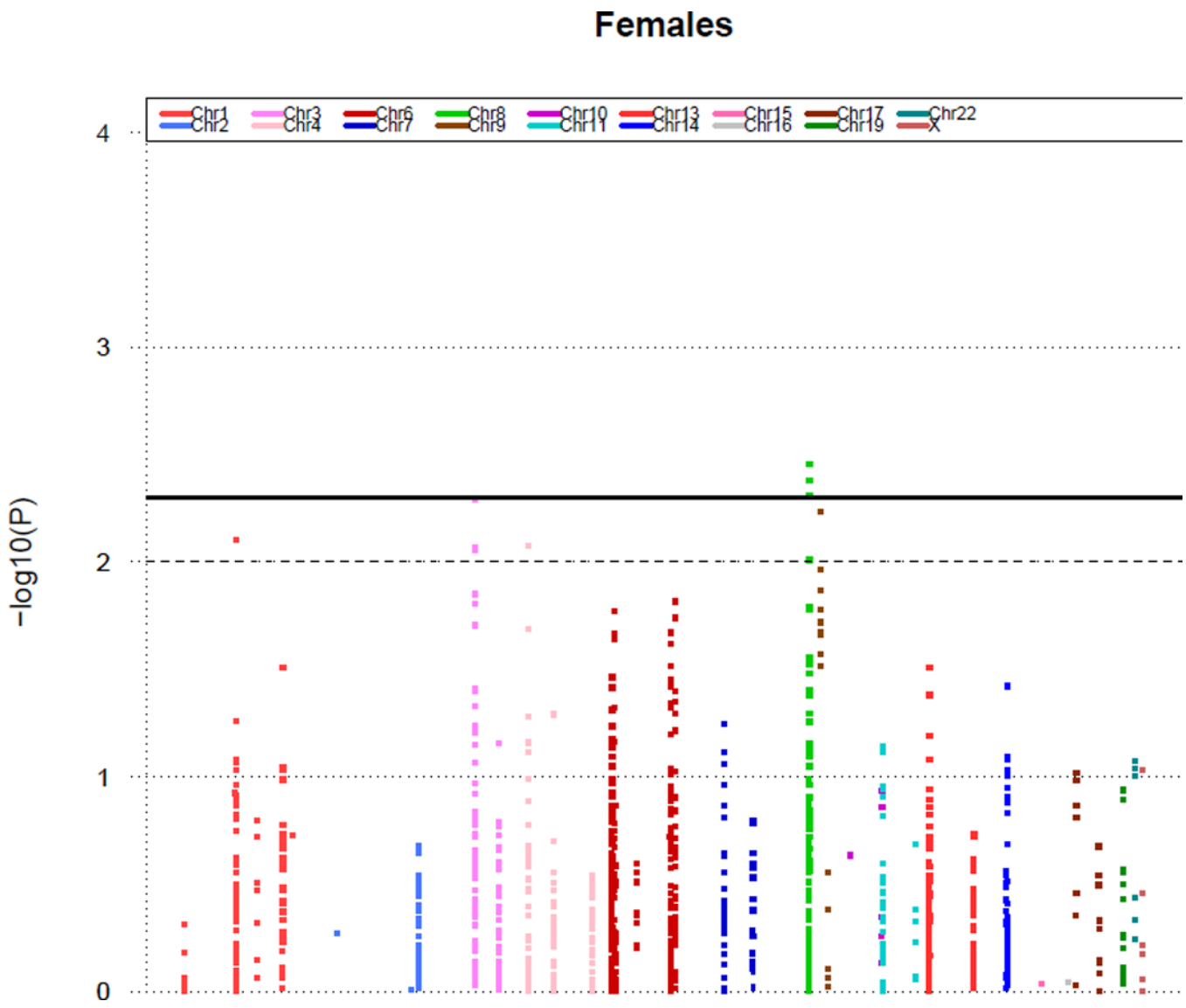

Figure 2.

P values (minus log-transformed) of women adjusting for age are shown in a signal intensity (Manhattan) plot relative to their genomic position. Each SNP is plotted with respect to its chromosomal location (*x* axis) and its *P* value (*y* axis on the left). The solid horizontal line marks the threshold for significance in our study (P=0.005). The dashed line represents a standard *P* value (P=0.01).

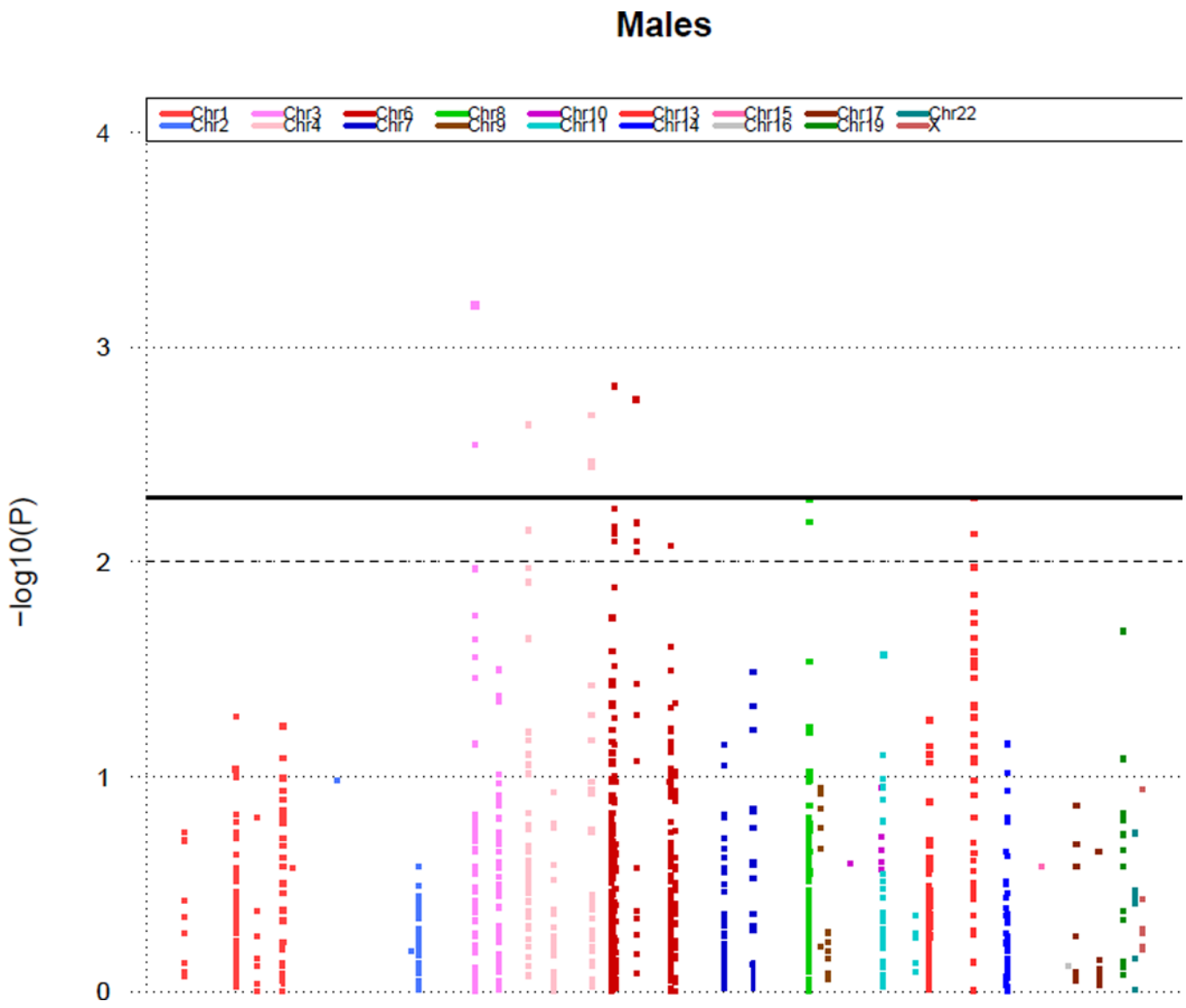

Figure 3.

P values (minus log-transformed) of men adjusting for age are shown in a signal intensity (Manhattan) plot relative to their genomic position. Each SNP is plotted with respect to its chromosomal location (*x* axis) and its *P* value (*y* axis on the left). The solid horizontal line marks the threshold for significance in our study (P=0.005). The dashed line represents a standard *P* value (P=0.01).

Table 1

Patient characteristics

	All (n=643)	Women (n=426)	Men (n=217)	P value
Age, years	49.7 (11.4)	51.3 (10.9)	46.5 (11.8)	<0.001
BMI, kg/m^2	29.0 (6.2)	29.1 (6.8)	28.9 (4.7)	0.57
Postmenopausal		247 (58%)		
CFR	3.0 (0.7)	2.8 (0.6)	3.2 (0.8)	<0.001
Risk factor				
Diabetes Mellitus	52 (8%)	28 (7%)	24 (11%)	0.049
Hypertension	264 (41%)	165 (39%)	99 (46%)	0.09
Dyslipidemia	352 (55%)	217 (51%)	135 (62%)	0.009
Family history	409 (65%)	272 (65%)	137 (66%)	0.91
Current smoking				<0.001
Never	326 (51%)	241 (57%)	85 (39%)	
Former	233 (36%)	147 (35%)	86 (40%)	
Current	82 (13%)	37 (9%)	45 (21%)	
Drugs				
Aspirin	325 (51%)	205 (48%)	120 (55%)	0.09
CCB	240 (37%)	150 (35%)	90 (42%)	0.12
ACE-I /ARB	104 (16%)	62 (15%)	42 (19%)	0.12
Beta-blocker	185 (29%)	127 (30%)	58 (27%)	0.41
Diuretics	105 (16%)	84 (20%)	21 (10%)	0.001
Lipid-lowering drugs	249 (39%)	151 (35%)	98 (45%)	0.015
ERT		119 (28%)		

Values are given as n (%) or mean (standard deviation). P value shows women vs. men. BMI, body mass index; CFR, Coronary flow reserve; CCB, Calcium channel blocker; ACE-I, Angiotensin-converting enzyme - inhibitor; ARB, Angiotensin II receptor blocker; ERT, estrogen replacement therapy

Table 2

Comparison of coronary risk factor between the 2 groups divided by CFR

Parameters	CFR<2.5 (n=184)	CFR≥2.5 (n=459)	P value
Age, years	53.4 (11.0)	48.2 (11.2)	<0.001
BMI	27.8 (5.5)	29.5 (6.3)	0.001
Postmenopausal	117 (64%)	248 (54%)	0.06
CFR	2.2 (0.3)	3.3 (0.6)	
Risk Factors			
Men, n (%)	36 (20%)	181 (39%)	<0.001
Diabetes, n (%)	18 (10%)	34 (7%)	0.32
Hypertension, n (%)	77 (42%)	187 (41%)	0.73
Dyslipidemia, n (%)	105 (57%)	247 (54%)	0.44
Family history, n (%)	118 (66%)	291 (65%)	0.73
Smoking, n (%)			0.63
Never	96 (52%)	230 (50%)	
Former	66 (36%)	169 (37%)	
Current	22 (12%)	60 (13%)	
Drugs			
Aspirin	99 (54%)	226 (49%)	0.30
Calcium channel blocker	70 (38%)	170 (37%)	0.79
ACE inhibitor/ARB	36 (20%)	68 (15%)	0.14
Beta-blocker	62 (34%)	123 (27%)	0.08
Diuretics	36 (20%)	69 (15%)	0.16
Lipid-lowering drugs	84 (46%)	165 (36%)	0.024
ERT	53 (29%)	124 (27%)	0.65

Values are given as n (%) or mean (standard deviation). BMI, body mass index; CFR, coronary flow reserve; ACE, Angiotensin-converting enzyme; ARB, Angiotensin II receptor blocker; ERT, estrogen replacement therapy

Table 3

SNP analysis for CFR <2.5 after adjusting for age, sex, and BMI

Chr.	SNP	Position	Gene region	Risk Allele X/Y	Affected genotype XX/XY/YY	Unaffected genotype XX/XY/YY	SNP annotation	OR	95% CI	P -value
6	rs3025039	43860514	*VEGFA*	A/G	5/57/122	7/105/347	3'UTR	1.68	1.18–2.39	0.0041
9	rs10757274	22086055	*CDKN2B-AS1*	G/A	48/94/35	95/209/138	intron	1.49	1.15–1.92	0.0028
9	rs2383206	22105026	*CDKN2B-AS1*	G/A	59/93/32	122/206/131	intron	1.43	1.12–1.83	0.0048
9	rs1004638	22105589	*CDKN2B-AS1*	T/A	61/91/32	125/205/129	intron	1.45	1.13–1.85	0.0036
9	rs2383207	22105959	*CDKN2B-AS1*	T/A	61/91/32	124/206/129	intron	1.46	1.14–1.87	0.0029
9	rs1333049	22115503	*CDKN2B-AS1*	G/C	52/94/38	102/219/138	3'downstream	1.44	1.12–1.86	0.0045

VEGFA, vascular endothelial growth factor A; *CDKN2B-AS1*, CDKN2B antisense RNA1, 3'UTR, 3' untranslated region

Table 4

Sex-specific estimates for the association of SNPs with CFR < 2.5

Chr.	SNP	Position	Gene region	Risk Allele X/Y	Affected Male genotype XX/XY/YY	Unaffected Male genotype XX/XY/YY	SNP annotation	OR Men	P value Men	OR Women	P value Women	P value SNP × SEX
3	rs4855559	109597726	*MYH15*	A/C	7/20/2009	16/70/95	intron	2.27	0.0029	1.22	0.2215	0.0008
3	rs7630352	109605691	*MYH15*	A/G	11/20/2005	24/83/74	intron	2.60	0.0006	1.25	0.1454	0.0002
6	rs3025028	43858529	*VEGFA*	C/G	21/13/2	53/97/30	intron	2.74	0.0015	1.06	0.6909	0.0017
6	rs6922	86262042	*NT5E*	C/A	24/10/2	60/96/25	3'UTR	2.85	0.0018	1.11	0.4794	0.0027

MYH15; myosin, heavy chain 15; *VEGFA*, vascular endothelial growth factor A; *NT5E*, 5'-nucleotidase, ecto (CD73)