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Association of Genetic Variants with the Metabolic Syndrome in 20,806 Caucasian Women: The Women's Health Genome Study

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Abstract

Background—candidate genes associated with cardiovascular disease (CVD) represent potential risk factors for the metabolic syndrome (MetS).

Methods—the association between prevalent MetS and a panel of 62 polymorphisms within 42 candidate genes, previously implicated in the pathophysiology of CVD, were investigated in 20,806 Caucasian participants of the Women's Health Study. All were free of known CVD and diabetes at baseline. Logistic regression was performed to investigate the relationship between genotype and the MetS assuming an additive model. Stratified analyses by hormone therapy use were also performed. Correction for multiple testing was performed using false discovery rate (FDR) for polymorphisms and false positive rate probability (FPRP) for stratified analysis, respectively.

Results—the prevalence of the MetS was 23%. In a marker-by-marker analysis, the *ADRB2 rs180088* (OR=1.22, 95%CI=1.01–1.48) and *PAII rs1799768* (OR=1.05, 95%CI=1.01–1.10) were associated with an increased MetS risk, while the *C5 rs17611* (OR=0.95, 95%CI=0.91–1.00) and the *CTLA4 rs5742909* (OR=0.91, 95%CI=0.84–0.99) were associated with a decreased risk. In postmenopausal women, an increased MetS risk was found for the *ADRB2 rs180088* (OR=1.28, 95%CI=0.99–1.65), *PAII rs1799768* (OR=1.07, 95%CI=1.01–1.14), *SCNNIA rs5742912* (OR=1.22, 95%CI=1.01–1.47), and *IL1A rs1800587* (OR=1.07, 95%CI=1.01–1.15), while the *AGTR1 rs5186* (OR=0.93, 95%CI=0.87–0.99) was associated with decreased risk. However, none remained significant after FDR correction. In a stratified analysis, one or more copies of the variant C-allele of *SCNNIA rs5742912* were associated with an increased MetS risk among the current users (OR=1.56, 95%CI=1.21–2.01, p-interaction=0.007, FPRP=0.13).

Conclusions—effect modification of the *SCNNIA rs5742912* on the MetS by hormone therapy use warrants further investigation.

Introduction

The metabolic syndrome (MetS) is a cluster of cardiovascular risk factors, which is associated with substantial risk of type 2 diabetes mellitus (DM) and cardiovascular disease (CVD) (1). Individuals with the MetS have a six-fold increased risk of DM and a two-fold increased risk of CVD, regardless of age, sex and ethnicity (1). While multiple definitions have been proposed, according to the ATP III definition, the MetS is characterized by ≥ 3 of the following:

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abdominal obesity, elevated triglycerides, low levels of high-density lipoprotein (HDL) cholesterol, high blood pressure, and elevated fasting glucose (2). The prevalence of MetS increases with aging (3), and some studies suggest that the prevalence of MetS is higher in middle-aged women than middle-aged men (4,5). The MetS may be also associated with greater cardiovascular risk in women than in men (6).

The etiology of metabolic syndrome is complex, being determined by interplay of environmental and genetic factors. However, heritability is a substantial contributor to the MetS ($r=0.61$) (7). Since the MetS is a cluster of conditions each of which has been associated with risk of cardiovascular disease (CVD), candidate genes previously implicated in the pathophysiology of CVD may represent potential candidates for the MetS. Genetic variants in pathways related to inflammation, thrombosis, homeostasis, neurohormonal activation and endothelial dysfunction may represent potential risk factors for the MetS (8–10). Although previous studies have shown genetic associations for many of the components of the MetS, few genome wide linkage studies have examined the full MetS (9–13). Recent candidate-gene association studies have studied polymorphisms in candidate genes for CVD in the context of the MetS and its components; (14–17) however, no polymorphisms have been consistently replicated. Insufficient statistical power, population stratification, heterogeneity between populations, genetic variants with modest effects or even gene-environment influences may contribute to limited progress in identifying candidate genes associated with complex traits such as the MetS (18). In this context, association-based studies may be more powerful than linkage analyses for identification of the genes that contribute to variation in complex traits.

In the current study, we assessed a panel of 62 polymorphisms from 42 candidate genes previously implicated in CVD (8,9) to determine their association with prevalent MetS in a large cohort of Caucasian women.

Material and Methods

Study design

We studied DNA samples from participants enrolled in the Women's Health Study (WHS), a recently completed, randomized, double-blinded, placebo-controlled trial of low-dose aspirin, beta-carotene and vitamin E for the primary prevention of cardiovascular disease and cancer (19,20). The WHS was initiated in 1992 among 39,876 female, predominantly Caucasian, U.S. health professionals, 45–89 years old who were free of prior myocardial infarction, stroke, transient ischemic attacks, cancer or any serious illness that might preclude participation at study entry (19). Details of the study design have been described previously (19,20).

In brief, before randomization, 28,345 participants provided an EDTA-anticoagulant blood sample that was stored for genetic analysis. Women enrolled in the WHS completed a baseline questionnaire, which included questions on demographics (age, race, marital status and level of education), health characteristics/behaviors [height, weight, alcohol use, smoking status, diet, physical activity, hormone therapy (HT) use], menopause (age at menopause, and type of menopause), and past medical history (history of hypertension, diabetes mellitus, elevated cholesterol and use of cholesterol drugs). Women were considered postmenopausal after report of permanent cessation of menstrual periods due to natural menopause, bilateral oophorectomy, radiation, or chemotherapy. Women, who had undergone hysterectomy and could therefore not report age at menopause, were considered postmenopausal after reaching the age of 60.

In the current investigation, 20,806 Caucasian women who did not have DM at baseline and who had all genotypes of interest determined were assessed for the presence or absence of metabolic syndrome. Since many of the components of metabolic syndrome are affected by menopause and hormonal status (21–23), we also examined potential gene-environment

interaction between genetic variants and hormone therapy use in postmenopausal women. Since definitions of metabolic syndrome have continued to evolve, an additional 636 women with type 2 DM at baseline were also included in secondary analyses. The Brigham and Women's Hospital Institutional Review Board for Human Subjects Research approved the study protocol.

Study Variables

The genetic marker selection of 42 candidate genes was based on previous evidence of their potential functionality in pathways of inflammation, thrombosis, homeostasis, neurohormonal activation and endothelial dysfunction (8,9). Further, a validated allele frequency and heterozygosity, and sequence-proven allelic variation were considered for this current analysis.

We utilized a modified definition of the MetS, which has been previously validated and shown to predict cardiovascular outcomes in this cohort (24). In addition this modified definition resulted in nearly identical rates of MetS among women in the WHS compared with NHANES data utilizing ATP III in the same time period (24). Since waist circumference was not available at baseline, we used a cut point for obesity of body mass index (BMI) ≥ 26.7 kg/m². This value corresponded to the same percentile for BMI; as did a waist circumference of 88 cm when it was measured at year 6 of follow up in the WHS. A Spearman correlation of 0.96 between self-reported and measured weights was found in validation study of a similar cohort of female health professionals (25). Because fasting glucose levels were not available, we used a diagnosis of diabetes during follow-up to identify impairment of glucose metabolism. The diagnosis of diabetes was determined by self-report on the basis of annual questionnaires. More than 90% of the self-reported diagnoses of diabetes was confirmed through the American Diabetes Association (ADA) diagnostic criteria (26) by telephone interview in the WHS (27). We also considered triglycerides levels more than 150mg/dl and HDL cholesterol levels more than 50mg/dl. Both were directly measured using stored baseline blood samples (Roche Diagnostics, Indianapolis, IN). Finally, we defined elevated blood pressure (BP) as a self-reported BP $\geq 130/85$ mmHg. Self-reported blood pressure has been shown to be highly correlated with measured systolic and diastolic blood pressures in health professionals (28).

Genotyping determination

Genotype determination was performed using previously described multimarker assay using an immobilized probe approach for candidate markers of cardiovascular disease, immune response and inflammation (Roche Molecular Systems, Alameda, CA, USA) (29). In brief, each DNA sample was amplified by polymerase chain reaction (PCR) with biotinylated primers. Each PCR product pool was then hybridized to a panel of sequence-specific oligonucleotide probes immobilized in a linear array. The colorimetric detection method was based on the use of streptavidin-horseradish peroxidase conjugate with hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine as substrates. Genotype assignment was performed using the proprietary Roche molecular systems strip scan image processing software. To confirm genotype assignment, scoring was carried out by two independent observers. Discordant results (<1%) were resolved by a joint reading, and where necessary, a repeat genotyping.

Statistical analysis

The distribution of baseline characteristics according to the metabolic syndrome status was examined. Based on nonparametric distribution all continuous variables were examined by Wilcoxon Rank-Sum test while Chi-square test was used for categorical variables. We calculated allele frequencies and performed a Hardy-Weinberg Equilibrium (HWE) test using the Fisher probability test statistics for each individual polymorphism amongst controls. Multivariable logistic regression model adjusted by age, smoking status (never, past and current), hormone therapy use (no/yes) and randomized treatment assignments (aspirin,

vitamin E and beta carotene) were performed under the additive model. Additional adjustment for a number of covariates [physical activity (rarely/never, <1/week, 1–3/week and $\geq$ 4/week), CRP and hemoglobin A1C levels] was also considered in multivariable models. Potential interactions between hormone therapy use and genotypes on the risk of the MetS were examined using a formal interaction term (genotype X hormone use), followed by a stratified analysis according to hormone therapy use. The association of each component of the MetS with each of the 62 polymorphisms was also evaluated.

As we simultaneously examined multiple gene polymorphisms, correction for multiple hypothesis testing was performed using the false discovery rate (FDR) for polymorphisms (30). Although no universal FDR significance threshold has been defined, previous candidate gene studies have used a value of 0.20 (31). As for the stratified analysis, we applied the false positive report probability (FPRP) (32). The FPRP approach is especially helpful for hypotheses with low prior probability, including subgroup analyses. A preset threshold value of 0.5 for noteworthiness has been advocated (32).

For each odds ratio, we calculated 95% confidence intervals (CIs). A two-tailed *p*-value of 0.05 was considered to represent a statistically significant result. All statistical analyses were conducted with the use of SAS software (version 9.1; SAS institute, Cary, NC).

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Results

The baseline characteristics of the 20,806 Caucasian healthy women, without known cancer, cardiovascular disease (myocardial infarction, revascularization or ischemic stroke) or diabetes, according to the presence or absence of the MetS are shown in (Table 1). As expected, among those participants with the MetS, higher prevalence of classic cardiovascular risk factors was observed (Table 1). Overall, the prevalence of the metabolic syndrome was 23%. Among those with the MetS 82% had a BMI $\geq$ 26.7 kg/m² compared with 17.2% of women without the MetS, *p* < 0.0001 (data not shown). Similar baseline characteristics were found among postmenopausal women; however, postmenopausal women had a higher percentage of blood pressure equal or greater than 130/85 mmHg than all sample (Table 1). Approximately 59.4% of postmenopausal women were HT users, and among these HT users 85.2% used oral hormone therapy. Minor allele frequencies did not differ between all sample and postmenopausal women (Online Supplementary Data Table 1). All 62 polymorphisms were in Hardy-Weinberg Equilibrium (HWE).

Table 2 presents those individual polymorphisms with a nominal *p*-value < 0.10 for association with the MetS in multivariable models. In a marker-by-marker analysis, the *ADRB2* *rs180088* (OR=1.22, 95%CI=1.01–1.48) and *PAII* *rs1799768* (OR=1.05, 95%CI=1.01–1.10) polymorphisms were associated with an increased risk of the MetS, while the *C5* *rs17611* (OR=0.95, 95%CI=0.91–1.00) and the *CTLA4* *rs5742909* (OR=0.91, 95%CI=0.84–0.99) were associated with a decreased risk. In postmenopausal women, an increased risk of the MetS was found for the *PAII* *rs1799768* (OR=1.07, 95%CI=1.01–1.14), *SCNN1A* *rs5742912* (OR=1.22, 95%CI=1.01–1.47), and *IL1A* *rs1800587* (OR=1.07, 95%CI=1.00–1.15), while the *AGTRI*

rs5186 (OR=0.93, 95%CI=0.87–0.99) was associated with decreased risk. However, none of these associations remained significant after correction for multiple testing using FDR. When each of significant genetic markers in postmenopausal women was examined, a significant interaction between *SCNN1A rs5742912* and hormone therapy use was found (*p-interaction*=0.007). In analyses stratified by hormone therapy use, one or more copies of the C allele were associated with decreased risk of obesity in white post-menopausal women. One or more copies of the variant C allele of *SCNN1A rs5742912* were associated with an increased risk of the MetS among the current hormone users compared to nonusers (OR=1.56, 95% CI=1.21–2.01, FPRP=0.13).

Additional adjustment for physical activity, C-reactive protein levels, and HbA1c levels did not materially change our results. We also assessed the association with each individual component of the MetS, and did not find any significant associations (data not shown). Furthermore, analyses with the inclusion of women with baseline type 2 DM showed virtually identical findings (data not shown).

Discussion

In this large study, we assessed the relationship of 62 candidate polymorphisms with the MetS. Overall, none of the polymorphisms tested were significantly associated with the MetS after correction for multiple testing. However, in a stratified analysis, significant effect modification of the Sodium Channel Nonvoltage-Gated 1-Alpha Subunit gene (*SCNN1A*) *rs5742912* polymorphism by hormone therapy use on the MetS was observed. This association remained significant after correction for multiple testing. To the best of our knowledge, this is the first large-scale study reporting an association between *SCNN1A* gene variation and the MetS.

The amiloride-sensitive epithelial sodium channel (ENaC), which is expressed in the distal nephron and regulated by aldosterone, is composed of three subunits: alpha (*SCNN1A*), beta (*SCNN1B*) and gamma (*SCNN1G*) (33). The alpha subunit supports sodium conductance, and when expressed by itself, and channel activity is enhanced beta and gamma subunits are also expressed (33). Two rare monogenetic diseases of ENaC demonstrate potential consequences of alterations in channel structure and function (34,35). Autosomal recessive pseudoaldosteronism type 1 (PHA type 1) is a severe salt wasting syndrome caused by loss-of-function mutations in all three ENaC subunits; whereas, autosomal dominant pseudohypoaldosteronism (Liddle's Syndrome), is a severe form of salt sensitive hypertension caused by gain-of function in beta and gamma subunits (34–36). The *SCNN1A* gene, which is located on chromosome 12p13.3 and consists of 13 exons spanning 17Kb, encodes the alpha subunit of the ENaC (37). The 12p region has previously been suggested as a new locus for the MetS in genome-wide linkage analyses (parametric LOD score 2.86) (13). Few associations of *SCNN1A* polymorphisms with cardiovascular risk factors have been reported (38). One study investigating *SCNN1A* as a candidate gene for essential hypertension in 3,898 Japanese individuals (38) found that the G(2139) allele was significantly associated with an increased risk of hypertension and proteinuria (38). In a Mexican-American cohort, an association between the *SCNN1A* gene A663T polymorphism was associated with decreased fasting insulin levels after adjusting for BMI(10). We found an increased risk of MetS among postmenopausal women who were carriers of the C minor allele of the *SCNN1A rs5742912* polymorphism and who used hormone therapy. This rare (2% minor allele frequency) (36) variant results in a missense mutation (Trp to Arg) in exon 10 of the *SCNN1A* gene. Although aldosterone can regulate the EnaC (33), estrogen has not previously been reported as interacting with ENaC function; It is possible that it interacts with other regulatory elements.

We examined the potential functional effect of *SCNN1A rs5742912* using two prediction programs, PolyPhen (<http://genetics.bwh.harvard.edu/pph/>) and SIFT

(<http://blocks.fhcrc.org/sift/SIFT.html>), and both prediction models suggested that the substitution would alter protein function and structural stability.

The candidate gene approach relies on prior knowledge of biological pathways and its associations with the phenotype of interest. In recent years, genome-wide association studies of common, complex diseases have become available, and have provided insights in the underlying pathophysiological mechanisms of several common disorders. Unfortunately, to date, no large genome-wide association investigations have been conducted in relation to the MetS, thus, highlighting the need for large-scale, prospective studies in this important clinical condition. In this context, in addition to the candidate gene set described here, the Women's Genome Health Study project (39) will eventually include full genome-wide scan data (estimated completion by the end of 2008); thus, more detailed results regarding other potential genetic predispositions to the MetS are expected in the future.

Strengths of the present study are the overall sample size, the biological relevance of the polymorphisms considered, the prospective design and the complete long-term follow-up. We also chose, on an *a priori* basis, to adjust for multiple comparisons, and to present all our data simultaneously rather than focusing on any one specific finding. Nonetheless, some potential limitations of our study require discussion. Limitations include generalizability and potential bias. We examined only Caucasian middle aged and older women and our findings may not be generalizable to other populations with diverse ethnicity. Since the MetS is a combined outcome of complex traits, including hypertension, obesity, dyslipidemia and insulin resistance, we cannot rule out selection bias or chance as an explanation of our findings. Finally, we utilized a previously validated modified definition of the MetS which could introduce some bias in our results, although it has been shown to predict cardiovascular outcomes in this cohort (40). Furthermore, based on the observed odds ratios and the corresponding confidence intervals, polymorphisms that are potentially false negatives after our multiple-testing correction warrant further investigation.

While there is no universal consensus on the FDR threshold for genetic association studies, we chose to use $FDR < 0.20$ (20%) as arbitrarily suggested by Smith et al. for hypothesis-generating genetic association studies (31). Thus, future methodological studies of comparing the FDR to standard approaches such as Bonferroni correction, and establishing threshold(s) for the q-statistics of FDR should be explored for genetic association studies. Of relevant note, a modified method of correction for multiple testing for single nucleotide polymorphisms in linkage disequilibrium has recently been proposed (41). Thus, its utility in genetic association studies with prespecified candidate single nucleotide polymorphisms should be further methodologically explored.

In conclusion, none of the 62 candidate polymorphisms tested were significantly associated with the MetS. However, possible effect modification of the *SCNN1A rs57429121* polymorphism by hormone therapy use on the MetS warrants further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics of Caucasian apparently healthy women with and without metabolic syndrome in the Women's Health Study.

Characteristics	All women (N=20,806)		Postmenopausal women (N=11,297)		p-value
	No metabolic syndrome (n =16,029)	Metabolic syndrome (n =4,777)	No metabolic syndrome (n =8,573)	Metabolic syndrome (n =2,724)	
Age ^d	52.7 (48.8–58.5)	53.9 (49.6–60.3)	57.4 (52.8–63.2)	58.8 (53.5–64.4)	<.0001
Aspirin use (%)	49.7	50.1	50.3	49.9	0.66
BMI ^d	23.7 (21.9–25.8)	29.9 (27.5–33.3)	23.8 (21.9–25.8)	29.3 (27.3–32.6)	<.0001
BMI ≥ 26.7 kg/m ² (%)	17.2	82.0	16.9	77.8	<.0001
Blood pressure $\geq 130/85$ mmHg (%)	21.2	73.1	26.6	77.1	<.0001
Diabetes during follow-up (%)	0.9	21.2	0.9	20.7	<.0001
Triglycerides ≥ 150 mg/dl	20.7	79.2	24.9	82.2	<.0001
HDL <50mg/dl(%)	30.2	89.4	26.7	87.2	<.0001
CRP (mg/dl)	1.55 (0.63–3.40)	3.92 (2.10–6.74)	1.92 (0.84–3.91)	4.03 (2.23–6.90)	<.0001
HbA1c (%) (IQR)	4.96 (4.81–5.13)	5.11 (4.93–5.31)	5.00 (4.84–5.20)	5.12 (4.94–5.33)	<.0001
Hormone therapy users (%)	45.9	39.0	62.4	49.9	<.0001
Smoking status (%)					
Never	51.1	51.8	49.1	49.9	
Past	37.6	36.0	38.8	37.7	
Current	11.3	12.2	12.2	12.4	
Exercise (%)	34.1	46.3	36.3	47.0	0.63
Rarely/never	34.1	46.3	36.3	20.1	
< 1/week	19.2	21.2	18.3	20.1	
1–3/week	33.6	26.1	32.0	25.6	
≥ 4 /week	13.2	6.4	13.4	7.3	<.0001

P values from Wilcoxon Rank Sum test for continuous variables and chi-square analysis for categorical variables.

^dMedian (Interquartile range) for continuous measures.

Table 2

Odds ratios for metabolic syndrome in apparently healthy Caucasian women in the Women's Health Study.

Polymorphisms	All sample		Postmenopausal women	
	OR (95%CI) *	p-value	OR (95%CI) *	p-value
SCNN1A rs5742912	1.14 (0.98–1.32)	0.09	1.22 (1.01–1.47)	0.04
AGTR1 rs5186	0.95 (0.90–1.00)	0.05	0.93 (0.87–0.99)	0.03
ADRB2 rs180088	1.22 (1.01–1.48)	0.04	1.28 (0.99–1.65)	0.06
CTLA4 rs5742909	0.91 (0.84–0.99)	0.02	0.91 (0.81–1.01)	0.07
PAL1rs1799768	1.05 (1.01–1.10)	0.03	1.07 (1.01–1.14)	0.02
TNF-rs1800750	0.84 (0.68–1.03)	0.09	1.10 (0.99–1.23)	0.07
ADRB2 rs1042714	0.96 (0.92–1.00)	0.07	0.94 (0.88–1.01)	0.08
C5 rs17611	0.95 (0.91–1.00)	0.03	1.07 (1.00–1.15)	0.04
IL1B rsrs16944	1.05 (1.00–1.10)	0.06	1.07 (1.00–1.15)	0.06
			1.16 (1.00–1.35)	0.05

* Multivariable adjustment by age, aspirin use, vitamin E, beta-carotene use, hormones therapy use, smoking status; OR, odds ratio; 95%CI, confidence interval. All of polymorphisms had false discovery rate (FDR) ≥ 0.25 .