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Association of the sirtuin and mitochondrial uncoupling protein genes with carotid intima-media thickness

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Sirtuins (SIRT)s are a family of nicotinamide adenine dinucleotide (NAD⁺)–dependent deacetylases involved in chromatin remodelling, cellular metabolism, and lifespan regulation.¹ Uncoupling proteins (UCPs) are a family of inner mitochondrial membrane proteins capable of driving the adenosine triphosphate synthase pathway via regulation of the proton electrochemical gradient.² SIRTs and UCPs have been implicated in atherosclerosis and cardiovascular disease (CVD) through the control of reactive oxygen species production.^{1,2} In our previous *in vivo* study, the up- or downregulation and the enzymatic activity of SIRT/UCP proteins have been related to the degree of tolerance to brain ischemia.³ Therefore, a different alteration in functionality of these proteins may have a different impact on CVD and subclinical atherosclerosis.

We have recently demonstrated a link between variants in the SIRTs and UCPs genes and risk of carotid plaque (CP) and reported the effect-modifications of these relationships by sex, smoking, diabetes, and hypertension in the Northern Manhattan Study (NOMAS).⁴ Genetic variants in the SIRTs and UCPs genes may have an important role in development of atherosclerosis, independent of, or synergistic with, common vascular risk factors. Given that CP and carotid intima-media thickness (cIMT) are mainly biologically and genetically distinct phenotypes of atherosclerosis,⁵ in the present study, we extend our investigations to the associations of genetic variations in the SIRTs and UCP genes with cIMT.

We analyzed 1018 participants with cIMT and genotype data from NOMAS.⁶ All participants provided written informed consents. The study was approved by the Institutional Review Boards of Columbia University and the University of Miami. cIMT was assessed by B-mode ultrasound according to standardized scanning and reading protocols.⁷

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A total of 85 single-nucleotide polymorphism (SNPs) in the 11 UCP and SIRT genes were available from the AffyMetrixGenome-Wide Human SNP array 6.0. Detailed genotyping procedures were described previously.⁴ Generalized linear modelling was performed to evaluate the association of each SNP with cIMT after adjustment for age, sex, ever smoking, and the top 2 principle components by EIGENSTRAT to adjust for population stratification.⁸ The effect modifications by sex and ever smoking were explored by including interaction terms to the regression models. Stratified analyses by sex and smoking were also performed.

The mean age of the participants was 70 ± 9 years, 61% were women, 67% Caribbean Hispanic, 17% Black, and 15% White. The mean cIMT was 0.96 ± 0.10 mm for men and 0.93 ± 0.09 mm for women. The prevalence of hypertension was 62%, diabetes 18%, hypercholesterolemia 27%, and ever smoking 52%. In univariate analysis, age, sex, and smoking were significantly associated with cIMT. Three SNPs showed an association with cIMT with a nominal $P < 0.05$, after adjustment for significant covariates and the top 2 principal components of ancestry (Table I): elevated cIMT for TT-carriers at rs1430583 ($\beta = 0.036$, $P = 0.006$) or CC-carriers at rs6818140 ($\beta = 0.036$, $P = 0.007$) in *UCP1*, and lower cIMT for CC-carriers allele at rs12363280 ($\beta = -0.041$, $P = 0.046$) in *SIRT3*. Significant effect-modification was observed by sex and smoking (Table II). Men homozygous for the minor alleles at rs6818140 (C) in *UCP1* had increased cIMT, whereas women homozygous for the T allele (minor allele) at rs3825075 in *SIRT3* had lower cIMT. Significantly elevated cIMT was found in smokers homozygous for any of the minor alleles at rs6818140 (C) in *UCP1*, rs7109266 (A) in *UCP2*, or rs4802998 (G) in *SIRT2*.

The present study demonstrates a sex-specific effect of genetic variants in *UCP1* and *SIRT3* on cIMT. Sex hormones have been shown to modulate *UCP1* expression.⁹ Recent reports have described the *SIRT3* sex differences in its expression after physical activity.¹⁰ These findings may in part explain sex-dependent associations of *UCP1* and *SIRT3* genetic variants with cIMT.

The present study also shows an effect-modification of *UCP1*, *UCP2*, and *SIRT2* with cIMT by smoking. A direct association between smoking and UCPs was found in mice where exposure to cigarette smoke increased UCPs in the brown fat.¹¹ The regulation of the *UCP1* transcription has been demonstrated in the development of atherosclerotic lesions through its effect on monocytes/macrophages, vascular endothelial cells, and vascular smooth muscle cells.¹² *UCP2* levels measured in peripheral blood lymphocytes were higher in smokers than that in nonsmokers, indicating a role of this protein in response to the oxidative stress induced by smoke.¹³ *SIRT2* is a predominantly cytoplasmic protein implicated in oxidative stress, cell cycle regulation, and apoptosis,¹ and its role in atherosclerosis may be exaggerated by smoking. Further studies including gene expression, proteomics, and deep sequencing are needed to clarify exact mechanisms of these interactions. Previously, using the same study population we demonstrated novel associations of genetic variants in *UCP1*, 3, 4, and 5 and *SIRT3*, 5, and 6 and CP.⁴ Several important effect modifications of these relationships were found by sex, smoking, hypertension, and diabetes. The lack of replication of these findings in the present study further confirms that plaque and cIMT are different subclinical phenotypes of atherosclerosis with different genetic mechanisms.⁵

Plaque is more strongly related to traditional risk factors and to coronary artery disease than is IMT.⁵ A combined approach using traditional vascular risk factors and validated genetic markers for atherosclerosis may offer a better risk-prediction of vascular diseases. In addition, it may be possible to identify alterations in pathways involved in CVD beyond the effect of traditional vascular risk factors.

Strength of this study is the cohort population with adequate representation of minority ethnic groups, which are usually far underrepresented in studies of this type. Our results however need to be taken with caution because of several limitations.⁴ We included a relative small convenience sample from our genome-wide association studies with available SNPs in the 7 SIRT and 5 UCP genes (a total of 3055 SNPs, <http://www.ncbi.nlm.nih.gov/snp>) and did not adjust for multiple testing.

Despite these limitations the present study reveals novel associations between the UCP and SIRT genes and cIMT with specific effect-modifications by sex and smoking. cIMT is a significant predictor of vascular events. Genetics may play an important role in the determination of atherosclerosis, detecting asymptomatic individuals at increased vascular risk and help developing new therapeutic approaches, which are specifically tailored to atherosclerotic pathways.

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Table 1

SNPs associated with cIMT

Gene	SNP	BP	Genotype (frequency, %)	Beta (95% CI)	P	Genetic model
UCP1	rs1430583 *	141706434	TT(5.3), TC(35.2), CC(59.5)	0.036 (0.010, 0.062)	0.006	Recessive (TT vs TC/CC)
UCP1	rs6818140 *	141707405	CC(5.5), CT(35.0), TT(59.5)	0.035 (0.010, 0.060)	0.007	Recessive (CC vs CT/TT)
SIRT3	rs12363280	221980	CC(2.2), CG(18.3), GG(79.5)	-0.041 (-0.082, -0.001)	0.046	Recessive (CC vs CG/GG)

Abbreviations: cIMT, carotid intima media thickness; UCP, uncoupling protein; SIRT, Sirtuin; SNP, single nucleotide polymorphism; BP, base-pair

* The two SNPs in linkage disequilibrium with $r^2 = 1$.⁴

Table II

UCP and SIRT SNPs showing interaction with sex and smoking

Gene	SNP	Genotype (frequency, %)	Interaction analysis [†]		Stratified analysis			
			Beta (95% CI)	P	Beta (95% CI)	P	Beta (95% CI)	P
UCP1	rs1430583 [*]	TT(5.3), TC(35.2), CC(59.5)	Sex [*] SNP	0.056 (0.003, 0.109)	0.040	Female		
						Male	0.070 (0.025, 0.115)	0.002
UCP1	rs6818140 [*]	CC(5.5), CT(35.0), TT(59.5)	0.067 (0.015, 0.118)	0.012	0.075 (0.032, 0.119)	0.0008 [‡]	0.001 (−0.022, 0.039)	0.593
SIRT3	rs3825075	TT(9.3), TC(41.6), CC(49.1)	0.049 (0.007, 0.090)	0.022	0.025 (−0.010, 0.061)	0.166	−0.026 (−0.049, −0.002)	0.036
UCP1	rs1430583 [*]	TT(5.3), TC(35.2), CC(59.5)	Smoking [*] SNP	0.053 (0.002, 0.105)	0.043	Smoker		
						Non-Smoker	0.064 (0.025, 0.103)	0.002
UCP1	rs6818140 [*]	CC(5.5), CT(35.0), TT(59.5)	0.054 (0.004, 0.104)	0.035	0.062 (0.024, 0.100)	0.001 [‡]	0.011 (−0.024, 0.042)	0.604
UCP2	rs7109266	AA(2.8), AG(29.2), GG(68.0)	0.111 (0.039, 0.183)	0.003	0.065 (0.013, 0.116)	0.014	−0.046 (−0.096, 0.004)	0.075
SIRT2	rs4802998	GG(6.1), GA(37.5), AA(56.4)	0.086 (0.038, 0.134)	0.0005 [‡]	0.052 (0.018, 0.085)	0.003	−0.035 (−0.070, −0.001)	0.047

Abbreviations: UCP, uncoupling protein; SIRT, sirtuin; SNP, single nucleotide polymorphism.

* The two SNPs in LD with $r^2 = 1^4$;

[‡] Based on recessive genetic model;

[‡] Bonferroni-corrected $P < 0.05$ was based on the sum of singleton SNPs and linkage disequilibrium blocks.⁴