Published in final edited form as:

Transl Res. 2015 February ; 165(2): 358–359. doi:10.1016/j.trsl.2014.08.007.

Relationship between Sirtuin and Mitochondrial Uncoupling Protein Genes and Carotid Artery Stiffness

David Della-Morte^{a,e,f,*}, Chuanhui Dong^{a,*}, Ashley Beecham^b, Liyong Wang^b, Digna Cabral^a, Matthew S. Markert^a, Susan H. Blanton^{b,d}, Ralph L. Sacco^{a,c,d}, and Tatjana Rundek^{a,c,d}

^aDepartment of Neurology, Miller School of Medicine, University of Miami, Miami, Florida, USA

^bDepartment of Human Genetics, John P Hussman Institute for Human Genomics, Miller School of Medicine, University of Miami, Miami, Florida, USA

^cPublic Health Sciences, Miller School of Medicine, University of Miami, Miami, Florida, USA

^dEvelyn McKnight Brain Institute, Miller School of Medicine, University of Miami, Miami, Florida, USA

^eDepartment of Systems Medicine, University of Rome Tor Vergata, Via Montpellier 1, 00133 Rome, Italy

^fIRCCS San Raffaele Pisana, Rome, Italy

Sirtuins (SIRT6) are a family of nicotinamide adenine dinucleotide (NAD⁺)–dependent deacetylases,¹ and mitochondrial Uncoupling Proteins (UCPs) are a family of inner mitochondrial membrane proteins capable of driving the ATP-synthase pathway via regulation of the proton electrochemical gradient.² SIRT6 and UCPs have been implicated in slowing vascular aging by reducing ROS.³ Previously we demonstrated a significant association between single nucleotide polymorphisms (SNPs) in SIRT6/UCP and risk for carotid plaque (CP), number of plaques,⁴ and carotid intima media thickness (cIMT)⁵ as well as risk modification of these associations by vascular risk factors (RFs). Carotid stiffness (STIFF) is a measure of the vessel wall's tendency to resist deformation by systolic blood pressure (BP) during the cardiac cycle, and is considered a biologically and genetically distinct phenotypes of atherosclerosis compared with CP and cIMT.^{6, 7} A previous genetic study on carotid atherosclerosis conducted among 3,300 American Indian participants, reported as the genetic linkage seen for different phenotypes of atherosclerosis

© 2014 Mosby, Inc. All rights reserved.

Corresponding Author: Tatjana Rundek, MD, PhD, Department of Neurology, Miller School of Medicine, University of Miami, 1120 NW 14th Street, Miami, FL 33136, USA, Tel. 305-243-7847, Fax. 305-243-7081, trundek@med.miami.edu.

*First two authors contributed equally to this work.

Conflict of Interest

All authors have read the journal's policy on disclosure of potential conflicts of interest. The authors have declared that no competing interests exist.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

was not replicated for STIFF, suggesting a different genetic influence among these functional and structural parameters.⁸ In the present study we investigate the association between variation in the SIRT/UCP genes and STIFF and its components, systolic (SD) and diastolic diameter (DD) in an urban and elderly multi-ethnic population.

We analyzed 1,143 participants with STIFF and genotype data from the Northern Manhattan Study (NOMAS).⁹ All participants provided written informed consents. The study was approved by the Institutional Review Boards of Columbia University and the University of Miami and conforms to the relevant ethical guidelines for human and genetic research. STIFF was assessed by B-mode ultrasound and derived as a dimensionless quantity that expresses the tendency of an individual's arteries to deform from a given change in BP or $[\ln(\text{SBP}-\text{DBP})]/\text{Strain}$. SBP and DBP are mean systolic and diastolic brachial BP and Strain is derived as a ratio of the amount of stress deformation relative to the unstressed state or $(\text{SD}-\text{DD})/\text{DD}$.¹⁰ A total of 85 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 To account for population stratification, we first performed principal component analysis to examine population substructure using EIGENSTRAT and included the top three principle components (PCAs) as genomic control variables in the genetic association analysis.⁴ To control for the potential confounders, we conducted univariate analysis to identify demographic characteristics and RFs associated with parameters of arterial function (STIFF and strain, both were log-transformed in the regression analysis to reduce skewness) and structure (carotid DD and SD) ($p < 0.05$), in order to include them as covariates in the genetic association analysis of the UCP and SIRT variants. For single SNP-based association analyses, we examined the additive genetic effects of the UCP/SIRT variants on STIFF using linear regression models, after adjusting for the significant demographic characteristics, and RFs and the top 3 principle components. Power calculation revealed that a sample size of 1143 subjects allow 80% power to detect an additive genetic effect of $\beta = 0.12$ at type 1 error rate of 0.05 given the minor allele frequency = 0.25 and SD = 0.9 for diameter measures. We also examined SNP-by-RFs interactions and performed stratified analyses if the interaction terms had $p < 0.005$.

The mean age of the participants was 68 ± 9 years, 61% were women, 71% Caribbean Hispanic, 15% Black, and 12% White. Overall, 29% had obesity, 19% had diabetes, 29% had hypercholesterolemia; 16% were current smokers, 35% former smokers, 40% moderate alcohol drinkers, and 55% had leisure-time physical activity. The mean STIFF was 8.35 ± 5.38 , mean strain was 0.08 ± 0.14 , mean DD was $6.20 \pm 0.95\text{mm}$, and mean SD was $6.68 \pm 0.95\text{mm}$. In univariate analysis, STIFF, strain or diameters were significantly associated with age, sex, race-ethnicity, current smoking, obesity, and diabetes, but not with moderate alcohol drinking, leisure-time physical activity and hypercholesterolemia. SNPs associated with STIFF ($p < 0.05$, adjusted for age, sex, current smoking, obesity, diabetes and the top three PCAs) are reported in Table 1. T allele carriers of rs10498683 in SIRT5 had higher STIFF ($\beta = 0.07$, $p = 0.045$) whereas G allele carriers of rs7895833 in SIRT1 had lower STIFF ($\beta = -0.06$, $p = 0.027$). Moreover, 4 SNPs in UCP1, which were in strong LD ($r^2 > 0.99$), showed an association with both SD and DD, with 0.10–0.11mm increase in DD or SD per copy of minor allele of these SNPs. Similar association was found between rs1800849 in UCP3 and DD ($\beta = 0.11$ per copy of A allele, $p = 0.046$). In contrast, minor allele (A) carriers

of rs5977238 in UCP5, showed a decrease in DD ($\beta = -0.21$, $p = 0.012$) and SD ($\beta = -0.23$, $p = 0.007$). Table 2 shows interactions between SNPs and modifiable RFs with a nominal $p < 0.005$ and genetic effects stratified by the status of specific vascular risk factors. Mainly, SNPs of SIRT1 gene had greater effects on DD and SD in current smokers than in non-smokers. Moreover, SNPs of SIRT5 gene had greater effect on STIFF in diabetic compared to non-diabetic, with an opposite effect on Strain in the same patients (Table 2).

Current evidence suggests that STIFF fulfils the criteria for a biomarker of vascular aging as a cumulative measure of the impact of cardiovascular risk factors on the arterial wall. Based on the present study and on our previous findings^{4, 5}, SIRT/UCP effects provide a balance between deleterious and protective atherosclerosis mechanisms. However, SNPs found to be associated with other atherosclerotic phenotypes in previous studies^{4, 5} were not found to be associated with STIFF in this study. These findings further confirm that STIFF, plaque and cIMT are different subclinical phenotypes of atherosclerosis with different genetic mechanisms. SIRT1, the most studied sirtuin protein, extends lifespan of all tested organisms by mimicking the effects of caloric restriction.¹ A reduced risk for STIFF was observed for rs7895833G/A allele carriers in SIRT1 gene. Supporting the present findings rs7895833 in SIRT1 was previously associated with variability in BP and lower BMI.¹¹ We found an increase in STIFF among individuals with the T allele of rs10498683 in SIRT5. Previously, we found that subjects with this allele have an increased risk for CP with a specific effect-modification by a presence of diabetes and hypertension.⁴ SIRT5 is a mitochondrial sirtuin that is up-regulated by caloric restriction and has a similar role to SIRT1 in controlling ROS production.¹ An interaction with diabetes was also observed in association between SIRT5 variants with strain and STIFF.

We have recently demonstrated race-ethnic differences in changes in STIFF and carotid diameters,¹⁰ indicating that genetics may influence arterial dilatation. We previously reported significant associations between variation in UCP1 (rs1430583 and rs6818140) and cIMT.⁵ Similarly, in this study we found these SNPs to be associated with DD and SD. In transgenic mice, UCP1 expression in aortic smooth muscle cells has been shown to cause hypertension and increased dietary atherosclerosis without affecting cholesterol levels.¹² We have previously reported a significant association between rs5977238 in UCP5 and decreased risk for CP and plaque number, particularly in subjects with hypertension.⁴ In this study, we found a similarly protective role involving the carotid diameters. UCP5 physiological function has not yet been fully established, although by regulating the proton gradient across the inner membrane UCP5 may be implicated in ROS formation and ATP synthesis.¹

The present study reveals novel associations between UCP and SIRT genes and arterial stiffness with specific effect modifications by smoking and diabetes. Given that arterial stiffness is a potential biomarker of vascular aging and a potential predictor of vascular events, understanding of its genetic basis may be of particular importance for risk stratification and development of new therapeutic approaches specifically tailored to atherosclerotic pathways.

Acknowledgments

This research was supported by the NIH/NINDS K24-NS062737 grant, James & Esther King Biomedical Research Program (2KN01), and Evelyn F. McKnight Brain Institute. All authors have read the journal's authorship agreement and that the manuscript has been reviewed by and approved by all named authors

References

1. Dali-Youcef N, Lagouge M, Froelich S, Koehl C, Schoonjans K, Auwerx J. Sirtuins: The 'magnificent seven', function, metabolism and longevity. *Ann Med*. 2007; 39:335–345. [PubMed: 17701476]
2. Rousset S, Alves-Guerra MC, Mozo J, et al. The biology of mitochondrial uncoupling proteins. *Diabetes*. 2004; 53 (Suppl 1):S130–135. [PubMed: 14749278]
3. Della-Morte D, Ricordi C, Rundek T. The fountain of youth: Role of sirtuins in aging and regenerative medicine. *Reg Med*. 2013; 8:681–683.
4. Dong C, Della-Morte D, Wang L, et al. Association of the sirtuin and mitochondrial uncoupling protein genes with carotid plaque. *PloS one*. 2011; 6:e27157. [PubMed: 22087257]
5. Della-Morte D, Dong C, Bartels S, et al. Association of the sirtuin and mitochondrial uncoupling protein genes with carotid intima-media thickness. *Transl Res*. 2012; 160:389–390. [PubMed: 22750084]
6. Rundek T, Brook RD, Spence JD. Letter by rundek et al regarding article, "prediction of clinical cardiovascular events with carotid intima-media thickness: A systematic review and meta-analysis". *Circulation*. 2007; 116:e317. [PubMed: 17724268]
7. Della-Morte D, Guadagni F, Palmirotta R, et al. Genetics of ischemic stroke, stroke-related risk factors, stroke precursors and treatments. *Pharmacogenomics*. 2012; 13:595–613. [PubMed: 22462751]
8. Bella JN, Cole SA, Laston S, et al. Genome-wide linkage analysis of carotid artery lumen diameter: The strong heart family study. *Int J Cardiol*. 2013; 168:3902–8. [PubMed: 23871337]
9. Sacco RL, Sabala EA, Rundek T, et al. Design of a family study among high-risk caribbean hispanics: The Northern Manhattan Family Study. *Ethn Dis*. 2007; 17:351–357. [PubMed: 17682370]
10. Markert MS, Della-Morte D, Cabral D, et al. Ethnic differences in carotid artery diameter and stiffness: The Northern Manhattan Study. *Atherosclerosis*. 2011; 219:827–832. [PubMed: 21906739]
11. Zillikens MC, van Meurs JB, Rivadeneira F, et al. Sirt1 genetic variation is related to BMI and risk of obesity. *Diabetes*. 2009; 58:2828–2834. [PubMed: 19741164]
12. Bernal-Mizrachi C, Gates AC, Weng S, et al. Vascular respiratory uncoupling increases blood pressure and atherosclerosis. *Nature*. 2005; 435:502–506. [PubMed: 15917810]

Table 1

SNPs associated with diameters and stiffness with a p value <0.05*

Gene	SNP	BP	Minor/Major allele (MAF)	Trait	Beta (95% CI)	P
SIRT1	rs7895833	69293063	G/A (0.24)	Stiffness, log-transformed	-0.06 (-0.12, -0.01)	0.027
	rs10498683	13668813	T/C (0.14)	Stiffness, log-transformed	0.07 (0.00, 0.13)	0.045
	rs7693034	141698129	G/C (0.48)	Diastolic diameter	0.11 (0.03, 0.19)	0.007
UCP1	rs12502572	141704584	A/G (0.49)	Systolic diameter	0.11 (0.03, 0.19)	0.007
	rs1430583	141706434	T/C (0.23)	Diastolic diameter	0.10 (0.02, 0.18)	0.011
	rs6818140	141707405	G/A (0.23)	Systolic diameter	0.10 (0.03, 0.18)	0.010
UCP3	rs1800849	73397813	A/G (0.15)	Diastolic diameter	0.12 (0.03, 0.21)	0.010
	rs5977238	129308417	A/G (0.06)	Systolic diameter	0.11 (0.02, 0.20)	0.017
				Diastolic diameter	0.12 (0.03, 0.20)	0.011
UCP5				Systolic diameter	0.11 (0.02, 0.20)	0.018
				Diastolic diameter	0.11 (0.00, 0.22)	0.046
				Diastolic diameter	-0.21 (-0.38, -0.05)	0.012
				Systolic diameter	-0.23 (-0.40, -0.06)	0.007

* adjusted for age, sex, current smoking, obesity, diabetes, and the top three PCAs.

Table 2

SNPs showing interaction with smoking and diabetes with a p value <0.005*

Gene	SNP	Minor allele	Trait	Interaction analysis		Stratified analysis			
				Beta (95% CI)	P	Beta (95% CI)	P	Beta (95% CI)	P
SIRT1	rs17712705	A	Diastolic diameter	Current smoking *SNP		Current smoker		Non-current smoker	
				0.34 (0.12, 0.55)	0.002	0.22 (0.03, 0.42)	0.025	-0.07 (-0.16, 0.02)	0.108
	rs10997841	G	Systolic diameter	0.33 (0.12, 0.54)	0.002	0.22 (0.03, 0.42)	0.025	-0.08 (-0.17, 0.01)	0.086
			Diastolic diameter	0.30 (0.10, 0.51)	0.004	0.16 (-0.04, 0.35)	0.115	-0.09 (-0.17, -0.00)	0.047
			Systolic diameter	0.30 (0.09, 0.50)	0.004	0.15 (-0.04, 0.34)	0.131	-0.09 (-0.18, -0.01)	0.035
SIRT5	rs7894483	A	Systolic diameter	Diabetes *SNP		Diabetic		Non-diabetic	
				0.29 (0.09, 0.50)	0.004	0.15 (-0.04, 0.34)	0.123	-0.09 (-0.18, 0.00)	0.042
	rs2253217	C	Strain, log-transformed	-0.20 (-0.32, -0.08)	0.001	-0.13 (-0.25, -0.01)	0.027	0.04 (-0.01, 0.09)	0.122
			Stiffness, log-transformed	0.19 (0.07, 0.31)	0.003	0.12 (0.00, 0.24)	0.053	-0.04 (-0.09, 0.02)	0.188
			Strain, log-transformed	-0.21 (-0.33, -0.08)	0.001	-0.11 (-0.24, 0.01)	0.070	0.06 (0.01, 0.11)	0.031
	rs9382227	T	Stiffness, log-transformed	0.20 (0.07, 0.33)	0.002	0.14 (0.01, 0.26)	0.037	-0.04 (-0.09, 0.02)	0.217
	rs2804918	A	Strain, log-transformed	0.16 (0.05, 0.27)	0.003	0.08 (-0.03, 0.18)	0.161	-0.07 (-0.12, -0.02)	0.007

* adjusted for age, sex, current smoking, obesity, diabetes, and the top three PCAs.