Published in final edited form as:

Int J Stroke. 2015 December ; 10(8): 1247–1252. doi:10.1111/ijss.12623.

Sirtuin/uncoupling protein gene variants and carotid plaque area and morphology

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Abstract

Background—Sirtuins and uncoupling proteins have been implicated in cardiovascular diseases by controlling oxidative stress.

Aims—We sought to investigate the association of sirtuins and uncoupling proteins single nucleotide polymorphisms with total carotid plaque area and morphology measured by ultrasonographic gray scale median.

Methods—We analyzed 1356 stroke-free subjects (60% women, mean age = 68 ± 9 years) from the Northern Manhattan Study. Multiple linear regression models were used to evaluate the association of 85 single nucleotide polymorphisms in 11 sirtuin/uncoupling protein genes with total plaque area and gray scale median after controlling for demographics, vascular risk factors (RFs), and population stratification. We investigated effect modifications of these relationships by gender and RFs and performed stratified analysis if the interaction effect had $P < 0.005$.

Results—Among individuals with present plaque (55%), the mean total plaque area was $20.3 \pm 20.8 \text{ mm}^2$ and gray scale median 90 ± 29 . After adjustment, SIRT6 rs107251 was significantly associated with total plaque area ($\beta = 0.30$ per copy of T allele increase, Bonferroni-corrected $P =$

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Authors' contributions

Chuanhui Dong: Conceived and performed the study, performed the statistical analysis, written and revised the manuscript. David Della-Morte: Conceived and performed the study, interpreted the data, written and revised the manuscript; Digna Cabral: Collected the ultrasound data; Liyong Wang: Supervised the genetic data analysis and made critical revision of the manuscript; Susan H. Blanton: Supervised the genetic data analysis and made critical revision of the manuscript; Seemant Chaturvedi: Made critical revision of the manuscript; Ralph L. Sacco: Conceived and performed the study, interpreted the data, supervised all data collections, made critical revision of the manuscript; Tatjana Rundek: Conceived and performed the study, interpreted the data, supervision of all data collections, made critical revision of the manuscript.

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Conflict of interest: None declared.

0-005). T allele carriers of rs1430583 in UCP1 showed a decreased gray scale median in women but not in men. The minor allele carriers of rs4980329 and rs12363280 in SIRT3 had higher gray scale median in men but not in women. Variants in UCP3 gene were significantly associated with higher mean gray scale median in individuals with dyslipidemia.

Conclusion—Our findings suggest that polymorphisms in SIRT6/UCP1 genes may be important for increased carotid plaque burden and echodensity, but translation of these findings to an individual risk of cerebrovascular events needs further investigation. Significant associations of rs1430583 in women, rs12363280 in men, and rs1685354 in those with dyslipidemia also deserve further investigations.

Keywords

atherosclerosis; carotid total plaque area; genetics; gray scale median; mitochondrial uncoupling proteins; sirtuins

Introduction

Atherosclerosis is a multifactorial disorder and underlying cause of most ischemic strokes and cardiovascular diseases (CVD) (1). Subclinical markers of atherosclerosis including carotid plaque (CP) and carotid intima media thickness (IMT) have been widely used to assess the risk of future clinical atherosclerotic disease (1). Interestingly, these markers may be distinct biological phenotypes of atherosclerosis (2). Recent studies have demonstrated that variation in carotid atherosclerosis is largely unexplained by known vascular risk factors (3,4), suggesting that other unaccounted factors, both environmental and genetic, play an important role in the determination of atherosclerosis.

Oxidative stress is clearly involved in mechanisms of atherosclerosis leading to vascular diseases mainly by the enhancement of endothelial dysfunction, and reduction of nitric oxide bioactivity (5). Therefore, genes controlling balance between production and removal of reactive oxygen species (ROS) such as sirtuin (SIRT) (6) and mitochondrial uncoupling proteins (UCPs) genes (7) have been proposed in atherosclerosis. These processes may be pivotal in the regulation of plaque morphology since ROS can increase the expression of cell surface receptors required for uptake of oxidized low-density lipoprotein (8). SIRT control the genetic expression of UCPs by binding directly to their promoters. The up- or down-regulation and the enzymatic activity of SIRT/UCP proteins have been related to the degree of tolerance to brain ischemia (9). Therefore, modulating SIRTs and UCPs functions may offer a promising strategy for combating atherosclerosis and CVD.

Previously, we demonstrated a significant association between several single nucleotide polymorphisms (SNPs) in SIRT/UCP genes and CP presence and number (10), IMT (11), and carotid artery stiffness (STIFF) (12), as well as effect modification of these associations by vascular risk factors. In this study, we extend these investigations to plaque burden measured by total plaque area (TPA) and plaque morphology measured by an ultrasonographic index of plaque echodensity or gray scale median (GSM).

TPA has been suggested as the most useful subclinical CVD measure to evaluate the effects of anti-atherosclerotic treatments (13,14). GSM represents a novel marker of plaque morphology with great potential for clinical use (15,16). Low GSM values correspond to echolucent plaques with features that include high lipid content, and a thin fibrous cap, whereas high GSM index correlates to echogenic plaques of high fibrous content and calcification (17). In our previous study, we demonstrated that subjects with CP calcification had a significant increased risk of the combined vascular events (18). Based on our previous discovered findings (10,12) and SIRT/UCP antioxidant properties, we hypothesize the significant role of SIRT/UCP genes in atherosclerosis burden and plaque morphology. We also hypothesized that these associations may be gender specific. Several reports have shown less lipid rich plaques in women (19,20), while others have reported more calcified plaques in men (21). In addition, major vascular risk factors may be significant modifiers of these associations.

Materials and methods

Study population

A sample of 1356 stroke-free participants was derived from the Northern Manhattan Study (NOMAS). We have reported the detailed ascertainment scheme of the NOMAS previously (22). Briefly, NOMAS participants were eligible if they had never been diagnosed with a stroke, were at least 40 years of age, and resided for at least three-months in a household with a telephone in northern Manhattan. At enrollment, demographic characteristics and RFs were collected through standardized questionnaires and laboratory tests. Hypertension was defined as a systolic BP ≥ 140 mmHg, diastolic BP ≥ 90 mmHg, or a history of hypertension and anti-hypertension treatment; diabetes mellitus was defined as fasting blood glucose ≥ 126 mg/dl or use of insulin or hypoglycemic medications; dyslipidemia was defined as total cholesterol ≥ 240 mg/dl or a history of taking lipid-lowering medications. Smoking was dichotomized as ever or never smoking, physical activity as any leisure-time activity or none, and alcohol drinking as light to moderate (≥ 1 drink/month but <2 drinks/day), none, or other. Body mass index (kg/m^2) was calculated based on the measured weight and height. All subjects provided written informed consent to participate, and the study was approved by the Institutional Review Boards of Columbia University and the University of Miami.

Assessment of CP GSM and TPA

High-resolution B-mode ultrasounds (GE LogIQ 700, GE Health-care, Milwaukee, WI, USA, 9- to 13-MHz linear-array transducer) were performed by trained and certified sonographers as previously detailed (23). Left and right carotid bifurcations and internal and common carotid arteries were examined for the presence of plaque. Plaque was defined as an area of focal wall thickening 50% greater than surrounding wall thickness confirmed by marking and comparing plaque thickness with the thickness of the surrounding wall during scanning by electronic calipers (3). The plaque boundaries were traced offline from the digitized images using an automatic edge detection system M' Ath (Imt, Inc., Paris, France). The sum of plaque areas in all carotid arteries from both sides of the neck was expressed as a total CP area (TPA) (3). After image normalization using linear scaling, GSM of an

operator-selected blood region inside the vessel lumen was mapped to 0, and the brightest region of the adventitia was mapped to 255 using automated software in M'ATH (24). Both of these reference regions were approximately 0.4 mm² in area and were selected on the first image of the image sequence. The reference GSM values calculated on the first frame were applied to that and all subsequent images and GSM was expressed for each plaque separately. The mean GSM of all plaques within an individual was calculated and used in the analyses.

Genotyping

We identified variants in SIRT and UCP genes available from a genome-wide screen performed on the Affymetrix Genome-Wide Human SNP Array 6.0 chip (Affymetrix, Inc., Santa Clara, CA, USA). The arrays were scanned on the Gene Chip Scanner 3000 7G (Affymetrix, Inc.), and image data were analyzed using the Genotyping Console™ (Affymetrix, Inc.). Subjects were removed from further analysis if they had call rates below 95%, relatedness, gender discrepancies, or were outliers beyond 6 SD from the mean using EIGENSTRAT (25). SNPs with severe deviation from Hardy–Weinberg equilibrium ($P < 1E^{-06}$) or a genotyping call rate less than 95% were also removed using PLINK1.05 (26). After quality control, a total of 85 UCP/SIRT SNPs in the 11 UCP/SIRT genes were included in the final analysis of this study (10).

Statistical analyses

To reduce potential bias due to 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. To control for potential confounders, we also included demographics (age, gender) and vascular factors (smoking, physical activity, moderate alcohol consumption, obesity, hypertension, diabetes mellitus, and hypercholesterolemia) in the model. For single SNP-based association analyses, we examined the additive genetic effects of the UCP/SIRT variants on TPA and GSM using linear regression models, after adjusting for the demographic characteristics, vascular factors, and the top three PCAs. Given a total of 16 LD blocks were identified and 14 SNPs were not located in any LD block, Bonferroni corrections were done to control for multiple testing based on an effective number of 30 (10,27). Alternatively, multiple testing corrections were conducted based on gene set tests with 5000 permutations (corrected P for gene set test = empirical $P \times 11$). To explore the effect modification of modifiable vascular factors on the associations between genetic variants and mean TPA and GSM, we examined SNP-by-gender and SNP-by-vascular factor interactions and performed stratified analyses if the interaction terms had $P < 0.005$. All the analyses were performed using PLINK1.05 and SAS9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Among 1356 subjects (mean age: 68 ± 9), 60% were women, 69% Hispanic, 19% Black, and 11% White. All subjects were stroke free and did not report any previous cardiovascular clinical events, including myocardial infarct and/or transient ischemic attack. Overall, 19% had diabetes, 52% were smokers (17% current smokers and 35% former smokers), 30% had

dyslipidemia, and 71% hypertension (Table 1). Among those with plaque (55%), the mean TPA was $20.3 \pm 20.8 \text{ mm}^2$ (interquartile range, 7.0–25.6) and mean GSM 89.5 ± 28.5 (interquartile range, 67.0–109.0).

Table 2 reports the SNPs associated with TPA or GSM with a nominal $P < 0.05$ after adjustment for demographics, vascular factors, and the top three PCAs. T allele carriers of rs107251 in SIRT6 were associated with higher mean GSM ($\beta = 8.66$, 95% CI = 2.99–14.63, $P = 0.003$) and larger TPA ($\beta = 0.30$, 95% CI = 0.14–0.45, $P = 0.0002$). After correction for multiple testing based on effective test number, rs107251 remained significantly associated with TPA (Bonferroni corrected $P = 0.005$). When the correction for multiple testing through permutation was performed, gene set analysis showed that SIRT6 gene was significantly associated with GSM (permutation corrected $P = 0.04$) and TPA (permutation corrected $P = 0.002$). In addition, C allele carriers of rs2841503 in SIRT5 tended to have higher mean GSM and larger TPA, and C allele carriers of rs5977233 in UCP5 appeared to have higher mean GSM, but none of these associations remained significant after correction for multiple testing.

Table 3 shows the interactions between SNPs and gender and vascular factors with a nominal $P < 0.005$ and the genetic effects stratified by gender and the status of the specific vascular risk factors. SNP-by-gender interaction was found for three potential regulatory SNPs, one in UCP1 (rs1430583), and two in SIRT3 gene (rs4980329 and rs12363280) in the association with mean GSM. Specifically, T allele carriers of rs1430583 in UCP1 showed a decreased GSM in women ($\beta = -8.87$, $P = 0.002$) but not in men ($P = 0.25$), while the minor allele carriers of rs4980329 ($\beta = 12.53$, $P = 0.005$) or rs12363280 ($\beta = 11.62$, $P = 0.01$) in SIRT3 had higher GSM in men but not in women (P for interaction = 0.0031). For the interactions between SNP and vascular risk factors, the strongest interaction was found between SNP rs1685354 in UCP3 gene and hypercholesterolemia. Specifically, A allele of rs2734827 and G allele of rs1685354 in UCP3 were significantly associated with higher mean GSM ($\beta = 9.5$, $P = 0.004$; $\beta = 11.56$, $P = 0.001$, respectively) in individuals with hypercholesterolemia, but not in those without hypercholesterolemia. Similarly, also, G allele in rs1685354 of UCP3 was associated with increased TPA ($\beta = 0.27$, $P = 0.004$) in individuals with hypercholesterolemia, but not in those without hypercholesterolemia (P for interaction = 0.0007).

Discussion

In this study, we report a significant association of SIRT6 SNP rs107251 with total TPA and morphology (GSM) and several potential effect modifications by gender and hypercholesterolemia. Lower GSM was associated with UCP1 among women but not among men. Greater GSM was associated with SIRT3 in men but not in women. Greater GSM was also associated with UCP3 among women and men with dyslipidemia. Present results provide further evidence that genetic variants in sirtuin and UCP genes contribute to atherosclerosis and that the SIRT/UCP pathway may be an attractive target for the anti-atherosclerotic therapies.

Both TPA and GSM have been proposed as novel markers of plaque atherosclerosis and predictive of incident CVD and stroke (14,16). Plaque size (TPA) rather than plaque presence (yes/no) may be more useful measure in targeting and monitoring individuals for more aggressive anti-atherosclerotic treatments (14). The ultrasonographic GSM index is a global measure of plaque echodensity and therefore an indicator of plaque morphology. Plaque composition is based on the hyperechoic or hypoechoic ultrasound signal (28). Similar to TPA, GSM has been recently used to evaluate the effects of anti-atherosclerotic therapies (15).

In our previous study (10), we observed that carriers of the SIRT6 rs107251 had an increased risk for CP. In the present study with a larger sample size, we provide further evidence that SIRT6 SNPs are associated with increased TPA. SIRT6 is one of the seven sirtuins, which functions as an ADP-ribosylase and NAD⁺-dependent deacylase of both acetyl groups and long-chain fatty-acyl groups (29). SIRT6 is predominantly localized in the cellular nucleus and is involved in DNA repair and lifespan extension (29). The role of SIRT6 in carotid atherosclerosis can be related to its proinflammatory as well as anti-inflammatory function depending on the context and cell type involved (29). SIRT6 deficiency causes a specific increase in glucose uptake in multiple cell types (30), and absence in SIRT6 results in accumulation of triglycerides (TG) (31). A recent study has shown that SIRT6 pathway mediates the switch of vascular smooth muscle cells between a quiescent contractile phenotype and a synthetic phenotype in response to cyclic strain (32). Therefore, the pleiotropic enzymatic activities of SIRT6 in atherosclerosis may be regulated by different SIRT6 gene variants. In particular, rs107251, that we reported in the association with CP presence (10), has also been associated with ulcerative colitis in a meta-analysis of 6 GWAS (33), emphasizing its proinflammatory role. In addition, subjects carrying rs107251 minor allele homozygotes (TT) have been shown to have a shorter lifespan (five-year mean survival) in a cohort of 3763 participants after controlling for aging-related risk factors, suggesting that this SIRT6 variant may predispose individuals for premature age-related diseases (34). Our results show an association of this SIRT6 SNP with atherosclerotic phenotypes regardless of age, which deserves further investigation.

Another finding of our study is the marginal association of rs2841503 in SIRT5, and rs5977233 in UCP5 with increased TPA and increased GSM. In our previous studies, we found the association between different SIRT5 and UCP5 variants with plaque presence, STIFF, and carotid diameters, suggesting a potential important role of these genes in carotid atherosclerosis structure and function (10,12). SIRT5 is a mitochondrial sirtuin that is upregulated by caloric restriction and is involved in ROS production (6). The physiological role of UCP5 has not been yet defined, but it has been proposed as a modulator of ATP production (35).

We observed gender-specific effect of genetic variants in UCP1 resulting in lower GSM in women but not in men. We found a gender interaction between UCP1 SNPs and CP presence in our previous study (10). Our results confirm the role of UCP1 gene in carotid atherosclerotic and extend them to associations with plaque morphology. Calcified plaque are more prevalent in men than in women suggesting a gender-specific interaction in this process (21), with smoking being most important factor associated with calcified plaque in

men (36). Gender-specific associations observed in current study could be explained, at least in part, by the sex hormone modulation of UCP1 expression, which have been already found to control body weight (37). In our study, women carrying specific UCP1 genetic variant have lower GSM. Even though our study population is comprised of elderly people, the different gender imprinting of sex steroid hormones on the effect of UCP1 in the mechanisms leading to the atherosclerosis during aging could have had an important effect. Our results may have several clinical implications. The American Heart Association and the European Stroke Organization guidelines suggest including age as well as gender into account when selecting patients with carotid stenosis for carotid revascularization (38). In a recent study conducted among individuals with unilateral asymptomatic internal carotid artery stenosis, different athero-sclerotic phenotypes including stenosis progression and irregular plaque surface have had a relevant gender-specific variability in the risk of ipsilateral stroke (39). Moreover, women with symptomatic high-grade carotid stenosis benefit from carotid surgery while asymptomatic women have less benefit (40). Taking these reports together with our results further emphasize the importance of understanding the gender-specific genetic and environmental interactions in the development of atherosclerosis that will drive the development of targeted and gender-specific CVD preventive strategies.

The UCP1 rs1430583 was also associated with variation in diastolic and systolic diameters of carotid artery in our previous study (12), suggesting its role in the regulation of carotid geometry and remodeling. The regulation of carotid artery diameter changes and carotid blood flow hemodynamics is pivotal in the development of atherosclerotic plaque (41).

We observed gender-specific effect of variants in SIRT3 resulting in greater GSM in men but not in women. The mitochondrial SIRT3 deacetylates numerous proteins involved in mechanisms associated with atherosclerosis including fatty acid β -oxidation, amino acid metabolism, the electron transport chain, and anti-oxidant defenses (6). A recent study however has demonstrated that SIRT3 deletion affected neither plaque burden nor features of plaque vulnerability (i.e., fibrous cap thickness and necrotic core diameter) (42). However, in the setting of increased oxidative stress (e.g. smoking that is more prevalent in men), SIRT3 effect may be present and deleterious in men.

Finally, we found an interaction between UCP3 SNPs and dyslipidemia. Specifically, genetic variances in UCP3 were associated with greater GSM and TPA only in individuals with increased cholesterol. UCP3 rs1685354 has been associated with a significant decrease in visceral fat mass in a Dutch men study (43). Other UCP3 genetic variants have been indicated as modifiers of increased serum lipid levels and indices of abdominal obesity (44).

Our results need to be taken with caution because of several limitations. We included a relatively small convenience sample of SNP from our genome-wide association study (85 SNPs in the SIRT and UCP genes) (10). We have not provided an independent validation of our results. However, SNPs that we found associated with CP burden and morphology in the present study have been associated with vascular diseases and risk factors in several other studies (43,45,46).

Conclusions

Despite the limitations previously reported, the results from our study may be particularly important. Variances in SIRT/UCP genes associated with CP burden and morphology may have a pivotal role in atherosclerosis. Some of these processes may be more pronounced among women or may be more expressed in different metabolic conditions such as dyslipidemia. Exploration of the specific role of SIRT/UCP variants in vascular disease may help develop more specific SIRT activator compounds that can restore impaired molecular pathways, and thereby prevent and reduce atherosclerosis and CVD. In addition, the significant SIRT/UCP variances associated with TPA and GSM observed in this study were not found in our previous analyses with IMT (11) and STIFF (12). These ‘inconsistent’ findings support the hypothesis that CP, IMT, and STIFF are different phenotypes of atherosclerosis and under different genetic control. Further research is imperative to confirm these results.

Funding:

This research was supported by the NIH/NINDS K24-NS062737 grant, James & Esther King Biomedical Research Program (2KN01), and Evelyn F. McKnight Brain Institute.

References

1. Johnsen SH, Mathiesen EB. Carotid plaque compared with intima-media thickness as a predictor of coronary and cerebrovascular disease. *Curr Cardiol Rep* 2009; 11:21–7. [PubMed: 19091171]
2. 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]
3. Kuo F, Gardener H, Dong C et al. Traditional cardiovascular risk factors explain the minority of the variability in carotid plaque. *Stroke* 2012; 43:1755–60. [PubMed: 22550054]
4. Rundek T, Blanton SH, Bartels S et al. Traditional risk factors are not major contributors to the variance in carotid intima-media thickness. *Stroke* 2013; 44:2101–8. [PubMed: 23704105]
5. Tousoulis D, Briasoulis A, Papageorgiou N et al. Oxidative stress and endothelial function: therapeutic interventions. *Recent Pat Cardiovas Drug Discov* 2011; 6:103–14.
6. Dali-Youcef N, Lagouge M, Froelich S, Koehl C, Schoonjans K, Auwerx J. Sirtuins: the ‘magnificent seven’, function, metabolism and longevity. *Ann Med* 2007; 39:335–45. [PubMed: 17701476]
7. Rousset S, Alves-Guerra MC, Mozo J et al. The biology of mitochondrial uncoupling proteins. *Diabetes* 2004; 53(Suppl. 1):S130–5. [PubMed: 14749278]
8. Fuhrman B, Volkova N, Aviram M. Oxidative stress increases the expression of the CD36 scavenger receptor and the cellular uptake of oxidized low-density lipoprotein in macrophages from atherosclerotic mice: protective role of antioxidants and of paraoxonase. *Atherosclerosis* 2002; 161:307–16. [PubMed: 11888513]
9. Della-Morte D, Dave KR, DeFazio RA, Bao YC, Raval AP, Perez-Pinzon MA. Resveratrol pretreatment protects rat brain from cerebral ischemic damage via a sirtuin 1-uncoupling protein 2 pathway. *Neuroscience* 2009; 159:993–1002. [PubMed: 19356683]
10. 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]
11. 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; 160:389–90. [PubMed: 22750084]

12. Della-Morte D, Dong C, Beecham A et al. Relationship between sirtuin and mitochondrial uncoupling protein genes and carotid artery stiffness. *Transl Res* 2015; 165:358–9. [PubMed: 25258298]
13. Mathiesen EB, Johnsen SH, Wilsgaard T, Bonna KH, Lochen ML, Njolstad I. Carotid plaque area and intima-media thickness in prediction of first-ever ischemic stroke: a 10-year follow-up of 6584 men and women: the Tromso Study. *Stroke* 2011; 42:972–8. [PubMed: 21311059]
14. Spence JD, Eliasziw M, DiCicco M, Hackam DG, Galil R, Lohmann T. Carotid plaque area: a tool for targeting and evaluating vascular preventive therapy. *Stroke* 2002; 33:2916–22. [PubMed: 12468791]
15. Della-Morte D, Moussa I, Elkind MS, Sacco RL, Rundek T. The short-term effect of atorvastatin on carotid plaque morphology assessed by computer-assisted gray-scale densitometry: a pilot study. *Neurol Res* 2011; 33:991–4. [PubMed: 22081003]
16. Sztajzel R, Momjian-Mayor I, Comelli M, Momjian S. Correlation of cerebrovascular symptoms and microembolic signals with the stratified gray-scale median analysis and color mapping of the carotid plaque. *Stroke* 2006; 37:824–9. [PubMed: 16456117]
17. Tegos TJ, Stavropoulos P, Sabetai MM, Khodabakhsh P, Sassano A, Nicolaides AN. Determinants of carotid plaque instability: echoicity versus heterogeneity. *Eur J Vasc Endovasc Surg* 2001; 22:22–30. [PubMed: 11461098]
18. Prabhakaran S, Singh R, Zhou X, Ramas R, Sacco RL, Rundek T. Presence of calcified carotid plaque predicts vascular events: the Northern Manhattan Study. *Atherosclerosis* 2007; 195:e197–201. [PubMed: 17482197]
19. Hellings WE, Pasterkamp G, Verhoeven BA et al. Gender-associated differences in plaque phenotype of patients undergoing carotid endarterectomy. *J Vasc Surg* 2007; 45:289–96, discussion 96–7. [PubMed: 17264005]
20. Ota H, Reeves MJ, Zhu DC et al. Sex differences in patients with asymptomatic carotid atherosclerotic plaque: in vivo 3.0-T magnetic resonance study. *Stroke* 2010; 41:1630–5. [PubMed: 20616325]
21. Zhao XQ, Hatsukami TS, Hippe DS et al. Clinical factors associated with high-risk carotid plaque features as assessed by magnetic resonance imaging in patients with established vascular disease (from the AIM-HIGH Study). *Am J Cardiol* 2014; 114:1412–9. [PubMed: 25245415]
22. 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; 17:351–7. [PubMed: 17682370]
23. Rundek T, Hundle R, Ratchford E et al. Endothelial dysfunction is associated with carotid plaque: a cross-sectional study from the population based Northern Manhattan Study. *BMC Cardiovasc Disord* 2006; 6:35. [PubMed: 16916467]
24. Ferguson GG, Eliasziw M, Barr HW et al. The North American Symptomatic Carotid Endarterectomy Trial : surgical results in 1415 patients. *Stroke* 1999; 30:1751–8. [PubMed: 10471419]
25. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; 38:904–9. [PubMed: 16862161]
26. Purcell S, Neale B, Todd-Brown K et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81:559–75. [PubMed: 17701901]
27. Nicodemus KK, Liu W, Chase GA, Tsai YY, Fallin MD. Comparison of type I error for multiple test corrections in large single-nucleotide polymorphism studies using principal components versus haplotype blocking algorithms. *BMC Genet* 2005; 6(Suppl. 1):S78. [PubMed: 16451692]
28. Alsulaimani S, Gardener H, Elkind MS, Cheung K, Sacco RL, Rundek T. Elevated homocysteine and carotid plaque area and densitometry in the Northern Manhattan Study. *Stroke* 2013; 44:457–61. [PubMed: 23287787]
29. Kugel S, Mostoslavsky R. Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem Sci* 2014; 39:72–81. [PubMed: 24438746]
30. Zhong L, D'Urso A, Toiber D et al. The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. *Cell* 2010; 140:280–93. [PubMed: 20141841]

31. Kim HS, Xiao C, Wang RH et al. Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. *Cell Metab* 2010; 12:224–36. [PubMed: 20816089]
32. Yao QP, Zhang P, Qi YX et al. The role of SIRT6 in the differentiation of vascular smooth muscle cells in response to cyclic strain. *Int J Biochem Cell Biol* 2014; 49:98–104. [PubMed: 24495875]
33. Anderson CA, Boucher G, Lees CW et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet* 2011; 43:246–52. [PubMed: 21297633]
34. TenNapel MJ, Lynch CF, Burns TL et al. SIRT6 minor allele genotype is associated with >5-year decrease in lifespan in an aged cohort. *PLoS ONE* 2014; 9:e115616. [PubMed: 25541994]
35. Kwok KH, Ho PW, Chu AC et al. Mitochondrial UCP5 is neuroprotective by preserving mitochondrial membrane potential, ATP levels, and reducing oxidative stress in MPP+ and dopamine toxicity. *Free Radic Biol Med* 2010; 49:1023–35. [PubMed: 20600837]
36. Wang L, Yanuck D, Beecham A et al. A candidate gene study revealed sex-specific association between the OLR1 gene and carotid plaque. *Stroke* 2011; 42:588–92. [PubMed: 21257822]
37. Rodriguez AM, Palou A. Uncoupling proteins: gender dependence and their relation to body weight control. *Int J Obes Relat Metab Disord* 2004; 28:500–2. [PubMed: 14758347]
38. Giannopoulos S, Katsanos AH, Vasdekis SN, Boviatsis E, Voumvourakis KI, Tsiygoulis G. Age and gender disparities in the risk of carotid revascularization procedures. *Neurol Sci* 2013; 34:1711–7. [PubMed: 23666437]
39. Buratti L, Balestrini S, Avitabile E et al. Sex-associated differences in the modulation of vascular risk in patients with asymptomatic carotid stenosis. *J. Cereb. Blood Flow Metab* 2015; 35:684–8. [PubMed: 25586143]
40. Kuy S, Seabrook GR, Rossi PJ, Lewis BD, Dua A, Brown KR. Management of carotid stenosis in women. *JAMA Surg* 2013; 148:788–90. [PubMed: 23804123]
41. Beaussier H, Masson I, Collin C et al. Carotid plaque, arterial stiffness gradient, and remodeling in hypertension. *Hypertension* 2008; 52:729–36. [PubMed: 18779439]
42. Winnik S, Gaul DS, Preitner F et al. Deletion of Sirt3 does not affect atherosclerosis but accelerates weight gain and impairs rapid metabolic adaptation in LDL receptor knockout mice: implications for cardiovascular risk factor development. *Basic Res Cardiol* 2014; 109:399. [PubMed: 24370889]
43. van Abeelen AF, de Krom M, Hendriks J, Grobbee DE, Adan RA, van der Schouw YT. Variations in the uncoupling protein-3 gene are associated with specific obesity phenotypes. *Eur J Endocrinol* 2008; 158:669–76. [PubMed: 18426825]
44. Salopuro T, Pulkkinen L, Lindstrom J et al. Variation in the UCP2 and UCP3 genes associates with abdominal obesity and serum lipids: the Finnish diabetes prevention study. *BMC Med Genet* 2009; 10:94. [PubMed: 19769793]
45. Albani D, Ateri E, Mazzucco S et al. Modulation of human longevity by SIRT3 single nucleotide polymorphisms in the prospective study ‘Treviso Longeva (TRELONG)’. *Age (Omaha)* 2014; 36:469–78.
46. Polito L, Kehoe PG, Davin A et al. The SIRT2 polymorphism rs10410544 and risk of Alzheimer’s disease in two Caucasian case-control cohorts. *Alzheimers Dement* 2013; 9:392–9. [PubMed: 22651940]

Table 1

Characteristics of participants by plaque size (total plaque area) and morphology (gray scale median)

	N	%	TPA, mm ²		GSM	
			Mean	SD	Mean	SD
All	1356	100	11	18	49	49
Age						
<70	744	55	7	15	40	48
70 +	612	45	16–1	21	62	47
Gender						
Male	540	40	14	22	52	49
Female	816	60	9	16	48	49
Race/Ethnicity						
White	153	11	14	17	57	47
Black	255	19	14	22	57	50
Hispanic	930	69	10	18	46	49
Other	18	1	9	14	44	50
Ever smoking						
No	655	48	9	16	43	48
Yes	701	51	13	20	56	50
Physical activity						
No	625	46	11	18	48	49
Yes	731	53	11	19	50	49
Moderate drinking						
No	821	60	11	18	49	49
Yes	535	39	11	18	50	49
Obesity						
No	946	70	12	19	52	50
Yes	410	30	10	16	44	46
Diabetes						
No	1105	81	10	17	47	49
Yes	251	18	15	23	59	48
Hypertension						
No	396	29	8	13	42	48
Yes	960	71	13	20	53	49
Dyslipidemia						
No	956	70	11	18	47	49
Yes	400	29	13	19	55	49

Table 2

SNPs associated with carotid plaque size (area, TPA) and morphology (GSM)

Gene	Chr	SNP	BP	Minor allele	MAF	Phenotype	Beta (95% CI)*	Nominal P	Bonferroni-corrected P
<i>SIRT5</i>	6	rs2841503	13548834	C	0-08	TPA	0.20 (0.02, 0.38)	0-0322	0-625
						GSM	7.10 (0.34, 13.87)	0-0399	0-705
<i>SIRT6</i>	19	rs107251	4176085	T	0-11	TPA	0.30 (0.14, 0.45)	0-00017	0-005
						GSM	8.81 (2.99, 14.63)	0-0031	0-088
<i>UCP5</i>	X	rs5977233	129454635	C	0-29	GSM	4.59 (0.23, 8.95)	0-0393	0-699

* Adjusted for age, gender, smoking, physical activity, moderate alcohol consumption, obesity, hypertension, diabetes mellitus, hypercholesterolemia, and the top three PCAs. The value reported in bold passed the Bonferroni's multiple testing corrections adjustment.

SNPs showing interaction with gender and vascular factors with $P < 0.005$ in the association with carotid plaque size (area, TPA) and morphology (GSM)

Table 3

Gene	Chr	SNP	BP	Minor allele	Phenotype	Stratified analysis				P for interaction	
						Beta (95% CI)*	Men	P	Beta (95% CI)*	Women	P
<i>UCP1</i>	4	rs1430583	141486984	T	GSM	4.04 (-2.77, 10.85)	0.2455	0.0047	-8.87 (-14.35, -3.39)	0.0016	0.0048
<i>SIRT3</i>	11	rs12363280	231980	G	GSM	12.53 (3.88, 21.17)	0.0047	0.0006	-3.84 (-11.34, 3.66)	0.3158	0.0031
		rs4980329	232598	T	GSM	11.62 (2.86, 20.38)	0.0006	0.0007	-5.39 (-12.62, 1.83)	0.1439	0.0024
					Hypercholesterolemia (+)						
<i>UCP3</i>	11	rs1685354	73713591	G	TPA	0.27 (0.09, 0.46)	0.0043	0.0011	-0.11 (-0.22, 0.01)	0.0693	0.0007
					GSM	11.56 (4.67, 18.44)	0.0011	0.0044	-2.89 (-7.28, 1.51)	0.1984	0.0009
		rs2734827	73716277	A	GSM	9.5 (3.01, 15.99)	0.0044	0.0034	-2.04 (-6.38, 2.31)	0.3587	0.0034

* Adjusted for age, gender if applicable, smoking, physical activity, moderate alcohol consumption, obesity, hypertension, diabetes mellitus, hypercholesterolemia if applicable, and the top three PCAs. Bold are reported the values that are statistically significant after the multiple adjustment.