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Polymorphic variation in choline transporter gene (CHT1) is associated with early, subclinical measures of carotid atherosclerosis in humans

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Abstract

Atherosclerosis is a heritable trait with little known about specific genetic influences on preclinical measures of plaque formation. Based on relations of parasympathetic-cholinergic function to atherosclerosis and to a choline transporter gene [CHT1 (G/T)] polymorphism, we investigated whether the same allelic variant predicts variation in carotid intima-media thickness (IMT) and plaque formation. Carotid IMT and plaque occurrence as well as genotyping for the CHT1 (G/T) variant were measured in a sample (N = 264) of generally healthy adults (age 30–55) of European

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ancestry. CHT1 GG homozygotes had greater IMT ($P < 0.005$) and plaque occurrence ($P < 0.020$) than T allele carriers. This is the first study showing polymorphic variation in the CHT1 gene to predict early, subclinical measures of carotid atherosclerosis which may aid in understanding cholinergic-vagal processes potentially underlying atherosclerotic risk.

Keywords

Atherosclerosis; Intima-media thickness; Plaque; Cholinergic function; Choline transport; Acetylcholine

Introduction

Atherogenesis is influenced by both environmental and genetic factors [1, 2], but relatively little is known regarding heritable contributions to interindividual variability in preclinical atherosclerosis. One approach to investigating potential heritable influences on interindividual variability in subclinical measures of atherosclerosis is to examine genes that regulate pathobiologic risk for atherosclerotic cardiovascular disease. In this regard, diminished variation in inter-beat intervals of heart rate or low heart rate variability (HRV), which reflects reduced autonomic (particularly parasympathetic-cholinergic) modulation of cardiac rhythm, predicts cardiovascular morbidity and mortality in both patient and non-patient populations [3, 4], and is also associated with psychosocial and biological risk factors for coronary heart disease, coronary atherosclerosis, risk of clinical cardiac events including premature myocardial infarction or sudden death [5–7] and with risk factors for cardiovascular disease, such as hypertension, abdominal adiposity, insulin resistance, and presence of the metabolic syndrome [6, 8–11]. Because these associations were obtained in diverse populations and were often independent of other risk factors (e.g., age, blood pressure, glucose and lipids, etc.), it is conceivable that reduced autonomic-cholinergic-cardiac function contributes, in part, to the development of atherosclerotic disease. Such speculation is consistent with the association of the autonomic nervous system in lesion development, in the mediation of stress-induced endothelial injury and atherogenesis in nonhuman primates [12], and in the regulation of systemic inflammatory responses [13], lipid mobilization, and altered platelet function in humans [14].

If diminished autonomic-cholinergic-cardiac activity is associated with increased risk of atherosclerotic cardiovascular disease, genetic variation contributing to individual differences in autonomic-cholinergic function may be related to atherosclerosis. Biometric family and twin studies report significant genetic influence on cholinergic/parasympathetic phenotypes, with heritability estimates of up to 65% [15–20]. Most readily interpreted in relation to autonomic control is high frequency (HF) spectral power, which measures HRV associated with respiration and is known to be parasympathetically (cholinergically) mediated [3, 21]. Because acetylcholine (ACh) is the primary neurotransmitter for parasympathetic function, genes encoding components of cholinergic neurotransmission contain a source of potential genetic variation influencing interindividual variability in HRV.

High-affinity choline uptake and transport into ACh-releasing neurons is affected by the choline transporter, and this uptake is the rate-limiting step in ACh biosynthesis [22]. High affinity choline transporter (CHT1) expression has been reported in the intima and media layers of the arterial vascular wall in humans [23]. A common base pair substitution (G → T single nucleotide polymorphism [SNP]) is located in the 3′ untranslated region (3′UTR) of the high affinity choline transporter gene (CHT1), and allelic variation at this site has been found to predict cardiac autonomic function in healthy, middle-aged adults [24]. Compared

to persons carrying any CHT1 T allele, individuals homozygous for the CHT1 G allele exhibited lower cholinergially-driven HF power. Predicated on the associations of low HRV with coronary and carotid artery atherosclerosis, evidence of expression of the high-affinity choline transporter (CHT1) in the intima and medial layers of the arterial vascular wall, and relations of cholinergically mediated HRV indices with genetic variation in the choline transporter, in the present study, we hypothesized that allelic variation in CHT1 may similarly predict variation in carotid intima-media thickness and plaque occurrence among middle-aged men and women without a clinical history of atherosclerotic cardiovascular disease.

Materials and methods

Participants

Subjects were participants in the University of Pittsburgh's Adult Health and Behavior (AHAB) Project and were recruited from Allegheny County, Pennsylvania, from February 2002 to August 2004 using mailed brochures. Participants were community volunteers of European ancestry (non-Hispanic to examine population specific relations), 30–55 years of age. Exclusion criteria included clinical history of atherosclerotic disease (e.g., stroke, myocardial infarction, angioplasty, or bypass surgery), cancer diagnosis, or treatment within the past year, chronic liver or kidney disease, as well as use of cardiovascular, lipid-lowering, diabetic, glucocorticoid, prescription weight-loss, or psychotropic medications. Women were excluded if they were not using reliable birth control, or were pregnant, lactating, or currently experiencing age-related menstrual period irregularities.

This study was approved by the University of Pittsburgh's Institutional Review Board. Participants received a general description of the study upon recruitment, and data collection procedures were fully explained. Informed consent was obtained from all participants, and safety and data monitoring procedures were followed in accordance with the University of Pittsburgh's Institutional Review Board guidelines. A total of 273 subjects completed the protocol (135 female), of which 264 were successfully genotyped and included in the analyses. Only two participants in the final sample were taking antihypertensive medications.

Carotid atherosclerotic measures

Participants underwent B-mode ultrasonography at the Department of Epidemiology ultrasound research laboratory at the University of Pittsburgh to assess mean carotid intima-media thickness (IMT) and carotid plaque occurrence, as defined by an index of plaque classification (see Table 1 for sample characteristics). A Toshiba SSA-270 scanner (Tobisha, Nasu, Japan) equipped with a 5-MHz linear array imaging probe was used by trained sonographers to image the right and left common carotid artery, carotid bifurcation, and the first centimeter of the internal carotid artery. Mean IMT was quantified from digitized images of the lumen-intima and media-adventitia interface across each carotid segment and were aggregated and averaged across the near and far walls of the right and left distal common carotid artery (1 cm proximal to the carotid bulb), the far wall of the carotid bulb (initiated where the near and far walls of the common carotid artery are unparallel and completed at the flow divider), and the far wall of the internal carotid artery (from the flow divider to the first centimeter distal to this point). Measures were obtained using software that uses an edge detection algorithm and generates one measurement for each pixel, yielding ~140 measures for each segment. Computerized readings were over-read by certified sonographers making adjustments as needed. Mean IMT was calculated as the average of the mean IMT measured at each location. Since the average IMT distribution was positively skewed, reciprocal transformations (1/Y) were performed to normalize the data

for final analysis. Plaque was defined as a focal area with IMT exceeding adjacent areas by more than 50%. Values were reduced to create a dichotomous measure of 0, absence of plaque and 1, presence of one or more plaques (ranged from 1 to 5 plaques). It is important to note that this IMT estimated measure of plaque does not necessarily distinguish between lesions with or without a necrotic core and may be better defined as an intermediate phenotype of plaque [25–27].

DNA extraction and analysis

Blood specimens for genetic analysis were collected as a part of the subjects' prior participation in the AHAB project. Blood was extracted into 10 mm EDTA vials, and DNA was isolated from lymphocytes using a salting out procedure [28]. Genotyping of the CHT1 (G/T) SNP [Position: Chromosome 2 (107111468); Band 2q12.3; Relative Position: 26772; GenBank™ accession number: AC009963:1; dbSNP: rs333229] was achieved through polymerase chain reaction (PCR) amplification and allele specific detection by fluorescence polarization [29]. Amplification used primers F: 5′-GTAGGGACGAATGAAGGA-3′ and R: 5′-GCTCTCTAGATACAATGG-3′. The reactions were performed using the following conditions: 95°C for 1 min; and 35 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s; and then 72°C for 1 min. The FP-TDI primer, 5′-TCACAAATCTATAGTGTGGGG-3′, was used for detection. Genetic sequencing of this site confirmed the validity of this variation.

By convention, the more common allele is designated "G" and the less common allele "T". Sample frequencies of the G and T alleles in the study population were 0.78 and 0.22, respectively. The resulting distribution of CHT1 (G/T) genotypes in the current study sample ($N = 264$; GG = 163, GT = 85, and TT = 16) conformed to Hardy–Weinberg equilibrium (Chi-square = 1.17, NS) and did not differ between men and women (Chi-square = 2.98, NS). Due to the low number of TT homozygotes, GT and TT genotypes were combined for analysis. Of the participants for which DNA amplification and geno-typing was successful, 2 had missing mean IMT data, yielding a final sample of 262 subjects to be included in the IMT analyses. All participants with CHT1 (G/T) genotypes were included in the plaque occur-rence analyses (see Table 1 for sample characteristics).

Cardiovascular risk factors

Self-reported smoking exposure (calculated as lifetime exposure to smoking cigarettes in pack-years), body mass index, fasting glucose, total and high-density lipoprotein (HDL) cholesterol concentration, and daytime systolic and diastolic blood pressure were evaluated as standard cardiovascular risk factors. Mean systolic and diastolic blood pressure was obtained at two separate sessions using a mercury sphygmomanometer after a 5-min resting period. BMI was calculated as weight (kg)/height (m^2). Fasting serum glucose was assayed by standard colorimetry, and serum total and HDL cholesterol levels were measured enzymatically by the Heinz Nutrition Laboratory, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, which has met the criteria of the Centers for Disease Control—National Heart, Lung, and Blood Institute Lipid Standardization Program since 1982. For women, menopausal status was recorded and defined as follows: (1) postmenopausal-has not menstruated for at least 12 months, has had a hysterectomy or removal of uterus (36%); (2) perimenopausal-currently going through menopause as indicated by their physician and/or taking hormone replacement therapy and or >40 years old with unpredictable menstrual cycles for more than a year (2%); and (3) premenopausal-currently cycling ($n = 62\%$).

Statistical analyses

All analyses were performed using SPSS for Windows (version 16). Univariate associations by bivariate correlational analysis was conducted between indices of carotid atherosclerosis and cardiovascular risk factors to select for covariates for subsequent analyses. Risk factors shown to be significantly associated with carotid measurements were controlled for in the final statistical models evaluating the relation of CHT1 genotypes with mean IMT and plaque. Hierarchical multiple regressions were performed with CHT1 genotypes as predictor of mean IMT, with sex, age, BMI, systolic blood pressure, glucose, and total and HDL cholesterol as covariates. Binary logistic regressions were computed to evaluate whether CHT1 genotype predicted plaque index, controlling for sex, age, glucose, diastolic blood pressure, smoking exposure, and cholesterol.

Results

Preliminary analyses

Participants averaged (mean ± SD: 44.99 ± 6.7) years of age, and 49.5% were female. The sample mean for BMI (26.39 ± 4.3 kg/m^2) fell within the range for the overweight classification (25.0–29.9 kg/m^2) according to clinical guidelines for obesity. Cholesterol values (201.18 ± 34.3 mg/dL) were considered borderline-high risk (>200 mg/dL) and HDL levels in men (47.8 ± 11.3 mg/dL) and women (59.9 ± 13.9 mg/dL) were about average (Men: 40–50 mg/dL; Women: 50–60 mg/ dL). Glucose values (94.05 ± 9.5 mg/dL) and blood pressure (114.86 ± 12.5/77.37 ± 8.9 mm Hg) were also within the normal range (70–99 mg/dL fasting and <120/80 mm Hg, respectively). Only 10.3% of the participants were current smokers. Compared with carotid intima-media thickness values in another healthy adult population [30], mean IMT (0.66 ± 0.9 mm; range = 0.49–1.15 mm) for this sample was considered moderately high-risk (0.6–0.8 mm), with 14.7% (n = 40) of the sample having at least one focal carotid plaque.

As expected, direct associations were found between age, BMI, systolic and diastolic blood pressure, cholesterol, and glucose levels and mean IMT (Ps < 0.019), while an inverse relationship was noted between HDL concentrations and mean IMT (P < 0.004). With respect to plaque, point-biserial correlations showed greater age, blood pressure, and glucose levels associated with presence of carotid plaque (Ps < 0.02). Higher cholesterol levels showed a marginal relationship (P = 0.059) with plaque occurrence and subsequently was included as a covariate in evaluating the association of CHT1 genotype with presence of carotid plaque. Men had greater mean IMT and plaque scores than women (Ps < 0.04).

Main analyses: CHT1 genotype and carotid atherosclerotic measures

Hierarchical Multiple and Logistic Regression analyses demonstrated that the carotid atherosclerotic risk-factors, carotid intima-media thickness and plaque, may be associated with the CHT1 genotype (see Tables 2, 3 for regression analyses). In step 1 of the linear, hierarchical multiple regression analysis, age, sex, BMI, cholesterol, HDL, glucose, and systolic blood pressure accounted for 35.6% of the variance in mean carotid intima-media thickness. An estimate of the effect size of each association is provided by a semi-partial correlation for IMT and a partial correlation for plaque. Among covariates in the final model of the regression equation, greater age, BMI, and systolic blood pressure were significant independent predictors of mean IMT (Ps < 0.05). In addition, men were associated with greater mean IMT (P = 0.002). In step 2 of the linear multivariate regression analysis, the CHT1 GG homozygotes predicted greater mean IMT (β = 0.059, P < 0.005), accounting for an additional 2% of the variance. Based on the untransformed IMT average, GG homozygosity corresponds to a 0.029 mm increase in mean IMT than those carrying a T

allele. Please note that the variance inflation factors for all variables in the above analysis were close to 1 suggesting that multicollinearity is not likely influencing the results.

In contrast with bivariate correlations showing male sex, age, blood pressure, and glucose levels to be associated significantly with plaque occurrence, multiple logistic regression analysis showed only age was associated independently with plaque occurrence ($P < 0.001$) in the final model. Our analysis indicates that compared to CHT1 T allele carriers, GG homozygotes are approximately 2.5 times more likely to exhibit detectable plaque after covariate adjustment for age, sex, diastolic blood pressure, cholesterol, smoking exposure and glucose (OR = 0.396; 95% CI = 0.18–0.86; $P < 0.02$). After adjusting for womens' menopausal status, these analyses remained essentially the same.

Discussion

In this report, we present evidence that allelic variation in CHT1, which has previously been shown to predict in vivo measures of parasympatheticcholinergic function [24], predicted carotid intima-media thickness and plaque occurrence among middle-aged men and women without a clinical history of atherosclerotic cardiovascular disease. As expected, we found that as compared to carriers of a T allele, GG homozygotes had significantly greater intima-media thickness and a 2.5 times greater likelihood of having plaque, independent of traditional cardiovascular risk factors for atherosclerosis; GG homozygotes have also been shown to have lower HRV [24], a significant predictor of cardiovascular morbidity and mortality [3, 4] and an in vivo measure of cholinergic function. Because this CHT1 polymorphism is located in the 3′UTR region, it could influence rate of protein synthesis via alteration of gene expression or, alternatively, vary in linkage disequilibrium with a functional polymorphism located elsewhere. The variance accounted for by these relations is about 2%, which is considered a fairly large effect for a polymorphism influencing a complex trait such as atherosclerosis. These findings are consistent with the understanding that atherosclerotic plaque formation is a complex process and likely influenced by multiple genes of small effect, as well as gene by gene and gene by environment interactions [1, 2]. In this regard, other genetic variants of various pathophysiologic pathways found to influence atherosclerosis such as, endothelial dysfunction (e.g., NOS3, MnSOD, KDR genes), inflammation (e.g., IL-1, IL-1Ra, IL-6, IL-10, TNF-α, TNF-receptor genes) and vascular remodeling (e.g., TGF-β1, MMP-1, MMP-3,MMP-7, MMP-9, MMP-12 genes) [1, 2], together with the CHT1 (G/T) variant may have additive or synergistic effects that yield significant clinical information.

To our knowledge, this is the first report that polymorphic variation in the CHT1 gene is associated with subclinical measures of carotid atherosclerosis. Taken together with previous work demonstrating an association of this CHT1 polymorphism with vagal function, these findings may contribute to our understanding of cholinergic-vagal modulation of the pathophysiologic processes underlying risk for atherosclerosis. In this regard, descending pathways of the vagus nerve involves cholinergic neurotransmission and has been shown to inhibit cellular activation of macrophages and inflammatory cytokines which have been implicated in atherosclerosis [31]. In addition, non-neuronal acetylcholine is expressed widely in the body and can be involved in basic cell functions, such as the regulation of gene expression and specific and nonspecific immune functions [32]. Future experiments should examine the cellular effects of acetylcholine to better understand the cholinergic system in the pathogenesis of diseases involving inflammation, including atherosclerosis, depression, and dementia. In the future, it may also prove beneficial to evaluate inflammatory markers in relation to this CHT1 (G/T) variant to further understand the development of atherosclerotic plaque formation. As alluded to above, this relation may

be pertinent to depression phenotypes [33] and to augmented risk of coronary disease events that is related to low vagal activity [3–5, 34].

These findings should be interpreted with some caution, however, since they are based on simple association analysis. As in all investigations that involve samples of unrelated individuals, spurious genetic association derived from unknown sources of population substructure remains a theoretical possibility [35]. Confirmatory and validation studies are warranted using more definitive genetic methodologies, such as family-based association designs [36] or statistical adjustment for population stratification by concurrent evaluation of multiple single-nucleotide polymorphisms [37]. Another important limitation to the interpretation of these findings is that it is unclear if these results could generalize to other age groups or ancestry. Finally, it is important to recognize that plaque as measured here may not represent necrotic plaques and is a less sensitive estimate of plaque as compared to measures of total plaque area. Future studies should consider including total plaque area measures, which would more closely approximate pathologic plaques in atherosclerosis progression.

Nonetheless, this is the first investigation to demonstrate that the CHT1 (G/T) genetic polymorphism, previously associated with an in vivo measure of cholinergic function suggesting functionality with this variant, to predict early, subclinical measures of carotid atherosclerosis. Our findings will hopefully aid in delineating cholinergic processes that may underlie risk for atherosclerosis and uncover new ways to prevent this life threatening illness.

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References

1. O'Donnell CJ, Cupples LA, D'Agostino RB, Fox CS, Hoffmann U, Hwang SJ, et al. Genome-wide association study for subclinical atherosclerosis in major arterial territories in the NHLBI's Framingham Heart Study. BMC Med Genet. 2007; 8(Suppl 1):S4. [PubMed: 17903303]
2. Roy H, Bhardwaj S, Yla-Herttuala S. Molecular genetics of atherosclerosis. Hum Genet. Mar 20.2009 125:467–491. [PubMed: 19301036]
3. Kleiger RE, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after myocardial infraction. Am J Cardiol. 1997; 73:845–849.
4. Tsuji H, Larson MG, Venditti FJ, Manders ES, Evans JC, Feldman CL, et al. Impact of reduced heart rate variability on risk for cardiac events—the Framingham Heart Study. Circulation. 1996; 94(11):2850–2855. [PubMed: 8941112]
5. Carney RM, Freedland KE, Veith RC. Depression, the autonomic nervous system, and coronary heart disease. Psychosom Med. 2005; 67:S29–S33. [PubMed: 15953797]
6. Eller NH, Malmberg B, Bruhn P. Heart rate variability and intima media thickness. Int J Behav Med. 2006; 13(3):201–213. [PubMed: 17078770]
7. Singh JP, Larson MG, O'Donnell CJ, Tsuji H, Evans JC, Levy D. Heritability of heart rate variability—the Framingham Heart Study. Circulation. 1999; 99(17):2251–2254. [PubMed: 10226089]
8. Antelmi I, de Paula RS, Shinzato AR, Peres CA, Mansur AJ, Grupi CJ. Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. Am J Cardiol. 2004; 93(3):381–385. [PubMed: 14759400]
9. Reims HM, Sevre K, Fossum E, Hoieggen A, Mellem H, Kjeldsen SE. Relations between insulin sensitivity, fitness and autonomic cardiac regulation in healthy, young men. J Hypertens. 2004; 22(10):2007–2015. [PubMed: 15361774]

10. Gottsater A, Ahlgren AR, Taimour S, Sundkvist G. Decreased heart rate variability may predict the progression of carotid atherosclerosis in type 2 diabetes. Clin Auton Res. 2006; 16(3):228–234. [PubMed: 16763752]
11. Schroeder EB, Chambless LE, Liao D, Prineas RJ, Evans GW, Rosamond WD, et al. Diabetes, glucose, insulin, and heart rate variability: the Atherosclerosis Risk in Communities (ARIC) study. Diabetes Care. 2005; 28(3):668–674. [PubMed: 15735206]
12. Clarkson TB, Kaplan JR, Adams MR, Manuck SB. Psychosocial influences on the pathogenesis of atherosclerosis among nonhuman primates. Circulation. 1987; 76(1 Pt 2):I29–I40. [PubMed: 3297407]
13. Marsland AL, Gianaros PJ, Prather AA, Jennings JR, Neumann SA, Manuck SB. Stimulated production of proinflammatory cytokines covaries inversely with heart rate variability. Psychosom Med. 2007; 69(8):709–716. [PubMed: 17942840]
14. Sheps DS, Sheffield D. Depression, anxiety, and the cardiovascular system: the cardiologist's perspective. J Clin Psychiatry. 2001; 62(Suppl 8):12–16. [PubMed: 12108816]
15. Singh JP, Larson MG, O'Donnell CJ, Levy D. Genetic factors contribute to the variance in frequency domain measures of heart rate variability. Auton Neurosci Basic Clin. 2001; 90(1–2): 122–126.
16. Busjahn A, Voss A, Knoblauch H, Knoblauch M, Jeschke E, Wessel N, et al. Angiotensin-converting enzyme and angiotensinogen gene polymorphisms and heart rate variability in twins. Am J Cardiol. 1998; 81(6):755–760. [PubMed: 9527087]
17. Kupper NH, Willemsen G, van den Berg M, de Boer D, Posthuma D, Boomsma DI, et al. Heritability of ambulatory heart rate variability. Circulation. 2004; 110(18):2792–2796. [PubMed: 15492317]
18. Snieder H, van Doornen LJ, Boomsma DI, Thayer JF. Sex differences and heritability of two indices of heart rate dynamics: a twin study. Twin Res Hum Genet. 2007; 10(2):364–372. [PubMed: 17564526]
19. Sinnreich R, Friedlander Y, Luria MH, Sapoznikov D, Kark JD. Inheritance of heart rate variability: the kibbutzim family study. Hum Genet. 1999; 105(6):654–661. [PubMed: 10647902]
20. Uusitalo AL, Vanninen E, Levalahti E, Battie MC, Videman T, Kaprio J. Role of genetic and environmental influences on heart rate variability in middle-aged men. Am J Physiol Heart Circul Physiol. 2007; 293(2):H1013–H1022.
21. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability—standards of measurement, physiological interpretation, and clinical use. Circulation. 1996; 93(5):1043–1065. [PubMed: 8598068]
22. Okuda T, Okamura M, Kaitsuka C, Haga T, Gurwitz D. Single nucleotide polymorphism of the human high affinity choline transporter alters transport rate. J Biol Chem. 2002; 277(47):45315–45322. [PubMed: 12237312]
23. Lips KS, Pfeil U, Reiners K, Rimasch C, Kuchelmeister K, Braun-Dullaeus RC, et al. Expression of the high-affinity choline transporter CHT1 in rat and human arteries. J Histochem Cytochem. 2003; 51(12):1645–1654. [PubMed: 14623932]
24. Neumann SA, Lawrence EC, Jennings JR, Ferrell RE, Manuck SB. Heart rate variability is associated with polymorphic variation in the choline transporter gene. Psychosom Med. 2005; 67(2):168–171. [PubMed: 15784779]
25. Spence JD, Hegele RA. Noninvasive phenotypes of atherosclerosis. Arterioscler Thromb Vasc Biol. 2004; 24(11):e188–e189. [PubMed: 15528487]
26. Spence JD. Measurement of intima-media thickness vs. carotid plaque: uses in patient care, genetic research and evaluation of new therapies. Int J Stroke. 2006; 1(4):216–221. [PubMed: 18706019]
27. Finn AV, Kolodgie FD, Virmani R. Correlation between carotid intimal/medial thickness and atherosclerosis: a point of view from pathology. Arterioscler Thromb Vasc Biol. 2010; 30(2):177–181. [PubMed: 19679833]
28. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. 1988; 16:12–15.
29. Chen XN, Levine L, Kwok PY. Fluorescence polarization in homogeneous nucleic acid analysis. Genome Res. 1999; 9(5):492–498. [PubMed: 10330129]

30. Veller MG, Fisher CM, Nicolaides AN, Renton S, Geroulakos G, Stafford NJ, et al. Measurement of the ultrasonic intima-media complex thickness in normal subjects. J Vasc Surg. 1993; 17(4): 719–725. [PubMed: 8464091]

31. Tracey K. The inflammatory reflex. Nature. 2002; 40:853–859. [PubMed: 12490958]

32. Kirkpatrick CJ, Bittinger F, Unger RE, Kriegsmann J, Kilbinger H, Wessler I. The non-neuronal cholinergic system in the endothelium: evidence and possible pathobiological significance. Jpn J Pharmacol. 2001; 85(1):24–28. [PubMed: 11243570]

33. Neumann SA, Flory JD, Ferrell RE, Manuck SB. Depression is related to polymorphic variation in the choline transporter gene. Psychosom Med. 2005; 67:A38.

34. Rozanski A, Blumenthal JA, Kaplan J. Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation. 1999; 99(16):2192–2217. [PubMed: 10217662]

35. Burmeister M. Basic concepts in the study of disease with complex genetics. Biol Psychiatry. 2007; 45:522–532. [PubMed: 10088042]

36. Allison D. Transmission-disequilibrium tests for quantitative traits. Am J Hum Genet. 1997; 60:676–690. [PubMed: 9042929]

37. Devlin B, Roeder K, Wasserman L. Genomic control for association studies: a semiparametric test to detect excess-haplotype sharing. Biostatistics. 2000; 1:369–387. [PubMed: 12933562]

Table 1

Sample characteristics

Variable	Mean IMT (*N* = 262)	PI (*N* = 264)
Age, years	45.08 (6.58)	45.11 (6.57)
Female, *n* (%)	132 (50.38)	132 (50.0)
Lifetime exposure to cigarettes (pack-years)	4.38 (10.29)	4.42 (10.29)
Body mass index, kg/m^2	26.41 (4.31)	26.40 (4.31)
Systolic blood pressure, mm Hg	114.68 (12.33)	114.77 (12.33)
Diastolic blood pressure, mm Hg	77.31 (8.89)	77.37 (8.89)
Total cholesterol, mg/dL	202.38 (34.14)	202.16 (34.26)
HDL cholesterol, mg/dL	53.83 (13.98)	53.73 (13.98)
Glucose, mg/dL	93.84 (9.51)	93.92 (9.52)
Average IMT, mm[a]	0.66 (0.09)	0.66 (0.09)
Carotid Plaque Present, *n* (%)	38 (14.5)	40 (15.15)

Values are reported as mean (SD) unless otherwise indicated

IMT intima-media thickness, *PI* plaque index, *HDL* high density lipids

[a]Values are non-transformed

Table 2

Correlations among cardiovascular risk factors and carotid atherosclerotic measures

Variable	Mean IMT (*N* = 262)[a]	1/Mean IMT (*N* = 262)	PI (*N* = 264)[b]
Age, years	0.384 (0.000)	–0.411 (0.000)	0.252 (0.000)
Sex, male < female	–0.313 (0.000)	0.324 (0.000)	–0.127 (0.040)
Lifetime smoking exposure (pack-years)	0.015 (0.804)	–0.015 (0.804)	0.144 (0.020)
Body mass index, kg/m^2	0.295 (0.000)	–0.315 (0.000)	0.000 (0.998)
Systolic blood pressure, mm Hg	0.348 (0.000)	–0.358 (0.000)	0.142 (0.021)
Diastolic blood pressure, mm Hg	0.234 (0.000)	–0.267 (0.000)	0.158 (0.010)
Total cholesterol, mg/dL	0.145 (0.019)	–0.149 (0.016)	0.116 (0.059)
HDL cholesterol, mg/dL	–0.176 (0.004)	0.182 (0.003)	–0.077 (0.214)
Glucose, mg/dL	0.235 (0.000)	–0.230 (0.000)	0.170 (0.006)

Values expressed as *r* (2-tailed *P* value)

IMT intima-media thickness, *PI* plaque index, *HDL* high density lipids

[a] Non-transformed variable

[b] Point-biserial correlations

Table 3

Regression model results of CHT1, cardiovascular risk factors and carotid atherosclerotic measures

	1/Mean IMT (N = 262) β (ρ)			PI (N = 264) B (ρ)	Odds ratio	95% CI
Step 1						
Age, years	–0.012 (0.000)			0.132 (0.001)	1.142	1.055–1.236
Sex, male < female	0.094 (0.000)			0.480 (0.279)	1.617	0.677–3.861
Body mass index, kg/m^2 [a]	–0.008 (0.002)					
Lifetime smoking exposure (pack-years)				0.023 (0.113)	1.023	0.995–1.053
Systolic blood pressure, mm Hg	–0.003 (0.033)			–0.009 (0.707)	0.991	0.946–1.039
Diastolic blood pressure, mm Hg	0.001 (0.531)			0.051 (0.128)	1.052	0.985–1.124
Total cholesterol, mg/dL	0.000 (0.890)			0.005 (0.342)	1.005	0.994–1.017
HDL cholesterol, mg/dL [a]	0.000 (0.704)					
Glucose, mg/dL	–0.001 (0.517)			0.033 (0.134)	1.033	0.990–1.078
Step 2 [b]		R^2	ΔR^2			
CHT1, GG Vs. GT, TT	0.059 (0.005)	0.376	0.020	–0.926 (0.020)	0.396	0.182–0.863

IMT intima-media thickness, *PI* plaque index, *CI* confidence interval, *HDL* high density lipids

[a] Variable not included in the PI regression analysis

[b] Values are reported for the final model of each regression equation