Traditional Risk Factors Are Not Major Contributors to the Variance in Carotid Intima-Media Thickness

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Background and Purpose—Carotid intima-media thickness (cIMT) was a widely accepted ultrasound marker of subclinical atherosclerosis in the past. Although traditional risk factors may explain ≈50% of the variance in plaque burden, they may not explain such a high proportion of the variance in IMT, especially when measured in plaque-free locations. We aimed this study to identify individuals with cIMT unexplained by traditional risk factors for future environmental and genetic research.

Methods—As part of the Northern Manhattan Study, 1790 stroke-free individuals (mean age, 69±9 years; 60% women; 61% Hispanic; 19% black; 18% white) were assessed for cIMT using B-mode carotid ultrasound. Multiple linear regression models were evaluated: (1) incorporating prespecified traditional risk factors; and (2) including less traditional factors, such as inflammation biomarkers, adiponectin, homocysteine, and kidney function. Standardized cIMT residual scores were constructed to select individuals with unexplained cIMT.

Results—Mean total cIMT was 0.92±0.09 mm. The traditional model explained 11% of the variance in cIMT. Age (7%), male sex (3%), glucose (<1%), pack-years of smoking (<1%), and low-density lipoprotein cholesterol (<1%) were significant contributing factors. The model, including inflammatory biomarkers, explained 16% of the variance in cIMT. Adiponectin was the only additional significant contributor to the variance in cIMT. We identified 358 individuals (20%) with cIMT unexplained by the investigated risk factors.

Conclusions—Vascular risk factors explain only a small proportion of variance in cIMT. Identification of novel genetic and environmental factors underlying unexplained subclinical atherosclerosis is of utmost importance for future effective prevention of vascular disease. (Stroke. 2013;44:00-00.)

Key Words: carotid intima-media thickness ■ carotid ultrasound ■ risk factors
atherosclerosis would allow for more efficient genetic studies and discoveries of therapeutic targets without loss of statistical power.25

The aim of this study was to assess the contribution of traditional and less traditional vascular risk factors to the variance in cIMT and to identify individuals whose cIMT is not explained by these factors to serve as a resource for future genetic and environmental research.

Methods

Subjects

Subjects were participants in the National Institutes of Health–funded Northern Manhattan Study (NOMAS), an ongoing, prospective, population-based study of stroke incidence and vascular risk factors and concurrently enrolled in the National Institutes of Health–funded Oral Infections and Vascular Disease Study (INVEST) cohort.26,27 Since 1998, 1790 consecutive stroke-free subjects have been enrolled in the carotid imaging ancillary study. These individuals underwent high-resolution 2-dimensional (2D) carotid ultrasound for assessment of cIMT. Details on subject ascertainment, extensive assessments, and methods used in NOMAS and INVEST are described elsewhere.5,13,19,21,26,27 The high reliability of cIMT measurements in our laboratory was reported previously.23

Both studies were approved by the Institutional Review Boards of Columbia University, New York, and the University of Miami, Florida. All subjects gave written consent.

Evaluation of Risk Factors

Data were collected through interviews of the participants using standardized data collection instruments, review of medical records, and physical and neurological examinations. Race-ethnicity was based on self-identification through a series of questions modeled after the US Census. Hypertension was defined as a systolic blood pressure (BP) ≥140 mm Hg or a diastolic BP ≥90 mm Hg or a patient’s self-report of a history of hypertension or use of antihypertensive medications. Cigarette smoking was categorized as nonsmoker, former, or current smoker (within the last year), and the pack-years of smoking were calculated. Completion of high school was used as a proxy for socioeconomic status. Fasting total cholesterol and high-density lipoprotein cholesterol were measured using a Hitachi 705 automated spectrophotometer (Boehringer Mannheim; Mannheim, Germany). Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dL or the patient’s self-report of such a history or use of insulin or hypoglycemic medications.5,21 Adiponectin was measured as previously described.19 Fasting serum homocysteine was measured by licensed methods for commercial use.28 Serum inflammatory markers (interleukin-6, C-reactive protein, serum amyloid A, and tumor necrosis factors) were measured using enzyme-linked immunosorbent assay
BMI indicates body mass index; cIMT, carotid intima-media thickness; CRP, C-reactive protein; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; N/A, not applicable; PP, pulse pressure; SBP, systolic blood pressure; TC, total cholesterol; WBC, white blood cell count; and WHR, waist-to-hip ratio.

Assessment of Carotid IMT

Carotid ultrasound was performed according to the standard scanning and reading protocols by a trained and certified sonographer as detailed previously. Our cIMT protocol is in the alignment with the Mannheim consensus, which recommends to measure cIMT in the segments free of plaque. The near and the far wall of the left and the right carotid bifurcations, and the internal and the common carotid arteries were measured off-line using an automated edge detection image analysis system M’Ath (Intelligence in Medical Technologies, Inc, Paris, France). cIMT was calculated as a composite measure of the mean IMT measured at each of the 12 carotid sites within an individual, averaged, and expressed in millimeters.

Statistical Analysis

Univariate analysis was performed using the F test for categorical variables and correlation scores for continuous variables to assess the associations of demographic and vascular risk factors with cIMT, whereas general linear regression modeling for categorical variables and partial correlation for continuous variables was conducted to evaluate their age-adjusted associations with cIMT. To validate the previously proposed model using traditional vascular risk factors, we first regressed cIMT on the traditional risk factors, including age, sex, glucose level, pack-years of smoking, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein cholesterol, BP, pulse pressure, and lipid-lowering and antihypertensive medications (Model 1: Traditional model), with forward stepwise modeling by setting the selection criterion of P<0.1 for each term in the model. We then performed a multiple regression using a similar approach to investigate whether more variation of cIMT can be explained by adding other potentially important factors. In addition to the factors in Model 1, Model 2 (Modified model) included socioeconomics (race-ethnicity and education), traditional factors (body mass index, waist-to-hip-ratio, waist, alcohol, and physical activity), and less traditional factors (adiponectin, homocysteine, kidney function, and inflammatory biomarkers: white blood cell count, C-reactive protein, interleukin-6, and serum amyloid A). To identify the individuals with largely unexplained cIMT, we have taken the approach from Spence et al6,12,25 and computed the standardized cIMT residual scores from Model 2. A predicted cIMT value was calculated by summing the product of each individual’s independent variables and the standardized parameter coefficients from a multiple linear regression. Subtracting an individual’s predicted cIMT value from actual cIMT yielded a residual cIMT value. All analyses were conducted using SAS version 9.2 (SAS Institute; Cary, NC).

Results

Carotid ultrasound was performed among 1790 stroke-free subjects. Demographics of this group did not differ from the characteristics of the parent cohort. The mean age in the carotid population was 69±9 years; 60% were women, 61% Caribbean Hispanics, 19% black, 18% white. Mean total cIMT was 0.92±0.09 mm.
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age-adjusted analyses, male sex, moderate alcohol intake, increase in waist-to-hip-ratio, pack-years of smoking, fasting glucose, white blood cell count, and lower levels of adiponectin remained significantly associated with cIMT.

After performing the stepwise multiple regression model with inclusion of traditional factors (Model 1; Table 3), the following factors were identified as significant contributors to the variance in cIMT: age (7%), male sex (3%), glucose (<1%), pack-years of smoking (<1%), and LDL-C (<1%). Overall, these factors explained 11% of the variance in cIMT (the coefficient of determination, $R^2 = 0.108$).

The modified model (Model 2; Table 3) was able to explain 16% of the variance ($R^2 = 0.157$). The contributing factors in this model were age (9%), male sex (3%), LDL-C (0.9%), body mass index (0.9%), and fasting glucose (0.7%). The contributions of adiponectin (0.4%), pack-years of smoking (0.4%), and black race-ethnicity (0.3%) were low but significant, whereas those of lipid (0.3%) and BP-lowering medication (0.2%) were marginally significant. The addition of less traditional risk factors, such as homocysteine, eGFR, and inflammatory markers, did not significantly contribute to the cIMT variance (not included in Table 3). The results remained the same after exclusion of 438 subjects with a history of coronary artery disease, peripheral artery disease, or myocardial infarction.

We have calculated the cIMT residual scores for each participant by regressing cIMT on the significant contributors in Model 2 and identified 358 individuals (20%) with cIMT unexplained by these factors (Figure). There is no significant difference in the risk factors between these 2 groups, except in observed cIMT (Table 4).

Discussion

In this large, urban, and multiethnic population, we report that traditional vascular risk factors explain only 11% of the variance in cIMT. The addition of other less traditional factors, including adiponectin, homocysteine, and inflammation, explained an additional 5% of the cIMT variance, resulting in a total of 16% of the cIMT variance explained by all these factors. Age and sex explain most of the variance in cIMT ($\approx 10\%$). Therefore, most of cIMT variance in our study is not explained by traditional vascular risk factors commonly investigated in cerebrovascular research or assessed in vascular preventive clinics.

Our results are similar to previous findings from the Cardiovascular Health Study, in which cholesterol levels, cigarette smoking, hypertension, diabetes mellitus, age, and sex contributed to 17% of the variance in cIMT in common carotid artery (CCA) and 18% in internal carotid artery (ICA); however, suggesting that cIMT less likely represents atherosclerosis. However, the contribution of traditional risk factors to the variance of cIMT in other populations differed from our results (Table 5). In the Framingham Offspring cohort, the risk factors in the Framingham score explained 28.6% of the cIMT variability in CCA and 27.5% in ICA, with age and sex being the strongest predictors of cIMT. In a population-based study from Mexico among low-income residents, there was a significant association of age, diabetes mellitus, systolic BP, total cholesterol, and high-density lipoprotein cholesterol, with cIMT accounting for 28% of cIMT variance in CCA, but only 12% in ICA. Despite the differences between cIMT protocols and population characteristics of these studies,
the majority of cIMT variance (>70%) is not explained by traditional vascular risk factors. Age and sex are the highest contributors reported, whereas other contributors vary most likely because of different study populations, study designs, and measurements of cIMT in different carotid sites (eg, CCA versus ICA, the near versus the far wall, inclusion of carotid plaques to cIMT measurements, or cIMT measured as a composite measure of all carotid segments outside a portion of plaque, such as in our study).

Besides age and sex, only a small part of the cIMT variance (∼1%–4%) is accounted by the remaining risk factors included in our study. Systolic BP, glucose, cholesterol, and smoking were also small contributors to the cIMT variance. Other reports also showed that LDL-C in our study was marginal, whereas high-density lipoprotein cholesterol, total cholesterol, triglycerides, and lipid-lowering medication did not have a significant effect. Other studies did not show a convincing contribution of LDL-C to the variance of cIMT either.14,31,33 This may be substantiated by the results from the recent Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) trial, in which the addition of ezetimibe to a statin did not show any reduction of cIMT, despite an obvious lowering effect on LDL-C.36 Numerous lipid-lowering interventional clinical trials have used cIMT as a surrogate measure of atherosclerosis with inconsistent and often conflicting results.36,37 cIMT has not been affected to a large extent by the lipid metabolism, which could have been responsible for the weak results of the lipid-lowering trials on cIMT.

Among the less traditional risk factors in our study, only adiponectin showed significant contribution to the cIMT variance, albeit a small one. Adiponectin was shown to be inversely correlated with cIMT.38 This evidence underlines the role of adiponectin, an insulin-sensitizing adipocyte-secreted plasma protein, in maintenance of vascular homoeostasis through its vasoprotective actions. Evidence on the association of kidney dysfunction and cardiovascular disease is strong.39,40 However, our results did not show a significant contribution of eGFR to cIMT variance. Accordingly, a relationship between eGFR and carotid plaque, but not IMT, has been documented, emphasizing again that cIMT and carotid plaque are different phenotypes.41 A significant relationship between inflammatory markers and cardiovascular risk was reported,42 but their contribution to the cIMT variance was not found to be substantial in our and in other studies.

Our results of no apparent strong contribution of traditional and less traditional markers to the cIMT variance suggest that cIMT largely may not be a direct measure of atherosclerotic process. Carotid IMT may represent adaptive changes to biomechanical parameters with aging and not an indicator of atherosclerotic changes.43,44 In addition, an increase in cIMT may be a consequence of hypertension with hypertrophy of the media layer of the arterial wall.41 Our study, BP parameters were not significant contributors to the variance in cIMT. Other vascular wall structure and

| Table 4. Traditional and Less Traditional Risk Factors Among Individuals With Unexplained cIMT |
|------------------|------------------|------------------|
| Characteristics   | Unexplained cIMT (Bottom 10%) | Unexplained cIMT (Top 10%) | P Value Bottom 10% vs. Top 10% |
| N                 | 179              | 179              |
| Mean age, y±SD    | 70.2±9.7         | 70.3±7.8         | 0.94                          |
| Men               | 67 (37)          | 74 (41)          | 0.45                          |
| LDL-C             | 132.8±32.5       | 132.9±34.9       | 0.96                          |
| BMI               | 28.2±4.8         | 28.3±5.3         | 0.83                          |
| Glucose           | 100.1±36.2       | 104.8±45.1       | 0.27                          |
| Adiponectin       | 10.3±4.9         | 9.9±4.0          | 0.44                          |
| Smoking, pack-years | 11.8±21.6       | 11.1±22.8        | 0.78                          |
| Race, black       | 40 (22)          | 37 (21)          | 0.70                          |
| Lipid-lowering meds | 28 (16)         | 30 (17)          | 0.77                          |
| BP-lowering meds  | 74 (41)          | 67 (37)          | 0.45                          |
| Predicted cIMT, mm | 0.91±0.03        | 0.91±0.03        | 0.49                          |
| Observed cIMT, mm | 1.05±0.06        | 0.80±0.05        | 2.37×10⁻¹²²                    |

BMI indicates body mass index; BP, blood pressure; cIMT, carotid intima-media thickness; and LDL-C, low-density lipoprotein cholesterol.
function parameters (e.g., arterial diameter, stiffness) may be important contributors. Although cIMT was associated with vascular disease in previous reports,2–4,9,31,34 recent studies have argued that carotid plaque, not cIMT, was responsible for this effect.43,45,46

Many unaccounted factors likely contribute to the variance of cIMT in a significant number of individuals, as shown in our analyses of residual scores. Using our previous knowledge of traditional vascular risk factors and adding some novel factors, we have identified individuals whose cIMT is significantly greater or lesser than predicted, representing individuals with unexplained cIMT. These individuals would be ideal candidates for further investigations of genetic, lifestyle, and novel environmental factors. Carotid IMT is a highly heritable trait,32,47 and genetic factors possibly attribute to a high proportion of the phenotypic variance of cIMT in CCA (66%) and in ICA (75%).32,47,48 Selective genotyping of extreme discordant phenotypes by identifying individuals with traits that cannot be explained by well-recognized risk factors may be a promising approach for discoveries of novel variants. With this approach, efficient and affordable genetic studies for identifying genetic variants and novel pathways of complex traits may be designed without loss of statistical power, as elegantly showed in a study on extreme phenotypes of atherosclerotic plaque.25 In addition, the influences of lifestyle factors, such as dietary habits, moderate alcohol intake, and physical activity, as well as occupational stress and psychosocial changes throughout life, also have to be addressed in relation to cIMT in future studies. Finally, the role of infection, inflammation, and innate immunity has to be further investigated.27,49,50

We acknowledge the limitations to our results. Our study included an elderly and predominantly Hispanic population and, therefore, our results may not be generalizable to other populations. Our results are cross-sectional, and causality, therefore, cannot be inferred. Our selection of investigated risk factors might have been limited, especially with respect to sociocultural or socioeconomic characteristics, but we wanted to include traditional vascular risk factors with addition of several biologically plausible factors for atherosclerosis, which were also available in our cohort.

### Table 5. Summary of the cIMT Protocols and Traditional Risk Factors Contributions in Selected Population-Based Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Carotid Segment Measured</th>
<th>cIMT Definition</th>
<th>Inclusion of Plaque in cIMT Measurements</th>
<th>Risk Factors Associated With IMT (And Their Contribution If Available)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIC2</td>
<td>CCA, ICA, bifurcation; far wall</td>
<td>Mean IMT</td>
<td>Yes</td>
<td>Age, LDL-C, HDL-C, hypertension, smoking, diabetes mellitus</td>
</tr>
<tr>
<td>CHS14</td>
<td>CCA, ICA, carotid bulb; near and far wall</td>
<td>Max IMT</td>
<td>No</td>
<td>CCA IMT 18% ICA IMT 17% from age, male sex, hypertension, diabetes mellitus, cholesterol levels, cigarette smoking</td>
</tr>
<tr>
<td>Epidemiological survey in Mexico City32</td>
<td>CCA, ICA; near and far wall</td>
<td>Max IMT</td>
<td>No</td>
<td>CCA IMT: age, sex, triglycerides, TC, diabetes mellitus, HDL-C, and SBP (all together 28%), ICA IMT: age, sex, triglycerides, TC, smoking, diabetes mellitus, and SBP (all together 12%)</td>
</tr>
<tr>
<td>Framingham offspring cohort31</td>
<td>CCA, ICA, carotid bulb; far wall</td>
<td>Max IMT</td>
<td>Yes</td>
<td>CCA IMT: Total: 28.6%; age (19.4%), gender (4.1%), systolic BP (1.9%), HDL-C (1.2%), smoking (0.9%), diabetes mellitus (0.8%), hypertension treatment (0.3%), and total cholesterol (0.002%). ICA IMT: total 27.5%; age (18.5%), sex (4%), smoking (1.6%), hypertension treatment (1.1%), systolic BP (0.8%), diabetes mellitus (0.8%), HDL-C (0.6%), and total cholesterol (0.1%).</td>
</tr>
<tr>
<td>INVEST27</td>
<td>CCA, ICA, bifurcation; near and far wall</td>
<td>Mean IMT</td>
<td>No</td>
<td>Cumulative periodontal burden associated with IMT</td>
</tr>
<tr>
<td>NOMAS23</td>
<td>CCA, ICA, bifurcation; near and far wall</td>
<td>Mean IMT</td>
<td>No</td>
<td>Stromelysin-1 (MMP3), interleukin-6 (IL-6), Hepatic lipase (HL; each 19%)</td>
</tr>
</tbody>
</table>

ARIC indicates Atherosclerosis Risk in Communities; BP, blood pressure; CCA, common carotid artery; CHS, Cardiovascular Health Study; cIMT, carotid intima-media thickness; HDL-C, high-density lipoprotein cholesterol; ICA, internal carotid artery; INVEST, Oral Infections and Vascular Disease Study; LDL-C, low-density lipoprotein cholesterol; NOMAS, Northern Manhattan Study; and TC, total cholesterol.
Conclusions
The variance of cIMT remains largely unknown. Traditional cardiovascular risk factors explain only a small part of the cIMT variance. Adiponectin is a novel factor, which has provided a small but significant contribution to the cIMT variance in our study. Even though just a small part of variance of cIMT can be explained by traditional risk factors, adequate reduction and control of these factors are still the most important part of vascular disease prevention programs.

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Disclosures
None.

References


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