

# Traditional Cardiovascular Risk Factors Explain the Minority of the Variability in Carotid Plaque

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**Background and Purpose**—Subclinical atherosclerotic plaque is an important marker of increased vascular risk. Identifying factors underlying the variability in burden of atherosclerotic carotid plaque unexplained by traditional vascular risk factors may help target novel preventive strategies.

**Methods**—As a part of the carotid substudy of the Northern Manhattan Study (NOMAS), 1790 stroke-free individuals (mean age, 69±9; 60% women; 61% Hispanic, 19% black, 18% white) were assessed for total plaque area (TPA) burden using 2-dimensional carotid ultrasound imaging. Multiple linear regression models were constructed. Model 1 used prespecified traditional risk factors: age, sex, low-density lipoprotein cholesterol, diabetes mellitus, pack-years of smoking, blood pressure, and treatment for blood pressure; and Model 2, an addition of socioeconomic and less traditional risk factors. The contributions of the components of the Framingham heart risk score and the NOMAS Global Vascular Risk Score to the TPA were explored.

**Results**—Prevalence of carotid plaque was 58%. Mean TPA was 13±19 mm<sup>2</sup>. Model 1 explained 19.5% of the variance in TPA burden ( $R^2=0.195$ ). Model 2 explained 21.9% of TPA burden. Similarly, the Framingham heart risk score explained 18.8% and NOMAS global vascular risk score 21.5% of the TPA variance.

**Conclusions**—The variation in preclinical carotid plaque burden is largely unexplained by traditional and less traditional vascular risk factors, suggesting that other unaccounted environmental and genetic factors play an important role in the determination of atherosclerotic plaque. Identification of these factors may lead to new approaches to prevent stroke and cardiovascular disease. (*Stroke*. 2012;43:1755-1760.)

**Key Words:** carotid plaque ■ carotid ultrasound ■ epidemiology ■ plaque area ■ preclinical atherosclerosis ■ risk factors

Carotid plaque is an important risk factor for stroke, myocardial infarction, and vascular death.<sup>1-3</sup> Asymptomatic and preclinical carotid plaque is biologically and genetically distinct from other phenotypic presentations of atherosclerosis.<sup>4,5</sup> Preclinical carotid plaque is a better predictor of vascular events than carotid intima-media thickness, another subclinical marker of atherosclerosis.<sup>2,6-10</sup> Assessment of carotid plaque and measurement of plaque area by 2-dimensional carotid ultrasound is a safe, inexpensive, easy, reliable, and reproducible method of detection of atherosclerosis. Measures of subclinical atherosclerosis are useful tools for assessing vascular risk beyond traditional vascular risk factors, risk factor management, genetic and environmental research, and evaluation of new therapies.<sup>6,7</sup>

The traditional vascular risk factors associated with carotid plaque area are age, sex, pack-years of smoking, systolic blood pressure (SBP), diastolic blood pressure (DBP), diabe-

tes mellitus (DM), lipid-lowering and blood pressure (BP)-lowering medication, and high-density lipoprotein (HDL) cholesterol. These risk factors explained approximately 50% of the variance in total plaque area in prior studies.<sup>1,11</sup> Data on the variability of carotid plaque area based on vascular risk factors in population-based cohorts are limited. Therefore, the aim of this study was to identify traditional and less traditional vascular risk factors associated with carotid plaque area, to estimate their contribution to the variance in carotid plaque area, and to validate the contribution of previously determined traditional vascular risk factors on carotid plaque burden<sup>1,11</sup> in an urban, multiethnic community cohort.

## Subjects and Methods

### Subjects

The Northern Manhattan Study (NOMAS) is an ongoing population-based study designed to determine the incidence of stroke and the

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role of novel risk factors in a multiethnic urban community.<sup>12</sup> The race-ethnic distribution in NOMAS is 61% Hispanics, 19% blacks, and 17% whites.

### Selection of the Prospective Cohort

The details of the NOMAS design have been described previously.<sup>13</sup> In brief, subjects were identified by random digit dialing and enrolled in a prospective study between 1993 and 2001 if they had never been diagnosed with stroke, age >39 years, and resided in northern Manhattan for >3 months. A total of 3298 individuals have been enrolled in the NOMAS. NOMAS was approved by the Institutional Review Boards of Columbia University Medical Center and the University of Miami. All participants gave written informed consent for participation in the study. The carotid plaque imaging ancillary study to NOMAS started in 1999. All NOMAS subjects who have been enrolled in NOMAS since 1999 received carotid ultrasound at the time of baseline enrollment to NOMAS. There were no separate visits to a Doppler laboratory at different time points and there were not specific selection criteria for the carotid ancillary study. Overall, 1790 stroke-free subjects have been enrolled into the NOMAS carotid imaging substudy.<sup>5</sup>

### Baseline Evaluation

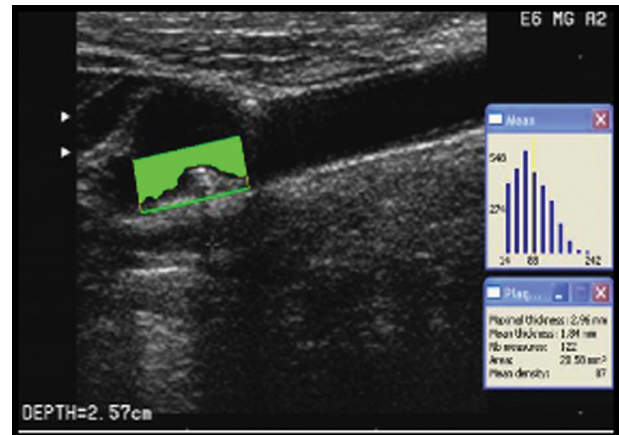
Baseline data were collected through interviews using standardized collection instruments, review of the medical records, and physical and neurological examinations.<sup>13</sup> Race-ethnicity was based on self-identification through a series of questions modeled after the US Census and the standard definitions outlined by Directive 15. Hypertension was defined as a SBP  $\geq 140$  mm Hg or a DBP  $\geq 90$  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). Pack-years of smoking were calculated. Mild to moderate alcohol use was defined as current drinking of >1 drink per month and  $\leq 2$  drinks per day. Diabetes was defined as fasting blood glucose  $\geq 126$  mg/dL or the patient's self-report of such a history or use of insulin or hypoglycemic medications. Completion of high school was a proxy for socioeconomic status.

### Assessment of Carotid Plaque Area

Carotid ultrasound was performed according to standard scanning and reading protocols by a trained and certified sonologist as detailed previously.<sup>5,14</sup> The left and right carotid bifurcations, the internal artery, and the common carotid artery were examined for wall thickness and the presence of plaque. Plaque was defined as an area of focal wall thickening 50% greater than surrounding wall thickness. All plaques were scanned in long axes from multiple angles and a digital sequence of 5 to 10 seconds was recorded in the cine loops. The plaque boundaries were traced offline from the digitized images using an automatic edge detection system M<sup>2</sup>Ath (Paris, France). Two-dimensional carotid plaque area ( $\text{mm}^2$ ) was measured for each plaque according to the validated protocol using M<sup>2</sup>Ath (Figure). The sum of plaque areas in all carotid arteries from both sides of the neck was expressed as a total carotid plaque area. High reproducibility of M<sup>2</sup>Ath measurements has been reported previously.<sup>15-17</sup> The image normalization and multiangle compound imaging method was performed to reduce angle dependence and random variation (speckle).<sup>18,19</sup> In addition, high validity of measurements was achieved by implementation of the M<sup>2</sup>Ath quality index.<sup>15</sup> Only images with M<sup>2</sup>Ath quality index of  $\geq 80\%$  were accepted for the analyses.

### Statistical Analysis

The primary assessment of total plaque area (TPA) burden was calculated as the sum of plaque area and values of 0 were assigned to those with no plaque area. The variable was then transformed using a cube root function ( $x^{1/3}$ ) to meet the normality assumption. Given that age is a well-known major risk factor for TPA, we explored the age-adjusted association of each demographic and vascular risk factor with TPA based on generalized linear regression



**Figure.** Carotid plaque area measurements (carotid plaque area in  $\text{mm}^2$  in the far wall of the carotid bifurcation measured by automated edge detection image software).

models for categorical factors and partial correlation for continuous factors. To validate the previously published plaque area model using traditional vascular risk factors,<sup>1</sup> we first regressed TPA on 9 traditional risk factors (age, sex, cigarette smoking pack-years, DM status, SBP, DBP, HDL, use of BP-lowering medications, and use of lipid-lowering medications). To investigate whether more variation of TPA can be explained by adding other potential risk factors, we then performed a multiple regression with a forward stepwise modeling by setting a selection criterion of probability value <0.1 for each term in the model. The modified model includes risk factors age, sex, pack-years smoking, SBP, DBP, DM, low-density lipoprotein (LDL):HDL ratio, homocysteine, high school completion, LDL, lipid-lowering medication, moderate alcohol drinking, and white blood cell (WBC) count. In addition, 2 separate models were constructed using the components of Framingham Risk Score<sup>20</sup> and the components of NOMAS Global Vascular Risk Score<sup>21</sup> as independent variables. We conducted all analyses using SAS Version 9.2 (SAS Institute, Cary, NC).

### Results

Carotid ultrasound was performed among 1790 stroke-free subjects at baseline. Demographics of this group did not differ from the characteristics of the parent cohort.<sup>10</sup> The mean age in the carotid sample was  $69 \pm 9$  years; 60% were women; 61% Caribbean Hispanics, 19% black, and 17% white. Clinical characteristics and the age-adjusted associations with TPA burden are shown in Table 1. The following factors were significantly associated with TPA burden in univariate models: age, sex, race-ethnicity, high school completion, diabetes, BP-lowering and lipid-lowering medication, waist-to-hip ratio, pack-years of smoking, SBP, LDL, HDL, triglycerides, LDL:HDL ratio, WBC count, and homocysteine.

Using the "traditional" risk factors model,<sup>1</sup> forward stepwise multiple regression model (Table 2) showed the  $R^2$  (coefficient of determination) of 0.195 (19.5%). Age (13.8%), smoking (2.2%), SBP (1.3%), and diabetes (0.9%) contributed the most to the variance in TPA burden. Of the variables in the "traditional" model, both HDL and lipid medication were not significantly associated with TPA burden ( $P=0.08$  and 0.80, respectively).

The modified model which included age, sex, pack-years of smoking, SBP, diabetes, LDL:HDL ratio, homocysteine levels, high school completion, LDL, lipid-lowering medication, and WBC count (Table 2), showed

**Table 1. Demographics and Clinical Characteristics of the Study Population and Relationships With the Total Plaque Area**

Characteristics	Sample No. (%)	TPA* Mean±SD	P Value
All	1790 (100)	1.45±1.38	
Sex			<0.0001
Female	1074 (60)	1.37±1.34	
Male	716 (40)	1.58±1.44	
Race-ethnicity			0.004
Black	341 (19)	1.63±1.44	
Hispanic	1094 (61)	1.28±1.33	
White	313 (17)	1.90±1.37	
High school completion			0.005
No	943 (53)	1.35±1.37	
Yes	847 (47)	1.56±1.39	
Moderate alcohol drinking			0.05
No	1081 (60)	1.44±1.39	
Yes	709 (40)	1.47±1.37	
Physical activity			0.08
No	767 (43)	1.46±1.38	
Yes	1003 (57)	1.46±1.38	
Diabetes			<0.0001
No	1443 (81)	1.38±1.37	
Yes	347 (19)	1.74±1.41	
Blood pressure medication			0.001
No	1062 (59)	1.33±1.34	
Yes	728 (41)	1.62±1.42	
Lipid medication			0.01
No	1493 (83)	1.41±1.37	
Yes	297 (17)	1.66±1.40	
History of sibling stroke or heart disease			0.87
No	1415 (0.79)	1.43±1.38	
Yes	375 (0.21)	1.54±1.37	
	Mean±SD	Correlation	
Age, y	69.40±9.26	0.37	<0.0001
Body mass index, kg/m <sup>2</sup>	28.16±5.03	0.01	0.71
Waist-to-hip ratio	0.90±0.09	0.09	<0.0001
Smoking, pack-years	12.16±23.06	0.16	<0.0001
Systolic blood pressure, mm Hg	140.97±20.21	0.11	<0.0001
Diastolic blood pressure, mm Hg	83.01±10.93	0.01	0.78
LDL-C, mg/dL	128.01±35.09	0.06	0.009
HDL-C, mg/dL	46.69±14.43	-0.06	0.01
TG, mg/dL	134.68±79.19	0.05	0.04
LDL/HDL ratio	2.98±1.20	0.08	0.0005
White blood cell count, 1000/mm <sup>3</sup>	6.20±2.01	0.11	<0.0001
Estimated glomerular filtration rate, mL/min	75.09±19.89	-0.02	0.35
Homocysteine, μmol/L	9.42±4.62	0.08	0.002
C-reactive protein, mg/L	4.68±7.21	0.02	0.46

TPA indicates total plaque area; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

\*TPA burden was cube root transformed; P values were adjusted for age for all variables.

**Table 2. Variation of Total Atherosclerotic Carotid Area Explained by Traditional Vascular Risk Factor Model and Modified Risk Factor Model**

	Parameter Estimate	SE	β	Partial R <sup>2</sup>	P Value
<b>Traditional model</b>					
Age	0.048	0.003	0.332	0.138	<0.0001
Smoking, pack-years	0.008	0.001	0.1128	0.022	<0.0001
SBP	0.011	0.002	0.161	0.013	<0.0001
Diabetes	0.269	0.077	0.077	0.009	<0.0001
DBP	-0.012	0.004	-0.095	0.006	0.0006
Male sex	0.238	0.066	0.084	0.004	0.002
Lipid medication	0.155	0.084	0.042	0.002	0.08
BP medication	0.115	0.066	0.041	0.001	0.02
HDL-C	0.000	0.002	-0.006	0.000	0.80
				R <sup>2</sup> =0.195	
<b>Modified model</b>					
Age	0.048	0.004	0.317	0.135	<0.0001
Smoking, pack-years	0.008	0.001	0.133	0.028	<0.0001
SBP	0.011	0.002	0.159	0.011	<0.0001
Diabetes	0.318	0.085	0.091	0.008	0.0003
LDL-C/HDL-C ratio	0.038	0.042	0.033	0.007	0.0005
Homocysteine	0.024	0.008	0.077	0.007	0.0005
DBP	-0.013	0.004	-0.104	0.006	0.001
Completed high school	0.161	0.068	0.059	0.005	0.003
White blood cell count	0.047	0.016	0.071	0.004	0.007
LDL-C	0.003	0.001	0.075	0.003	0.03
Male sex	0.168	0.075	0.060	0.002	0.09
Lipid medication	0.193	0.092	0.050	0.002	0.06
Moderate alcohol drinking	0.107	0.068	0.038	0.002	0.05
				R <sup>2</sup> =0.219	

P values are based on the multiple linear regression models with a forward stepwise selection.

β indicates standardized parameter estimate; R<sup>2</sup>, coefficient of determination; SBP, systolic blood pressure; DBP, diastolic blood pressure; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

the R<sup>2</sup> of 0.219 (21.9%) with most contribution by age (13.5%), smoking (2.8%), SBP (1.1%), diabetes (0.8%), LDL:HDL ratio (0.7%), and homocysteine (0.7%). All variables included in this model were significantly associated with TPA burden (P<0.05) except male sex, lipid medication, and moderate alcohol drinking (P=0.09, 0.06, and 0.06, respectively).

Clustering of risk factors within an individual did not improve prediction models. The Framingham Risk Score (age, sex, smoking, BP, DM, and total cholesterol) explained 18.8% and the NOMAS Global Vascular Risk Score (which includes all the Framingham Risk Score variables plus race-ethnicity, waist circumference, physical activity, alco-

hol consumption, peripheral arterial disease symptoms, and fasting glucose instead of DM) explained 21.5% of the variance in TPA burden.

### Discussion

In an urban multiethnic cohort from northern Manhattan, traditional vascular risk factors explain only 19.5% of the variance in the TPA burden. The explanatory risk factors include age, sex, pack-years of smoking, SBP, DBP, BP-lowering and lipid-lowering medications, and diabetes. An inclusion of less traditional risk factors such as LDL:HDL ratio, homocysteine, high school completion, WBC count, moderate alcohol drinking, and LDL cholesterol to the traditional model contributed an additional 2.4%, explaining 21.9% of the variance in TPA burden. Therefore, variation in subclinical carotid plaque burden is largely unexplained by many known vascular risk factors. Our results suggest that unaccounted factors, both environmental and genetic, play an important role in the determination of subclinical atherosclerosis. Identification of these factors is of great importance for successful prevention of stroke and cardiovascular disease (CVD).

Age, sex, pack-years of smoking, SBP, DBP, DM, HDL, BP-lowering, and lipid-lowering medication were the most significant determinants of carotid plaque area in a previous study,<sup>1</sup> explaining 52% of the TPA variance. Our results, however, found considerably lower contribution of these risk factors (19.5%) to TPA burden. This difference is most likely attributed to different characteristics of the study populations and study designs. Our cohort is a population-based sample as opposed to the subjects enrolled to a hospital-based clinic in the Premature Atherosclerosis Clinic (PAC) registry,<sup>1</sup> which represents a highly selected patient population at high risk for CVD. Furthermore, subjects in PAC were predominantly white men with a significant proportion of symptomatic carotid disease and had a strong family history of premature atherosclerosis. Our population, however, was comprised of predominately Hispanic women who were free of stroke or other CVD. The TPA burden also differed between our study and PAC (after cube root transformation, 1.45 mm<sup>3</sup> in our study versus 8.73 mm<sup>3</sup> in PAC). Nevertheless, traditional vascular risk factors could not explain approximately half of the variance in plaque burden even in the PAC registry.

Age is the primary contributing factor to the atherosclerotic plaque burden in both the traditional and modified model in our as well as in prior studies.<sup>1,11</sup> A marked increase in carotid plaque area was found between the ages of 45 to 70 years, leveling off after age 70 years<sup>11</sup> most likely due to survival bias. Smoking is another important risk factor for plaque burden.<sup>2,22,23</sup> Systolic BP and DBP have been significantly associated with TPA and plaque progression.<sup>8,24,25</sup> Diabetes mellitus provides an environment that enhances the effect of other environmental and genetic risk factors, and therefore is an important risk factor for increased atherosclerotic plaque burden.<sup>26</sup> Increased total cholesterol is associated with plaque burden and plaque progression, and intensive lipid-lowering reduces plaque volume and improves carotid plaque stability and morphology.<sup>2,27,28</sup> Moreover, the LDL:HDL ratio is

associated with increased plaque burden, whereas LDL may have the strongest relation with carotid plaque<sup>29</sup>; although contributing only 0.2% of TPA variation in our study, LDL remains an important risk factor to treat clinically. Men have more plaque compared with women and are at higher risk of stroke and CVD.<sup>30</sup> In addition to having less plaque, women have more stable and less inflammatory carotid plaques compared with men.<sup>31</sup> Low socioeconomic status has been associated with subclinical atherosclerosis.<sup>32</sup> Individuals with lower education have larger carotid plaques even after adjusting for vascular risk factors.<sup>32,33</sup> Despite the fact that traditional risk factors explain only a small proportion of the variance in plaque burden, most of them are modifiable and their adequate control is still of utmost importance for prevention of atherosclerosis and its devastating clinical consequences.

Several factors previously identified that influence carotid atherosclerosis were also noted in our study. Increased levels of homocysteine are associated with greater risk of plaque burden<sup>4,34</sup> and vitamin B6 and B12 therapy may be effective in lowering homocysteine and plaque progression.<sup>35</sup> Another novel vascular risk factor is increased WBC count. Relative elevations of WBC levels have been associated with presence of subclinical carotid plaque in our population<sup>36</sup> and with plaque progression in other populations<sup>37</sup>; however, from a practical point of view, although elevated WBC count is relevant to development of TPA, its potential for modification is limited. Although several studies have found positive associations with serum C-reactive protein<sup>38</sup> and estimated glomerular filtration rate,<sup>39</sup> we have not observed an association between these factors and carotid plaque burden. The exact role of these novel risk factors in atherosclerosis is yet to be fully elucidated.

If only 19.5% of the carotid plaque burden can be explained by the contribution of traditional and less traditional vascular risk factors, what explains the remaining variance in plaque burden? Clustering of risk factors within an individual may provide a better explanation of the variation in atherosclerotic plaque burden. However, our results remained relatively unchanged when using Framingham Risk Score<sup>20</sup> or NOMAS Global Vascular Risk Score.<sup>21</sup> The effects of some unaccounted and less known factors such as inflammation and infection, innate immunity, psychosocial factors, or behavioral factors may contribute to plaque burden, but it is less likely that they would account for a large proportion of the plaque burden variance. Recently, we have identified a contribution of variation in genes involved in inflammation, endothelial function, and lipid metabolism to carotid plaque burden.<sup>40,41</sup> The investigations of novel genetic factors and their effects in a combination with environmental factors hold the promise of future research, treatment, and prevention of atherosclerosis, stroke, and CVD.

Limitations of our study need to be acknowledged. The study population consists of an elderly and predominantly Hispanic population limiting generalizability of our results. The cross-sectional nature of the current findings does not allow inference of causality. Our study mainly included well-known traditional atherosclerotic risk factors along with some behavioral and less traditional factors, whereas other

factors of possible importance for atherosclerosis such as diet or endothelial function were not considered.

### Summary

Carotid plaque burden is a significant predictor of CVD and 2-dimensional ultrasound measurement of plaque area is an inexpensive tool to identify individuals with increased atherosclerotic burden at risk for CVD, evaluate the effects of current and novel therapies, and investigate new contributing factors. Modifiable behaviors such as smoking and physical activity can influence BP, glycemic control, and cholesterol levels, which are important risk factors for plaque burden. Their modification should be strongly recommended. Our study also suggests that many unaccounted factors play an important role in the determination of atherosclerosis, underscoring the importance of further research.

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### Disclosures

F.K. analyzed, interpreted the data, and drafted and revised the article. H.G. and C.D. performed statistical analyses and analyzed and interpreted the data. D.C. acquired the data. D.D.-M. analyzed and interpreted the data. S.H.B. interpreted the data and made revisions of the article. R.L.S., M.S.V.E., and T.R. conceived and designed the research, supervised data collection, statistical analyses, handled funding, and made critical revision of the article for important intellectual content.

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