Carotid Artery Intima-media Thickness, Carotid Plaque and Coronary Heart Disease and Stroke in Chinese

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Accessibility
Introduction

Cardiovascular disease remains the leading cause of death worldwide. International studies have demonstrated that Asian and Western populations have different patterns of cardiovascular disease events[1,2]. In Western countries, coronary heart disease (CHD) is more common than stroke, whereas in Asian-Pacific countries, stroke outnumbers CHD [3]. Because of ethnic differences in the atherosclerosis process[4], it is important to investigate the role of subclinical atherosclerosis in the development of CHD and stroke in Asian populations.

Intima-media thickness (IMT) measurements of the common carotid artery (CCA) and internal carotid artery (ICA) are considered as useful indicators of carotid atherosclerosis[5–10]. Previous studies have only focused on common measurements of carotid arteries and did not discriminate the potential differences between CCA and ICA[10,11]. Evidence showed that CCA and ICA narrowing may reflect different pathophysiology and thus may be differentially related to cardiovascular events[12]. Moreover, the carotid plaque severity has also been associated with increased risk of cardiovascular disease [11,13–16]. Therefore, we conducted a prospective community-based cohort study to examine the associations of carotid artery IMT and carotid plaque with incidence of CHD and stroke in Chinese in Taiwan.

Results

Higher IMT was associated with higher prevalence of obesity, hypertension, and the metabolic syndrome (table 1). The correlations between IMT and other risk factors ranged from 0.07 for triglycerides to 0.20 for systolic blood pressure (Table 2). The IMT measurements were correlated with the carotid plaque score (0.34–0.57). The carotid plaque score was minimally correlated with other risk factors, ranging from 0.06 for body mass index to 0.06 for serum cholesterol.

The incidence of CHD and stroke events increased progressively with increasing quartiles for IMT measurements (Table 3). After multivariate adjustments, the relative risks (RRs) associated with a change of 1 standard deviation of maximal common carotid IMT were 1.38 (95% confidence interval [CI], 1.12–1.70) for CHD and 1.47 (95% CI, 1.28–1.69) for stroke. The corresponding RRs with internal carotid IMT were 1.47 (95% CI, 1.21–1.79) for CHD and 1.52 (95% CI, 1.31–1.76) for stroke. Carotid plaque measured by the degree of diameter stenosis was also significantly associated with increased risk of CHD (p for trend<0.0001) and stroke (p for trend<0.0001). However, these associations were largely attenuated when adjusting for IMT measurements.

Conclusions: This prospective study indicates a significant association between carotid IMT and incidence of CHD and stroke in Chinese adults. These measurements may be useful for cardiovascular risk assessment and stratification in Chinese.
**Table 1.** Characteristics of the 2190 study participants at enrollment according to quartiles of maximal ICA intima media thickness and plaque stenosis severity measurements.

| ICA | 1  | 2  | 3  | 4  | Plaque | 0  | 1  | 2  | > = 3 |
|-----|----|----|----|----|--------|----|----|----|-------|
| N   | 560| 796| 416| 418| N=1876 | N=93 | N=70| N=151|
| (%) | (%)| (%)| (%)| (%)| (%)    | (%) | (%)| (%)| (%)  |
| Gender | <.0001 | Gender | <.0001 |
| Men | 34.8 | 41.1 | 52.4 | 58.1 | Men | 43.0 | 51.6 | 51.4 | 61.6 |
| Women | 65.2 | 58.9 | 47.6 | 41.9 | Women | 57.0 | 48.4 | 48.6 | 38.4 |
| Current smoker (yes) | 21.1 | 31.8 | 38.2 | 48.8 | <.0001 | Current smoker (yes) | 31.5 | 36.6 | 47.1 | 50.3 | <.0001 |
| Alcohol drinking (yes) | 23.0 | 28.8 | 32.7 | 31.8 | 0.0029 | Alcohol drinking (yes) | 28.1 | 25.8 | 27.1 | 37.1 | 0.11 |
| Married status | <.0001 | Married status | <.0001 |
| Single | 1.4 | 2.6 | 4.1 | 2.6 | Single | 2.4 | 2.2 | 10.0 | 2.0 |
| Lived with spouse | 92.5 | 87.4 | 84.3 | 79.3 | Married status | 88.3 | 79.6 | 64.3 | 79.5 |
| Divorced | 6.1 | 10.0 | 11.6 | 18.0 | Divorced | 9.3 | 18.3 | 25.7 | 18.5 |
| Education level | 0.25 | Education level | 0.23 |
| <9 yr | 91.8 | 93.2 | 93.8 | 95.0 | <9 yr | 92.9 | 96.8 | 94.3 | 96.0 |
| >= 9 yr | 8.2 | 6.8 | 6.3 | 5.0 | >= 9 yr | 7.1 | 3.2 | 5.7 | 4.0 |
| Job status | <.0001 | Job status | <.0001 |
| No job | 36.4 | 48.0 | 50.7 | 61.5 | No job | 44.5 | 68.8 | 71.4 | 69.5 |
| Labor work | 38.0 | 34.9 | 33.2 | 27.5 | Labor work | 36.0 | 22.6 | 21.4 | 21.9 |
| Professional | 25.5 | 17.1 | 16.1 | 11.0 | Professional | 19.5 | 8.6 | 7.1 | 8.6 |
| Regular exercise habit | 12.1 | 15.6 | 16.6 | 19.4 | 0.019 | Regular exercise habit | 14.8 | 16.1 | 27.1 | 19.9 | 0.0181 |
| Family history of CHD | 12.0 | 10.1 | 8.2 | 9.1 | 0.23 | Family history of CHD | 10.7 | 6.5 | 4.3 | 6.6 | 0.08 |
| Hypertension | 14.0 | 25.9 | 32.2 | 44.1 | <.0001 | Hypertension | 25.0 | 35.5 | 40.0 | 48.3 | <.0001 |
| Diabetes | 9.7 | 10.8 | 14.2 | 18.1 | <.0001 | Diabetes | 11.4 | 247 | 15.7 | 17.9 | 0.0021 |
| Metabolic syndrome | 16.0 | 22.2 | 29.2 | 31.9 | <.0001 | Metabolic syndrome | 22.5 | 29.0 | 37.1 | 30.2 | 0.0041 |
| Mean | | | | | Mean | | | | |
stroke. Carotid plaque was also significantly associated with cardiovascular events; the multivariate RRs with 1 unit increment in carotid plaque were 1.15 (95% CI, 1.07–1.24) for CHD and 1.11 (95% CI, 1.05–1.18) for stroke events. Analyses based on the quartiles of common and internal carotid IMT and carotid plaque showed similar results as those from the continuous analyses.

In multivariate analyses including both IMT and carotid plaque score in the same models (Table 4), the association of common carotid IMT with CHD becomes nonsignificant (multivariate RR, 1.16, 95% CI, 0.86–1.57), but its association with stroke risk remained significant (RR, 1.48, 95% CI, 1.21–1.80). The internal carotid IMT remained significant for both CHD and stroke. However, the relative risk of carotid plaque diminished appreciably and became nonsignificant after adjusting for IMT in the models.

In stratified analyses, common and internal carotid IMT predicted CHD risk in most subgroup analyses defined by cardiovascular risk factors, and only the tests for interaction between IMT and diabetes were statistically significant (P for interaction = 0.03 for CCA) (data not shown). Common and internal carotid IMT was also significantly associated with the risk of stroke in most subgroups (all P for interaction >0.05).

Adding IMT provided only a slight improvement in predicting the risk of CHD and stroke beyond the standard risk factors (Table 5). First, the increase in the AUC (from 0.787 to 0.798 for CHD, from 0.822 to 0.829 for stroke) reached a borderline significant level after adding IMT to the models with traditional risk factors for CHD. Second, adding IMT information resulted in a slightly better integrated discrimination improvement (IDI) for stroke (IDI = 0.022, P = .011) and CHD (IDI = 0.0035, P = .09). These findings showed non-significant improvement by adding IMT information.

**Discussion**

The results of this large prospective study of middle-aged and older Chinese indicate that elevated carotid IMT measurements significantly predict an increase risk of CHD and stroke in healthy Chinese, independent of other cardiovascular risk factors. These data provide useful information on the potential utility of IMT measurements and carotid sonography in screening subclinical cardiovascular disease in populations with relatively low CHD but high stroke risk.

Carotid sonography has been recommended as a screening tool for future cardiovascular events among high-risk populations, such as elderly adults[17], type 2 diabetics[18] or stable CHD patients[19]. Updated consensus has proposed the standards for measurement of IMT and plaque in the carotid artery[20,21]. However, there is a disagreement regarding the use of these measurements as screening tool in the general population[20]. And it has been recommended that more data need to be collected for different ethnic groups. Our study has partially filled this gap.

Carotid artery IMT and plaque stenosis, as markers of subclinical atherosclerosis, reflect not only early atherosclerosis but also compensatory enlargement with medial hypertrophy as a result of smooth muscle cell proliferation reactions[20,22]. Because atherosclerosis develops in men at an earlier stage, carotid IMT is greater in men than in women. Common and internal carotid artery IMT progression has been related to several cardiovascular risk factors including smoking, hypertension and hyperglycemia [23]. In the stiffer arteries such as ICA, systolic blood pulse is augmented by fast travel of pulse wave and the blood flow velocity is reduced in diastole, further accelerating lipid
deposition and local inflammation and results in increasing thickness of intima medial layers in ICA[24].

Clinical observations suggested that the higher blood pressure and vascular wall shear stress on the left carotid artery resulted in higher common carotid IMT on left side[25,26]; however, the side difference was only limited to CCA and did not affect the prediction of subsequent cardiovascular events[27]. Our study did not show differential effects of measurements from different sides on CVD risk. Also, there were no appreciable gender, age, smoking, hypertension, obesity and hyperlipidemia differences in the role of IMT for predicting risk of CHD and stroke. Nevertheless, our findings showed a slightly higher risk for CHD and stroke for ICA than for CCA, but the difference was small.

Several cohort studies have explored the association between carotid artery IMT and the incidence of CHD and stroke in Western populations[5,6,10,28,29]. After one year follow-up among 1257 middle-aged Finnish men, common carotid IMT was associated with a 3.3-fold increased risk for CHD event[28]. In the Rotterdam Elderly Study including 7983 participants older than 55 years and follow up for 6 years, common carotid IMT was a significant predictor for stroke and CHD[6,30]. Chambless and colleagues demonstrated that the combined CCA and ICA measurements were significant predictors of CHD among 15792 middle-aged adults in the Atherosclerosis Risk in Community cohort[29]. In another study based on 5858 older adults (65 years of age or older) and 6 year of follow-up, O’Leary and colleagues demonstrated that both common and internal carotid IMT measurements were significant predictors of CHD and stroke[10].

Few studies have examined the role of carotid IMT in predicting CVD events in Asian populations[31–34]. In a study of Japanese diabetic patients, carotid IMT was associated with increased CHD events during a 3-year follow up [32]. Among 298 elderly Japanese (older than 75 years, average 80 years), carotid IMT was associated with increased cardiovascular death and total mortality during 3 years of follow-up[34]. Our study provided strong evidence that carotid IMT significantly predicts CHD and stroke in a community-based healthy Chinese population. Furthermore, our findings were compatible with a recent meta-analysis results which showed one standard deviation of IMT difference increased a 1.26-fold risk for CHD and a 1.32-fold risk for stroke [35].

Carotid plaque provided additional information for cardiovascular risk prediction because the plaque score reflects the severity of irregular morphology and lumen narrowing[14,36]. Carotid plaque was reported to be associated with local inflammation and biomechanical stress[36] and was considered as a marker of advanced atherosclerosis. Furthermore, ethnic variation in carotid plaque severity has been demonstrated and African Americans men appear to have appreciably lower carotid plaque than white men.[37] Cross-sectional studies found that carotid plaque was significantly associated with prevalence of CHD[38], and the prospective cohort data suggested that carotid plaque predicted future risk of ischemic stroke among 1939 U.S. adults[15] and among 1289 elderly Japanese men[11]. Our findings suggested that the carotid plaque was significantly associated with risk of CHD and stroke, but the association was largely explained by IMT.

To our knowledge, this is the first extensive investigation of carotid artery structure and risk of CHD and stroke among Chinese. Because of the prospective cohort design, the baseline measurements of our cohort members were unlikely to be affected by disease status. Furthermore, the use of a community-based population could reduce the possibility of selection bias. We also included important covariates including socioeconomic status, lifestyle factors, and well-established CVD risk factors including hypertension, diabetes, blood lipid profiles and the metabolic syndrome. Adjustment for these variables did not diminish the role of IMT in predicting CHD or stroke.

Our study had several potential limitations. First, the number of incident cases of CHD and stroke events was relatively small, even with more than a decade’s follow up, which would reduce the power to detect the subtle differences between common and internal carotid artery IMT and make the relative risk estimation unstable. However, the 95% confidence intervals for the estimated relative risks were narrow and tests for linear trends were significant for our exposure variables. Second, we did not measure functional parameters such as resistance index, which might be useful for further risk stratification. In addition, there was no formal comparison of our results to those from other racial/ethnic groups. Nonetheless, our findings added to the existing literature about the role of carotid atherosclerosis for further cardiovascular risk.

| Carotid Artery in Chinese | Table 2. Adjusted Spearman Correlation coefficients of carotid-artery intima-media thickness, plaque scores and various atherosclerotic risk factors. |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                           | **CCA, IMT** | **ICA, IMT** | **Plaque** | **BMI** | **SBP** | **DBP** | **Cholesterol** | **TG** | **HDL** | **LDL** | **Glucose** |
| CCA, IMT                  | 0.77        | 0.37        | 0.12       | 0.21    | 0.14    | 0.09    | 0.07           | −0.07  | 0.11    | 0.06    |
| ICA, IMT                  | 0.34        | 0.11        | 0.18       | 0.13    | 0.08    | 0.07    | 0.07           | −0.09  | 0.10    | 0.07    |
| CCA & ICA, IMT            | 0.94        | 0.94        | 0.36       | 0.12    | 0.20    | 0.14    | 0.09           | 0.07   | −0.08   | 0.11    | 0.07    |
| Carotid plaque            | −0.06       | 0.05        | 0.01       | 0.06    | 0.03    | −0.01   | 0.06           | 0.00   |
| BMI                       | 0.30        | 0.30        | 0.16       | 0.35    | 0.25    | 0.21    |
| SBP                       | 0.72        | 0.10        | 0.24       | −0.15   | 0.14    | 0.11    |
| DBP                       | 0.12        | 0.22        | −0.13      | 0.15    | 0.11    |
| Cholesterol               | 0.27        | 0.10        | 0.94       | 0.14    |
| TG                        | −0.48       | 0.39        | 0.23       |
| HDL-cholesterol           | −0.16       | −0.17       |
| LDL-cholesterol           | 0.19        |

Abbreviation: CCA, common carotid artery; ICA, internal carotid artery; IMT, intima-media thickness; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein.

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In conclusion, we demonstrate that IMT and carotid plaque were associated with the risks of CHD and stroke among Chinese. Because of only moderate correlation coefficients between carotid artery measurements and traditional vascular risk factors, the carotid artery measurements especially IMT can be useful for comprehensive evaluation of cardiovascular risk in Asian populations.

### Methods

#### Study Design and Study Participants

Details of this cohort study have been published previously[39–41]. Briefly, the Chin-Shan Community Cardiovascular Cohort Study (CCCG) began in 1990 by recruiting 1703 men and 1899 women of Chinese ethnicity aged 35 years old and above from the Chin-Shan township, 30 km north of metropolitan Taipei, Taiwan. Lifestyle information and medical conditions were assessed by interview questionnaires at 2-year cycles for the initial 6 years, and the validity and reproducibility of these data and anthropometric measurements have been reported in detail elsewhere[42]. Two thousand and two hundred forty-four participants had complete carotid ultrasonography measurements in 1994–1995. After excluding those with previous history of cardiac disease (n = 16) and cerebrovascular disease (n = 38), a total of 2190 participants were included in this study.

#### Table 3

| Maximal CCA IMT | Quartile 1 | 2 | 3 | 4 | Median (mm) | 0.55 | 0.65 | 0.80 | 1.00 |
|-----------------|-----------|---|---|---|--------------|------|------|------|------|
| **Coronary Heart Disease** | Rate/1,000 | 1.5 | 1.8 | 3.8 | 8.2 | P, trend | Per 1 SD (0.26 mm) increase | P |
| Model 1 | 1 | 0.87 | 0.35 | 2.19 | 1.42 | 0.62 | 3.26 | 2.47 | 1.11 | 5.47 | 0.006 | 1.41 | 1.17 | 1.70 | 0.0003 |
| Model 2 | 1 | 0.78 | 0.31 | 1.96 | 1.26 | 0.55 | 2.90 | 2.08 | 0.94 | 4.63 | 0.018 | 1.41 | 1.15 | 1.72 | 0.001 |
| Model 3 | 1 | 0.65 | 0.25 | 1.70 | 1.08 | 0.46 | 2.51 | 1.78 | 0.79 | 3.97 | 0.037 | 1.38 | 1.13 | 1.70 | 0.002 |
| Model 4 | 1 | 0.66 | 0.25 | 1.74 | 1.08 | 0.47 | 2.52 | 1.75 | 0.78 | 3.94 | 0.045 | 1.38 | 1.12 | 1.70 | 0.003 |
| Stroke | Rate/1,000 | 1.5 | 3.7 | 5.3 | 11.1 | P, trend | Per 1 SD (0.26 mm) increase | P |
| Model 1 | 1 | 1.66 | 0.75 | 3.67 | 1.52 | 0.70 | 3.33 | 2.36 | 1.12 | 4.97 | 0.023 | 1.54 | 1.35 | 1.75 | <0.0001 |
| Model 2 | 1 | 1.69 | 0.76 | 3.75 | 1.53 | 0.70 | 3.36 | 2.50 | 1.19 | 5.28 | 0.014 | 1.61 | 1.41 | 1.83 | <0.0001 |
| Model 3 | 1 | 1.68 | 0.73 | 3.87 | 1.57 | 0.70 | 3.54 | 2.27 | 1.04 | 4.96 | 0.046 | 1.49 | 1.30 | 1.70 | <0.0001 |
| Model 4 | 1 | 1.66 | 0.72 | 3.83 | 1.57 | 0.70 | 3.54 | 2.21 | 1.01 | 4.85 | 0.055 | 1.47 | 1.28 | 1.69 | <0.0001 |

Carotid Plaque

| Score | 0 | 1 | 2 | > =3 |
|-------|---|---|---|------|
| **Coronary Heart Disease** | Rate/1,000 | 2.4 | 4.9 | 10.1 | 13.9 | P, trend | Per 1 increase | P |
| Model 1 | 1 | 1.76 | 0.62 | 5.03 | 2.97 | 1.20 | 7.35 | 3.59 | 1.85 | 6.95 | <0.0001 | 1.11 | 1.04 | 1.18 | 0.001 |
| Model 2 | 1 | 1.77 | 0.62 | 5.11 | 2.99 | 1.16 | 7.70 | 3.95 | 2.02 | 7.71 | <0.0001 | 1.13 | 1.05 | 1.20 | 0.001 |
| Model 3 | 1 | 1.69 | 0.59 | 4.88 | 2.61 | 0.99 | 6.88 | 3.91 | 1.98 | 7.71 | <0.0001 | 1.15 | 1.06 | 1.23 | 0.000 |
| Model 4 | 1 | 1.67 | 0.58 | 4.82 | 2.67 | 1.01 | 7.11 | 3.85 | 1.91 | 7.74 | <0.0001 | 1.15 | 1.07 | 1.24 | 0.000 |
| Stroke | Rate/1,000 | 3.5 | 6.2 | 11.8 | 17.6 | P, trend | Per 1 increase | P |
| Model 1 | 1 | 1.07 | 0.43 | 2.68 | 1.53 | 0.68 | 3.42 | 2.19 | 1.28 | 3.76 | 0.005 | 1.13 | 1.08 | 1.18 | <0.0001 |
| Model 2 | 1 | 1.21 | 0.48 | 3.05 | 1.40 | 0.61 | 3.21 | 2.39 | 1.38 | 4.13 | 0.003 | 1.15 | 1.09 | 1.20 | <0.0001 |
| Model 3 | 1 | 1.16 | 0.46 | 2.96 | 1.32 | 0.56 | 3.14 | 2.09 | 1.18 | 3.68 | 0.014 | 1.11 | 1.05 | 1.18 | 0.000 |
| Model 4 | 1 | 1.18 | 0.46 | 3.01 | 1.27 | 0.53 | 3.02 | 2.06 | 1.10 | 3.49 | 0.029 | 1.11 | 1.05 | 1.18 | 0.001 |

Model 1: adjusted for age groups (35–44, 45–54, 55–64, 65–74, > = 75 years old) and gender.
Model 2: Model 1 plus body mass index (<18, 18 to 20.9, 21 to 22.9, 23 to 24.9, or > = 25 kg/m²), alcohol intake (nondrinker/regular), exercise (yes/no), marital status (single, married or divorced), education level (<9 years, ≥9 years), occupation (no work, manual work, or professional), and family history of coronary heart disease (yes/ no).
Model 3: Model 2, adding baseline hypertension, diabetes, continuous HDL-C and LDL-C variables.
Model 4: Model 3, adding metabolic syndrome (yes/no).

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In conclusion, we demonstrate that IMT and carotid plaque were associated with the risks of CHD and stroke among Chinese. Because of only moderate correlation coefficients between carotid artery measurements and traditional vascular risk factors, the carotid artery measurements especially IMT can be useful for comprehensive evaluation of cardiovascular risk in Asian populations.
Review Board approved the study protocol. The participants gave
National Taiwan University College of Public Health Committee
included in this study. The cases were confirmed by internists. The
from the image study. Transient ischemic attacks were not
origin that lasted longer than 24 hours, with supporting evidence
available. Incident stroke cases were ascertained according to the
most plausible cause of death, or if evidence of previous CHD was
as the cause of death on the death certificate as the underlying and
infarction was confirmed by hospital records or if CHD was listed
certificate documents, further verified by house-to-house visits.
Deaths were identified from official
coronary bypass surgery. Deaths were identified from official
nonfatal myocardial infarction, fatal coronary heart disease and
documented 68 incident cases of CHD, 94 incident cases of stroke
years, median 10.5 years, inter-quartile range: 9.5–10.6 years), we
follow up from 1994 to the end of 2005 (a total of 20,102.7 person-
Model 1: adjusted for age groups (35–44, 45–54, 55–64, 65–74, 75 years old)
and gender.
Model 2: Model 1 plus body mass index (<18, 18 to 20.9, 21 to 22.9, 23 to 24.9,
or > 25 kg/m²), alcohol intake (nondrinker/regular), exercise (yes/no), marital
status(single, married or divorced), education level (<9 years, ≥9 years),
occupation (no work, manual work, or professional), and family history of
coronary heart disease (yes/no).
Model 3: Model 2, adding baseline hypertension, diabetes, continuous HDL-C
and LDL-C variables.
Model 4: Model 3, adding metabolic syndrome (yes/no).
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| Table 5. Summary statistics comparing risk prediction for the
models without and with IMT for the risk of CHD and stroke. |
|-----------------|--------------------|--------------------|--------------------|--------------------|
|                 | AUC                | IDI*               | P                  | NRI*               | P                  |
| CHD              |                    |                    |                    |                    |                    |
| Without IMT     | 0.10               | 0.004              | 0.09               | 0.086              | 0.09               |
| With IMT        | 0.787              |                    |                    |                    |                    |
| Stroke          | 0.28               | 0.022              | 0.011              | 0.079              | 0.12               |
| Without IMT     | 0.822              |                    |                    |                    |                    |
| With IMT        | 0.829              |                    |                    |                    |                    |

| Abbreviation: AUC, area under receiver operative characteristic curve; CHD, coronary heart disease; IMT, intima-media thickness; IDI, integrated
discrimination improvement; NRI, Net reclassification improvement.
Integrated discrimination improvement.

| Measurement of biochemical markers |
|-----------------------------------|
The procedures of blood sample collection were reported
elsewhere [43,44]. Briefly, all venous blood samples drawn after
a 12-hour overnight fast were immediately refrigerated and
transported within 6 hours to the National Taiwan University
Hospital. Serum samples were then stored at −70°C before batch
assay for levels of total cholesterol, triglycerides, and high density
lipoprotein cholesterol (HDL-C). Standard enzymatic tests for
serum cholesterol and triglycerides were used (Merck 14354 and
14366, Germany, respectively). HDL-C levels were measured in
supernatants after the precipitation of specimens with magnesium
chloride phosphotungstate reagents (Merck 14993). LDL-C
concentrations were calculated as total cholesterol minus
cholesterol in the supernatant by precipitation method [Merck
14992][45].

| Carotid artery ultrasonographic measurements |
|---------------------------------------------|
The measurements of IMT were obtained by using a Hewlett-
Packard SONO 1500 ultrasound system, equipped with a
7.5 MHz real-time B-mode scanner to examine the patient’s
carotid arteries. Patients were asked to lie supine with the neck
extended in a slightly lateral rotation. Then we scanned the carotid
artery and found the lumen of the carotid artery beneath the
surface of the neck. We defined IMT as the distance from the front
edge of the first echogenic line (lumen-intima interface) to the front
deck of the second line (media-adventitia interface) in the far wall
of the vessel. We performed the same procedures on the other side
of the neck. The maximal IMT was defined by averaging maximal
measurement on both sides[46]. The inter-observer reliability of
these measurements using the inter-rater and intra-rater correla-
tion reliability ranged from 0.70 to 0.95[47].
The quantification of carotid plaque was described elsewhere
[39,47]. Briefly, carotid artery segment, including proximal CCA
(>20 mm proximal to the bulb bifurcation), distal CCA, bulb,
ICA, and external carotid artery were examined bilaterally. A
grade was assigned to each chosen segment: grade 0 for normal or
no observable plaque; grade 1 for one small plaque with diameter
stenosis <30%; grade 2 for one medium plaque with 30% to 49%
diameter stenosis or multiple small plaques; grade 3 for one large

| Table 4. Relative risk and 95% confidence intervals for
jointed analysis of per 1 SD increase in IMT and 1 score
increase in plaque score for CHD and stroke. |
|---------------------------------------------|

| Coronary Heart Disease                     | Relative risk and 95% CI | Relative risk and 95% CI |
|---------------------------------------------|--------------------------|--------------------------|
| CCA Plaque                                 |                          |                          |
| Model 1                                     | 1.31 1.00 1.71           | 0.05 1.04 1.14           |
| Model 2                                     | 1.25 0.95 1.64           | 0.11 1.07 1.17           |
| Model 3                                     | 1.18 0.88 1.59           | 0.27 1.10 1.22           |
| Model 4                                     | 1.16 0.86 1.57           | 0.34 1.11 1.24           |
| ICA Plaque                                 |                          |                          |
| Model 1                                     | 1.41 1.13 1.77           | 0.003 1.04 1.13          |
| Model 2                                     | 1.40 1.11 1.76           | 0.004 1.06 1.15          |
| Model 3                                     | 1.34 1.05 1.70           | 0.019 1.08 1.18          |
| Model 4                                     | 1.33 1.04 1.69           | 0.023 1.08 1.19          |

| Stroke                                      |                          |                          |
| CCA Plaque                                 |                          |                          |
| Model 1                                     | 1.47 1.22 1.78           | <0.001 1.02 1.10          |
| Model 2                                     | 1.53 1.26 1.85           | <0.001 1.03 1.14          |
| Model 3                                     | 1.49 1.23 1.81           | <0.001 1.00 1.13          |
| Model 4                                     | 1.48 1.21 1.80           | <0.001 1.00 1.12          |
| ICA Plaque                                 |                          |                          |
| Model 1                                     | 1.42 1.19 1.70           | <0.001 1.05 1.12          |
| Model 2                                     | 1.44 1.21 1.73           | <0.001 1.06 1.13          |
| Model 3                                     | 1.50 1.24 1.82           | <0.001 1.01 1.14          |
| Model 4                                     | 1.50 1.23 1.81           | <0.001 1.01 1.13          |

Model 1: adjusted for age groups (35–44, 45–54, 55–64, 65–74, > = 75 years old)
and gender.
Model 2: Model 1 plus body mass index (<18, 18 to 20.9, 21 to 22.9, 23 to 24.9,
or > 25 kg/m²), alcohol intake (nondrinker/regular), exercise (yes/no), marital
status(single, married or divorced), education level (<9 years, ≥9 years),
occupation (no work, manual work, or professional), and family history of
coronary heart disease (yes/no).
Model 3: Model 2, adding baseline hypertension, diabetes, continuous HDL-C
and LDL-C variables.
Model 4: Model 3, adding metabolic syndrome (yes/no).
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plaque with 50% to 99% diameter stenosis or multiple plaques with at least one medium plaque; and grade 4 for 100% occlusion. The carotid plaques on the proximal and distal CCA, the bulb, and the internal and external carotid arteries, were measured and given numerical values; measurements were tallied and an individual was assigned a composite score for the severity of carotid plaque. Reproducibility of plaque grade scores was good, with a kappa value of 0.70[47].

Statistical analysis
Participants were categorized into quartiles of common and internal carotid artery IMT measurements. Continuous variables were presented by mean (standard deviation) or median levels, and categorical data were presented in contingency tables. Correlations between baseline IMT and other cardiovascular risk factors were estimated by Spearman’s partial correlation coefficients adjusted for age and gender.

Incidence rates of CHD and stroke were calculated by dividing the number of cases by the person-years of follow up for each quartile of IMT. Relative risk of CHD and stroke was calculated by dividing the incidence rate of each quartile by the rate in the first quartile. In addition, we estimated the relative risks associated with a change of one standard deviation in the IMT. We used Cox proportional-hazards models to adjust for potential confounding variables. We specified four models to estimate relative risk of CHD and stroke. In model 1, we adjusted for age groups (35–44, 45–54, 55–64, 65–74, > 75 years old) and gender. In Model 2, we additionally adjusted for body mass index (<18, 18 to 20.9, 21 to 22.9, 23 to 24.9, or ≥ 25 kg/m²) and lifestyle factors, including alcohol intake (nondrinker/current), smoking, (yes/no) and exercise(yes/no), as well as socioeconomic status, including marital status (single, married, or divorced), educational level (<9 years, ≥9 years), occupation (no work, manual work, or professional), and family history of CHD (yes/no). In Model 3, we adjusted further for the presence or absence of hypertension and diabetes and continuous variables including LDL and HDL cholesterol levels. In model 4, we further adjusted for the presence or absence of the metabolic syndrome defined by the NCEP ATP III criteria[48]. To test for linear trend across IMT quartiles, we used the median IMT value for each quartile. The goodness of fit for each model was tested by the Hosmer and Lemeshow test[49]. We conducted stratified analyses to evaluate a potential effect modification by baseline hypertension (absence or presence), diabetes (absence or presence), total cholesterol, and body mass index using median values as the cutoffs.

We compared the performance of the models without and with CCA IMT information using the area under the receiver operating characteristic curve (AUC). The curve is a graph of sensitivity versus 1-specificity (or false-positive rate) for various cutoff definitions of a positive diagnostic test result[50]. Statistical differences in the AUCs were compared using the method of DeLong et al[51]. However, the AUC value is not the best discriminatory statistics for prediction power[52–54]. Therefore, we provided several additional statistics, including integrated discrimination improvement (IDI) and net reclassification improvement (NRI) [53] for the comparison of nested models with and without IMT. The IDI is considered the difference between improvement in average sensitivity and any potential increase in average ‘one minus specificity’[53], and is estimated as the difference in Yates discrimination slopes between the nested models[53,56]. The reclassification table as a tool for comparing the models was suggested by Ridker and colleagues[54]. Pencina and colleagues constructed the reclassification tables and developed a NRI (net reclassification improvement) statistic according to a sum of differences between the ‘upward’ movement in categories for event subjects and the ‘downward’ movement in those for nonevent subjects[53]. A priori risk categories were defined as 0–5%, 5–10%, 10–20%, and > 20%.

All statistical tests were two-tailed with a type I error of 0.05, and P values<0.05 were considered statistically significant. Analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC) and Stata version 9.1 (Stata Corporation, College Station, Texas).

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Author Contributions
Conceived and designed the experiments: KLC MFC YTL. Performed the experiments: JSJ HCH WTC. Analyzed the data: KLC TCS HCH FH. Contributed reagents/materials/analysis tools: TCS WTC YTL. Wrote the paper: FH.

References
1. Rosamond W, Flegal K, Friday G, Furie K, Go A, et al. (2007) Heart disease and stroke statistics—2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 115: e69–171.
2. Reddy KS (2004) Cardiovascular disease in non-Western countries. New England Journal Medicine 350: 2438–2440.
3. van den Hoogen PC, Feskens EJ, Nagelkerke NJ, Menotti A, Nissinen A, et al. (2000) The relation between blood pressure and mortality due to coronary heart disease among men in different parts of the world. Seven Countries Study Research Group. N Engl J Med 342: 1–8.
4. Bild DE, Detrano R, Peterson D, Guerci A, Liu K, et al. (2005) Ethnic differences in coronary calcification: the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation 111: 1313–1320.
5. Houdin RN, Mack WJ, Laljee L, Selzer RH, Liu CR, et al. (1998) The role of carotid arterial intima-media thickness in predicting clinical coronary events. Ann Intern Med 128: 262–269.
6. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE (1997) Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 96: 1432–1437.
7. Touboul PJ, Elbaz A, Koller C, Lucas C, Adrai V, et al. (2000) Common carotid artery intima-media thickness and brain infarction : the Etude du Profil Genetique de l’Infarctus Cerebral (GENIC) case-control study. The GENIC Investigators. Circulation 102: 313–318.
8. Scuteri A, Manolio TA, Marino EK, Arnold AM, Lakatta EG (2004) Prevalence of specific variant carotid geometric patterns and incidence of cardiovascular events in older persons. The Cardiovascular Health Study (CHS-E-131). J Am Coll Cardiol 43: 187–193.
9. Nichols WW, Pepine CJ, O’Rourke MF (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke. N Engl J Med 340: 1762–1763.
10. O’Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, et al. (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 340: 14–22.
11. Kitamura A, Iso H, Imano H, Ohira T, Okada T, et al. (2004) Carotid intima-media thickness and risk of stroke and myocardial infarction in Japanese elderly men. Stroke 35: 2780–2789.
12. Silvestrini M, Vernieri F, Pasqualetti P, Matteis M, Passarelli F, et al. (2000) Impaired cerebral vasoreactivity and risk of stroke in patients with asymptomatic carotid artery stenosis. JAMA 283: 2122–2127.
13. Brook RD, Bard RL, Patel S, Rubenfire M, Clarke NS, et al. (2006) A negative carotid plaque area test is superior to other noninvasive atherosclerosis studies for reducing the likelihood of having underlying significant coronary artery disease. Arterioscler Thromb Vasc Biol 26: 656–662.
14. Prati P, vanuzzo D, Casaroli M, Bader G, Mos L, et al. (2006) Determinants of carotid plaque occurrence. A long-term prospective population study: the San Daniele Project. Cerebrovasc Dis 22: 416–422.
15. Prabhakaran S, Rundek T, Ramas R, Elkink MS, Paik MC, et al. (2006) Carotid plaque surface irregularity predicts ischemic stroke: the northern Manhattan study. Stroke 37: 2696–2701.

16. Rubin MR, Rundek T, McMahon DJ, Lee HS, Sacco RL, et al. (2006) Carotid artery plaque thickness is associated with increased serum calcium levels: The Northern Manhattan study. Atherosclerosis.

17. Jacobowitz GR, Rockman CB, Gagne PJ, Adelman MA, Lamparello PJ, et al. (2003) A model for predicting occult carotid artery stenosis: screening is justified in a selected population. J Vasc Surg 38: 705–709.

18. Lacroux P, Aboyans V, Grigori MH, Bertin F, Bouhamed T, et al. (2006) Type-2 diabetes and carotid stenosis: a proposal for a screening strategy in asymptomatic patients. Vasc Med 11: 95–99.

19. Rockman CB, Jacobowitz GR, Gagne PJ, Adelman MA, Lamparello PJ, et al. (2004) Focused screening for occult carotid artery disease: patients with known heart disease are at high risk. J Vasc Surg 39: 44–51.

20. Toutou PJ, Hennerici MG, Meairs S, Adams H, Amarreco P, et al. (2007) Mannheim carotid intima-media thickness consensus (2004–2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis 23: 73–80.

21. Stein JH, Korczaz GE, Hurst RT, Lonn E, Kendall CB, et al. (2008) Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 21: 93–111. quiz 189–190.

22. Pruissen DM, Gerritsen SA, Prinsen TJ, Dijk JM, Kappelle LJ, et al. (2007) Is there a side predilection for cerebrovascular disease? Hypertension 49: 189–190.

23. Johnson HM, Douglas PS, Sinivasan SR, Bond MG, Tang R, et al. (2007) Predictors of carotid intima-media thickness progression in young adults: the Bogalusa Heart Study. Stroke 38: 900–905.

24. Irace C, Cortese F, Fiaschi E, Carallo C, Farinario E, et al. (2004) Wall shear stress is associated with intima-media thickness and carotid atherosclerosis in subjects at low coronary heart disease risk. Stroke 35: 464–468.

25. Gnasso A, Irace C, Carallo C, De Franceschi MS, Monti C, et al. (1997) In vivo association between low wall shear stress and plaque in subjects with asymmetrical carotid atherosclerosis. Stroke 28: 993–998.

26. Rodriguez Hernandez SA, Kreon AA, van Beekel MP, Mees WH, Lodder J, et al. (2003) Is there a side predilection for cerebrovascular disease? Hypertension 42: 56–60.

27. Foerch C, Buehler A, von Kegler S, Sitzer M (2003) Intima-media thickness side differences are limited to the common carotid artery. Hypertension 42: e17. author reply e18.

28. Salonen JT, Saaristo R (1991) Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. Arterioscler Thromb 11: 1245–1249.

29. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, et al. (1997) Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987–1993. Am J Epidemiol 146: 483–494.

30. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M (2007) Prediction of carotid atheromatous plaque inflammation with biomechanical stress: Utility of USPIO enhanced MR imaging and biomechanical analysis. Atherosclerosis.

31. Freedman BI, Hsu FC, Langefeld DJ, Rich SS, Herrington DM, et al. (2005) The impact of ethnicity and sex on subclinical cardiovascular disease: the Diabetes Heart Study. Diabetologia 48: 2511–2518.

32. Kanadani M, Gayli M, San M, Akimbaek K, Ahlan CC, et al. (2006) The presence of a calcific plaque in the common carotid artery as a predictor of coronary atherosclerosis. Angiology 57: 585–592.

33. Su TC, Chien KL, Jeng JS, Chang CJ, Hu HC, et al. (2006) Pulse pressure, aortic regurgitation and carotid atherosclerosis: a comparison between hypertensives and normotensives. Jpn Circ J 60: 134–140.

34. Chien KL, Sung FC, Hsu HC, Su TC, Lin RS, et al. (2002) Apolipoprotein AI & B, and stroke events in a community-based cohort in Taiwan. Report of Chin-Shan Community Cardiovascular Study. Stroke 33: 39–44.

35. Lee YT, Lin RS, Sung FC, Yang CY, Chien KL, et al. (2000) Chin-Shan Community Cardiovascular Cohort in Taiwan: baseline data and five-year follow-up morbidity and mortality. Journal of Clinical Epidemiology 53: 836–846.

36. Lee YT, Sung FC, Lin RS, Hsu HC, Chien KL, et al. (2001) Peripheral blood cells among community residents living near nuclear power plants. Science of the Total Environment 290: 165–172.

37. Chien KL, Lee YT, Sung FC, Su TC, Hsu HC, et al. (1999) Lipoprotein(a) level in the population in Taiwan: relationship to sociodemographic and atherosclerotic risk factors. Atherosclerosis 143: 267–273.

38. Chien KL, Sung FC, Hsu HC, Su TC, Chang WD, et al. (2003) Relative importance of atherosclerotic risk factors for coronary heart disease in Taiwan. Eur J Cardiovasc Prev Rehabil 12: 95–101.

39. Wieland H, Siegel D (1983) A simple specific method for precipitation of low density lipoproteins. Journal of Lipid Research 24: 904–909.

40. Su TC, Jeng JS, Chien KL, Torng PL, Sung FC, et al. (1999) Measurement reliability of common carotid artery intima-media thickness by ultrasonographic assessment. J Med Ultrasound 7: 73–79.

41. Su TC, Jeng JS, Chien KL, Sung FC, Hsu HC, et al. (2001) Hypertension status is the major determinant of carotid atherosclerosis: A community-based study in Taiwan. Stroke 32: 2265–2271.

42. (2001) Executive Summary of The Third Report Of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 285: 2486–2497.

43. Hosmer DW Jr, Lemeshow S (1989) The multiple logistic regression model. Applied logistic regression. 1 ed. New York: John Wiley & Sons. pp 25–37.

44. Hanley JA, McNeil BJ (1983) A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 148: 839–843.

45. DeLong ER, DeLong DM, Clarke-Pearson DL (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44: 837–843.

46. Cook NR (2007) Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 115: 929–935.

47. Perucchini MJ, D'Agostino RBS, D'Agostino RB Jr, Vasan RS (2008) Evaluating the added predictive ability of a new marker: From area under the ROC curve to reclassification and beyond. Stat Med 27: 157–172.

48. Kühler PM, Buring JE, Kifun N, Cook NR (2007) Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. Jama 297: 611–619.

49. Schmidt C, Griffith J (1998) Multivariable classification rules: calibration and discrimination. In: Armitage P, Colton T, eds. Encyclopedia of Bostastics. Chichester, U.K., Wiley.

50. Yates J (1982) External correspondence: decomposition of the mean probability score. Organizational Behavior and Human Performance 30: 132–156.