Retinal Microvascular Signs and Risk of Stroke
The Multi-Ethnic Study of Atherosclerosis (MESA)

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Background and Purpose—Small-vessel disease contributes to the pathophysiology of stroke, and retinal microvascular signs have been linked to the risk of stroke. We examined the relationship of retinal signs with incident stroke in a multiethnic cohort.

Methods—The Multi-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort study that enrolled participants without clinical cardiovascular diseases from 6 US communities between 2000 and 2002. Of the participants, 4849 (71.2%) had fundus photography performed in 2002 to 2004. Retinopathy and retinal vessel caliber were assessed from retinal images. Stroke risk factors including high-sensitivity C-reactive protein, carotid artery intima-media thickness, and coronary artery calcium were measured using standardized protocols. Incident stroke was confirmed from medical record review and death certificates.

Results—After 6 years of follow-up, there were 62 incident strokes. Narrower retinal arteriolar caliber was associated with increased risk of stroke after adjusting for conventional cardiovascular risk factors (adjusted incidence rate ratio, 2.83; 95% CI, 1.34—5.95; P=0.006; adjusted hazard ratio, 3.01; 95% CI, 1.29—6.99; P=0.011). Retinopathy in persons without diabetes was associated with increased risk of stroke (adjusted adjusted incidence rate ratio, 2.96; 95% CI, 1.50—5.84; P=0.002; adjusted hazard ratio, 3.07; 95% CI, 1.17—8.09; P=0.023). These associations remained significant after adjusting for high-sensitivity C-reactive protein, carotid intima-media thickness, or coronary artery calcium.

Conclusions—Narrower retinal arteriolar caliber and retinopathy in nondiabetic persons were associated with increased risk of stroke in this relatively healthy multiethnic cohort independent of traditional risk factors and measures of atherosclerosis. The association between narrower retinal arteriolar caliber and stroke warrants further investigation. *(Stroke. 2012;43:3245-3251.)*

Key Words: retinal vessel ■ microvascular network ■ retinopathy ■ stroke

Several epidemiologic studies have linked retinal microvascular signs with stroke and its related mortality.1-9 Prospective data from these studies suggest that people with retinopathy signs have 2- to 3-fold higher risk of stroke than those without retinopathy signs.1,2 In addition, by using new computer-based technologies to measure retinal vessel caliber, widened retinal venular caliber has similarly been associated with increased risk of stroke.3-7

However, most of the previous population-based studies were conducted in white populations, and some included people with clinical cardiovascular disease, a major potential confounder. Importantly, it remains uncertain whether the reported associations between retinal signs and stroke risk were related to subclinical large artery atherosclerosis.

In the Multi-Ethnic Study of Atherosclerosis (MESA), we have previously reported cross-sectional associations between retinal microvascular signs and measures of subclinical cardiovascular disease.10-12 In this study, we examined prospectively the relationship between retinal microvascular signs and stroke incidence at the same time as adjusting for traditional and novel cardiovascular risk factors including high-sensitivity C-reactive protein (hsCRP), carotid intima-media thickness (IMT), and coronary artery calcium (CAC).

Methods

Study Participants

The MESA is a prospective study of adults without a history of clinical cardiovascular disease. Subjects were recruited from 6...
Assessment of Retinal Microvascular Signs

Fundus photography was performed according to a standardized protocol using a CR6-45NM fundus camera with a digital Canon D-60 camera back (Canon Inc.). Both eyes of each participant were photographed for 2 photographic fields: the first centered on the optic disc (Early Treatment Diabetic Retinopathy Study Field 1) and the second centered on the fovea (Field 2). Images were sent to the Ocular Epidemiology Reading Center at the University of Wisconsin (Madison, WI). Retinopathy was considered to be present if there are any lesion as defined by the Early Treatment Diabetic Retinopathy Study severity scale (ie, microaneurysms/vascular diameter could not be measured in the right eye, the left eye was 45 to 84 years.

Assessment of Cardiovascular Risk Factors

Participants underwent interviews and assessments of cardiovascular risk factors during the course of the study. Cardiovascular risk factors used for this analysis were collected at the second examination (baseline of this analysis) of the MESA. Resting BP was measured 3 times with participants in the seated position (Dinamap model Pro-100 automated oscillometric sphygmomanometer; Critikon, General Electric Healthcare, Piscataway, NJ). The MESA personnel-assessed medication use was confirmed by taking a medication inventory. Hypertension was defined as a systolic BP ≥140 mm Hg, diastolic BP ≥90 mm Hg, or use of medication prescribed for hypertension. Diabetes mellitus was defined as being present if the fasting glucose was ≥7.0 mmol/L or use of insulin or oral hypoglycemic medication. Dyslipidemia was defined as having lipid-lowering medications or those who qualified for treatment recommended in the Third report of the National Cholesterol Education Program Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Body mass index was calculated as weight (kg) divided by height (m) squared. HsCRP was determined by a BNII nephelometer (N-High Sensitivity; Dade Behring Inc, Deerfield, IL; minimum detection level 0.17 mg/L) in 2000 to 2002. Trained technicians in each field center performed B-mode ultrasonography of the right and left near and far walls of the internal carotid and common carotid arteries using the Logiq 700 ultrasound device (General Electric Medical Systems, Waukesha, WI). maximal IMT of the internal and common carotid sites as the mean of the maximum IMT of the near and far walls of the right and left sides was measured at an ultrasound reading center (Department of Radiology, Tufts–New England Medical Center, Boston, MA). Scanning centers assessed CAC by chest CT using either a cardiac-gated electron-beam CT scanner or a multidetector CT system. A phantom of known
physical calcium concentration was included in the field of view; a radiologist or cardiologist read all CT scans at a central reading center (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA) using an interactive scoring system similar to that used by Yaghoubi et al. The Agatston CAC score was computed based on plaque densities and their areas in coronary arteries. We used the natural logarithm (ln) of (CAC score+1) following our previous analysis because this transformation better normalized the CAC distribution.

Assessment of Incident Stroke Events

New occurrences of stroke were recorded over 6 years of follow-up. In brief, a telephone interviewer contacted each participant every 9 to 12 months. Information about all new cardiovascular conditions, hospital admissions, cardiovascular outpatient diagnoses, treatments, and deaths were obtained. To verify self-reported diagnoses, information was collected from death certificates and medical records for all hospitalizations and outpatient cardiovascular diagnoses. For nonfatal events, International Classification of Diseases, 9th Revision codes for procedures (36, 37, 38, 39, 84.1, and 88.5) and diagnoses (402, 410, 411, 412, 413, 414, 425, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 440, 441, 443.8, 443.9, and 518.4) were eligible for further investigation and data abstraction. For charts that include International Classification of Diseases, 9th Revision procedure code 35 or diagnosis codes 250, 390–459, 745 to 747, 794.3, or 798 to 799, physicians disagreed on the event classification, they adjudicated differences. If disagreements persisted, the full events committee made the final determination. The reviewers were blinded to the study data. If the reviewing physicians disagreed on the event classification, they adjudicated differences. If disagreements persisted, the full events committee made the final classification. Neurologists reviewed and classified stroke as present if there was a focal neurologic deficit lasting 24 hours or until death, or if <24 hours, there was a clinically relevant lesion on brain imaging and no nonvascular cause. Patients with focal neurological deficits secondary to brain trauma, tumor, infections, or other nonvascular cause were excluded.

Statistical Analysis

Comparison of continuous variables and categorical variables were analyzed with the analysis of variance and χ² test, respectively; Kruskal-Wallis rank test was used for comparing variables with skewed distribution. We compared absolute event rates of stroke by sex and estimated incidence rate ratios using multiple Poisson regression models. To confirm our analysis, we also used the Kaplan-Meier curves and Cox proportional hazard models to estimate hazards ratios. Covariates used in adjustment were: Model 1 adjusting for age, sex, study site, race/ethnicity, hypertension, diabetes, dyslipidemia, and history of smoking; Model 2-1 adjusting for variables in Model 1 plus hsCRP; Model 2-2 adjusting for variables in Model 1 plus carotid IMT; and Model 2-3 adjusting for variables in Model 1 plus CAC. Retinopathy and retinal vessel calibers were included together in all models. We calculated the Harrell C-discrimination index (c-index), which is an extension of the area under the receiver operating curve to the case of survival data and compared the predictive value of the models. All statistical analysis was performed using Stata 12.1 (StataCorp, College Station, TX) P<0.05 was considered statistically significant.

Results

Table 1 summarizes the baseline characteristics of the study cohort. Compared with participants without retinopathy, those with retinopathy were older, had higher systolic BP, greater carotid IMT, higher CAC, and were more likely to be men and have diabetes. Compared with participants with wider (highest tertile) retinal arteriolar caliber, those with narrower (lowest tertile) retinal arterioles were older, had higher systolic and diastolic BP, higher carotid IMT, higher CAC, and were less likely to be female, have diabetes, and to be a current smoker. Participants with larger retinal venular caliber were younger,

Table 1. Baseline Characteristics of Participants by Retinal Microvascular Signs

<table>
<thead>
<tr>
<th>Retinopathy</th>
<th>Central Retinal Artery Equivalent</th>
<th>Central Retinal Vein Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent (n=4304)</td>
<td>Tertile 1 &lt;139 μm</td>
<td>Tertile 2 139–150 μm</td>
</tr>
<tr>
<td>Age, y</td>
<td>62.4</td>
<td>63.7</td>
</tr>
<tr>
<td>Women, %</td>
<td>53.5</td>
<td>48.8</td>
</tr>
<tr>
<td>Caucasian, %</td>
<td>44.7</td>
<td>31.2</td>
</tr>
<tr>
<td>African Americans, %</td>
<td>27.2</td>
<td>37.1</td>
</tr>
<tr>
<td>Hispanic, %</td>
<td>19.2</td>
<td>21.1</td>
</tr>
<tr>
<td>Chinese Americans, %</td>
<td>8.9</td>
<td>10.6</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122.8</td>
<td>129.9</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>70.6</td>
<td>71.5</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>42.0</td>
<td>58.8</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>10.4</td>
<td>37.1</td>
</tr>
<tr>
<td>Dyslipidemia, %</td>
<td>30.3</td>
<td>35.1</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>11.6</td>
<td>12.9</td>
</tr>
<tr>
<td>Serum hsCRP, mg/dL*</td>
<td>1.92</td>
<td>1.97</td>
</tr>
<tr>
<td>Carotid IMT, mm</td>
<td>1.02</td>
<td>1.17</td>
</tr>
<tr>
<td>ln (CAC score+1), unit</td>
<td>1.93</td>
<td>2.52</td>
</tr>
</tbody>
</table>

hsCRP indicates high-sensitivity C-reactive protein; IMT, intima-media thickness; CAC, Agatston coronary calcium score.
*Median is shown.
had lower systolic BP, higher hsCRP, lower CAC, and more likely to be have diabetes and to be a current smoker than those with narrower retinal venular caliber.

There were 62 cases of incident stroke (mean age [±SE] 68.6 ± 1.3 years old and 58.1% were male; incidence rate [IR], 2.2% per person-year; 95% CI, 1.8%–2.9%) during 6-year follow-up (median, 5.9 years; interquartile range, 5.7–6.1 years). There were 56 brain infarctions, 5 intraparenchymal hemorrhages, and 1 without detailed information on stroke subtype. There were 45 incident stroke cases in persons with hypertension (IR, 3.8%; 95% CI, 2.8%–5.1%) compared with 17 incident strokes in persons without hypertension (IR, 1.1%; 95% CI, 0.7%–1.8%). There were 9 incident strokes in persons with diabetes (IR, 2.5%; 95% CI, 1.3%–4.8%) compared with 53 incident strokes in persons without diabetes (IR, 2.2%; 95% CI, 1.7%–2.9%). For non-Hispanic whites, blacks, Hispanics, and Chinese Americans, there were 33 (IR, 2.7%; 95% CI, 2.0%–3.9%), 17 (IR, 2.2%; 95% CI, 1.4%–3.5%), 11 (IR, 2.1%; 95% CI, 1.1%–3.7%), and 1 incident stroke (IR, 0.4%; 95% CI, 0.1%–2.8%), respectively.

Kaplan-Meier curves are shown in Figures 2–4. The log-rank test for equality of survivor function showed significant difference for retinopathy and tertile of central retinal artery equivalent (both P<0.001) but not for tertile of central retinal vein equivalent (P=0.597). After adjusting for age, sex, study sites, race/ethnicity, hypertension, diabetes, and smoking, the presence of retinopathy in nondiabetic participants was associated with an approximately 3-fold higher risk of stroke (Model 1; Tables 2 and 3). The presence of retinopathy in overall participants was significantly associated with increased risk of stroke with Poisson regression model (P<0.001; Table 2) but marginally significant with Cox regression model (P=0.081; Table 3). Specific retinopathy signs of retinal hemorrhages/microaneurysms and cotton wool spots were significantly associated with higher risk of stroke (Table 2).

Narrower retinal arteriolar caliber was significantly associated with stroke with Poisson regression models (Model 1; Table 4). This association was consistently observed with Cox regression models (Model 1; Table 3). Wider retinal venular caliber was not associated with increased risk of incident stroke in this study. These associations of nondiabetic retinopathy and smaller central retinal artery equivalent with stroke remained significant after adjusting for hsCRP, carotid IMT, or CAC (Models 2–1 to 2–3; Tables 2–4).

The association between retinopathy and stroke was consistently observed in both men and women (P=0.010 0.013, respectively) and both non-Hispanic white participants and in other 3 ethnic groups studied (ie, blacks, Hispanics, and Chinese Americans; P=0.003 and 0.002, respectively). There was no significant interaction between retinopathy and sex (P for interaction=0.898) or racial/ethnic groups (P for interaction=0.977).

The Harrell c-index for the stroke prediction model with traditional cardiovascular risk factors of age, sex, study site, race/ethnicity, hypertension, diabetes, dyslipidemia, and smoking was 0.78 (95% CI, 0.72–0.83). The model with retinopathy had a comparable c-index (0.79) to those with hsCRP (0.78, P=0.208), carotid IMT (0.77, P=0.235), and CAC (0.78, P=0.650). There was no significant difference by adding retinopathy (c-index 0.79 versus 0.80; P=0.159) or both retinopathy and retinal vessel caliber (c-index 0.79 versus 0.81, P=0.059) in c-index between the model with traditional risk factors plus hsCRP, carotid IMT, and CAC.
Discussion

In this prospective analysis of the MESA cohort, the presence of retinopathy signs in persons without diabetes was associated with an approximately 3-fold higher risk of 6-year incident stroke independent of traditional risk factors. This association remained significant after further adjustment for measures of subclinical atherosclerosis in the carotid and coronary arteries (ie, carotid IMT and CAC). Our finding of an association between retinopathy signs and stroke is consistent with previous population-based studies. Mild retinopathy signs such as retinal hemorrhages and cotton wool spots are common in general populations (prevalence 7%–11%) and are considered to be related to small-vessel arteriolosclerosis, retinal ischemia, and breakdown of the blood retinal barrier. The presence of retinopathy has been associated with the prevalence of white matter lesions. Moreover, our data showed associations between individual retinopathy lesions and stroke risk. Cotton wool spots in the retina are thought to be due to focal retinal capillary obstruction, which potentially reflects similar pathophysiological processes occurring in the cerebral circulation related to stroke. This is in line with the Atherosclerosis Risks In Communities study reporting that cotton wool spots were associated with subclinical cerebral infarct detected by the MRI and risk of stroke in a nondiabetic population.

We found that narrower retinal arteriolar caliber, but not wider retinal venular caliber, was associated with risk of stroke. Decreased retinal arteriolar caliber was hypothesized to be associated with risk of stroke. Earlier studies found

Table 2. Risk Associations of Retinopathy and Incident Stroke (Poisson regression analysis)

<table>
<thead>
<tr>
<th>Retinopathy in overall participants</th>
<th>Model 1 (n=4849)</th>
<th>Model 2-1 (n=4757)</th>
<th>Model 2-2 (n=4657)</th>
<th>Model 2-3 (n=4783)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy in non-diabetic participants (n=4169)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.8 (1.3–2.5)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>Present</td>
<td>5.7 (3.2–10.3)</td>
<td>2.96 (1.50–5.84)</td>
<td>0.002</td>
<td>3.29 (1.67–6.50)</td>
</tr>
</tbody>
</table>

Table 3. Risk Associations of Retinal Vessel Calibers and Incident Stroke (Poisson regression analysis)

<table>
<thead>
<tr>
<th>Central Retinal Artery Equivalent (CRAE)</th>
<th>Model 1 (n=4849)</th>
<th>Model 2-1 (n=4757)</th>
<th>Model 2-2 (n=4657)</th>
<th>Model 2-3 (n=4783)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 3 (&gt;150 μm)</td>
<td>1.2 (0.6–2.2)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>Tertile 2 (139–150 μm)</td>
<td>1.6 (1.0–2.7)</td>
<td>1.20 (0.54–2.68)</td>
<td>0.659</td>
<td>1.20 (0.54–2.68)</td>
</tr>
<tr>
<td>Tertile 1 (&lt;139 μm)</td>
<td>3.9 (2.8–5.5)</td>
<td>2.83 (1.34–5.95)</td>
<td>0.006</td>
<td>2.85 (1.35–6.01)</td>
</tr>
<tr>
<td>Per -1SD decrease</td>
<td>—</td>
<td>1.45 (1.07–1.95)</td>
<td>0.016</td>
<td>1.45 (1.07–1.96)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Central Retinal Vein Equivalent (CRVE)</th>
<th>Model 1 (n=4849)</th>
<th>Model 2-1 (n=4757)</th>
<th>Model 2-2 (n=4657)</th>
<th>Model 2-3 (n=4783)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 1 (&lt;204 μm)</td>
<td>2.6 (1.7–3.8)</td>
<td>1.45 (1.07–1.95)</td>
<td>0.016</td>
<td>1.45 (1.07–1.96)</td>
</tr>
<tr>
<td>Tertile 2 (204–223 μm)</td>
<td>2.3 (1.5–3.5)</td>
<td>1.38 (0.74–2.55)</td>
<td>0.308</td>
<td>1.38 (0.74–2.55)</td>
</tr>
<tr>
<td>Tertile 3 (&gt;223 μm)</td>
<td>1.9 (1.2–3.0)</td>
<td>1.25 (0.60–2.38)</td>
<td>0.548</td>
<td>1.27 (0.61–2.62)</td>
</tr>
<tr>
<td>Per +1SD increase</td>
<td>—</td>
<td>1.12 (0.83–1.51)</td>
<td>0.473</td>
<td>1.12 (0.83–1.52)</td>
</tr>
</tbody>
</table>

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that smaller artery-to-vein ratio (AVR) was associated with incident stroke, although the Blue Mountains Eye Study did not confirm this association. Smaller AVR can reflect either narrower arteriolar caliber or wider venular caliber; the Rotterdam study and the Cardiovascular Health Study found that wider retinal venular caliber is associated with stroke. This was confirmed in a meta-analysis based on 6 cohort studies with 20,798 subjects. In this meta-analysis, wider venular caliber was significantly associated with stroke (pooled hazard ratio per 20 μm, 1.15; 95% CI, 1.05–1.25); however, retinal arteriolar narrowing was not associated with stroke (pooled hazard ratio per 20 μm, 1.00; 95% CI, 0.92–1.08), which is not consistent with the findings in MESA reported here. The reason for the difference in arteriolar findings in the current study versus the meta-analysis is not apparent, but the possibility of chance finding cannot be excluded given the relatively small number of events in our generally healthy cohort without a history of cerebrovascular disease at baseline.

Although retinopathy signs, carotid IMT, and CAC have all been associated with incident cardiovascular events independently, it is unclear whether retinopathy signs can contribute to improve predictive value for stroke risk. Our data suggest that the predictive value of retinopathy for stroke risk was comparable to that of other novel risk factors like the hsCRP, carotid IMT, and CAC. However, when retinopathy was added to the model including these novel risk factors, it did not significantly improve the discriminating capacity.

Strengths of this study include its multiethnic sample, longitudinal cohort study design, standardized and detailed assessment of retinal microvascular signs and cardiovascular risk factors including measures of subclinical atherosclerosis, and stroke based on symptomatic end points validated with medical records. Limitations should also be noted. First, the precision of risk estimates was limited due to the small number of incident stroke cases (n=62) in our study, which is likely related to the relatively healthy cohort, which, by design, was free of clinical cardiovascular disease at enrollment. Second, we cannot exclude the possibility of residual confounding effects from other factors or unmeasured factors (eg, long-term hypertension) and chance finding (Type 1 error) for the association between narrower arteriolar caliber and stroke. Additional studies are needed to verify this finding.

Conclusions
Our study showed that middle-aged persons free of clinical cardiovascular disease with retinal microvascular signs were more likely to develop clinical stroke over a 6-year period than those without retinal microvascular signs independent of traditional cardiovascular risk factors and measures of subclinical atherosclerosis. The association between narrower retinal arteriolar caliber and stroke risk in this multiethnic population warrants further investigation.

Acknowledgments
We thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at www.mesa-nhlbi.org.

Sources of Funding
This research was supported by contracts N01-HC-95159 through N01-HC-95169 from the National Heart, Lung, and Blood Institute and by grants UL1-RR-024156 and UL1-RR-025005 from National Center for Research Resources (NCRR).

Disclosures
None.

References
1. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, Wofford MR, Sharrett AR. Retinal microvascular abnormalities and


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*Stroke.* 2012;43:3245-3251; originally published online October 30, 2012; doi: 10.1161/STROKEAHA.112.673335

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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