N-Terminal Pro–B-type Natriuretic Peptide and Stroke Risk
The Reasons for Geographic and Racial Differences in Stroke Cohort

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Background and Purpose—Improved identification of those at risk of stroke might improve prevention. We evaluated the association of the cardiac function biomarker N-terminal pro–B-type natriuretic peptide (NT-proBNP) with stroke risk in the 30,239 black and white participants of the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort.

Methods—During 5.4 years of follow-up after enrollment in 2003 to 2007, NT-proBNP was measured in baseline blood samples of 546 subjects with incident ischemic stroke and 956 without stroke.

Results—NT-proBNP was higher with older age and in those with heart disease, kidney disease, atrial fibrillation, and lower low-density lipoprotein-cholesterol. Adjusting for age, race, sex, income, education, and traditional stroke risk factors, there was an increased risk of stroke across quartiles of NT-proBNP; participants with NT-proBNP in the top versus the bottom quartile had a hazard ratio of 2.9 (95% confidence interval, 1.9–4.5). There was no impact of added adjustment for kidney function and heart failure. Among pathogenetic stroke subtypes, the association was largest for cardioembolic stroke, with a hazard ratio of 9.1 (95% confidence interval, 2.9–29.2). Associations did not differ by age, sex, or race, or after excluding those with baseline heart failure or atrial fibrillation. Predicted stroke risk was more accurate in 27% of participants if NT-proBNP was considered after traditional stroke risk factors (P<0.001).

Conclusions—NT-proBNP was a major independent risk marker for stroke. Considering this and other data for stroke, coronary disease, and atrial fibrillation, the clinical use of NT-proBNP measurement in primary prevention settings should be considered. (Stroke. 2014;45:1646-1650.)

Key Words: natriuretic peptides ■ risk factors ■ stroke

We evaluated the association of baseline NT-proBNP with the risk of future ischemic stroke in a large population-based cohort study of black and white Americans followed for 5.4 years. Because there is little information on racial differences in NT-proBNP, we also assessed whether the levels were higher in blacks and thus might contribute to racial disparities in stroke risk.8

Subjects
The Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort is a national population-based cohort study evaluating racial and geographic disparities in stroke.6 Between January 2003 and October 2007, 30,239 individuals ≥45 years of age were enrolled by telephone. The telephone response rate was 33% and cooperation rate was 49%, similar to other cohort studies.10 Blacks and residents of the stroke belt were oversampled: 45% men, 55% women; 58%...
whites, 42% blacks; and 56% stroke belt residents, 44% non–stroke belt residents. Demographics, socioeconomic factors, medical history, and verbal informed consent were obtained by computer-assisted telephone interview. At an in-home examination, written informed consent, blood pressure, anthropomorphic measures, blood samples, ECG, and medication inventory were obtained. Study methods were reviewed and approved by the institutional review board at each study institution.

Measurements and Definitions
Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic pressure ≥90 mm Hg, or self-reported hypertension with use of antihypertensive medications. Diabetes mellitus was defined by self-report with use of antidiabetic medications, fasting glucose >126 mg/dL, or nonfasting glucose >200 mg/dL. Left ventricular hypertrophy (LVH) was classified by ECG. Atrial fibrillation was defined as self-report or presence on ECG. Prebaseline heart disease was defined as self-reported myocardial infarction, bypass, angioplasty or stenting, or myocardial infarction on ECG. Prebaseline stroke was defined by self-report. Heart failure was defined as presence of orthopnea or paroxysmal nocturnal dyspnea.

Stroke Ascertainment
The outcome was first ischemic stroke through September 1, 2011. Participants or their proxies were contacted every 6 months by telephone to update health status. Medical records were obtained in the case of death, suspected cerebrovascular event, or occurrence of stroke symptoms elucidated using the Questionnaire for Verifying Stroke-Free Status. Prior to prereview by a stroke nurse, records were reviewed and validated by ≥2 physicians. Stroke was defined as focal neurological symptoms lasting >24 hours or nonfocal symptoms with positive imaging for stroke. Strokes were classified as ischemic or hemorrhagic, and ischemic into pathogenetic subtypes of small vessel, large vessel, cardioembolic, or unclassified as in other studies. For analysis by pathogenetic subtype, when >1 cause was considered present, that case was counted in each subtype group.

Case–Cohort Sample
We used a case–cohort study design with a mean follow-up of 5.4 years. Cases were 576 participants with incident ischemic stroke and no prebaseline stroke. The cohort random sample was selected using stratified sampling to ensure sufficient representation of high-risk groups. Participants were given a random number and divided into 20 strata based on age (45–54, 55–64, 65–74, 75–84, ≥75 years) and sex. In each stratum, participants were randomly selected to fulfill the desired distribution: 50% black, 50% white; 50% women, 50% men; and age groups 45 to 54 (20%), 55 to 64 (20%), 65 to 74 (25%), 75 to 84 (25%), and ≥85 (10%) years. Of 1104 selected participants, we excluded 87 with prebaseline stroke.

Laboratory Methods
Fasting baseline blood samples were drawn and stored using standardized methods. NT-proBNP was measured in the case–cohort sample in August 2012 using an electrochemiluminescence immunoassay (Roche Elecsys 2010; Roche Diagnostics; coefficient of variation <5%). There were 88 participants with missing NT-proBNP, leaving 546 stroke cases and 956 in the cohort random sample.

Statistical Methods
Analysis used SAS 9.3 (Cary, NC). Levels of stroke risk factors were displayed as means or proportions in quartiles of NT-proBNP in the cohort random sample. Differences among quartiles were compared by χ² tests or ANOVA using sampling weights. Independent associations of NT-proBNP with race were evaluated in a multivariable linear regression model including only factors significantly associated with NT-proBNP at P < 0.05.

HRs of stroke by NT-proBNP quartiles were calculated using Cox proportional hazards models for case–cohort studies, with weighting to account for the sampling design. Participants without incident stroke were censored at death or last follow-up. The first model included age, sex, race, and an age×race interaction term because associations of race with stroke are larger at young ages. Model 2 added income, education, and Framingham stroke risk factors (antihypertensive medication use, systolic blood pressure, diabetes mellitus, LVH, history of heart disease, atrial fibrillation, smoking). Model 3 added heart failure status, estimated glomerular filtration rate (eGFR), and albuminuria to Model 2. Interactions of NT-proBNP with age, sex, and race were modeled using cross-product terms with statistical significance for an interaction of P < 0.10. Associations of NT-proBNP with each ischemic stroke subtype were estimated using Model 2, censoring participants when they developed ischemic stroke of another subtype.

To assess robustness of the findings, we conducted sensitivity analyses using Model 2. First, we excluded participants with NT-proBNP above the cut-off of the manufacturer for heart failure diagnosis (>125 pg/mL if <75 years of age and >450 pg/mL if ≥75 years of age). Second, we excluded participants with heart failure, LVH, eGFR <60 ml/min per 1.73 m², or atrial fibrillation.

Using Framingham stroke risk variables, we calculated the category-free net reclassification improvement for stroke by the measurement of NT-proBNP in addition to Framingham stroke risk variables.

Results
The values of NT-proBNP were 5 to 29284 pg/mL in the cohort random sample. Table 1 shows risk factor levels by NT-proBNP quartile in the cohort sample. In the multivariable model, there was no association of NT-proBNP with race, and factors significantly associated with NT-proBNP are shown in Table 2. The strongest correlates were older age, atrial fibrillation, heart disease, and kidney measures. Lower low-density lipoprotein-cholesterol was also associated with higher NT-proBNP. Statin use was not associated with NT-proBNP but was retained in the model to ensure that the association of NT-proBNP with low-density lipoprotein-cholesterol was not because of statin use. There were no independent associations of NT-proBNP with other factors listed in Table 1, and the inclusion of these variables in the multivariable model did not change the interpretations of the findings.

In Model 1, the HR of incident stroke increased with each increasing quartile of baseline NT-proBNP (Table 3); subjects in the fourth versus first quartile had a 3.9-fold increased risk. Adjustment for income, education, and stroke risk factors decreased this HR to 2.9 (Model 2). None of the individual covariates explained the majority of this decline in HR. Added adjustment for eGFR, albuminuria, and heart failure did not alter the HR (Model 3). Associations did not differ by age, sex, or race (interaction P values with NT-proBNP: 0.14, 0.31, and 0.38, respectively). Because NT-proBNP was not higher in blacks than in whites, mediation analysis of the racial disparity in stroke by NT-proBNP was not pursued. Analysis by stroke subtype revealed similar associations as for overall stroke except that the HR for cardioembolic stroke for NT-proBNP in the top quartile was higher at 9.1 and that for unclassified stroke was slightly lower at 2.1.

In sensitivity analysis excluding participants with NT-proBNP above the cut point for heart failure diagnosis (leaving 335 strokes and 988 in the cohort random sample), the fourth quartile HR was 2.0 (95% confidence interval [CI], 1.1–3.7). Individual exclusion of participants with baseline heart failure, LVH, eGFR <60 ml/min per 1.73 m², or atrial fibrillation had no material influence on associations (data not shown).

Because they were strongly associated with NT-proBNP, we assessed whether NT-proBNP explained the associations of heart disease and albuminuria with stroke risk. Other
NT-proBNP correlates, heart failure and eGFR <60 mL/min per 1.73 m², could not be assessed because they were not stroke risk factors in this study. In Model 2, the \( \beta \)-coefficients for heart disease before and after adjustment for NT-proBNP were 0.57 and 0.38, indicating 38\% reduction. The coefficients for albuminuria were 0.37 and 0.17, indicating 54\% reduction.

The Figure shows joint associations of NT-proBNP with stroke risk. Participants with NT-proBNP in the top quartile plus LVH, hypertension, or smoking had a stroke HR (95\% CI) of 1.8 for atrial fibrillation, 4\% for atrial fibrillation, and 29\% for hypertension. These observations were larger than in some other studies, even after controlling for a larger number of covariates (kidney function, atrial fibrillation, LVH). The Rotterdam Study demonstrated a 2.9-fold increased risk of ischemic stroke in the analyses adjusting for risk factors including atrial fibrillation and coronary disease, but not kidney disease.7

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To provide a perspective on NT-proBNP and stroke prediction, in this cohort, the HR of stroke was 1.5 for atrial fibrillation and 1.8 for hypertension.20 Based on their prevalences in the cohort, the population-attributable risk percentages for these 3 risk factors are 33\% for NT-proBNP in the fourth quartile, 4\% for atrial fibrillation, and 29\% for hypertension.

Results of this contemporary cohort were almost identical to an ARIC report,7 most notably for cardioembolic stroke. ARIC enrolled a younger cohort in 1987 to 1989: among 10902 stroke-free participants, with 444 ischemic stroke outcomes, NT-proBNP in the top quintile was associated with a 2.9-fold increased risk of ischemic stroke in the analyses adjusting for risk factors including atrial fibrillation and coronary disease, but not kidney disease.7 Our observed associations were larger than in other studies, even after controlling for a larger number of covariates (kidney function, atrial fibrillation, LVH). The Rotterdam Study demonstrated a 2.9-fold increased stroke risk in elderly men and women for BNP in the top tertile.4 The Framingham Offspring Study assessed only 53 outcomes of combined stroke plus transient ischemic attack and reported a 2.1-fold increased risk for BNP above the 80th percentile. In a Japanese study, associations were present for men but not women, a finding we did not confirm with our much larger sample size. Only ARIC included substantial numbers of black
participants, and they also found no racial difference in the association of NT-proBNP with stroke.

An association of NT-proBNP with the risk of cardioembolic stroke may not be surprising given the correlation of NT-proBNP with cardiac function. The association of NT-proBNP with other types of stroke may be because of shared risk factors, unknown mechanisms for these strokes, or unknown effects of NT-proBNP. Increasing evidence suggests causal relationships of natriuretic peptides with endothelial permeability, which might predispose to atherosclerosis, for example.21

There may be applications for NT-proBNP measurement in stroke prediction in select populations such as those with atrial fibrillation. In Randomized Evaluation of Long-term Anticoagulation Therapy (RE-LY), among 6189 patients with atrial fibrillation, 183 had stroke or systemic embolism during follow-up, and those with baseline NT-proBNP levels in the top quartile had a 2.4-fold increased risk of this outcome.22 Although we had few stroke events among those with baseline atrial fibrillation, heart disease, eGFR <60 mL/min per 1.73 m², and albuminuria. One other study recently reported higher NT-proBNP with both albuminuria and lower eGFR, independent of each other.26 In that study, NT-proBNP was also associated with the risk of future coronary events, independent of albuminuria or kidney function. Authors speculated that renal function impairment causes cardiac dysfunction and higher NT-proBNP. We observed no impact of adjustment for kidney function markers on the association of NT-proBNP with stroke, suggesting separate causal pathways. Adjustment for NT-proBNP reduced the risk estimate for albuminuria and stroke risk by 54%, suggesting that both biomarkers reflect vascular dysfunction, but that NT-proBNP more likely represents a factor involved in the causal path biology for stroke. This causal pathway may be related to clinical or subclinical atherosclerosis, not kidney disease, as supported by the reduction in the risk of stroke related to heart disease when NT-proBNP was accounted for. More research is needed.

Strengths of this study include the large geographically dispersed cohort of blacks and whites followed prospectively after extensive baseline data collection. Events were carefully adjudicated using medical records, minimizing misclassification bias and allowing accurate pathogenetic subtyping. Cohort retention was high, with only 12.7% cumulative dropout as of January 2011. We used measured levels of potential confounders. Limitations must be considered. Results only generalize to black and white Americans. NT-proBNP was measured once, so we could not control for regression dilution bias, although this would only bias results to the null hypothesis, making observed association underestimates. Prevalent heart

| Table 3. Hazard Ratio (95% Confidence Interval) of Ischemic Stroke by Baseline NT-ProBNP* |
|-------------------------------------|-----|-----|-----|-----|-----|-----|
| NT-ProBNP Quartiles†               | 1   | 2   | 3   | 4   | P for Trend |
| All ischemic stroke, n             | 60  | 82  | 110 | 294 |             |
| Model 1 of cohort                  | Reference | 1.3 (0.9–1.9) | 1.7 (1.1–2.5) | 3.9 (2.6–5.8) | <0.001 |
| Model 2                            | Reference | 1.1 (0.7–1.7) | 1.3 (0.8–2.0) | 2.9 (1.9–4.5) | <0.001 |
| Model 3                            | Reference | 1.1 (0.7–1.9) | 1.2 (0.8–1.9) | 2.9 (1.8–4.5) | <0.001 |
| Small vessel stroke, n             | 8   | 10  | 2   | 47  |             |
| Model 2                            | Reference | 2.2 (0.97–5.1) | 1.7 (0.7–5.3) | 2.8 (0.99–5.3) | 0.17  |
| Large vessel stroke, n             | 12  | 7   | 24  | 47  |             |
| Model 2                            | Reference | 1.0 (0.3–3.0) | 1.5 (0.5–4.4) | 3.5 (1.3–9.0) | 0.02  |
| Cardioembolic stroke, n            | 5   | 9   | 18  | 110 |             |
| Model 2                            | Reference | 1.2 (0.3–4.7) | 1.8 (0.5–6.4) | 9.1 (2.9–28.2) | <0.001 |
| Unclassified stroke, n             | 34  | 43  | 53  | 124 |             |
| Model 2                            | Reference | 1.1 (0.6–1.9) | 1.0 (0.6, 1.8) | 2.1 (1.2, 3.8) | 0.01  |

*Model 1 adjusted for age, race, age–race interaction, and sex. Model 2 added income, education, smoking, hypertension medication use, systolic blood pressure, atrial fibrillation, left ventricular hypertrophy, diabetes mellitus, and prevalent heart disease. Model 3 added estimated glomerular filtration rate, albuminuria, and heart failure. NT-proBNP indicates N-terminal pro–B-type natriuretic peptide.
†Quartile cut points as in Table 1.

Figure. Joint associations of N-terminal pro–B-type natriuretic peptide (NT-proBNP) in the fourth quartile and other risk factors with ischemic stroke. LVH indicates left ventricular hypertrophy.
failure was not associated with higher NT-proBNP, possibly because of the proximal definition used. We could not account for postbaseline atrial fibrillation to determine whether this was the cause of the large association between NT-proBNP and cardioembolic stroke, although this only partly explained the large association observed by the ARIC investigators.7

Conclusions

There was a substantial association of higher NT-proBNP with risk of ischemic stroke in this study. The attributable risk for NT-proBNP in the fourth quartile was similar to that of hypertension. Along with other findings,6-7,22,25,26 and data on risk prediction of other cardiovascular diseases,2,12,17-30 there is potential for wide clinical application of NT-proBNP testing.

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Disclosures

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