C-Reactive Protein and Risk of First-Ever Ischemic and Hemorrhagic Stroke in a General Japanese Population

The Hisayama Study

Yoshiyuki Wakugawa, MD; Yutaka Kiyohara, MD, PhD; Yumihiro Tanizaki, MD, PhD; Michiaki Kubo, MD, PhD; Toshiharu Ninomiya, MD, PhD; Jun Hata, MD; Yasufumi Doi, MD, PhD; Ken Okubo, MD; Yoshinori Oishi, MD; Kentaro Shikata, MD; Koji Yonemoto, PhD; Daisuke Maebuchi, MD; Setsuro Ibayashi, MD, PhD; Mitsuo Iida, MD, PhD

Background and Purpose—The role of high-sensitivity C-reactive protein (hsCRP) in the development of stroke is not clearly understood. We investigated the relationship between serum hsCRP levels and stroke occurrence in a general Japanese population.

Methods—We followed 2692 subjects ≥40 years of age for 12 years. The relative risks and 95% CIs for ischemic and hemorrhagic stroke occurrence were calculated according to the hsCRP quintiles.

Results—During the follow-up, 129 first-ever ischemic and 59 hemorrhagic strokes occurred. In men, the age-adjusted incidence of ischemic stroke significantly increased with elevated serum hsCRP levels; the difference between the first and fifth quintiles was statistically significant (1.4 versus 6.6 per 1000 person-years; \( P = 0.02 \)). This association remained significant even after adjustment for other confounding factors, such as age, systolic blood pressure, ECG abnormalities, diabetes, body mass index, total cholesterol, high-density lipoprotein cholesterol, smoking habits, alcohol intake, and regular exercise (adjusted relative risks, 3.11; 95% CI, 1.04 to 9.32; \( P = 0.04 \)). However, such associations were not observed for ischemic stroke in women or in hemorrhagic stroke in either sex. Among male subjects who were both in the fifth hsCRP level and had hypertension, diabetes, obesity, hypercholesterolemia, or a smoking habit, the risk of ischemic stroke was extremely increased, even after adjustment for other risk factors.

Conclusions—Our findings suggest that elevated serum hsCRP levels are an independent risk factor for future ischemic stroke in Japanese men and that the coexistence of a high hsCRP level with another risk factor extremely increases the risk of ischemic stroke. (Stroke. 2006;37:27-32.)

Key Words: C-reactive protein ■ hemorrhage, brain ■ ischemic stroke

C-reactive protein (CRP), an acute-phase reactant, increases significantly in inflammatory disorders and enhances immune reactivity. Recently, the role of endothelial cells and monocytes in the inflammatory process has become better understood, and inflammation has emerged as an important factor in atherosclerosis. Consequently, high-sensitivity CRP (hsCRP) levels have attracted clinical attention as a predictive marker of atherosclerosis. Several epidemiological studies have reported that hsCRP levels were positively associated with the risk of cardiovascular disease. Most of those studies examined coronary heart disease or combined end points of coronary heart disease and ischemic stroke, whereas only a few studies examined ischemic stroke. The subjects of the latter studies were limited to the elderly or men, and we found no studies on hemorrhagic stroke.

The purpose of the present study was to examine the relationship between serum hsCRP levels and the development of ischemic and hemorrhagic stroke in a prospective study of a general population consisting of middle-aged and elderly Japanese men and women.

Study Population

Since 1961, we have been conducting a long-term prospective cohort study of cardiovascular disease in the town of Hisayama, a suburb of Fukuoka City in Southern Japan. In 1988, a screening survey for the present study was performed in the town. A total of 2742 residents ≥40 years of age (80.9% of the total population of this age group) consented to participate in the examination. After excluding 96 subjects with a history of stroke or myocardial infarction and 54...
subjects whose frozen blood samples were insufficient for the measurement of serum hsCRP, the remaining 2592 individuals were enrolled in this study.

Follow-Up Survey
This population was followed up for 12 years, from December 1988 through November 2000, by repeated health examinations or by a daily monitoring system established by the study team and local physicians or members of the Health and Welfare Office for the town. A detailed description of the study methods was published previously.14,15

During the follow-up period, 188 subjects were moved out of town, and only 1 subject declined to be followed up. For subjects who did not undergo regular examinations or who moved out of town, their health status was checked by mail or telephone once a year. When new neurological symptoms were suspected, study-team physicians evaluated the subject’s detailed diagnostic information. The clinical diagnosis of stroke was based on the detailed history, neurological examinations, and ancillary laboratory examinations.

Stroke Classification
Stroke was defined as a sudden onset of nonconvulsive and focal neurological deficit persisting for ≥24 hours and was classified as either ischemic or hemorrhagic (cerebral hemorrhage or subarachnoid hemorrhage). Rare causes of cerebrovascular disease, such as collagen disease, hematologic disorder, trauma, chronic subdural hematoma, or moyamoya disease, were not considered in stroke cases. The diagnosis and classification of stroke were based on clinical information, ancillary laboratory examinations (such as brain imaging including computed tomography and MRI, cerebral angiography, echocardiography, and carotid duplex imaging), and autopsy findings.

During the follow-up period, 188 subjects developed first-ever stroke. During the follow-up, 92 of the 188 first-stroke cases died, and, of these, 71 (77.2%) underwent autopsy examination. The first-stroke cases were classified as 129 ischemic strokes (56 men and 73 women) and 59 hemorrhagic strokes (25 men and 34 women).

Risk Factors
Plasma glucose levels were determined by the glucose-oxidase method, and diabetes mellitus was defined by a 75-g oral glucose tolerance test and by fasting (≥7.0 mmol/L) or postprandial blood glucose level (≥11.1 mmol/L) or by the use of hypoglycemic agents. Total cholesterol and high-density lipoprotein (HDL) cholesterol levels were determined enzymatically. Hypercholesterolemia was defined as a serum cholesterol level of ≥5.69 mmol/L. Serum specimens collected at the time of CRP measurement were stored at −20°C until they were used in 2002. Serum hsCRP levels were analyzed using a modification of the Behring latex-enhanced CRP assay on a Behring nephelometer BN-100 with a 2% interassay coefficient of variation.

Sitting blood pressure was measured 3 times at the right upper arm using a sphygmomanometer after ≥5 minutes of rest; the average of the 3 measurements was used in the analysis. Hypertension was defined as systolic blood pressure of ≥140 mm Hg and diastolic blood pressure of ≥90 mm Hg and current treatment with antihypertensive agents. Height and weight were measured in light clothes without shoes, and the body mass index (BMI, kg/m²) was calculated. Obesity was defined as a BMI of ≥25 kg/m². ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code,16 3-1) and ST depression (4-1,2,3) and atrial fibrillation (8-3).

Information on smoking habits, alcohol intake, and physical activity during leisure time was obtained with the use of a standard questionnaire. Smoking habits and alcohol intake were classified as either current or not. Those subjects engaging in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group.

Statistical Analysis
In both men and women combined, we found a significant interaction between sex and hsCRP levels on the risk of ischemic stroke, so the additional analyses were performed separately for men and women by using sex-specific quintiles of hsCRP: Q1, 0.05 to 0.20; Q2, 0.21 to 0.40; Q3, 0.41 to 0.71; Q4, 0.72 to 1.56; and Q5, 1.57 to 14.20 mg/L for men and 0.05 to 0.17, 0.18 to 0.30, 0.31 to 0.53, 0.54 to 1.09, and 1.10 to 13.00 mg/L, respectively, for women. The incidence rates were calculated by the person-year method and adjusted for age by the direct method using 10-year age groupings. The multivariate-adjusted relative risks (RRs) and 95% CIs were calculated according to the hsCRP quintile distribution, using the stepwise Cox proportional hazards model with P<0.2 required for entering or remaining in the model. The interaction between 2 risk factors on the risk of stroke was tested by the χ² test. A P<0.05 was considered to indicate statistical significance.

Results
The baseline characteristics of the subjects are shown in Table 1. The mean age was 58 years for men and 59 years for women. Compared with women, men had higher mean levels of serum hsCRP and systolic and diastolic blood pressures, as well as higher frequencies of hypertension, ECG abnormalities, diabetes mellitus, current smoking, current drinking, and regular exercise, whereas women had higher mean levels of BMI, total cholesterol, and HDL cholesterol.

Figure 1 shows the age-adjusted incidence rates of first-ever ischemic stroke according to quintiles of baseline serum hsCRP. The incidence rates of ischemic stroke were 1.4, 1.9, 5.8, 4.2, and 6.6 per 1000 person-years from the first to fifth quintiles of hsCRP for men and 2.0, 3.4, 5.4, 2.9, and 2.7 per 1000 person-years, respectively, for women. In men, the incidence of stroke rose significantly with rising serum hsCRP levels (P<0.01 for trend), and the incidence for subjects in the fifth quintile was 5-fold that of subjects in the first quintile (P=0.02). However, such an association was not seen in women (P=0.71 for trend). On the other hand, the age-adjusted incidence rates of first-ever hemorrhagic stroke were 2.4, 1.1, 2.2, 1.9, and 2.7 per 1000 person-years, respectively, for men, and 1.1, 2.6, 1.0, 1.3, and 1.6 per 1000 person-years, respectively, for women, and there were no significant trends in either sex (Figure 2).
Table 2 shows the multivariate-adjusted RRs and their 95% CIs for the development of ischemic and hemorrhagic stroke according to hsCRP quintile categories. In men, the risk of ischemic stroke significantly increased with rising hsCRP levels even after adjustment for age, systolic blood pressure, ECG abnormalities, diabetes, BMI, total cholesterol, HDL cholesterol, smoking habits, alcohol intake, and physical activity ($P_{\text{trend}}<0.02$), and the multivariate-adjusted RR of subjects in the fifth quintile was significantly higher than that of subjects in the first quintile (RR, 3.11; 95% CI, 1.04 to 9.32; $P_{\text{trend}}=0.04$). However, such associations were not observed for ischemic stroke in women or for hemorrhagic stroke in either sex (Table 2). To examine the combined effects of elevated hsCRP levels and other cardiovascular risk factors on ischemic stroke occurrence, we estimated the age-adjusted RRs of ischemic stroke among 4 groups of male subjects according to the presence or absence of a high hsCRP level (the fifth quintile, ≥1.57 mg/L) and each risk factor (Table 3). Compared with the reference group having neither high hsCRP levels nor hypertension, the risk of ischemic stroke for the groups with either high hsCRP levels or hypertension was not significant, but the risk for the group having both high hsCRP levels and hypertension was significantly higher (RR, 2.77; 95% CI, 1.31 to 5.83; $P<0.01$). A similar pattern was observed for the coexistence of high hsCRP levels and diabetes (RR, 4.30; 95% CI, 1.89 to 9.79; $P<0.01$), obesity (RR, 4.00; 95% CI, 1.53 to 10.46; $P<0.01$), hypercholesterolemia (RR, 3.74; 95% CI, 1.71 to 8.19; $P<0.01$), or smoking habits (RR, 2.29; 95% CI, 1.78 to 4.87; $P=0.03$). There were significant interactions between high hsCRP levels and diabetes ($\chi^2=5.370; P=0.02$), as well as hypercholesterolemia ($\chi^2=6.052; P=0.01$), and a marginally significant interaction ($\chi^2=3.39; P=0.06$) between high hsCRP levels and hypertension. However, interactions for obesity and smoking were not significant. These associations were substantially unchanged even after adjustment for other risk factors in the multivariate analysis.

### Discussion

In a 12-year follow-up examination of a general Japanese population, we demonstrated that elevation of serum hsCRP levels was an independent risk factor for future ischemic stroke in men but not in women, whereas there was no association between serum hsCRP levels and the risk of future hemorrhagic stroke in either sex. Moreover, the coexistence of a high hsCRP level and another risk factor, such as hypertension, obesity, diabetes, hypercholesterolemia, or smoking, extremely increased the risk of future ischemic stroke in our male subjects.

Recently, the Framingham Study and Cardiovascular Health Study, both which had elderly subjects (mean age,
69.8 and 72.6 years, respectively), and a nested case-control study of Japanese-American men in Hawaii have investigated the association between hsCRP level and the risk of future ischemic stroke. In those studies, the elevation of serum hsCRP was clearly associated with ischemic stroke in men, which support our findings. For women, on the other hand, the effects of high levels of serum hsCRP on ischemic stroke were ambiguous. In the Framingham study women, hsCRP levels were significantly associated with the risk of ischemic stroke, whereas no significant association was observed for the women in the Cardiovascular Health Study, which was in accord with the findings of our study. Recent clinical evidence has shown that endogenous estrogen protects the development of atherosclerosis and that estrogen induces the elevation of hsCRP levels. In women, such conflicting effects of sex hormone might weaken the association of hsCRP elevation with ischemic stroke. Another reason for the sex difference in the risk of ischemic stroke might stem from the difference in the atherosclerotic process between men and women. Generally, it is considered that atherosclerosis is more severe in men than in women. Thus, it may be easier to detect the association between hsCRP levels and ischemic stroke in men.

In our subjects, we did not find a clear association between hsCRP levels and hemorrhagic stroke occurrence. Because cerebral hemorrhage develops from the rupture of small vessels, such as cerebral perforating arteries, damaged by hypertension causing lipohyalinosis, or by amyloid angiopathy, it is suggested that elevated hsCRP levels have little or no association with small vessel disease. Although hypertension and smoking may accelerate the development and growth of intracranial aneurysm, which is a main cause of subarachnoid hemorrhage, the association between atherosclerosis and intracranial aneurysm is considered weak. Thus, our finding that there is no association between serum hsCRP levels and hemorrhagic stroke is reasonable.

Our stratified analysis showed an extremely increased risk of ischemic stroke in men who have both a high-hsCRP level and another risk factor. Although the mechanism underlying this phenomenon is not clearly understood, several possible explanations have been proposed. Because inflammation is strongly related to atherosclerosis, elevated hsCRP levels may reflect the existence of advanced atherosclerosis induced by other cardiovascular risk factors. Accordingly, it is conceivable that the coexistence of elevated hsCRP levels and other risk factors is a marker of a group at high risk of atherosclerosis, and, thus, the risk of ischemic stroke is considerably high in that group. Additionally, recent clinical evidence has shown that endogenous estrogen protects the development of atherosclerosis and that estrogen induces the elevation of hsCRP levels. In women, such conflicting effects of sex hormone might weaken the association of hsCRP elevation with ischemic stroke. Another reason for the sex difference in the risk of ischemic stroke might stem from the difference in the atherosclerotic process between men and women. Generally, it is considered that atherosclerosis is more severe in men than in women. Thus, it may be easier to detect the association between hsCRP levels and ischemic stroke in men.

In our subjects, we did not find a clear association between hsCRP levels and hemorrhagic stroke occurrence. Because cerebral hemorrhage develops from the rupture of small vessels, such as cerebral perforating arteries, damaged by hypertension causing lipohyalinosis, or by amyloid angiopathy, it is suggested that elevated hsCRP levels have little or no association with small vessel disease. Although hypertension and smoking may accelerate the development and growth of intracranial aneurysm, which is a main cause of subarachnoid hemorrhage, the association between atherosclerosis and intracranial aneurysm is considered weak. Thus, our finding that there is no association between serum hsCRP levels and hemorrhagic stroke is reasonable.

Our stratified analysis showed an extremely increased risk of ischemic stroke in men who have both a high-hsCRP level and another risk factor. Although the mechanism underlying this phenomenon is not clearly understood, several possible explanations have been proposed. Because inflammation is strongly related to atherosclerosis, elevated hsCRP levels may reflect the existence of advanced atherosclerosis induced by other cardiovascular risk factors. Accordingly, it is conceivable that the coexistence of elevated hsCRP levels and other risk factors is a marker of a group at high risk of atherosclerosis, and, thus, the risk of ischemic stroke is considerably high in that group. Additionally, recent clinical evidence has shown that endogenous estrogen protects the development of atherosclerosis and that estrogen induces the elevation of hsCRP levels. In women, such conflicting effects of sex hormone might weaken the association of hsCRP elevation with ischemic stroke. Another reason for the sex difference in the risk of ischemic stroke might stem from the difference in the atherosclerotic process between men and women. Generally, it is considered that atherosclerosis is more severe in men than in women. Thus, it may be easier to detect the association between hsCRP levels and ischemic stroke in men.
reviews, as well as experimental and clinical studies, have shown that inflammation is directly associated with the development of atherosclerosis and instability of atheroma. It is, therefore, speculated that chronic inflammation directly and extremely enhances the risk of ischemic stroke by such atherogenic effects of inflammation in people whose arterial walls have already been damaged by other risk factors.

Several limitations of our study should be discussed. The primary limitation is that our findings are based on a 1-time measurement of serum hsCRP, which may not accurately reflect the status of the study participants. However, this source of variability could not account for the relationship observed in the present study, because a random misclassification of such nature would tend to underestimate study findings and bias the results toward the null hypothesis. Thus, the true association may be stronger than that observed in our study. A second limitation is that the serum samples were measured after being stored at $-20^\circ C$ for a long period. However, the Reykjavik Study confirmed the stability of CRP concentrations in serum preserved at this temperature for an average of 12 years. The last limitation is that our study lacked information on drug use, which could affect serum CRP levels. It is known that several medications, including statin, angiotensin-converting enzyme inhibitors, fibrates, niacin, thiazolidinedione, and estrogen/progestogen hormone can alter CRP levels. However, these medications were rarely used in our country in 1988, when the serum samples for our study were collected. This suggests that such a bias did not invalidate the present findings.

In conclusion, our study found that, in a general Japanese population, the elevation of serum hsCRP levels was an independent risk factor for future ischemic stroke in men but not for hemorrhagic stroke in either sex. The addition of elevated serum hsCRP levels to the risk factor profile may significantly increase the predictability of ischemic stroke. Moreover, our study revealed that the risk of future ischemic stroke was considerably high in subjects who had both high-hsCRP levels and another risk factor. For such individuals, an elevated serum hsCRP level may provide additional

| TABLE 3. Age-Adjusted RRs of First-Ever Ischemic Stroke according to High-Sensitivity C-Reactive Protein Levels and Risk Factors in Men |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Risk Factor     | CRP Levels      | Events/Populations (n) | RR  | 95% CI | $P$ Value |
| Hypertension    |                 |                 |     |       |            |
| No              | Low             | 16/472          | 1.00|       |            |
| Yes             | Low             | 22/363          | 1.34| 0.69 to 2.56 | 0.39 |
| No              | High            | 5/105           | 1.27| 0.46 to 3.47 | 0.65 |
| Yes             | High            | 13/96           | 2.77| 1.31 to 5.83 | <0.01 |
| Diabetes mellitus|                 |                 |     |       |            |
| No              | Low             | 30/719          | 1.00|       |            |
| Yes             | Low             | 8/116           | 1.65| 0.75 to 3.59 | 0.21 |
| No              | High            | 11/167          | 1.42| 0.71 to 2.84 | 0.32 |
| Yes             | High            | 7/34            | 4.30| 1.89 to 9.79 | <0.01 |
| Obesity         |                 |                 |     |       |            |
| No              | Low             | 47/635          | 1.00|       |            |
| Yes             | Low             | 11/200          | 1.91| 0.93 to 3.93 | 0.08 |
| No              | High            | 13/162          | 1.69| 0.87 to 3.29 | 0.12 |
| Yes             | High            | 5/39            | 4.00| 1.53 to 10.46| <0.01 |
| Hypercholesterolemia |              |                 |     |       |            |
| No              | Low             | 31/617          | 1.00|       |            |
| Yes             | Low             | 7/218           | 0.77| 0.34 to 1.75 | 0.54 |
| No              | High            | 10/145          | 1.15| 0.56 to 2.35 | 0.71 |
| Yes             | High            | 5/56            | 3.74| 1.71 to 8.19 | <0.01 |
| Current smoking |                 |                 |     |       |            |
| No              | Low             | 21/432          | 1.00|       |            |
| Yes             | Low             | 17/403          | 1.11| 0.59 to 2.12 | 0.74 |
| No              | High            | 8/87            | 1.48| 0.65 to 3.36 | 0.35 |
| Yes             | High            | 10/114          | 2.29| 1.78 to 4.87 | 0.03 |

CRP levels: “high” indicates the fifth quintile; low, the first to fourth quintiles. Hypertension: systolic blood pressure $\geq 140$ mm Hg, or diastolic blood pressure $\geq 90$ mm Hg, or current use of antihypertensive agents. Diabetes: fasting blood glucose $\geq 7.0$ mmol/L, or postprandial blood glucose level $\geq 11.1$ mmol/L, or current use of hypoglycemic agents. Obesity: BMI $\geq 25$ kg/m². Hypercholesterolemia: total cholesterol level $\geq 5.69$ mmol/L.
motivation for both the treating physician and the patient to control these risk factors strictly.

Acknowledgments
This study was supported in part by a Special Coordination Fund for Promoting Science; a Fund for Technology and Innovative Development Project in Life Sciences from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; and by a Grant from the Takeda Medical Research Foundation. We thank the residents of the town of Hisayama for their cooperation in the study, as well as the staff of the Division of Health and Welfare Office of Hisayama for their cooperation in the study.

References
C-Reactive Protein and Risk of First-Ever Ischemic and Hemorrhagic Stroke in a General Japanese Population: The Hisayama Study
Yoshiyuki Wakugawa, Yutaka Kiyohara, Yumihiro Tanizaki, Michiaki Kubo, Toshiharu Ninomiya, Jun Hata, Yasufumi Doi, Ken Okubo, Yoshinori Oishi, Kentaro Shikata, Koji Yonemoto, Daisuke Maebuchi, Setsuro Ibayashi and Mitsuo Iida

Stroke. 2006;37:27-32; originally published online November 23, 2005;
doi: 10.1161/01.STR.0000194958.88216.87
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/37/1/27

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/