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Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular hyperintensities: a cross-sectional study

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Keywords: Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity, White matter intensities, stroke

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Abstract

Objective: Brain white matter hyperintensities (WMHs) can be divided into periventricular hyperintensity (PVH) and deep-and-subcortical white matter hyperintensity (DSWMH), and the former contribute more to cognitive dysfunction and infarction risk. We conducted the present investigation to define the relationship between PVH and DSWMH.

Design: Cross-sectional study.

Setting: University hospital

Participants: We prospectively enrolled 228 healthy Japanese volunteer subjects with relative risk values (RRVs) > 0.5.

Primary outcome measures: We investigated whether it is possible to use the RRV to predict PVH and DSWMH.

Results: Among 228 subjects, 103 (45.1%) and 157 (68.8%) exhibited PVH and DSWMH, respectively. Age, body mass index (BMI), and PVH were significant independent determinants of RRV. A significant odds ratio (OR) for PVH was noted in the highest RRV tertile compared with the lowest one after adjusting for potential confounding factors. A significant OR for high predicted PVH risk was also found for RRV level as well.

Conclusion: Elevated RRV levels were significantly associated with increased predicted PVH, suggesting that measuring the plasma PCAcro, IL-6, and CRP levels may be useful for identifying Japanese at high risk for PVH.

Strengths and limitations of this study:

Using the RRV at clinical level, we investigate to evaluate WMHs.

We provided the first evidence that RRV is associate with PVH rather than DSWMH.

These data are obtained from cases of cautious health care in Asian people and may not be applicable to populations of poor health.

Keywords: Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity

1. Introduction

A number of large-scale clinical studies have demonstrated that white matter hyperintensities (WMHs) are associated with high stroke risk [1-3]. The results of the large-scale, multicenter open trial PICA study[4] conducted in Japan suggest that the Fazekas-classified periventricular hyperintensities (PVHs) and deep-and-subcortical white matter hyperintensities (DSWMHs)[5] are related to the risk of symptomatic brain infarction (SBI). In the Rotterdam Scan study on elderly subjects with no history of stroke, conducted by magnetic resonance imaging (MRI) for 4.2 years, the proportional hazard ratio of stroke occurrence after adjustment of comorbid factors was 4.7 (95% confidence interval [CI], 2.0–11.2) in PVH and 3.6 (CI, 1.4–9.2) in DSWMH[6]. Unlike DSWMH, PVH is associated with cognitive dysfunction[7]. In other studies, associations were separately assessed for PVH and DSWMH and was significant only for PVH, which was related to decreased processing speed and executive

function [8 9]. Additionally, PVH predicted poorer functional outcome after stroke both in the acute and chronic phases, independently of DSWMH [10 11]. A Chiba University group reported that the relative risk value (RRV) measured based on protein-conjugated acrolein (PC-Acro) together with interleukin-6 (IL-6) and C-reactive protein (CRP) can be used to predict the stroke risk factors of silent brain infarction (SBI), carotid atherosclerosis (CA), and WMH with high sensitivity and specificity [12]. We measured plasma PCAcro, IL-6 and CRP, analyzed the measurements in conjunction with age to determine whether it is possible to use the RRV to predict PVH and DSWMH.

2. Materials and methods

2.1. Subjects and blood sampling

We examined 228 elderly volunteers (78 women and 150 men, age 65.0 ± 7.0 years, range 31–83 years). All these subjects were healthy volunteers living independently at home without apparent history of stroke, cardiovascular disease, or malignancy. Subjects with RRV > 0.5 were enrolled prospectively. Informed consent was provided by each subject, and our study protocol was approved by the Ethics Committees of Nippon Medical School Hospital. Experiments were carried out in accordance with the Declaration of Helsinki principles. Blood samples were collected into tubes containing 3 U/mL heparin and centrifuged at 1500 × *g* for 10 min at 4°C.

2.2. PC-Acro, IL-6, and CRP Measurements

Blood samples were drawn from the antecubital vein after overnight fasting. PC-Acro [*N*-(3-formyl-3,4-dehydropiperidino)-lysine (FDPlysine) in protein] was determined as previously described [13] using an ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation, Tokyo, Japan) and 0.01 mL plasma. IL-6 and CRP were quantified using an Endogen Human IL-6 ELISA kit (Pierce Biotechnology, Inc., Rockford, IL, USA) and a human CRP ELISA kit (Alpha Diagnostic International, San Antonio, TX, USA), respectively, according to the manufacturers' protocols. After the reaction was terminated, absorbance was measured at 450 nm using a microplate reader (MTP-800APC, Hitachi, Tokyo, Japan). The biochemical markers from each subject were measured by an investigator who was blinded to the MRI results (Amine Pharma Research Institute, Chiba, Japan). Relative risk value (RRV) was calculated with artificial neural networks by back propagation method using NEUROSIM/L software version 4 (Fujitsu, Tokyo, Japan) [14]. Using the report by a Chiba University group, we worked out predictive RRV in the range of 0–1 [15 16], with the nil as the lowest value as an index of the degree of tissue damage. Values > 0.5 were considered to indicate WMH risk.

Standard enzymatic methods were used to measure the levels of serum total cholesterol, triglycerides, creatinine, and plasma glucose. Serum high-density lipoprotein (HDL) cholesterol level was measured with a direct method, and serum low-density lipoprotein (LDL) cholesterol level was calculated using Friedewald's formula in the 228 subjects with serum triglyceride levels < 400 mg/dL [17]. Diabetes was defined as a fasting plasma glucose level ≥126 mg/dL or the use of

glucose-lowering medications. Dyslipidemia was defined as total cholesterol level ≥220 mg/dL, HDL cholesterol level < 40 mg/dL and a triglyceride level ≥150 mg/dL, as well as the use of lipid-lowering medications. The estimated glomerular filtration rate (eGFR) was calculated for Japanese men as recommended by the Japanese Society of Nephrology [18] and represented as: eGFR (mL/min/1.73 m^2) = 193 × serum $creatinine^{-1.094}$ × $age^{-0.287}$.

2.3. Imaging

All 228 subjects underwent T1- and T2-weighted MRI and fluid-attenuated inversion recovery (FLAIR) at the Nippon Medical School Hospital, Japan, within 1 month after blood sampling. MRI was performed as described previously [10]. PVH and DSWMH were defined as hyperintense areas on T2 and FLAIR images without any abnormality on T1 [Fazekas,16] in subjects without neurological signs and/or symptoms. The 228 subjects were classified into 103 PVH subjects (38 women and 65 men, aged 68.2 ± 6.0 years, RRV 0.75 ± 0.11) and 157 WMH subjects (61 women and 96 men, aged 66.7 ± 5.8 years, RRV 0.71 ± 0.12).

2.4. Statistics

All statistical tests were performed using the JMP9.02 software program (SAS Institute, Cary, NC, USA). Continuous variables except for triglyceride levels were expressed as means ± standard deviation (SD). Triglyceride levels were transformed to the common logarithm for statistical analysis and are expressed as the geometric mean because of their skewed distribution. Categorical data are

expressed as the number of subjects (percent of total). The clinical characteristics for each RRV tertile were compared by analysis of variance (ANOVA) for continuous variables and χ^2 test for categorical variables. The RRVs between the two groups were compared by Student's t-tests or by ANOVA followed by multiple comparisons with the Bonferroni correction between the two groups. Correlations between RRV and other variables were evaluated with the Pearson's moment correlation coefficient. Factors with a P value < 0.20 as determined by Pearson's correlation analysis were included in a multiple linear regression analysis to identify independent determinants of the RRV. Logistic regression analysis was performed to obtain the odds ratios (ORs) for PVH and DSWMH in the three tertiles. All statistical tests were two-sided, and a P value < 0.05 was considered as significant.

3. Results

The study subjects were divided into tertiles according to RRV (0.50–0.62, 0.63–0.79, and 0.80–0.90 from the lowest to highest tertile, respectively). The subjects' clinical characteristics are summarized in Table 1. The mean RRV of the entire subject population was 0.71 ± 0.13, and the mean age was 65 ± 7 years. Age, body mass index (BMI), diastolic blood pressure (BP), HDL cholesterol level, triglyceride level, eGFR, and current smoking status were significantly different among the groups. RRVs were significantly higher in subjects with older age, lower eGFR, or PVH (Table 1). A simple correlation analysis showed that RRV was significantly correlated with age, systolic BP, eGFR, and

PVH (Table 2). Multiple linear regression analysis indicated that BMI (β = 0.0026, P = 0.044) and PVH (β = 0.0380, P < 0.0001) were significant independent determinants of RRV.

The results of logistic regression analysis of the association between PVH and RRV are shown in Table 3. Significant, unadjusted ORs for PVH were noted in the third RRV tertile (5.26 [95% CI, 2.66–10.78], P < 0.0001), compared to the first tertile. After adjusting for model 1 (BMI, systolic BP, triglycerides, eGFR, and current smoking status) the ORs in the third RRV tertile remained significant (4.75 [95% CI, 2.33–10.05], P < 0.0001). After adjusting for model 2, we found that the OR in the third RRV tertile 3 was significant (5.26 [95% CI, 2.65–10.83], P < 0.0001). A significant relationship was observed between RRV and PVH (P < 0.05) but no such significance was found between RRV and DSWMH (figure 1).

4. Discussion

The present study demonstrated a significant, positive correlation between RRV and PVH in healthy Japanese volunteers. Notably, the highest RRV level tertile showed a significantly higher OR for a high predicted PVH risk in comparison to the lowest tertile, even after adjusting for multiple confounding factors. These results suggest that RRV is associated with the estimated risk of PVH in healthy Japanese volunteers. A number of clinical and epidemiological studies have examined the predictive value of RRV for the presence of WMH[15 16]. However, those studies assessed WMH prevalence; no studies have shown any significant association of RRV with PVH and DSWMH

separately. In this regard, our results raise the possibility that RRV predicts the risk of PVH in the healthy Japanese population. With respect to age, these biochemical markers provide a good index of the presence of tissue damage related to PVH.

More recent studies focused on WMH location have reported that functional impairment within 1–3 months after stroke correlated with PVH but not with DSWMH [10 19]. PVH WMH, especially PVH, has impacts on early functional recovery after ischemic stroke regardless of the initial stroke severity and other cardiovascular risk factors[11]. Other groups found a significant association between PVH and decreases in processing speed and executive function, but there was no such relationship with DSWMH[8 9]. Why PVH and DSWMH have different relationships with stroke outcome remains unclear, but several theories have been put forward. DSWMH predominantly disrupt short association fibers that link adjacent gyri, while PVH affects long association fibers that connect the more distant cortical areas [20]. Thus, lesions in various white matter locations may disconnect from different neural networks that affect neural repair processes after stroke [21]. In addition, PVHs are related to diminished cerebral vasomotor reactivity and subsequent occurrence of cerebral hypoperfusion [22], while DSWMHs are generally associated with microangiopathy [23]. It is clear that regional hypoperfusion is a good predictor of functional outcome [24]. These findings shed light on why PVH can predict functional stroke outcome and specific cognitive functions [11].

Acrolein induces IL-6 production in astrocytes, macrophages, and endothelial cells, while IL-6 induces CRP production in hepatocytes. Then, CRP stimulates IL-6 production, and IL-6 decreases

acrolein toxicity [25]. Acrolein was thought to be one of the toxic compounds produced from unsaturated fatty acids by active oxygen species such as superoxide anion radical, hydrogen peroxide, and hydroxyl radical[13]. These findings may partially explain the pathophysiological mechanisms underlying the association between PVH and the three biomarkers assessed in the present study. Further investigation will be needed for a better understanding of their interrelationship.

Our multiple linear regression analysis showed that RRV was independently associated with BMI and PVH. Although obesity is believed to be an independent cardiovascular risk factor [26], it is still controversial whether BMI is a significant risk factor for stroke [27 28]. BMI was previously reported to be correlated with high RRV [29] which may be caused by vascular degeneration and endothelial dysfunction associated with hypertension and metabolic disorders. Subjects with metabolic syndrome are generally defined as those who have abdominal obesity and two additional metabolic disorders including hypertension, dyslipidemia, and hyperglycemia [30 31].

Our study has some potential limitations. Because it was a cross-sectional investigation, we could not determine a causal relationship between increased RRV and PVH risk. In addition, the population included healthy Japanese volunteers only. Therefore, it is unclear whether the results can be extrapolated to other populations of poor health, patients with cardiovascular diseases, or other ethnic groups. Despite these potential limitations, our findings support the conclusion that elevated

RRV is significantly associated with PVH in healthy Japanese volunteers. These results suggest that RRV measurement may be useful for identifying PVH in the general population. This would allow clinicians to follow patients who may be at risk for stroke and cognitive dysfunction.

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Contributorship Statement

Conception and design: AA

Analysis and interpretation: YN, MHA, SO, MU

Writing the article: AA

Critical revision of the article: YN, MM, YK

Final approval of the article: YN, MHA, SO, MU, MM, YK

Statistical analysis: YN, MM

Overall responsibility: AA

Competing Interests

None

Data Sharing Statement

No additional data available

Table 1. Characteristics of the study subjects due to their stroke risk marker level tertile

Item	All	Silent brain infarction RRV tertile			P value*
		Tertile 1	Tertile 2	Tertile 3	
Subjects (n)	228	76	73	79	-
Silent brain infarction relative risk value	0.71 ± 0.13	0.51–0.07**	0.69–0.05**	0.86–0.03**	-
Age (years)	65 ± 7	59 ± 7	66 ± 3	70 ± 6	<0.0001
Male Sex	150 (65.7)	51 (67.1)	52 (71.2)	47 (59.4)	0.299
Body mass index, kg/m^2	24.2 ± 6.4	23.2 ± 2.9	24.6 ± 6.3	24.8 ± 8.6	0.133
Systolic BP, mmHg	123 ± 15	121 ± 14	122 ± 14	126 ± 17	0.275
Diastolic BP, mmHg	75 ± 11	75 ± 9	75 ± 10	75 ± 13	0.947
Hypertension, n (%)	81 (35.6)	23 (30.6)	20 (27.4)	38 (48.1)	0.015
Total cholesterol, mg/dL	208± 32	208 ± 30	208 ± 32	207 ± 34	0.903
LDL cholesterol†, mg/dL	123 ± 29	123 ± 25	123 ± 29	122 ± 33	0.848
HDL cholesterol, mg/dL	58 ± 15	59 ± 17	57 ± 13	60 ± 16	0.385
Triglycerides††, mg/dL	125 (113, 138)	141 (111, 172)	123 (105, 142)	112 (99, 124)	0.425

Dyslipidemia, n (%)	55 (24.1)	19 (25.0)	13 (17.8)	23 (29.1)	0.259
Fasting plasma glucose, mg/dL	100 ± 16	100 ± 14	99 ± 18	100 ± 15	0.754
Diabetes, n (%)	18 (7.8)	3 (3.9)	6 (8.2)	9 (11.3)	0.226
eGFR (mL/min/1.73 m^2)	67.6 ± 12.5	70.2 ± 15.3	67.3 ± 10.6	65.4 ± 10.6	0.027
Current smoking status, n (%)	40 (17.5)	16 (21.0)	12 (16.4)	12 (15.1)	0.603
PVH, n (%)	103 (45.1)	18 (23.6)	36 (49.3)	49 (62.0)	<0.0001
DSWMH, n (%)	157 (68.8)	52 (68.4)	50 (68.4)	55 (69.6)	0.983

*Analysis of variance or chi-square test among the ADMA tertile. **Range of a minimum-to-maximum stroke risk marker in each tertile. †n = 228. ††Geometric mean (95% confidence intervals). ‡Statistical analysis was not conducted because of the extremely small number of subjects in each category. BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; RRV, relative risk value

Table 2. Correlation coefficients and multiple linear regression analysis of relative risk value with the clinical parameters

Item	Simple correlation analysis		Multiple linear regression analysis	
	Correlation coefficient (r)	P-value	Standardized regression coefficient (β)	P value
Age	0.60	<0.0001	†	
Male Sex	0.05	0.347	††	
BMI	0.11	0.075	0.0026	0.044
Systolic BP	0.14	0.026	0.0009	0.075
Diastolic BP	0.05	0.382	††	
Total cholesterol	-0.03	0.618	††	
LDL cholesterol*	-0.02	0.669	††	
HDL cholesterol	0.02	0.731	††	
Triglycerides**	-0.10	0.130	-0.0001	0.083
Fasting plasma glucose	0.01	0.841	††	
eGFR	-0.13	0.041	-0.0008	0.235
Current smoking status (Yes = 1)	-0.10	0.115	-0.0161	0.158
PVH (Yes = 1)	0.26	<0.0001	0.0380	<0.0001
DSWMH (Yes = 1)	0.02	0.689	††	

*n = 228. **Log-transformed value. †Not included in the multiple linear regression analysis to avoid multicollinearity with PVH relative risk value. ††Not included in the multiple linear regression analysis because their P values were ≥0.20 in the simple correlation analysis. BMI, body mass index; BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Unadjusted and adjusted PVH ORs in each silent brain infarction relative risk value tertile

Item		Unadjusted			Adjusted*			Adjusted**		
		OR	95% CI	P-value	OR	95% CI	P value	OR	95% CI	P value
	Tertile 1	1.00	reference	-	1.00	reference	-	1.00	reference	-
	Tertile 2	3.13	1.57–6.41	0.0014	3.68	1.77–7.97	0.0004	2.95	1.11–8.35	0.02
	Tertile 3	5.26	2.66–10.78	<0.0001	4.75	2.33–10.05	<0.0001	5.26	2.65–10.83	<0.0001

* Adjusted for BMI, systolic BP, triglycerides, eGFR, and current smoking status
** Adjusted for BMI
BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; OR, odds ratio;
PVH, periventricular hyperintensity

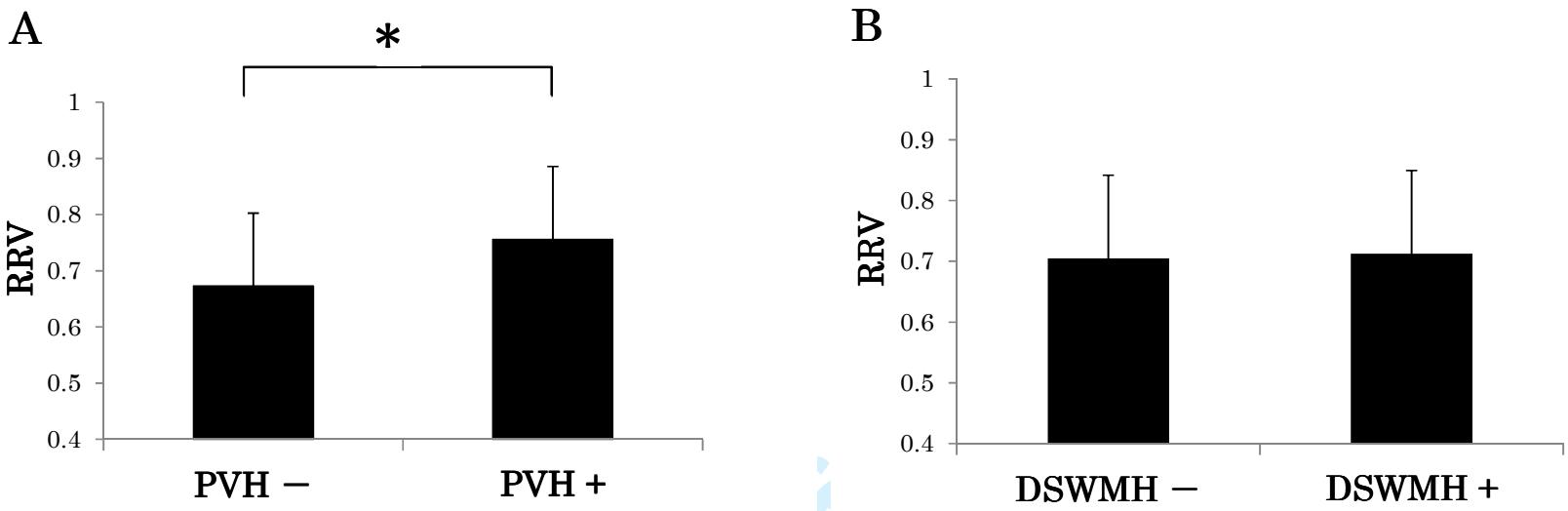

Figure 1. Correlationship between the RRV and the PVH (A). Correlationship between the RRV and DSWMH (B)

*Significant at $P < 0.05$

References

1. Wong TY, Klein R, Sharrett AR, Couper DJ, Klein BE, Liao DP, et al. Cerebral white matter lesions, retinopathy, and incident clinical stroke. *Jama* 2002;288(1):67-74.
2. Gouw AA, van der Flier WM, Fazekas F, van Straaten EC, Pantoni L, Poggesi A, et al. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke* 2008;39(5):1414-20.
3. Bokura H, Yamaguchi S, Kobayashi S. Electrophysiological correlates for response inhibition in a Go/NoGo task. *Clin Neurophysiol* 2001;112(12):2224-32.
4. Shinohara Y, Tohgi H, Hirai S, Terashi A, Fukuuchi Y, Yamaguchi T, et al. Effect of the Ca antagonist nilvadipine on stroke occurrence or recurrence and extension of asymptomatic cerebral infarction in hypertensive patients with or without history of stroke (PICA Study). 1. Design and results at enrollment. *Cerebrovasc Dis* 2007;24(2-3):202-9.
5. Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. *Neurology* 1999;53(1):132-9.
6. Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. *Stroke* 2003;34(5):1126-9.
7. Fukuda H, Kobayashi S, Okada K, Tsunematsu T. Frontal white matter lesions and dementia in lacunar infarction. *Stroke* 1990;21(8):1143-9.
8. van den Heuvel DM, ten Dam VH, de Craen AJ, Admiraal-Behloul F, Olofsen H, Bollen EL, et al. Increase in periventricular white matter hyperintensities parallels decline in mental processing speed in a non-demented elderly population. *J Neurol Neurosurg Psychiatry* 2006;77(2):149-53.
9. Debette S, Bombois S, Bruandet A, Delbeuck X, Lepoittevin S, Delmaire C, et al. Subcortical hyperintensities are associated with cognitive decline in patients with mild cognitive impairment. *Stroke* 2007;38(11):2924-30.
10. Kissela B, Lindsell CJ, Kleindorfer D, Alwell K, Moomaw CJ, Woo D, et al. Clinical prediction of functional outcome after ischemic stroke: the surprising importance of periventricular white matter disease and race. *Stroke* 2009;40(2):530-6.
11. Liou LM, Chen CF, Guo YC, Cheng HL, Lee HL, Hsu JS, et al. Cerebral white matter hyperintensities predict functional stroke outcome. *Cerebrovasc Dis* 2010;29(1):22-7.
12. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr. 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* 1999;340(1):14-22.
13. Uchida K, Kanematsu M, Morimitsu Y, Osawa T, Noguchi N, Niki E. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J Biol Chem* 1998;273(26):16058-66.
14. Ellenius J, Groth T, Lindahl B, Wallentin L. Early assessment of patients with suspected acute myocardial infarction by biochemical monitoring and neural network analysis. *Clin Chem* 1997;43(10):1919-25.
15. Yoshida M, Tomitori H, Machi Y, Katagiri D, Ueda S, Horiguchi K, et al. Acrolein, IL-6 and CRP as markers of silent brain infarction. *Atherosclerosis* 2009;203(2):557-62.
16. Yoshida M, Higashi K, Kobayashi E, Saeki N, Wakui K, Kusaka T, et al. Correlation between images of silent brain infarction, carotid atherosclerosis and white matter hyperintensity, and plasma levels of acrolein, IL-6 and CRP. *Atherosclerosis* 2010;211(2):475-9.

17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502.
18. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53(6):982-92.
19. Kang HJ, Stewart R, Park MS, Bae KY, Kim SW, Kim JM, et al. White matter hyperintensities and functional outcomes at 2 weeks and 1 year after stroke. *Cerebrovasc Dis* 2013;35(2):138-45.
20. Brodal P. *The central nervous system : structure and function*. 3rd ed. Oxford: Oxford University Press, 2004.
21. Chollet F, DiPiero V, Wise RJ, Brooks DJ, Dolan RJ, Frackowiak RS. The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography. *Ann Neurol* 1991;29(1):63-71.
22. Gerdes VE, Kwa VI, ten Cate H, Brandjes DP, Buller HR, Stam J. Cerebral white matter lesions predict both ischemic strokes and myocardial infarctions in patients with established atherosclerotic disease. *Atherosclerosis* 2006;186(1):166-72.
23. Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* 1993;43(9):1683-9.
24. Giubilei F, Lenzi GL, Di Piero V, Pozzilli C, Pantano P, Bastianello S, et al. Predictive value of brain perfusion single-photon emission computed tomography in acute ischemic stroke. *Stroke* 1990;21(6):895-900.
25. Saiki R, Hayashi D, Ikuo Y, Nishimura K, Ishii I, Kobayashi K, et al. Acrolein stimulates the synthesis of IL-6 and C-reactive protein (CRP) in thrombosis model mice and cultured cells. *J Neurochem* 2013.
26. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006;113(6):898-918.
27. Haapaniemi H, Hillbom M, Juvela S. Lifestyle-associated risk factors for acute brain infarction among persons of working age. *Stroke* 1997;28(1):26-30.
28. Rexrode KM, Hennekens CH, Willett WC, Colditz GA, Stampfer MJ, Rich-Edwards JW, et al. A prospective study of body mass index, weight change, and risk of stroke in women. *Jama* 1997;277(19):1539-45.
29. Yoshida M, Mizoi M, Saiki R, Kobayashi E, Saeki N, Wakui K, et al. Relationship between metabolic disorders and relative risk values of brain infarction estimated by protein-conjugated acrolein, IL-6 and CRP together with age. *Clin Chim Acta* 2011;412(3-4):339-42.
30. Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. *Circulation* 2004;109(1):42-6.
31. Kwon HM, Kim BJ, Lee SH, Choi SH, Oh BH, Yoon BW. Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke* 2006;37(2):466-70.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page4
Methods			
Study design	4	Present key elements of study design early in the paper	Page5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Page5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page6
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page4
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page8-9
		(*b*) Report category boundaries when continuous variables were categorized	Page8-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page8-9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page8-9
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page11
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular hyperintensities: a cross-sectional study

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Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular hyperintensities: a cross-sectional study

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Keywords: Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity, White matter intensities, stroke

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Abstract

Objective: Brain white matter hyperintensities (WMHs) can be divided into periventricular hyperintensity (PVH) and deep-and-subcortical white matter hyperintensity (DSWMH), and the former contribute more to cognitive dysfunction and infarction risk. We conducted the present investigation to define the relationship between PVH and DSWMH.

Design: Cross-sectional study.

Setting: University hospital

Participants: We prospectively enrolled 228 healthy Japanese volunteer subjects with relative risk values (RRVs) > 0.5.

Primary outcome measures: We investigated whether it is possible to use the RRV to predict PVH and DSWMH.

Results: Among 228 subjects, 103 (45.1%) and 157 (68.8%) exhibited PVH and DSWMH, respectively. Age, body mass index (BMI), and PVH were significant independent determinants of RRV. A significant odds ratio (OR) for PVH was noted in the highest RRV tertile compared with the lowest one after adjusting for potential confounding factors. A significant OR for high predicted PVH risk was also found for RRV level as well.

Conclusion: Elevated RRV levels were significantly associated with increased predicted PVH, suggesting that measuring the plasma PCAcro, IL-6, and CRP levels may be useful for identifying

Japanese at high risk for PVH.

Strengths and limitations of this study:

Using the RRV at clinical level, we investigate to evaluate WMHs.

We provided the first evidence that RRV is associate with PVH rather than DSWMH.

These data are obtained from cases of cautious health care in Asian people and may not be applicable to populations of poor health.

1. Introduction

A number of large-scale clinical studies have demonstrated that white matter hyperintensities (WMHs) are associated with high stroke risk [1-3]. The results of the large-scale, multicenter open trial PICA study[4] conducted in Japan suggest that the Fazekas-classified periventricular hyperintensities (PVHs) and deep-and-subcortical white matter hyperintensities (DSWMHs)[5] are related to the risk of symptomatic brain infarction (SBI). In the Rotterdam Scan study on elderly subjects with no history of stroke, conducted by magnetic resonance imaging (MRI) for 4.2 years, the proportional hazard ratio of stroke occurrence after adjustment of comorbid factors was 4.7 (95% confidence interval [CI], 2.0–11.2) in PVH and 3.6 (CI, 1.4–9.2) in DSWMH[6]. Unlike DSWMH, PVH is associated with cognitive dysfunction[7]. In other studies, associations were separately assessed for PVH and DSWMH and was significant only for PVH, which was related to decreased processing speed and executive function [8 9]. Additionally, PVH predicted poorer functional outcome after stroke both in the acute and chronic phases, independently of DSWMH [10 11]. A Chiba University group reported that the relative risk value (RRV) measured based on protein-conjugated acrolein (PC-Acro) together with interleukin-6 (IL-6) and C-reactive protein (CRP) can be used to predict the stroke risk factors of silent brain infarction (SBI), carotid atherosclerosis (CA), and WMH with high sensitivity and specificity[12]. We measured plasma PCAcro, IL-6 and CRP, analyzed the measurements in conjunction with age to determine whether it is possible to use the RRV to predict PVH and

DSWMH.

2. Materials and methods

2.1. Subjects and blood sampling

We examined 228 adult volunteers (78 women and 150 men, age 65.0 ± 7.0 years, range 31–83 years). All these subjects were healthy volunteers living independently at home without apparent history of stroke, cardiovascular disease, or malignancy. Subjects with RRV > 0.5 were enrolled prospectively. Informed consent was provided by each subject, and our study protocol was approved by the Ethics Committees of Nippon Medical School Hospital. Experiments were carried out in accordance with the Declaration of Helsinki principles. Blood samples were collected into tubes containing 3 U/mL heparin and centrifuged at 1500 × *g* for 10 min at 4°C.

2.2. PC-Acro, IL-6, and CRP Measurements

Blood samples were drawn from the antecubital vein after overnight fasting. PC-Acro [*N*-(3-formyl-3,4-dehydropiperidino)-lysine (FDPlysine) in protein] was determined as previously described [13] using an ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation, Tokyo, Japan) and 0.01 mL plasma. IL-6 and CRP were quantified using an Endogen Human IL-6 ELISA kit (Pierce Biotechnology, Inc., Rockford, IL, USA) and a human CRP ELISA kit (Alpha Diagnostic International, San Antonio, TX, USA), respectively, according to the manufacturers' protocols. After the reaction was terminated, absorbance was measured at 450 nm using a microplate reader

(MTP-800APC, Hitachi, Tokyo, Japan). The biochemical markers from each subject were measured by an investigator who was blinded to the MRI results (Amine Pharma Research Institute, Chiba, Japan). Relative risk value (RRV) was calculated with artificial neural networks by back propagation method using NEUROSIM/L software version 4 (Fujitsu, Tokyo, Japan) [14]. Using the report by a Chiba University group, we worked out predictive RRV in the range of 0–1 [12 15], with the nil as the lowest value as an index of the degree of tissue damage. Values > 0.5 were considered to indicate WMH risk.

Standard enzymatic methods were used to measure the levels of serum total cholesterol, triglycerides, creatinine, and plasma glucose. Serum high-density lipoprotein (HDL) cholesterol level was measured with a direct method, and serum low-density lipoprotein (LDL) cholesterol level was calculated using Friedewald's formula in the 228 subjects with serum triglyceride levels < 400 mg/dL [16]. Diabetes was defined as a fasting plasma glucose level ≥126 mg/dL or the use of glucose-lowering medications. Dyslipidemia was defined as total cholesterol level ≥220 mg/dL, HDL cholesterol level < 40 mg/dL and a triglyceride level ≥150 mg/dL, as well as the use of lipid-lowering medications. The estimated glomerular filtration rate (eGFR) was calculated for Japanese men as recommended by the Japanese Society of Nephrology [17]and represented as: eGFR ($mL/min/1.73\ m^2$) = $193 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287}$.

2.3. Imaging

All 228 subjects underwent T1- and T2-weighted MRI and fluid-attenuated inversion recovery (FLAIR) at the Nippon Medical School Hospital, Japan, within 1 month after blood sampling. MRI was performed as described previously[10]. PVH and DSWMH were defined as hyperintense areas on T2 and FLAIR images without any abnormality on T1[18] in subjects without neurological signs and/or symptoms. The 228 subjects were classified into 103 PVH subjects (38 women and 65 men, aged 68.2 ± 6.0 years, RRV 0.75 ± 0.11) and 157 WMH subjects (61 women and 96 men, aged 66.7 ± 5.8 years, RRV 0.71 ± 0.12). In more detail, 76 among all the subjects had both PVH and DSWMH, the other 23 having silent brain infarction. Also, 22 subjects had a complication of PVH and silent brain infarction, while in 20 subjects there was complication of DSWMH and silent brain infarction.

2.4. Statistics

All statistical tests were performed using the JMP9.02 software program (SAS Institute, Cary, NC, USA). Continuous variables except for triglyceride levels were expressed as means ± standard deviation (SD). Triglyceride levels were transformed to the common logarithm for statistical analysis and are expressed as the geometric mean because of their skewed distribution. Categorical data are expressed as the number of subjects (percent of total). The clinical characteristics for each RRV tertile were compared by analysis of variance (ANOVA) for continuous variables and χ^2 test for categorical variables. The RRVs between the two groups were compared by Student's t-tests or by ANOVA followed by multiple comparisons with the Bonferroni correction between the two groups.

Correlations between RRV and other variables were evaluated with the Pearson's moment correlation coefficient. Factors with a P value < 0.05 as determined by Pearson's correlation analysis were included in a multiple linear regression analysis to identify independent determinants of the RRV. Logistic regression analysis was performed to obtain the odds ratios (ORs) for PVH and DSWMH in the three tertiles. All statistical tests were two-sided, and a P value < 0.05 was considered as significant.

3. Results

The study subjects were divided into tertiles according to RRV (0.50–0.62, 0.63–0.79, and 0.80–0.90 from the lowest to highest tertile, respectively). The subjects' clinical characteristics are summarized in Table 1. The mean RRV of the entire subject population was 0.71 ± 0.13, and the mean age was 65 ± 7 years. Age, body mass index (BMI), diastolic blood pressure (BP), HDL cholesterol level, triglyceride level, eGFR, and current smoking status were significantly different among the groups. RRVs were significantly higher in subjects with older age, lower eGFR, or PVH (Table 1). A simple correlation analysis showed that RRV was significantly correlated with age, systolic BP, eGFR, and PVH (Table 2). Multiple linear regression analysis indicated that BMI ($\beta = 0.0026$, $P = 0.044$) and PVH ($\beta = 0.0380$, $P < 0.0001$) were significant independent determinants of RRV.

The results of logistic regression analysis of the association between PVH and RRV are shown in Table 3. Significant, unadjusted ORs for PVH were noted in the third RRV tertile (5.26 [95% CI,

2.66–10.78], P < 0.0001), compared to the first tertile. After adjusting for model 1 (BMI, systolic BP, triglycerides, eGFR, and current smoking status) the ORs in the third RRV tertile remained significant (4.75 [95% CI, 2.33–10.05], P < 0.0001). After adjusting for model 2, we found that the OR in the third RRV tertile 3 was significant (5.26 [95% CI, 2.65–10.83], P < 0.0001). A significant relationship was observed between RRV and PVH (P < 0.05) but no such significance was found between RRV and DSWMH (figure 1).

4. Discussion

The present study demonstrated a significant, positive correlation between RRV and PVH in healthy Japanese volunteers. Notably, the highest RRV level tertile showed a significantly higher OR for a high predicted PVH risk in comparison to the lowest tertile, even after adjusting for multiple confounding factors. These results suggest that RRV is associated with the estimated risk of PVH in healthy Japanese volunteers. A number of clinical and epidemiological studies have examined the predictive value of RRV for the presence of WMH[12 15]. However, those studies assessed WMH prevalence; no studies have shown any significant association of RRV with PVH and DSWMH separately. In this regard, our results raise the possibility that RRV predicts the risk of PVH in the healthy Japanese population. With respect to age, these biochemical markers provide a good index of the presence of tissue damage related to PVH.

More recent studies focused on WMH location have reported that functional impairment within 1–3

months after stroke correlated with PVH but not with DSWMH [10 19]. PVH WMH, especially PVH, has impacts on early functional recovery after ischemic stroke regardless of the initial stroke severity and other cardiovascular risk factors[11]. Other groups found a significant association between PVH and decreases in processing speed and executive function, but there was no such relationship with DSWMH[8 9]. Why PVH and DSWMH have different relationships with stroke outcome remains unclear, but several theories have been put forward. DSWMH predominantly disrupt short association fibers that link adjacent gyri, while PVH affects long association fibers that connect the more distant cortical areas [20]. Thus, lesions in various white matter locations may disconnect from different neural networks that affect neural repair processes after stroke [21]. In addition, PVHs are related to diminished cerebral vasomotor reactivity and subsequent occurrence of cerebral hypoperfusion [22], while DSWMHs are generally associated with microangiopathy [23]. It is clear that regional hypoperfusion is a good predictor of functional outcome [24]. These findings shed light on why PVH can predict functional stroke outcome and specific cognitive functions [11].

Acrolein induces IL-6 production in astrocytes, macrophages, and endothelial cells, while IL-6 induces CRP production in hepatocytes. Then, CRP stimulates IL-6 production, and IL-6 decreases acrolein toxicity [25]. Acrolein was thought to be one of the toxic compounds produced from unsaturated fatty acids by active oxygen species such as superoxide anion radical, hydrogen peroxide, and hydroxyl radical[13]. These findings may partially explain the pathophysiological mechanisms underlying the association between PVH and the three biomarkers assessed in the present study.

Further investigation will be needed for a better understanding of their interrelationship.

Our multiple linear regression analysis showed that RRV was independently associated with BMI and PVH. Although obesity is believed to be an independent cardiovascular risk factor [26], it is still controversial whether BMI is a significant risk factor for stroke [27 28]. BMI was previously reported to be correlated with high RRV [29] which may be caused by vascular degeneration and endothelial dysfunction associated with hypertension and metabolic disorders. Subjects with metabolic syndrome are generally defined as those who have abdominal obesity and two additional metabolic disorders including hypertension, dyslipidemia, and hyperglycemia [30 31].

Our study has some potential limitations. Because it was a cross-sectional investigation, we could not determine a causal relationship between increased RRV and PVH risk. In addition, the population included healthy Japanese volunteers only. Therefore, it is unclear whether the results can be extrapolated to other populations of poor health, patients with cardiovascular diseases, or other ethnic groups. Despite these potential limitations, our findings support the conclusion that elevated RRV is significantly associated with PVH in healthy Japanese volunteers. These results suggest that RRV measurement may be useful for identifying PVH in the general population. This would allow clinicians to follow patients who may be at risk for stroke and cognitive dysfunction.

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Contributorship

Conception and design: AA
Analysis and interpretation: YN, MHA, SO, MU
Writing the article: AA
Critical revision of the article: YN, MM, YK
Final approval of the article: YN, MHA, SO, MU, MM, YK
Statistical analysis: YN, MM
Overall responsibility: AA

Competing Interests

None

Data Sharing Statement

No additional data available

References

1. Wong TY, Klein R, Sharrett AR, et al. Cerebral white matter lesions, retinopathy, and incident clinical stroke. *Jama* 2002;288(1):67-74.
2. Gouw AA, van der Flier WM, Fazekas F, et al. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke* 2008;39(5):1414-20.
3. Bokura H, Yamaguchi S, Kobayashi S. Electrophysiological correlates for response inhibition in a Go/NoGo task. *Clin Neurophysiol* 2001;112(12):2224-32.
4. Shinohara Y, Tohgi H, Hirai S, et al. Effect of the Ca antagonist nilvadipine on stroke occurrence or recurrence and extension of asymptomatic cerebral infarction in hypertensive patients with or without history of stroke (PICA Study). 1. Design and results at enrollment. *Cerebrovasc Dis* 2007;24(2-3):202-9.
5. Schmidt R, Fazekas F, Kapeller P, et al. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. *Neurology* 1999;53(1):132-9.
6. Vermeer SE, Hollander M, van Dijk EJ, et al. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. *Stroke* 2003;34(5):1126-9.
7. Fukuda H, Kobayashi S, Okada K, et al. Frontal white matter lesions and dementia in lacunar infarction. *Stroke* 1990;21(8):1143-9.
8. van den Heuvel DM, ten Dam VH, et al. Increase in periventricular white matter hyperintensities parallels decline in mental processing speed in a non-demented elderly population. *J Neurol Neurosurg Psychiatry* 2006;77(2):149-53.
9. Debette S, Bombois S, Bruandet A, et al. Subcortical hyperintensities are associated with cognitive decline in patients with mild cognitive impairment. *Stroke* 2007;38(11):2924-30.
10. Kissela B, Lindsell CJ, Kleindorfer D, et al. Clinical prediction of functional outcome after ischemic stroke: the surprising importance of periventricular white matter disease and race. *Stroke* 2009;40(2):530-6.
11. Liou LM, Chen CF, Guo YC, et al. Cerebral white matter hyperintensities predict functional stroke outcome. *Cerebrovasc Dis* 2010;29(1):22-7.
12. Yoshida M, Tomitori H, Machi Y, et al. Acrolein, IL-6 and CRP as markers of silent brain infarction. *Atherosclerosis* 2009;203(2):557-62.
13. Uchida K, Kanematsu M, Morimitsu Y, et al. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J Biol Chem* 1998;273(26):16058-66.
14. Ellenius J, Groth T, Lindahl B, et al. Early assessment of patients with suspected acute myocardial infarction by biochemical monitoring and neural network analysis. *Clin Chem* 1997;43(10):1919-25.

15. Yoshida M, Higashi K, Kobayashi E, et al. Correlation between images of silent brain infarction, carotid atherosclerosis and white matter hyperintensity, and plasma levels of acrolein, IL-6 and CRP. *Atherosclerosis* 2010;211(2):475-9.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502.
17. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53(6):982-92.
18. Fazekas F, Kleinert R, Offenbacher H, et al. The morphologic correlate of incidental punctate white matter hyperintensities on MR images. *AJNR Am J Neuroradiol* 1991;12(5):915-21.
19. Kang HJ, Stewart R, Park MS, et al. White matter hyperintensities and functional outcomes at 2 weeks and 1 year after stroke. *Cerebrovasc Dis* 2013;35(2):138-45.
20. Brodal P. *The central nervous system : structure and function*. 3rd ed. Oxford: Oxford University Press, 2004.
21. Chollet F, DiPiero V, Wise RJ, et al. The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography. *Ann Neurol* 1991;29(1):63-71.
22. Gerdes VE, Kwa VI, ten Cate H, et al. Cerebral white matter lesions predict both ischemic strokes and myocardial infarctions in patients with established atherosclerotic disease. *Atherosclerosis* 2006;186(1):166-72.
23. Fazekas F, Kleinert R, Offenbacher H, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* 1993;43(9):1683-9.
24. Giubilei F, Lenzi GL, Di Piero V, et al.Predictive value of brain perfusion single-photon emission computed tomography in acute ischemic stroke. *Stroke* 1990;21(6):895-900.
25. Saiki R, Hayashi D, Ikuo Y, et al.Acrolein stimulates the synthesis of IL-6 and C-reactive protein (CRP) in thrombosis model mice and cultured cells. *J Neurochem* 2013.
26. Poirier P, Giles TD, Bray GA, et al.Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006;113(6):898-918.
27. Haapaniemi H, Hillbom M, Juvela S. Lifestyle-associated risk factors for acute brain infarction among persons of working age. *Stroke* 1997;28(1):26-30.
28. Rexrode KM, Hennekens CH, Willett WC, et al. A prospective study of body mass index, weight change, and risk of stroke in women. *Jama* 1997;277(19):1539-45.
29. Yoshida M, Mizoi M, Saiki R, et al. Relationship between metabolic disorders and relative risk values of brain infarction estimated by protein-conjugated acrolein, IL-6 and CRP together with age. *Clin Chim Acta* 2011;412(3-4):339-42.
30. Ninomiya JK, L'Italien G, Criqui MH, et al. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination

Survey. *Circulation* 2004;109(1):42-6.

31. Kwon HM, Kim BJ, Lee SH, et al. Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke* 2006;37(2):466-70.

Table 1. Characteristics of the study subjects due to their stroke risk marker level tertile

Item	All	Silent brain infarction RRV tertile			P value*
		Tertile 1	Tertile 2	Tertile 3	
Subjects (n)	228	76	73	79	-
Silent brain infarction relative risk value	0.71 ± 0.13	0.51 ± 0.07**	0.69 ± 0.05**	0.86 ± 0.03**	-
Age (years)	65 ± 7	59 ± 7	66 ± 3	70 ± 6	<0.0001
Male Sex	150 (65.7)	51 (67.1)	52 (71.2)	47 (59.4)	0.299
Body mass index, kg/m^2	24.2 ± 6.4	23.2 ± 2.9	24.6 ± 6.3	24.8 ± 8.6	0.133
Systolic BP, mmHg	123 ± 15	121 ± 14	122 ± 14	126 ± 17	0.275
Diastolic BP, mmHg	75 ± 11	75 ± 9	75 ± 10	75 ± 13	0.947
Hypertension, n (%)	81 (35.6)	23 (30.6)	20 (27.4)	38 (48.1)	0.015
Total cholesterol, mg/dL	208± 32	208 ± 30	208 ± 32	207 ± 34	0.903
LDL cholesterol†, mg/dL	123 ± 29	123 ± 25	123 ± 29	122 ± 33	0.848
HDL cholesterol, mg/dL	58 ± 15	59 ± 17	57 ± 13	60 ± 16	0.385
Triglycerides††, mg/dL	125 (113, 138)	141 (111, 172)	123 (105, 142)	112 (99, 124)	0.425

Dyslipidemia, n (%)	55 (24.1)	19 (25.0)	13 (17.8)	23 (29.1)	0.259
Fasting plasma glucose, mg/dL	100 ± 16	100 ± 14	99 ± 18	100 ± 15	0.754
Diabetes, n (%)	18 (7.8)	3 (3.9)	6 (8.2)	9 (11.3)	0.226
eGFR (mL/min/1.73 m^2)	67.6 ± 12.5	70.2 ± 15.3	67.3 ± 10.6	65.4 ± 10.6	0.027
Current smoking status, n (%)	40 (17.5)	16 (21.0)	12 (16.4)	12 (15.1)	0.603
PVH, n (%)	103 (45.1)	18 (23.6)	36 (49.3)	49 (62.0)	<0.0001
DSWMH, n (%)	157 (68.8)	52 (68.4)	50 (68.4)	55 (69.6)	0.983

*Analysis of variance or chi-square test among the ADMA tertile. **Range of a minimum-to-maximum stroke risk marker in each tertile. †n = 228. ††Geometric mean (95% confidence intervals). ‡Statistical analysis was not conducted because of the extremely small number of subjects in each category. BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; RRV, relative risk value

Table 2. Correlation coefficients and multiple linear regression analysis of relative risk value with the clinical parameters

Item	Simple correlation analysis		Multiple linear regression analysis	
	Correlation coefficient (r)	P-value	Standardized regression coefficient (β)	P value
Age	0.60	<0.0001	†	
Male Sex	0.05	0.347	††	
BMI	0.11	0.075	††	
Systolic BP	0.14	0.026	0.0009	0.078
Diastolic BP	0.05	0.382	††	
Total cholesterol	-0.03	0.618	††	
LDL cholesterol*	-0.02	0.669	††	
HDL cholesterol	0.02	0.731	††	
Triglycerides**	-0.10	0.130	††	
Fasting plasma glucose	0.01	0.841	††	
eGFR	-0.13	0.041	-0.0009	0.173
Current smoking status (Yes = 1)	-0.10	0.115	††	
PVH (Yes = 1)	0.26	<0.0001	0.0384	<0.0001
DSWMH (Yes = 1)	0.02	0.689	††	

*n = 228. **Log-transformed value. †Not included in the multiple linear regression analysis to avoid multicollinearity with PVH relative risk value. ††Not included in the multiple linear regression analysis because their P values were ≥0.05 in the simple correlation analysis. BMI, body mass index; BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Unadjusted and adjusted PVH ORs in each silent brain infarction relative risk value tertile

Item		Unadjusted			Adjusted*		
		OR	95% CI	P-value	OR	95% CI	P value
	Tertile 1	1.00	reference	-	1.00	reference	-
	Tertile 2	3.13	1.57–6.41	0.0014	3.01	1.50–6.20	0.0018
	Tertile 3	5.26	2.66–10.78	<0.0001	4.87	2.43–10.08	<0.0001

* Adjusted for systolic BP and eGFR

BP, blood pressure; eGFR, estimated glomerular filtration rate; OR, odds ratio; PVH, periventricular hyperintensity

Figure 1. Correlationship between the RRV and the PVH (A). Correlationship between the RRV and DSWMH (B)

*Significant at $P < 0.05$

Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular hyperintensities: a cross-sectional study

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Keywords: Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity, White matter intensities, stroke

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Abstract

Objective: Brain white matter hyperintensities (WMHs) can be divided into periventricular hyperintensity (PVH) and deep-and-subcortical white matter hyperintensity (DSWMH), and the former contribute more to cognitive dysfunction and infarction risk. We conducted the present investigation to define the relationship between PVH and DSWMH.

Design: Cross-sectional study.

Setting: University hospital

Participants: We prospectively enrolled 228 healthy Japanese volunteer subjects with relative risk values (RRVs) > 0.5.

Primary outcome measures: We investigated whether it is possible to use the RRV to predict PVH and DSWMH.

Results: Among 228 subjects, 103 (45.1%) and 157 (68.8%) exhibited PVH and DSWMH, respectively. Age, body mass index (BMI), and PVH were significant independent determinants of RRV. A significant odds ratio (OR) for PVH was noted in the highest RRV tertile compared with the lowest one after adjusting for potential confounding factors. A significant OR for high predicted PVH risk was also found for RRV level as well.

Conclusion: Elevated RRV levels were significantly associated with increased predicted PVH, suggesting that measuring the plasma PCAcro, IL-6, and CRP levels may be useful for identifying Japanese at high risk for PVH.

Strengths and limitations of this study:

Using the RRV at clinical level, we investigate to evaluate WMHs.

We provided the first evidence that RRV is associate with PVH rather than DSWMH.

These data are obtained from cases of cautious health care in Asian people and may not be applicable to populations of poor health.

Keywords: Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity

1. Introduction

A number of large-scale clinical studies have demonstrated that white matter hyperintensities (WMHs) are associated with high stroke risk [1-3]. The results of the large-scale, multicenter open trial PICA study[4] conducted in Japan suggest that the Fazekas-classified periventricular hyperintensities (PVHs) and deep-and-subcortical white matter hyperintensities (DSWMHs)[5] are related to the risk of symptomatic brain infarction (SBI). In the Rotterdam Scan study on elderly subjects with no history of stroke, conducted by magnetic resonance imaging (MRI) for 4.2 years, the proportional hazard ratio of stroke occurrence after adjustment of comorbid factors was 4.7 (95% confidence interval [CI], 2.0–11.2) in PVH and 3.6 (CI, 1.4–9.2) in DSWMH[6]. Unlike DSWMH, PVH is associated with cognitive dysfunction[7]. In other studies, associations were separately assessed for PVH and DSWMH and was significant only for PVH, which was related to decreased processing speed and executive

function [8 9]. Additionally, PVH predicted poorer functional outcome after stroke both in the acute and chronic phases, independently of DSWMH [10 11]. A Chiba University group reported that the relative risk value (RRV) measured based on protein-conjugated acrolein (PC-Acro) together with interleukin-6 (IL-6) and C-reactive protein (CRP) can be used to predict the stroke risk factors of silent brain infarction (SBI), carotid atherosclerosis (CA), and WMH with high sensitivity and specificity[12]. We measured plasma PCAcro, IL-6 and CRP, analyzed the measurements in conjunction with age to determine whether it is possible to use the RRV to predict PVH and DSWMH.

2. Materials and methods

2.1. Subjects and blood sampling

We examined 228 adult volunteers (78 women and 150 men, age 65.0 ± 7.0 years, range 31–83 years). All these subjects were healthy volunteers living independently at home without apparent history of stroke, cardiovascular disease, or malignancy. Subjects with RRV > 0.5 were enrolled prospectively. Informed consent was provided by each subject, and our study protocol was approved by the Ethics Committees of Nippon Medical School Hospital. Experiments were carried out in accordance with the Declaration of Helsinki principles. Blood samples were collected into tubes containing 3 U/mL heparin and centrifuged at 1500 × *g* for 10 min at 4°C.

2.2. PC-Acro, IL-6, and CRP Measurements

Blood samples were drawn from the antecubital vein after overnight fasting. PC-Acro [*N*-(3-formyl-3,4-dehydropiperidino)-lysine (FDPlysine) in protein] was determined as previously described [13] using an ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation, Tokyo, Japan) and 0.01 mL plasma. IL-6 and CRP were quantified using an Endogen Human IL-6 ELISA kit (Pierce Biotechnology, Inc., Rockford, IL, USA) and a human CRP ELISA kit (Alpha Diagnostic International, San Antonio, TX, USA), respectively, according to the manufacturers' protocols. After the reaction was terminated, absorbance was measured at 450 nm using a microplate reader (MTP-800APC, Hitachi, Tokyo, Japan). The biochemical markers from each subject were measured by an investigator who was blinded to the MRI results (Amine Pharma Research Institute, Chiba, Japan). Relative risk value (RRV) was calculated with artificial neural networks by back propagation method using NEUROSIM/L software version 4 (Fujitsu, Tokyo, Japan) [14]. Using the report by a Chiba University group, we worked out predictive RRV in the range of 0–1 [12 15], with the nil as the lowest value as an index of the degree of tissue damage. Values > 0.5 were considered to indicate WMH risk.

Standard enzymatic methods were used to measure the levels of serum total cholesterol, triglycerides, creatinine, and plasma glucose. Serum high-density lipoprotein (HDL) cholesterol level was measured with a direct method, and serum low-density lipoprotein (LDL) cholesterol level was calculated using Friedewald's formula in the 228 subjects with serum triglyceride levels < 400 mg/dL [16]. Diabetes was defined as a fasting plasma glucose level ≥126 mg/dL or the use of

glucose-lowering medications. Dyslipidemia was defined as total cholesterol level ≥220 mg/dL, HDL cholesterol level < 40 mg/dL and a triglyceride level ≥150 mg/dL, as well as the use of lipid-lowering medications. The estimated glomerular filtration rate (eGFR) was calculated for Japanese men as recommended by the Japanese Society of Nephrology [17] and represented as: eGFR (mL/min/1.73 m^2) = 193 × serum $creatinine^{-1.094}$ × $age^{-0.287}$.

2.3. Imaging

All 228 subjects underwent T1- and T2-weighted MRI and fluid-attenuated inversion recovery (FLAIR) at the Nippon Medical School Hospital, Japan, within 1 month after blood sampling. MRI was performed as described previously [10]. PVH and DSWMH were defined as hyperintense areas on T2 and FLAIR images without any abnormality on T1 [18] in subjects without neurological signs and/or symptoms. The 228 subjects were classified into 103 PVH subjects (38 women and 65 men, aged 68.2 ± 6.0 years, RRV 0.75 ± 0.11) and 157 WMH subjects (61 women and 96 men, aged 66.7 ± 5.8 years, RRV 0.71 ± 0.12). In more detail, 76 among all the subjects had both PVH and DSWMH, the other 23 having silent brain infarction. Also, 22 subjects had a complication of PVH and silent brain infarction, while in 20 subjects there was complication of DSWMH and silent brain infarction.

2.4. Statistics

All statistical tests were performed using the JMP9.02 software program (SAS Institute, Cary, NC, USA). Continuous variables except for triglyceride levels were expressed as means ± standard

deviation (SD). Triglyceride levels were transformed to the common logarithm for statistical analysis and are expressed as the geometric mean because of their skewed distribution. Categorical data are expressed as the number of subjects (percent of total). The clinical characteristics for each RRV tertile were compared by analysis of variance (ANOVA) for continuous variables and χ^2 test for categorical variables. The RRVs between the two groups were compared by Student's t-tests or by ANOVA followed by multiple comparisons with the Bonferroni correction between the two groups. Correlations between RRV and other variables were evaluated with the Pearson's moment correlation coefficient. Factors with a P value < 0.05 as determined by Pearson's correlation analysis were included in a multiple linear regression analysis to identify independent determinants of the RRV. Logistic regression analysis was performed to obtain the odds ratios (ORs) for PVH and DSWMH in the three tertiles. All statistical tests were two-sided, and a P value < 0.05 was considered as significant.

3. Results

The study subjects were divided into tertiles according to RRV (0.50–0.62, 0.63–0.79, and 0.80–0.90 from the lowest to highest tertile, respectively). The subjects' clinical characteristics are summarized in Table 1. The mean RRV of the entire subject population was 0.71 ± 0.13, and the mean age was 65 ± 7 years. Age, body mass index (BMI), diastolic blood pressure (BP), HDL cholesterol level, triglyceride level, eGFR, and current smoking status were significantly different among the groups.

RRVs were significantly higher in subjects with older age, lower eGFR, or PVH (Table 1). A simple correlation analysis showed that RRV was significantly correlated with age, systolic BP, eGFR, and PVH (Table 2). Multiple linear regression analysis indicated that BMI ($\beta = 0.0026$, $P = 0.044$) and PVH ($\beta = 0.0380$, $P < 0.0001$) were significant independent determinants of RRV.

The results of logistic regression analysis of the association between PVH and RRV are shown in Table 3. Significant, unadjusted ORs for PVH were noted in the third RRV tertile (5.26 [95% CI, 2.66–10.78], $P < 0.0001$), compared to the first tertile. After adjusting for model 1 (BMI, systolic BP, triglycerides, eGFR, and current smoking status) the ORs in the third RRV tertile remained significant (4.75 [95% CI, 2.33–10.05], $P < 0.0001$). After adjusting for model 2, we found that the OR in the third RRV tertile 3 was significant (5.26 [95% CI, 2.65–10.83], $P < 0.0001$). A significant relationship was observed between RRV and PVH ($P < 0.05$) but no such significance was found between RRV and DSWMH (figure 1).

4. Discussion

The present study demonstrated a significant, positive correlation between RRV and PVH in healthy Japanese volunteers. Notably, the highest RRV level tertile showed a significantly higher OR for a high predicted PVH risk in comparison to the lowest tertile, even after adjusting for multiple confounding factors. These results suggest that RRV is associated with the estimated risk of PVH in healthy Japanese volunteers. A number of clinical and epidemiological studies have examined the

predictive value of RRV for the presence of WMH[12 15]. However, those studies assessed WMH prevalence; no studies have shown any significant association of RRV with PVH and DSWMH separately. In this regard, our results raise the possibility that RRV predicts the risk of PVH in the healthy Japanese population. With respect to age, these biochemical markers provide a good index of the presence of tissue damage related to PVH.

More recent studies focused on WMH location have reported that functional impairment within 1–3 months after stroke correlated with PVH but not with DSWMH [10 19]. PVH WMH, especially PVH, has impacts on early functional recovery after ischemic stroke regardless of the initial stroke severity and other cardiovascular risk factors[11]. Other groups found a significant association between PVH and decreases in processing speed and executive function, but there was no such relationship with DSWMH[8 9]. Why PVH and DSWMH have different relationships with stroke outcome remains unclear, but several theories have been put forward. DSWMH predominantly disrupt short association fibers that link adjacent gyri, while PVH affects long association fibers that connect the more distant cortical areas [20]. Thus, lesions in various white matter locations may disconnect from different neural networks that affect neural repair processes after stroke [21]. In addition, PVHs are related to diminished cerebral vasomotor reactivity and subsequent occurrence of cerebral hypoperfusion [22], while DSWMHs are generally associated with microangiopathy [23]. It is clear that regional hypoperfusion is a good predictor of functional outcome [24]. These findings shed light on why PVH can predict functional stroke outcome and specific cognitive functions [11].

Acrolein induces IL-6 production in astrocytes, macrophages, and endothelial cells, while IL-6 induces CRP production in hepatocytes. Then, CRP stimulates IL-6 production, and IL-6 decreases acrolein toxicity [25]. Acrolein was thought to be one of the toxic compounds produced from unsaturated fatty acids by active oxygen species such as superoxide anion radical, hydrogen peroxide, and hydroxyl radical[13]. These findings may partially explain the pathophysiological mechanisms underlying the association between PVH and the three biomarkers assessed in the present study. Further investigation will be needed for a better understanding of their interrelationship.

Our multiple linear regression analysis showed that RRV was independently associated with BMI and PVH. Although obesity is believed to be an independent cardiovascular risk factor [26], it is still controversial whether BMI is a significant risk factor for stroke [27 28]. BMI was previously reported to be correlated with high RRV [29] which may be caused by vascular degeneration and endothelial dysfunction associated with hypertension and metabolic disorders. Subjects with metabolic syndrome are generally defined as those who have abdominal obesity and two additional metabolic disorders including hypertension, dyslipidemia, and hyperglycemia [30 31].

Our study has some potential limitations. Because it was a cross-sectional investigation, we could not determine a causal relationship between increased RRV and PVH risk. In addition, the population included healthy Japanese volunteers only. Therefore, it is unclear whether the results can be

extrapolated to other populations of poor health, patients with cardiovascular diseases, or other ethnic groups. Despite these potential limitations, our findings support the conclusion that elevated RRV is significantly associated with PVH in healthy Japanese volunteers. These results suggest that RRV measurement may be useful for identifying PVH in the general population. This would allow clinicians to follow patients who may be at risk for stroke and cognitive dysfunction.

Acknowledgments

We thank Drs. Mari Adachi and Sadaji Kura at Katsushika Health Center for assisting with the data analyses.

Table 1. Characteristics of the study subjects due to their stroke risk marker level tertile

Item	All	Silent brain infarction RRV tertile			P value*
		Tertile 1	Tertile 2	Tertile 3	
Subjects (n)	228	76	73	79	-
Silent brain infarction relative risk value	0.71 ± 0.13	0.51 ± 0.07**	0.69 ± 0.05**	0.86 ± 0.03**	-
Age (years)	65 ± 7	59 ± 7	66 ± 3	70 ± 6	<0.0001
Male Sex	150 (65.7)	51 (67.1)	52 (71.2)	47 (59.4)	0.299
Body mass index, kg/m^2	24.2 ± 6.4	23.2 ± 2.9	24.6 ± 6.3	24.8 ± 8.6	0.133
Systolic BP, mmHg	123 ± 15	121 ± 14	122 ± 14	126 ± 17	0.275
Diastolic BP, mmHg	75 ± 11	75 ± 9	75 ± 10	75 ± 13	0.947
Hypertension, n (%)	81 (35.6)	23 (30.6)	20 (27.4)	38 (48.1)	0.015
Total cholesterol, mg/dL	208± 32	208 ± 30	208 ± 32	207 ± 34	0.903
LDL cholesterol†, mg/dL	123 ± 29	123 ± 25	123 ± 29	122 ± 33	0.848
HDL cholesterol, mg/dL	58 ± 15	59 ± 17	57 ± 13	60 ± 16	0.385
Triglycerides††, mg/dL	125 (113, 138)	141 (111, 172)	123 (105, 142)	112 (99, 124)	0.425

Dyslipidemia, n (%)	55 (24.1)	19 (25.0)	13 (17.8)	23 (29.1)	0.259
Fasting plasma glucose, mg/dL	100 ± 16	100 ± 14	99 ± 18	100 ± 15	0.754
Diabetes, n (%)	18 (7.8)	3 (3.9)	6 (8.2)	9 (11.3)	0.226
eGFR (mL/min/1.73 m^2)	67.6 ± 12.5	70.2 ± 15.3	67.3 ± 10.6	65.4 ± 10.6	0.027
Current smoking status, n (%)	40 (17.5)	16 (21.0)	12 (16.4)	12 (15.1)	0.603
PVH, n (%)	103 (45.1)	18 (23.6)	36 (49.3)	49 (62.0)	<0.0001
DSWMH, n (%)	157 (68.8)	52 (68.4)	50 (68.4)	55 (69.6)	0.983

*Analysis of variance or chi-square test among the ADMA tertile. **Range of a minimum-to-maximum stroke risk marker in each tertile. †n = 228. ††Geometric mean (95% confidence intervals). ‡Statistical analysis was not conducted because of the extremely small number of subjects in each category. BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; RRV, relative risk value

Table 2. Correlation coefficients and multiple linear regression analysis of relative risk value with the clinical parameters

Item	Simple correlation analysis		Multiple linear regression analysis	
	Correlation coefficient (r)	P-value	Standardized regression coefficient (β)	P value
Age	0.60	<0.0001	†	
Male Sex	0.05	0.347	††	
BMI	0.11	0.075	††	
Systolic BP	0.14	0.026	0.0009	0.078
Diastolic BP	0.05	0.382	††	
Total cholesterol	-0.03	0.618	††	
LDL cholesterol*	-0.02	0.669	††	
HDL cholesterol	0.02	0.731	††	
Triglycerides**	-0.10	0.130	††	
Fasting plasma glucose	0.01	0.841	††	
eGFR	-0.13	0.041	-0.0009	0.173
Current smoking status (Yes = 1)	-0.10	0.115	††	
PVH (Yes = 1)	0.26	<0.0001	0.0384	<0.0001
DSWMH (Yes = 1)	0.02	0.689	††	

*n = 228. **Log-transformed value. †Not included in the multiple linear regression analysis to avoid multicollinearity with PVH relative risk value. ††Not included in the multiple linear regression analysis because their P values were ≥0.05 in the simple correlation analysis. BMI, body mass index; BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Unadjusted and adjusted PVH ORs in each silent brain infarction relative risk value tertile

Item		Unadjusted			Adjusted*		
		OR	95% CI	P-value	OR	95% CI	P value
	Tertile 1	1.00	reference	-	1.00	reference	-
	Tertile 2	3.13	1.57–6.41	0.0014	3.01	1.50–6.20	0.0018
	Tertile 3	5.26	2.66–10.78	<0.0001	4.87	2.43–10.08	<0.0001

* Adjusted for systolic BP and eGFR

BP, blood pressure; eGFR, estimated glomerular filtration rate; OR, odds ratio; PVH, periventricular hyperintensity

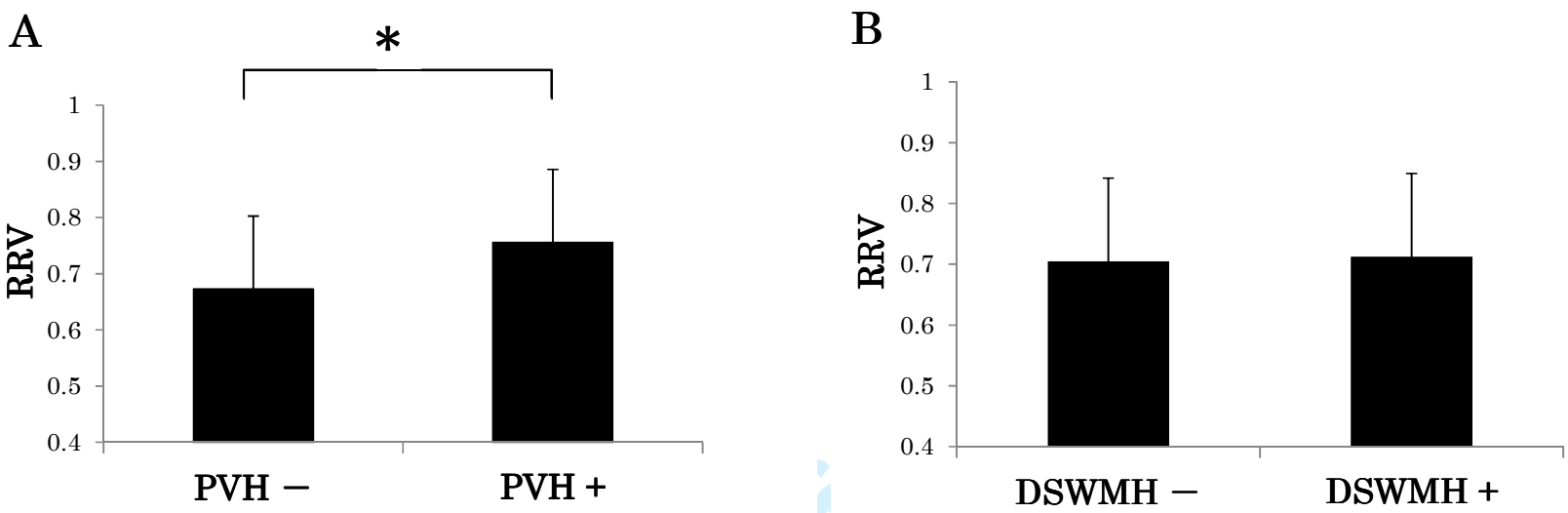


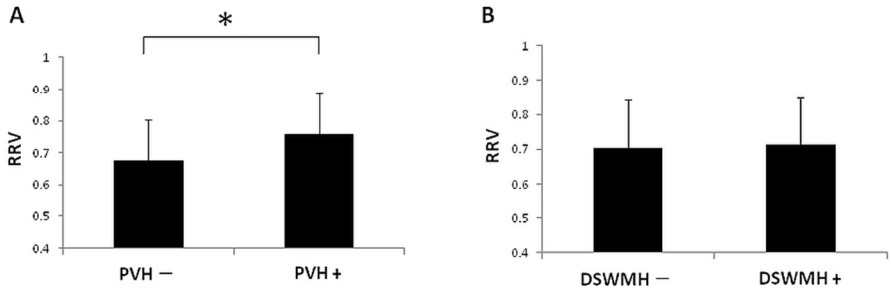
Figure 1. Correlationship between the RRV and the PVH (A). Correlationship between the RRV and DSWMH (B)

*Significant at $P < 0.05$

References

1. Wong TY, Klein R, Sharrett AR, Couper DJ, Klein BE, Liao DP, et al. Cerebral white matter lesions, retinopathy, and incident clinical stroke. *Jama* 2002;288(1):67-74.
2. Gouw AA, van der Flier WM, Fazekas F, van Straaten EC, Pantoni L, Poggesi A, et al. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke* 2008;39(5):1414-20.
3. Bokura H, Yamaguchi S, Kobayashi S. Electrophysiological correlates for response inhibition in a Go/NoGo task. *Clin Neurophysiol* 2001;112(12):2224-32.
4. Shinohara Y, Tohgi H, Hirai S, Terashi A, Fukuuchi Y, Yamaguchi T, et al. Effect of the Ca antagonist nilvadipine on stroke occurrence or recurrence and extension of asymptomatic cerebral infarction in hypertensive patients with or without history of stroke (PICA Study). 1. Design and results at enrollment. *Cerebrovasc Dis* 2007;24(2-3):202-9.
5. Schmidt R, Fazekas F, Kapeller P, Schmidt H, Hartung HP. MRI white matter hyperintensities: three-year follow-up of the Austrian Stroke Prevention Study. *Neurology* 1999;53(1):132-9.
6. Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. *Stroke* 2003;34(5):1126-9.
7. Fukuda H, Kobayashi S, Okada K, Tsunematsu T. Frontal white matter lesions and dementia in lacunar infarction. *Stroke* 1990;21(8):1143-9.
8. van den Heuvel DM, ten Dam VH, de Craen AJ, Admiraal-Behloul F, Olofsen H, Bollen EL, et al. Increase in periventricular white matter hyperintensities parallels decline in mental processing speed in a non-demented elderly population. *J Neurol Neurosurg Psychiatry* 2006;77(2):149-53.
9. Debette S, Bombois S, Bruandet A, Delbeuck X, Lepoittevin S, Delmaire C, et al. Subcortical hyperintensities are associated with cognitive decline in patients with mild cognitive impairment. *Stroke* 2007;38(11):2924-30.
10. Kissela B, Lindsell CJ, Kleindorfer D, Alwell K, Moomaw CJ, Woo D, et al. Clinical prediction of functional outcome after ischemic stroke: the surprising importance of periventricular white matter disease and race. *Stroke* 2009;40(2):530-6.
11. Liou LM, Chen CF, Guo YC, Cheng HL, Lee HL, Hsu JS, et al. Cerebral white matter hyperintensities predict functional stroke outcome. *Cerebrovasc Dis* 2010;29(1):22-7.
12. Yoshida M, Tomitori H, Machi Y, Katagiri D, Ueda S, Horiguchi K, et al. Acrolein, IL-6 and CRP as markers of silent brain infarction. *Atherosclerosis* 2009;203(2):557-62.
13. Uchida K, Kanematsu M, Morimitsu Y, Osawa T, Noguchi N, Niki E. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J Biol Chem* 1998;273(26):16058-66.
14. Ellenius J, Groth T, Lindahl B, Wallentin L. Early assessment of patients with suspected acute myocardial infarction by biochemical monitoring and neural network analysis. *Clin Chem* 1997;43(10):1919-25.
15. Yoshida M, Higashi K, Kobayashi E, Saeki N, Wakui K, Kusaka T, et al. Correlation between images of silent brain infarction, carotid atherosclerosis and white matter hyperintensity, and plasma levels of acrolein, IL-6 and CRP. *Atherosclerosis* 2010;211(2):475-9.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502.
17. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for

estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53(6):982-92.
18. Fazekas F, Kleinert R, Offenbacher H, Payer F, Schmidt R, Kleinert G, et al. The morphologic correlate of incidental punctate white matter hyperintensities on MR images. *AJNR Am J Neuroradiol* 1991;12(5):915-21.
19. Kang HJ, Stewart R, Park MS, Bae KY, Kim SW, Kim JM, et al. White matter hyperintensities and functional outcomes at 2 weeks and 1 year after stroke. *Cerebrovasc Dis* 2013;35(2):138-45.
20. Brodal P. *The central nervous system : structure and function*. 3rd ed. Oxford: Oxford University Press, 2004.
21. Chollet F, DiPiero V, Wise RJ, Brooks DJ, Dolan RJ, Frackowiak RS. The functional anatomy of motor recovery after stroke in humans: a study with positron emission tomography. *Ann Neurol* 1991;29(1):63-71.
22. Gerdes VE, Kwa VI, ten Cate H, Brandjes DP, Buller HR, Stam J. Cerebral white matter lesions predict both ischemic strokes and myocardial infarctions in patients with established atherosclerotic disease. *Atherosclerosis* 2006;186(1):166-72.
23. Fazekas F, Kleinert R, Offenbacher H, Schmidt R, Kleinert G, Payer F, et al. Pathologic correlates of incidental MRI white matter signal hyperintensities. *Neurology* 1993;43(9):1683-9.
24. Giubilei F, Lenzi GL, Di Piero V, Pozzilli C, Pantano P, Bastianello S, et al. Predictive value of brain perfusion single-photon emission computed tomography in acute ischemic stroke. *Stroke* 1990;21(6):895-900.
25. Saiki R, Hayashi D, Ikuo Y, Nishimura K, Ishii I, Kobayashi K, et al. Acrolein stimulates the synthesis of IL-6 and C-reactive protein (CRP) in thrombosis model mice and cultured cells. *J Neurochem* 2013.
26. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006;113(6):898-918.
27. Haapaniemi H, Hillbom M, Juvela S. Lifestyle-associated risk factors for acute brain infarction among persons of working age. *Stroke* 1997;28(1):26-30.
28. Rexrode KM, Hennekens CH, Willett WC, Colditz GA, Stampfer MJ, Rich-Edwards JW, et al. A prospective study of body mass index, weight change, and risk of stroke in women. *Jama* 1997;277(19):1539-45.
29. Yoshida M, Mizoi M, Saiki R, Kobayashi E, Saeki N, Wakui K, et al. Relationship between metabolic disorders and relative risk values of brain infarction estimated by protein-conjugated acrolein, IL-6 and CRP together with age. *Clin Chim Acta* 2011;412(3-4):339-42.
30. Ninomiya JK, L'Italien G, Criqui MH, Whyte JL, Gamst A, Chen RS. Association of the metabolic syndrome with history of myocardial infarction and stroke in the Third National Health and Nutrition Examination Survey. *Circulation* 2004;109(1):42-6.
31. Kwon HM, Kim BJ, Lee SH, Choi SH, Oh BH, Yoon BW. Metabolic syndrome as an independent risk factor of silent brain infarction in healthy people. *Stroke* 2006;37(2):466-70.



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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page4
Methods			
Study design	4	Present key elements of study design early in the paper	Page5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Page5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page6
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page4
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page8-9
		(*b*) Report category boundaries when continuous variables were categorized	Page8-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page8-9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page8-9
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page11
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.