Table 6. *p53* mutations in 64 cases of myxoid/round cell liposarcoma

Case number	Age/Sex	Location	Type	Exon	Codon	Base change	IHC	Prognosis
M35-MX	33/F	Lower leg	M/R	7	242	TGC(Cys) ≫ TAC(Tyr)	(−)	NA
M36-MX	44/M	Abdominal cavity	M	6	214	CAT(His) ≫ TAT(Tyr)	(−)	46M DOD
M39-RC	28/M	Thigh	M/R	7	225	GTT(Val) ≫ GCT(Ala)	(−)	22M DOD
					247	AAC(Asn) ≫ AGC(Ser)		
M67-RC	19/M	Paravertebra	M/R	7	238	TGT(Cys) ≫ TAT(Tyr)	(−)	16M DOD
M41-RC	44/M	Thigh	M/R	6	219	CCC(Pro) ≫ CCT(Pro)	(+)	18M NED
M30-MX	18/M	Cheek	M	6	210	AAC(Asn) ≫ AAT(Asn)	(−)	48M DOD
M44-MX	57/F	Thigh	M	5	167	CAC(Gln) ≫ TAG(stop)	(−)	144M DOD
M83-RC	44/M	Retroperitoneum	M/R	8	282	CGG(Arg) ≫ CGA(Arg)	(−)	6M DOD

MX, myxoid component; RC, round cell component; M/R, tumour with >5% round cells; M, tumour with <5% round cells; IHC, immunohistochemistry; NA, data not available; DOD, died of disease; NED, no evidence of disease.

RC components that comprised >25% of the tumour was found to be an adverse prognostic factor by both univariate and multivariate analysis.

A few studies have evaluated the prevalence of p53 nuclear immunoreactivity in MLS/RCLS, regardless of the methods used [9,11,12,34]. Smith *et al* [34] demonstrated that only 2/30 tumours (6.7%) showed positive immunoreaction for p53, and this was only in the RC areas, using the antibody DO7. In contrast, Dei Tos *et al* [12] reported that accumulation of p53 protein was observed in all of the 21 cases examined, in both the MX and RC areas, using the same antibody. No authors have directly compared the frequency of p53 expression between MX and RC components. In the current study, the frequency of p53 expression in RC components was found to be significantly higher than that in MX components, although the antibody used in our study was different from that of previously described studies (Pab 1801). Moreover, p53 expression was more frequently observed in tumours with >5% RC components than in tumours with <5%, as Antonescu *et al* [9] have reported.

As for *p53* gene alterations, only two authors have analysed such alterations in this tumour [12,35]. Dei Tos *et al* [12] reported that aberrations of the *p53* gene were observed in 28.5% of cases, whereas Pilotti *et al* [35] showed that they were present in only 2.8% of cases, all of which demonstrated aggressive histological findings. In the current study, 12.5% of the cases examined had *p53* gene point mutations. In the series of Dei Tos *et al* [12] *p53* mutation was distributed

Table 7. Correlation between immunohistochemistry and gene alteration

		p53 IHC	+	−	
p53 mutation	+(*n* = 8)		1	7	*p* = 0.7058
	−(*n* = 66)		9	57	
p53 missense mutation	+(*n* = 4)		0	4	*p* = 0.5522
	−(*n* = 70)		10	60	
		MDM2 IHC	+	−	
MDM2 amp	+(*n* = 8)		5	3	*p* < 0.0001*
	−(*n* = 51)		1	50	
		p14 IHC	Red.	Pres.	
p14 methylation	+(*n* = 8)		4	4	*p* = 0.0176*
	−(*n* = 62)		7	55	
p14 HD	+(*n* = 4)		1	3	*p* = 0.5373
	−(*n* = 66)		11	55	
p14 mutation	+(*n* = 14)		5	9	*p* = 0.0971
	−(*n* = 52)		8	44	
p14 missense mutation	+(*n* = 10)		2	8	*p* = 0.5438
	−(*n* = 56)		11	55	
		p16 IHC	Red.	Pres.	
p16 HD	+(*n* = 6)		2	4	*p* = 0.401
	−(*n* = 48)		10	38	
p16 mutation	+(*n* = 10)		2	8	*p* = 0.6364
	−(*n* = 60)		13	47	
p16 missense mutation	+(*n* = 8)		2	6	*p* = 0.5486
	−(*n* = 62)		13	49	

IHC, immunohistochemistry; HD, homozygous deletion; Red, reduced; amp, amplification; Pres, preserved. * Statistically significant.

Table 8. Prognostic factors in myxoid/round cell liposarcoma

Variable	*p* Value on survival analysis: Univariate	Multivariate
Clinicopathological		
Age (years: ≤40 vs >40)	0.0165*	0.4771
Location (extremity or trunk vs others)	<0.0001*	0.0251*
Tumour size (≤5 cm vs >5 cm)	0.125	0.6072
Depth (superficial vs deep)	NE	
Round cell components (≤5% vs >5%)	0.0485*	
Round cell components (≤25% vs >25%)	0.039*	0.0113*
Necrosis (absent vs present)	0.0474*	0.1648
Mitosis (≤5/50 HPFs vs >5/50 HPFs)	0.2678	0.2997
Histological grade (FNCLCC) (1 vs 2, 3)	0.0177*	0.0318*
AJCC stage (I, II vs III, IV)	0.0369*	0.0318*
Immunohistochemical		
p53 (cut-off 10%)	0.1692	0.0477*
MDM2 (cut-off 10%)	0.5138	0.1792
MIB-1 (cut-off 8.4%)	<0.0001*	0.0005*
p14 (preserved or reduced)	0.0338*	0.4266
p16 (preserved or reduced)	0.1627	0.2592
Molecular genetic		
p53 mutation (− vs +)	0.0328*	
p53 missense mutation (− vs +)	0.0111*	0.0036*
MDM2 amplification (− vs +)	0.3468	0.1792
p14 methylation (− vs +)	0.9646	0.9781
p14 HD (− vs +)	0.3785	NE
p14 mutation (− vs +)	0.5418	
p14 missense mutation (− vs +)	0.3958	0.1739
p16 HD (− vs +)	0.4424	0.3761
p16 mutation (− vs +)	0.27	
p16 missense mutation (− vs +)	0.1813	0.4536

* Statistically significant. NE, not evaluable.

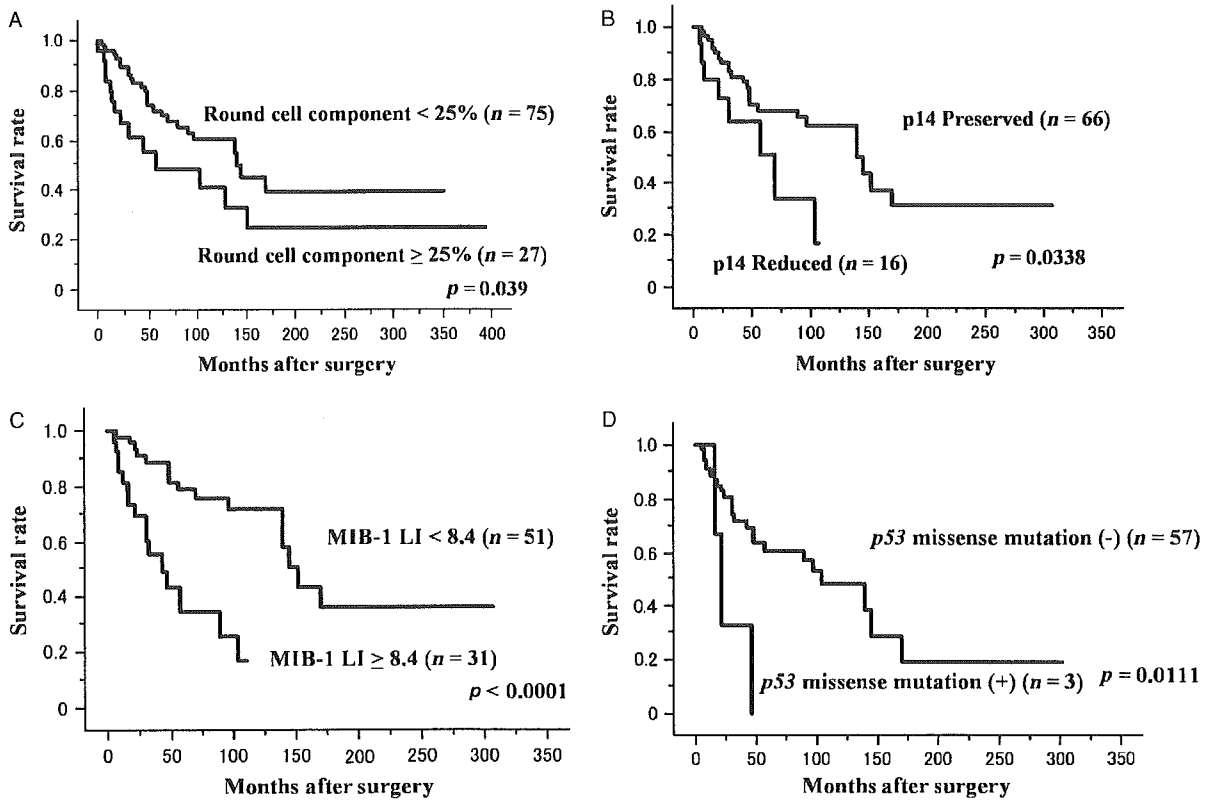

Figure 6. Kaplan–Meier curves for the patients with myxoid/round cell liposarcoma. (A) Curves for 75 patients with tumours containing <25% of round cell components and for 27 patients with tumours containing ≥ 25% round cell components (log rank $p = 0.039$). (B) Curves for 66 patients with tumours exhibiting preserved p14 expression and for 16 patients with reduced p14 expression (log rank $p = 0.0338$). (C) Curves for 51 patients with tumours exhibiting an MIB-1 LI of <8.4 and for 31 patients exhibiting an MIB-1 LI of ≥ 8.4 (log rank $p < 0.0001$). (D) Curves for three patients with tumours exhibiting *p53* gene missense mutation and 57 patients without *p53* missense mutation (log rank $p = 0.0111$)

equally in both the RC and MX components. On the other hand, in our current series, the frequency of *p53* point mutations in the RC components (21.5%) was higher than that in the MX components (6.8%), but this finding was not statistically significant. *p53* missense mutation was found to be one of the adverse prognostic factors by multivariate analysis. In sarcomas with specific translocation, such as synovial sarcoma [36], MLS/RCLS [9] and Ewing's sarcoma/PNET [37], p53 pathway alterations are a rather rare event, but when present they have been a strong prognostic factor. Our results support this phenomenon.

A few authors have reported homozygous deletion or hypermethylation of the $p16^{INK4a}$ gene and their correlation with loss of p16 protein and poor prognosis in bone and soft tissue sarcoma [19,21]. Kawaguchi *et al* [19] demonstrated that promoter hypermethylation of the $p16^{INK4a}$ gene correlated closely with decreased expression of the p16 protein and poor prognosis in soft tissue leiomyosarcoma.

In chondrosarcoma, van Beerendonk *et al* [23] showed that loss of p16 protein correlated significantly with high-grade tumours. In the current study, reduced expression of p16 was more frequent in RC components than in MX components and this reduced expression may play an important role in tumour progression in MLS/RCLS, as van Beerendonk *et al* [23] have demonstrated in chondrosarcoma. On the other hand, Olofsson *et al* [38] reported consistent immunohistochemical expression of p16 protein in MLS/RCLS. This discrepancy may be due to the use of different antibodies. They used polyclonal anti-p16 antibody, whereas we used a monoclonal antibody. Dei Tos *et al* [12] found $p16^{INK4a}$ gene mutations in 3/21 cases (14.3%) of MLS/RCLS and all the mutation cases were of MX histology. In our series, $p16^{INK4a}$ gene mutation was detected in 14.3% of the examined samples and it was observed in both MX and RC components.

Several studies have shown recently that $p14^{ARF}$ protein binds to the p53/MDM2 complex and inhibits the MDM2-mediated degradation of p53, which indicates that $p14^{ARF}$ is an upstream regulator of p53 through MDM2 [39,40]. In this study, reduced expression of p14 protein correlated significantly with p53 protein expression, which had no relationship to *p53* gene alteration, and which was therefore considered to be wild-type p53. We found that hypermethylation of the $p14^{ARF}$ gene correlated significantly with reduced p14 protein expression. In addition, promoter hypermethylation of $p14^{ARF}$ occurred independently of $p16^{INK4a}$ methylation status. Therefore, the main mechanism of inactivation of $p14^{ARF}$ may be hypermethylation of the promoter region. Furthermore, reduced expression of p14 was more frequently observed in RC components compared with MX components

and it was found to be an adverse prognostic factor by univariate analysis. In MLS/RCLS, reduced expression of p14 may have a larger contribution to make to its malignant progression, compared with reduced expression of p16. These deregulations of the p14–MDM2–p53 pathway have been reported in the progression of low-grade diffuse astrocytoma [25] or meningioma [26].

In conclusion, our results suggest that loss of p16INK4/p14ARF protein expression, especially repressed p14 protein, may be one of the important events during tumour progression in MLS/RCLS.

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

The Hisayama Study

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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[1] and enhances immune reactivity.[2] Recently, the role of endothelial cells and monocytes in the inflammatory process has become better understood,[3] 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.[4–9] Most of those studies examined coronary heart disease[4–6] or combined end points of coronary heart disease and ischemic stroke,[7–9] whereas only a few studies examined ischemic stroke.[10–12] The subjects of the latter studies were limited to the elderly[10,11] or men,[12] 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.

Methods

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.[13] 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

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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/m^2) was calculated. Obesity was defined as a BMI of ≥25 kg/m^2. 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 χ^2 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 1. Baseline Characteristics of Study Subjects, the Hisayama Study, 1988

Characteristic	Men (n=1092)	Women (n=1500)
Age, y	58.1±11.4	59.4±11.9
High-sensitivity C-reactive protein, mg/L		
Median	0.54	0.40
Mean	2.07±8.31	1.30±5.45
Systolic blood pressure, mm Hg	134.7±20.1	132.9±22.2
Diastolic blood pressure, mm Hg	80.5±11.4	75.8±10.8
Hypertension, %	45.2%	38.5%
Use of antihypertensive agents, %	14.2%	15.4%
ECG abnormalities, %	20.7%	14.7%
Diabetes mellitus, %	15.1%	9.6%
BMI, kg/m^2	22.8±2.9	22.9±3.3
Total cholesterol, mmol/L	5.09±1.07	5.54±1.07
HDL-cholesterol, mmol/L	1.25±0.31	1.33±0.30
Current smoking, %	49.8%	6.7%
Current drinking, %	60.6%	9.0%
Regular exercise, %	11.8%	9.1%

Data are mean±1 SD or percent, unless otherwise specified.

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=0.02 for trend), 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=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 (χ^2=5.370; P=0.02), as well as hypercholesterolemia (χ^2=6.052; P=0.01), and a marginally significant interaction (χ^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[10] and Cardiovascular Health Study,[11] both which had elderly subjects (mean age,

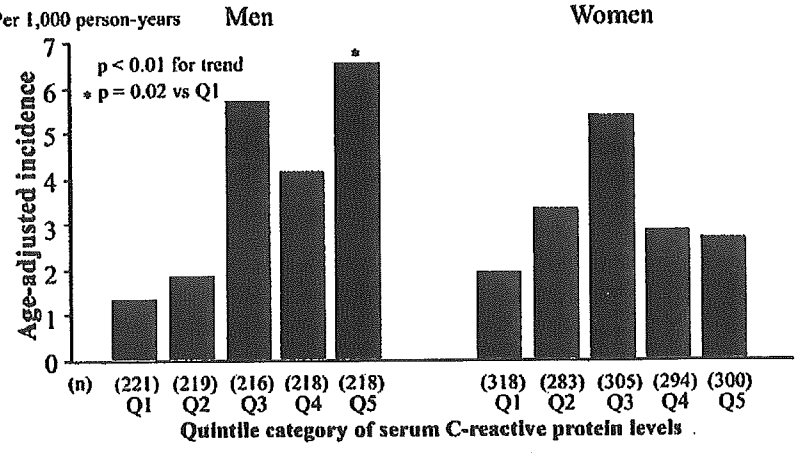

Figure 1. Age-adjusted incidence rates of first-ever ischemic stroke according to serum high-sensitivity C-reactive protein levels.

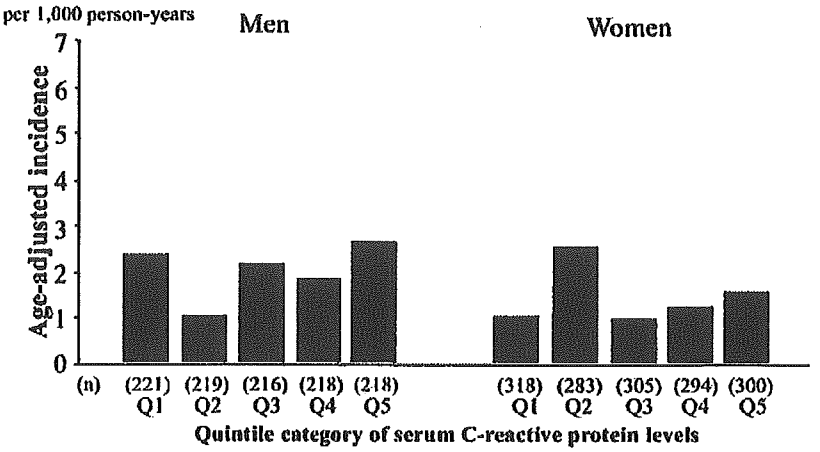

Figure 2. Age-adjusted incidence rates of first-ever hemorrhagic stroke according to serum high-sensitivity C-reactive protein levels.

Quintile range (mg/l)
Men:Q1= ≤ 0.20, Q2=0.21 to 0.40, Q3=0.41 to 0.71, Q4=0.72 to 1.56, Q5=1.57
Women:Q1= ≤ 0.17, Q2=0.18 to 0.30, Q3=0.31 to 0.53, Q4=0.54 to 1.09, Q5=1.10

69.8 and 72.6 years, respectively), and a nested case-control study of Japanese-American men[12] 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,[10] whereas no significant association was observed for the women in the Cardiovascular Health Study,[11] which was in accord with the findings of our study. Recent clinical evidence has shown that endogenous estrogen protects the development of atherosclerosis[17,18] and that estrogen induces the elevation of hsCRP levels.[19] 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.

TABLE 2. Multivariate-Adjusted RRs of First-Ever Ischemic and Hemorrhagic Stroke according to Serum High-Sensitivity C-Reactive Protein Levels

Quintiles of Men/Women	RR	95% CI	*P* Value	RR	95% CI	*P* Value
Ischemic stroke						
Q1	1.00	1.00				
Q2	1.08	0.29 to 4.03	0.91	1.27	0.55 to 2.94	0.58
Q3	2.81	0.93 to 8.51	0.07	1.56	0.71 to 3.39	0.27
Q4	2.24	0.73 to 6.92	0.16	1.05	0.46 to 2.42	0.90
Q5	3.11	1.04 to 9.32	0.04	1.34	0.61 to 2.91	0.46
P for trend	0.02	0.65				
Hemorrhagic stroke						
Q1	1.00			1.00		
Q2	0.33	0.07 to 1.65	0.18	2.66	0.82 to 8.61	0.10
Q3	0.58	0.17 to 1.91	0.37	1.00	0.24 to 4.06	0.99
Q4	0.78	0.26 to 2.37	0.67	2.10	0.63 to 7.04	0.23
Q5	0.68	0.21 to 2.26	0.53	1.74	0.51 to 5.85	0.37
P for trend	0.92	0.64				

Men, mg/L: Q1=≤0.20, Q2=0.21 to 0.40, Q3=0.41 to 0.71, Q4=0.72 to 1.56, Q5=≥1.57. Women, mg/L: Q1=≤0.17, Q2=0.18 to 0.30, Q3=0.31 to 0.53, Q4=0.54 to 1.09, Q5=≥1.10. Multivariate adjustment was made for age, systolic blood pressure, ECG abnormalities, diabetes, BMI, total cholesterol, HDL cholesterol, smoking habits, alcohol intake, and physical activity.

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,[20] or by amyloid angiopathy,[21] 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,[22] which is a main cause of subarachnoid hemorrhage, the association between atherosclerosis and intracranial aneurysm is considered weak.[23] 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

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 ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive agents. Diabetes: fasting blood glucose ≥7.0 mmol/L, or postprandial blood glucose level ≥11.1 mmol/L, or current use of hypoglycemic agents. Obesity: BMI ≥25 kg/m^2. Hypercholesterolemia: total cholesterol level ≥5.69 mmol/L.

reviews, as well as experimental and clinical studies, have shown that inflammation is directly associated with the development of atherosclerosis[24] and instability of atheroma.[25,26] 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°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.[27] 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.[28] 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

motivation for both the treating physician and the patient to control these risk factors strictly.

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Angiotensin I-converting enzyme gene polymorphism modifies the smoking–cancer association: the Hisayama Study

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We examined the long-term contribution of smoking and angiotensin I-converting enzyme (ACE) gene I/D polymorphism to total cancer deaths in a prospective study of a general Japanese population. A total of 937 subjects aged 40 years or older were selected from an original cohort of 1621 subjects and were followed up for 32 years. During the follow-up period, 176 subjects died of cancer. Cancer mortality increased significantly with increasing current smoking levels. Although no clear relationship was observed between ACE genotypes and fatal cancer, the interaction term between current smoking and ACE genotype DD was found to be significant. In stratified analysis by ACE genotype after controlling for age, sex, alcohol intake, body mass index, glucose intolerance, serum total cholesterol and systolic blood pressure, the risk of fatal cancer in currently smoking subjects with genotype DD was twofold greater than that in subjects with genotypes II and ID. Among current smokers, subjects with genotype DD also showed a significantly greater risk of death due to cancer compared with those with genotypes II and ID combined (hazard ratio (HR) 1.77; 95% confidence interval (CI) 1.04–3.00; $P=0.03$). In conclusion, our findings suggest that ACE genotype DD enhances the association between smoking and cancer death in the general population. *European Journal of Cancer Prevention* **15:000–000 © 2006 Lippincott Williams & Wilkins.**

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Keywords: angiotensin I-converting enzyme, cancer, cohort study, polymorphism, smoking

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Introduction

Cancer kills about 7 million people each year, making malignant neoplasm one of the leading causes of death worldwide (World Health Organization, 2002). Malignant respiratory neoplasm is the leading cause of cancer death, representing one-sixth of cancer mortalities, and approximately 80% of the respiratory malignant neoplasm burden is attributable to smoking (World Health Organization, 2002). Tobacco also causes malignant neoplasms in other sites, such as the oral cavity, pharynx, larynx, oesophagus, stomach, bile ducts, liver, pancreas, bladder, etc. (World Health Organization 2002; Doll *et al.*, 2004; Jee *et al.*, 2004).

The products of tobacco combustion cause DNA base modifications and adducts, leading to an accumulation of mutational changes in oncogenes and in tumour suppressor genes (Denissenko *et al.*, 1996; Pryor, 1997) and to the worsening of the biological properties of tumours (Suzuki *et al.*, 1992). Thus, it is likely that smoking is closely involved in the initiation and promotion steps of carcinogenesis. On the other hand, an enhanced renin–angiotensin system has been suggested to be involved in neoplastic cell proliferation (Muscella *et al.*, 2002), angiogenesis and metastasis (Fujita *et al.*, 2002). It is well known that angiotensin I-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism influences circulating ACE levels, and that the DD genotype induces the highest ACE levels in blood (Rigat *et al.*, 1990). Thus, the ACE genotype DD may modify and enhance the association between smoking habits and malignant neoplasm.

In order to clarify this issue, we examined the long-term prospective contribution of smoking and ACE gene I/D polymorphism to fatal cancer in a cohort study of a general Japanese population.

Subjects and methods

Study design and participants

The Hisayama Study is an ongoing population-based epidemiological study designed to investigate the mor-

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bidity and mortality of cardiovascular disease and its risk factors in a community living in the town of Hisayama in Japan (Katsuki, 1966; Arima *et al.*, 2003). At the initial screening in 1961, 1621 subjects aged 40 years or older were registered as a cohort population; this group of subjects included almost 90% of the total population of this age group (Fig. 1). Of the initial cohort population, 1309 had died by 2001, and of these, 1033 (78.9%) underwent autopsy examination. In the autopsy cases, tissue samples of the main organs, such as the brain, heart, lung, liver, spleen, gastrointestinal tract and kidney, were formalin-fixed, paraffin-embedded and stored until 2000, after which they were fresh-frozen. With regard to the surviving subjects, blood samples have been collected from 77 participants. Paraffin-embedded tissues, fresh-frozen tissues or blood samples were therefore available for a total of 1110 subjects, who were then selected for ACE gene I/D genotyping. Among those, however, 170 subjects had samples not suitable for genotyping, and another three subjects were excluded because of missing data on smoking habits. Thus, 937 subjects, representing 58% of the original cohort, were included in the present analysis.

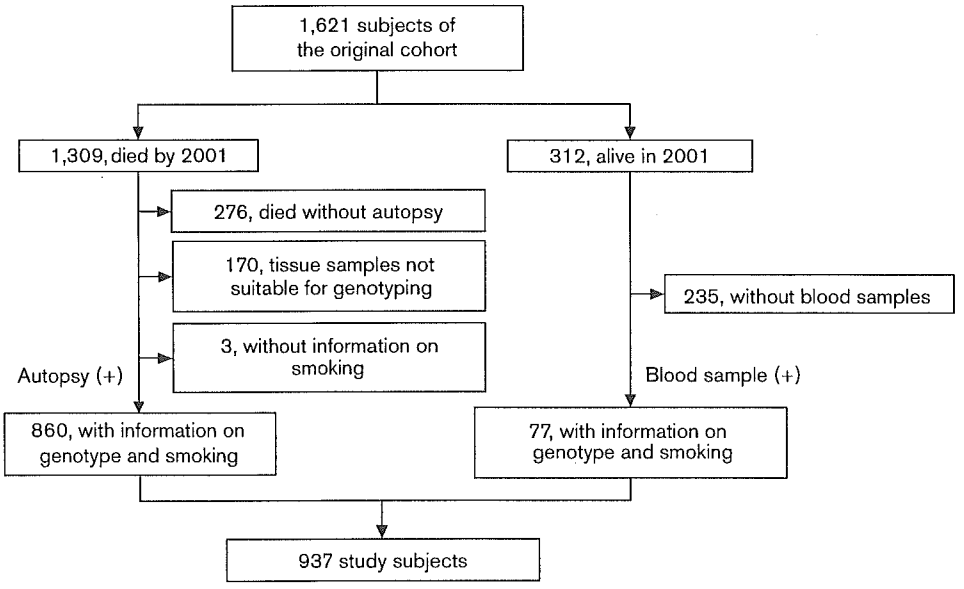
Risk factors

At baseline examination, information on smoking habits and alcohol intake was obtained using a standard questionnaire. Smoking habits were classified into three categories: never-, ex- and current-smoking. Subjects who had stopped smoking 1 year or more before the start of the study were classified as ex-smokers. Current smoking was subdivided into a further three categories: 1–9, 10–19 and 20 or more cigarettes per day. Alcohol intake was classified as either habitual use or no use. Body mass index (kg/m^2) was used as an indicator of obesity. Serum cholesterol level was determined using the Zak–Henly method following the modification described by Yoshikawa *et al.* (1960). Glucose intolerance was determined by an oral glucose tolerance test in subjects with glycosuria, as well as by obtaining the subjects' medical history regarding diabetes (Arima *et al.*, 2003). Recumbent blood pressures were obtained four times and the average values of the last three measurements were used in the analyses.

ACE I/D genotyping

Extraction of DNA from blood samples and fresh-frozen tissue samples was performed as described by Lahiri and Nurnberger (1991) and Tsukada and Ikari (1995), respectively. The ACE I/D genotype was determined using the polymerase chain reaction method as described by Evans *et al.* (1994). In the case of paraffin-embedded tissues, DNA was extracted using an automatic nucleic acid isolation system (NA-2000; Kurabo Inc., Osaka, Japan) and the ACE I/D genotype was determined using the double polymerase chain reaction method, as described previously (Arima *et al.*, 2002). All tissue samples obtained were examined microscopically to determine the presence or absence of tumour tissue, and only tumour-free tissues were used for ACE I/D genotyping. The accuracy of these genotyping methods was demonstrated in a previous study (Arima *et al.*, 2002).

Fig. 1



Flow diagram of the selection of study subjects.

Follow-up survey

The outcome of interest in the cases analysed in the present investigation was death due to any malignant neoplasm ($n = 176$). The participants were followed prospectively from November 1961 to October 1993 by means of repeated health examinations, or by a daily monitoring system established by the study team and local physicians, or by members of the Division of Health and Welfare of the town. The diagnosis of death due to malignant neoplasm was made based on clinical history, laboratory data, radiographs such as fluoroscopy of the gastrointestinal tract, cholangiography, urography, angiography, computed tomography, ultrasonograms, scintigrams, surgical findings, histopathological findings and disease courses. Clinical diagnoses were corrected by autopsy findings when necessary.

Statistical analysis

Genotype frequencies were compared with the values predicted by the Hardy–Weinberg equilibrium using the chi-square test. The mortality rates from malignant neoplasm were estimated by the person-year approach and were compared using the Cox's proportional hazards model. Hazard ratios (HRs) and 95% confidence intervals (CIs) of risk factors were also estimated using the Cox's proportional hazards model, in which significant interaction terms were included.

Ethical consideration

This study was conducted with the approval of the ethics committee of Kyushu University, and written informed consent was obtained from participants or from the families of each deceased participant.

Results

The baseline characteristics of our subjects are shown in Table 1. Mean age was 58 years, and 53% of the participants were women. The frequency of ACE genotypes was 38% for II, 49% for ID, and 13% for DD. This distribution was in the range of the Hardy–Weinberg equilibrium (chi-square, 1.7; df, 2; $P = 0.6$) and was similar to those found in previous studies on Japanese populations (Higaki *et al.*, 2000). Forty-four per cent of the subjects were smoking at the time of the baseline.

During the follow-up period, 176 subjects died of malignant neoplasm. The effects of smoking status on fatal cancer are shown in Table 2. Although ex-smoker status was not clearly associated with cancer deaths, mortality rates due to cancer increased significantly with increasing current smoking levels. These results were substantially unchanged even after controlling for age, sex, ACE genotype, alcohol intake, body mass index, glucose intolerance, serum total cholesterol and systolic blood pressure. In the following analyses, subjects who never smoked and ex-smokers were combined as non-smokers, and current smokers were also combined into a single group regardless of the number of cigarettes smoked daily.

The effects of current smoking and other risk factors on fatal cancer are shown in Table 3. When adjustment was made for age and sex, current smoking was found to be a significant risk factor for cancer deaths; however, ACE genotype DD and other risk factors were not significantly associated with fatal cancer. In the multivariate analysis, age, current smoking and body mass index were found to

Table 1 Baseline characteristics of subjects ($n = 937$)

Characteristic	
Age, years (SD)	58 (11)
Women, *n* (%)	492 (53)
ACE genotype	
II, *n* (%)	360 (38)
ID, *n* (%)	459 (49)
DD, *n* (%)	118 (13)
Smoking habits	
Never-smokers, *n* (%)	493 (53)
Ex-smokers, *n* (%)	27 (3)
Current smokers	
1–9 cigarettes/day, *n* (%)	98 (10)
10–19 cigarettes/day, *n* (%)	208 (22)
≥ 20 cigarettes/day, *n* (%)	111 (12)
Alcohol intake, *n* (%)	331 (35)
Body mass index, kg/m^2 (SD)	21.5 (2.6)
Glucose intolerance, *n* (%)	89 (9)
Serum total cholesterol, mmol/l (SD)	4.0 (0.9)
Systolic blood pressure, mmHg (SD)	136 (26)
Diastolic blood pressure, mmHg (SD)	78 (14)

SD, standard deviation; ACE, angiotensin I-converting enzyme; I, insertion; D, deletion.

Table 2 Mortality and age- and sex-adjusted and multivariate-adjusted hazard ratios with 95% confidence intervals for fatal cancer according to smoking status

	Smoking status (cigarettes per day)					*P*-value for trend
	Never	Ex	1–9	10–19	≥ 20	
Number of fatal cancers/person-years	68/10590	3/475	22/1610	44/4338	39/2314	
Crude mortality, per 1000 person-years	6.4	6.3	13.7	10.1	16.9	<0.0001
Age- and sex-adjusted HR	1.00[a]	0.81	1.90	1.73	3.02	<0.0001
(95% CI)		(0.25–2.62)	(1.16–3.12)	(1.06–2.83)	(1.79–5.08)	
Multivariate-adjusted HR[b]	1.00[a]	0.51	2.07	1.80	2.78	<0.0001
(95% CI)		(0.12–2.14)	(1.25–3.43)	(1.08–3.00)	(1.62–4.78)	

Never, never-smoker; Ex, ex-smoker; HR, hazard ratio; 95% CI, 95% confidence interval.
[a]This group served as the reference group.
[b]Adjustment has been made for age, sex, angiotensin I-converting enzyme genotype, alcohol intake, body mass index, glucose intolerance, serum total cholesterol and systolic blood pressure.

be independent significant risk factors for cancer deaths. The interaction term between current smoking and ACE genotype DD was also significantly associated with fatal cancer (HR 3.31; 95% CI 1.15–9.51; $P = 0.03$), suggesting that this genotype significantly modified the effects of current smoking on the risk of death due to malignant neoplasm.

We further estimated the effects of current smoking on fatal cancer according to ACE genotype after controlling for age, sex, alcohol intake, body mass index, glucose intolerance, serum total cholesterol and systolic blood pressure (Table 4). Compared with non-smokers, current smokers had an increased risk of fatal cancer regardless of their ACE genotypes. However, the risk of fatal cancer in currently smoking subjects with the DD genotype (HR 4.51; 95% CI 1.38–14.79; $P = 0.01$) was twofold greater than that in subjects with genotype II (HR 2.00; 95% CI 1.11–3.59; $P = 0.02$) or ID (HR 2.24; 95% CI 1.20–4.18; $P = 0.01$). Among current smokers, subjects with genotype DD also showed a significantly greater risk of cancer death compared with subjects with genotypes II and ID combined (HR 1.77; 95% CI 1.04–3.00; $P = 0.03$).

Table 3 Age- and sex-adjusted and multivariate-adjusted hazard ratios with 95% confidence intervals of risk factors for fatal cancer

Variable	Age- and sex-adjusted HR[a] (95% CI)	Multivariate-adjusted HR[a] (95% CI)
Age, per 11-year increase	–	2.54 (2.09–3.10)
Sex, women versus men	–	0.72 (0.47–1.12)
Smoking habits, current versus non-smokers	2.06 (1.41–3.00)	1.91 (1.27–2.86)
ACE genotype, DD versus II and ID	1.20 (0.78–1.85)	0.53 (0.21–1.33)
Interaction (smoking habits × ACE genotype), yes versus no	–	3.31 (1.15–9.51)
Alcohol intake, yes versus no	0.91 (0.63–1.30)	0.96 (0.66–1.38)
Body mass index, per 2.6 kg/m^2 increase	1.13 (0.98–1.32)	1.19 (1.02–1.40)
Glucose intolerance, yes versus no	1.25 (0.77–2.03)	1.19 (0.71–1.98)
Serum total cholesterol, per 0.9 mmol/l increase	0.97 (0.83–1.13)	0.95 (0.80–1.12)
Systolic blood pressure, per 26 mmHg increase	0.84 (0.71–1.00)	0.84 (0.70–1.01)

ACE, angiotensin I-converting enzyme; D, deletion; I, insertion; HR, hazard ratio; 95% CI, 95% confidence interval.
[a]Hazard ratios for continuous variables represent a difference of a standard deviation.

Discussion

In a prospective study of a general population, we found greater effects of current smoking on the instance of fatal cancer among subjects with ACE genotype DD compared with those with other genotypes. To the best of our knowledge, this is the first paper to suggest that ACE genotype DD enhances the association between smoking and the risk of cancer death. The strengths of the present study are that the causes of death were verified by autopsy in almost 80% of the deceased subjects, and that the tissue samples were stored for a long time. These circumstances provided us the opportunity to examine the long-term prospective contribution of smoking and ACE genotype to fatal cancer in a general population.

Previous studies have provided evidences linking the renin–angiotensin system with malignant neoplasm. Angiotensin II, which is a key peptide of the renin–angiotensin system, induces a mitogenic response in cancer cells (Muscella *et al.*, 2002), and blockade of the renin–angiotensin system reduces tumour growth (Volpert *et al.*, 1996; Small *et al.*, 1997; Fujita *et al.*, 2002), angiogenesis (Volpert *et al.*, 1996; Fujita *et al.*, 2002) and metastasis (Fujita *et al.*, 2002). The activities of the renin–angiotensin system are determined by genetic factors as well as by environmental ones. Plasma levels of ACE, which generates angiotensin II, are highly genetically determined, and a substantial proportion of interindividual variability is determined by the presence (I) or absence (D) of a 287-bp *Alu* sequence in intron 16 of the ACE gene (Rigat *et al.*, 1990). The D allele leads to higher expression of ACE mRNA than the I allele (Suehiro *et al.*, 2004), and therefore the DD genotype induces the highest circulating ACE levels and enhances the renin–angiotensin system (Rigat *et al.*, 1990). Through these mechanisms, ACE genotype DD may advance carcinogenesis.

The development of cancer is a multistep process, which is generally described as involving three steps: initiation, promotion and progression. Smoking is believed to be largely involved in the initiation and promotion steps of carcinogenesis (Suzuki *et al.*, 1992; Denissenko *et al.*, 1996; Pryor, 1997), while ACE genotype DD might be involved in the progression step (Volpert *et al.*, 1996;

Table 4 Effects of current-smoking on fatal cancer according to angiotensin I-converting enzyme genotype

ACE genotype	Non-smoker			Current smoker			
	Person-years	*N*	Mortality	Person-years	*N*	Mortality	Adjusted HR[a] (95% CI)
II	4254	25	5.9	3181	40	12.6	2.00 (1.11–3.59)
ID	5463	39	7.1	4150	48	11.6	2.24 (1.20–4.18)
DD	1348	7	5.2	931	17	18.3	4.51 (1.38–14.79)

ACE, angiotensin I-converting enzyme; D, deletion; I, insertion; HR, hazard ratio; 95% CI, 95% confidence interval.
N: Number of fatal cancers. Mortality: per 1000 person-years.
[a]Adjustment has been made for age, sex, alcohol intake, body mass index, glucose intolerance, serum total cholesterol and systolic blood pressure.

Small *et al.*, 1997; Fujita *et al.*, 2002; Muscella *et al.*, 2002). The combination of smoking and the DD genotype has the potential to accelerate all steps of carcinogenesis and then to increase the risk of fatal cancer. Smoking has also been shown to increase plasma renin activity (Laustiola *et al.*, 1988). Elevated plasma renin activity (Laustiola *et al.*, 1988) together with elevated ACE levels (Rigat *et al.*, 1990) might accelerate the production of angiotensin II and thereby advance carcinogenesis, particularly among smokers with ACE genotype DD.

With regard to clinical studies using ACE inhibitors, Lever *et al.* (1998) report clinical evidence supporting the concept that long-term use of ACE inhibitors may protect against incident and fatal cancer, while other studies have shown no clear associations (Lindholm *et al.*, 2001; ALLHAT Officers and Coordinators, 2002). In these studies, however, smoking habits were more frequent (48%) among the subjects in Lever's report (Lever *et al.*, 1998) than among the subjects in the other two studies (9–22%) (Lindholm *et al.*, 2001; ALLHAT Officers and Coordinators, 2002). The present results indicate the possibility that ACE increases the risk of cancer primarily among smokers. Thus, protective effects of ACE inhibitors against cancer might have been detected by Lever *et al.* (1998). The different results regarding the effects of ACE genotype DD on malignant neoplasm reported in several other clinical studies (Cheon *et al.*, 2000; Usmani *et al.*, 2000; Haiman *et al.*, 2003; Hajek *et al.*, 2003; Koh *et al.*, 2003; Medeiros *et al.*, 2004) might also be due to differences in smoking status as well as in study design.

There are several potential limitations to the findings of the present study. The primary limitation is the small number of subjects in the study population; this makes further stratified analyses (e.g. by tumour sites) difficult. Thus, the generalizability of the present results may be somewhat limited. Nonetheless, we believe that the findings of our study represent an actual effect of ACE genotype on the association between smoking and cancer since we used a highly accurate method of determining all cases of cancer death. Another limitation is that our results might be biased by the exclusion of subjects whose genotype could not be determined. Among the 1606 subjects who were involved in the original cohort of 1621 participants and for whom data on smoking habits were available, 669 subjects without genotype information were younger than the 937 subjects with genotype information (54 versus 58 years, $P < 0.0001$) and a larger proportion of the population was female (62% versus 53%, $P = 0.0002$) at baseline. When adjustments were made for age and sex, subjects without information were more obese (body mass index 21.8 versus 21.5 kg/m^2, $P = 0.02$) and were more likely to use alcohol (41 versus 35%, $P = 0.03$). However, no significant differences were observed between these groups in terms of other risk factors including current smoking (45% versus 44%, $P = 0.6$) and cancer mortality (8.0 versus 9.1 per 1000 person-years, $P = 0.2$). Finally, samples for genotyping were obtained from almost 80% of the deceased and our subject group is considered not to be biased due to deaths that occurred during the follow-up period. That is, this bias is not likely to have the potential to alter the present findings.

In conclusion, the present population-based prospective study suggests greater effects of current smoking on fatal cancer among subjects with ACE genotype DD than on those with genotype II or ID. These results raise the possibility that smoking cessation may have the greatest impact for reducing cancer mortality among people with ACE genotype DD. Further prospective studies of larger populations are needed to verify these findings.

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