Original Article

Angiotensin I-Converting Enzyme Gene Polymorphism Enhances the Effect of Hypercholesterolemia on the Risk of Coronary Heart Disease in a General Japanese Population: The Hisayama Study

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Aim: The angiotensin I-converting enzyme (ACE) insertion (I)/deletion (D) polymorphism has been reported to be implicated in susceptibility to coronary heart disease (CHD). However, this association remains inconclusive. The purpose of this study was to clarify the association between the I/D polymorphism of the ACE gene and the development of CHD in a Japanese general population and investigate whether the effects of traditional risk factors on the risk of CHD are heterogeneous among ACE genotypes.

Methods: The subjects included 2,125 community-dwelling individuals 40 years of age or older without cardiovascular disease for whom genetic information was available. All patients were prospectively followed for 19 years, and the association between the ACE polymorphism and the incidence of CHD was examined based on the interactions with traditional risk factors, such as hypercholesterolemia, hypertension, diabetes and smoking.

Results: A total of 161 CHD events occurred during the follow-up period. The age- and sex-adjusted incidence of CHD was not significantly different among the genotypes (5.8, 5.2, and 6.9 per 1,000 person-years for genotypes II, ID and DD, respectively). In a stratified analysis, however, the ACE DD genotype was found to significantly accelerate the risk of developing CHD by hypercholesterolemia (hazard ratio [HR]=4.50, 95% confidence interval=2.02-10.04 for hypercholesterolemia with the DD genotype; HR=1.48, 95% CI=1.04-2.12 for hypercholesterolemia with the ID+II genotype; P for interaction=0.01), even after adjusting for other confounding factors, whereas no such associations were observed for hypertension, diabetes or smoking.

Conclusions: The current findings suggest that the ACE DD genotype enhances the effect of hypercholesterolemia on the development of CHD in the general Japanese population.

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Key words: ACE polymorphism, Coronary heart disease, Cohort study, Hypercholesterolemia

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Introduction

Coronary heart disease (CHD) is a major cause of morbidity and mortality in industrialized countries[1]. Accumulated evidence has identified a number of risk factors, including hypercholesterolemia, hypertension, diabetes and smoking, for the development of

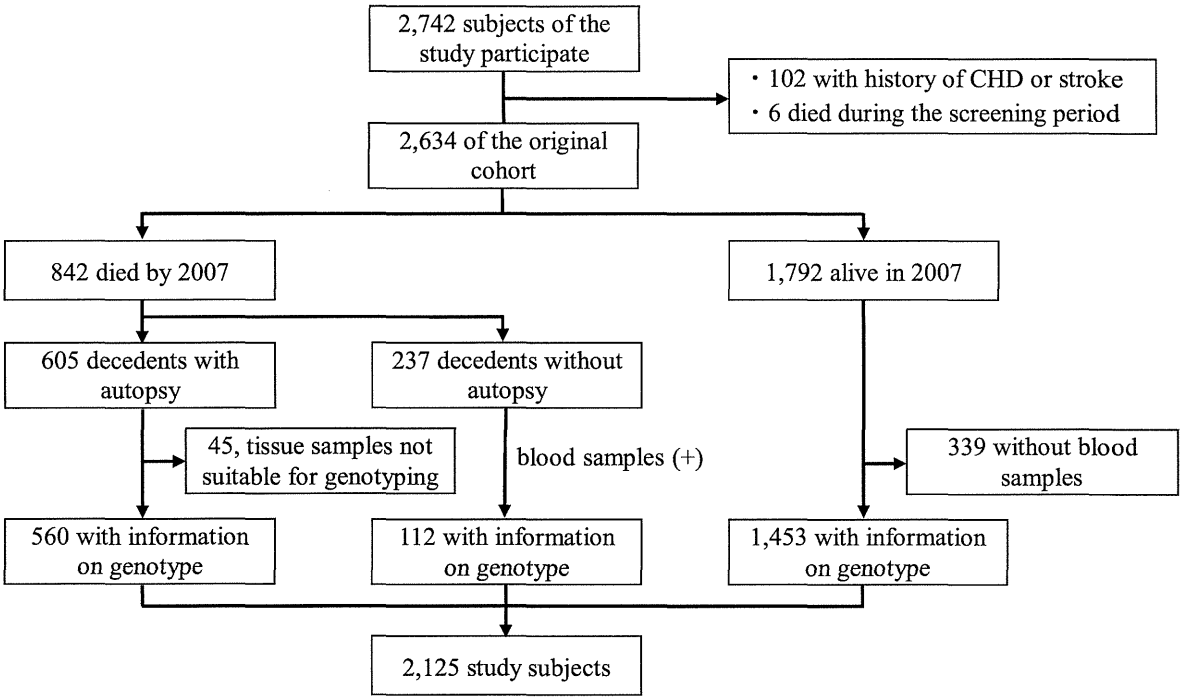

Fig. 1. Flow diagram of the selection of the study subjects.

CHD[2-4]. The renin-angiotensin system has also been acknowledged to be involved in the pathogenetic mechanisms of CHD. The plasma activity of angiotensin-I converting enzyme (ACE) regulates the production of angiotensin II, which has been shown to promote inflammation, reactive oxygen species generation, cell proliferation/apoptosis, fibrosis and oxidized lipid production[5].

The ACE gene contains a polymorphism characterized by either the insertion (I) or deletion (D) of a 287-base pair *Alu* repetitive sequence in intron 16. The D allele of this gene site has been reported to be associated with an increased plasma ACE activity[6]. Since being first reported by Cambien *et al.*[7], this polymorphism has received much attention as a susceptibility gene for CHD[8]. On the other hand, contrary evidence suggesting that there is no association between this polymorphism and the risk of CHD has also been reported[9, 10]. In addition, recent findings of observational studies and clinical trials have raised the possibility that the ACE gene polymorphism modifies the effects of cardiovascular risk factors and their treatment on the risk of CHD[11-15].

In order to clarify these issues, we established a community-based prospective cohort study to explore both environmental and genetic risk factors for cardiovascular disease in Japan. The aim of this study was to elucidate the association between the I/D polymorphism of the ACE gene and the development of CHD in a general Japanese population and investigate whether the influence of traditional risk factors on the risk of CHD is heterogeneous among ACE genotypes.

Methods

Study Protocol

The Hisayama Study is a prospective population-based cohort study of cerebro- and cardiovascular diseases established in 1961 in the town of Hisayama, a suburban community adjacent to Fukuoka City, a metropolitan area on Kyushu Island in Japan. Full community surveys of the health status of the residents have been repeated since that time[16]. In 1988, a screening survey was performed in the town for the present study. A detailed description of this survey has been published previously[17]. Briefly, a total of 2,742 residents 40 years of age or older (80.9% of the total population in that age group) consented to participate in the examination and underwent comprehensive assessments. After excluding 102 subjects with a history of CHD or stroke and six subjects who died during the screening period, a total of 2,634 subjects were registered as the original cohort (**Fig. 1**). In this population, 842 subjects died by 2007, of whom 605 (71.9%) underwent autopsies. In the autopsy cases, tissue samples of the main organs, such as the brain, heart,

lungs, liver, spleen, gastrointestinal tract and kidneys, were formalin fixed, paraffin embedded and stored until 2000, after which they were fresh frozen. Among these cases, 45 patients had tissue samples not suitable for genotyping; thus, genotyping was performed in the remaining 560 cases. Among the deceased subjects who did not undergo an autopsy, 112 provided blood samples before their death. In the surviving subjects, blood samples were gathered from 1,453 participants. Hence, suitable paraffin-embedded tissues, fresh-frozen tissues or blood samples were available for a total 2,125 subjects (866 men and 1,259 women, 80.7% of the original cohort), who were then selected for ACE gene I/D genotyping and enrolled in the present analysis.

Follow-up Survey

The subjects were followed prospectively for 19 years, from December 1988 to November 2007, using repeated health examinations. The health status of subjects who did not undergo regular examinations or who moved out of the town was checked yearly by mail or telephone. We also established a daily monitoring system incorporating the study team and local physicians or members of the town's Health and Welfare office. When CHD was diagnosed or suspected, physicians on the study team examined the subject and evaluated his/her detailed clinical information. The clinical diagnosis of CHD was made based on the patient's history, physical examination findings and ancillary laboratory examination data. Furthermore, when a subject died, an autopsy was performed at the Department of Pathology at Kyushu University. There was no true loss to follow-up during the follow-up period (autopsy rate: 71.9%).

Definition of Coronary Heart Disease

The criteria for a diagnosis of CHD included first-ever acute myocardial infarction, silent myocardial infarction, sudden cardiac death within one hour after the onset of acute illness or coronary artery disease followed by coronary artery bypass grafting or angioplasty[18-20]. Acute myocardial infarction was diagnosed in subjects who met at least two of the following criteria: (1) typical symptoms, including prolonged severe anterior chest pain; (2) abnormal cardiac enzymes more than twice the upper limit of the normal range; (3) evolving diagnostic electrocardiographic changes; and (4) morphological changes, including local asynergy of cardiac wall motion on echocardiography, persistent perfusion defects on cardiac scintigraphy and/or myocardial necrosis or scars ≥ 1 cm long accompanied by coronary atherosclerosis at autopsy. Silent myocardial infarction was defined as myocardial scarring without historical indications of clinical symptoms or abnormal changes in cardiac enzymes. The clinical diagnosis was corrected according to the autopsy findings when necessary.

Definition of the ACE Polymorphism

Extraction of DNA from the blood samples and fresh-frozen tissue samples was performed as described previously, respectively[21, 22]. The ACE I/D genotype was determined using the polymerase chain reaction method, as described by Evans *et al.*[23]. For 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[24]. The accuracy of these genotyping methods was demonstrated in our previous study[24].

Risk Factor Measurement

At baseline, each participant completed a self-administered questionnaire covering their medical history, treatment for hypertension or diabetes, alcohol intake and smoking and exercise habits. The questionnaires were reviewed by trained interviewers at the time of screening. Smoking and alcohol intake were classified as currently habitual or not. Subjects engaging in sports or other forms of exertion ≥ 3 times a week during their leisure time made up the regular exercise group. Blood pressure was measured three times using a standard mercury sphygmomanometer in the sitting position after at least five minutes of rest. The mean of the three measurements was used for the analysis. Hypertension was defined as a blood pressure of ≥ 140/90 mmHg and/or the current use of antihypertensive agents. Body height and weight were measured in light clothing without shoes, and the body mass index (kg/m^2) was calculated. Obesity was defined as a body mass index of ≥ 25 kg/m^2. Electrocardiogram abnormalities included left ventricular hypertrophy (Minnesota Code, 3-1), ST depression (4-1, 2, 3) or atrial fibrillation (8-3). The blood samples were collected from the antecubital vein after an overnight fast to determine the lipid and blood glucose levels. The serum total cholesterol concentrations were determined enzymatically. Hypercholesterolemia was defined as a serum cholesterol level of ≥ 5.69 mmol/L (220 mg/dL). The plasma glucose levels were measured based on the glucose oxidase method. Diabetes was defined according to the criteria recommended by the American Diabetes Association, in addition to a medical history of diabetes, using 75 g oral glucose tolerance tests in 1,992 subjects (93.7%)[25]

Table 1. Clinical characteristics of the study population according to the ACE genotype in 1988

Variable	ACE genotype			*p* value for trend
	II (n=918)	ID (n=927)	DD (n=280)	
Age, y	59±11	60±12	60±11	0.06
Men, %	40.3	41.8	38.2	0.80
Systolic blood pressure, mmHg	133±21	134±22	135±21	0.44
Diastolic blood pressure, mmHg	78±12	77±11	78±11	0.62
Antihypertensive medication, %	13.1	14.7	14.8	0.22
Hypertension, %	39.2	41.4	42.6	0.24
Diabetes, %	9.8	12.1	13.1	0.06
Serum total cholesterol, mmol/L	5.33±1.09	5.37±1.05	5.39±1.10	0.32
Hypercholesterolemia, %	36.2	36.1	33.4	0.42
Body mass index, kg/m^2	23.0±3.2	22.7±3.1	22.8±3.1	0.37
Obesity, %	24.2	22.6	22.5	0.54
Electrocardiogram abnormalities, %	17.0	15.6	17.8	0.93
Smoking habits, %	24.9	23.2	25.6	0.63
Alcohol intake, %	30.0	29.7	31.3	0.70
Regular exercise, %	9.4	11.4	13.7	0.03

ACE, angiotensin I-converting enzyme; HDL, high-density lipoprotein.
The values are presented as the mean ± standard deviation or percentage.

and the fasting and postprandial glucose concentrations in the 133 remaining subjects.

Statistical Analysis

The SAS computer package version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. The age- and sex-adjusted mean values of the possible risk factors were calculated and tested according to the analysis of covariance method, and trends in these parameters across the ACE polymorphisms were tested using a multiple regression analysis. The frequency of each risk factor was adjusted for age and sex using the direct method, and trends were examined using a logistic regression analysis. The incidence of CHD was calculated according to the person-year method and adjusted for age and sex using the direct method with 10-year age groups. Differences in the incidence of CHD between the subjects with and without the ACE genotype were analyzed by means of a Cox proportional hazards regression analysis after adjusting for age and sex. Cumulative incidence curves for CHD were estimated according to the Kaplan-Meier method, and differences in incidence were assessed using the log-lank test. Age- and sex-adjusted or multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were also estimated using a Cox proportional hazards model. The interactions in the associations between subgroups were tested by adding a multiplicative interaction term to the relevant Cox model. Two-tailed p values of <0.05 were considered to be statistically significant in all analyses.

Ethics Approval

This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research. Written informed consent for medical research was obtained from each participant or their family members if deceased.

Results

The frequency of each ACE genotype was 43.2% for II, 43.6% for ID and 13.2% for DD; these genotype frequencies were in agreement with the Hardy-Weinberg equilibrium (chi-square, 2.14; *df*, 2; p= 0.66) and are similar to those found in previous studies of Japanese populations[26, 27]. **Table 1** shows the baseline clinical and demographic characteristics of the study subjects according to the ACE genotypes of II, ID and DD. The frequency of regular exercise was higher in the subjects with the DD genotype than in those with the ID or II genotype. There was no evidence of differences in the mean values or frequencies of other risk factors across the ACE genotypes.

During the 19-year follow-up period, 161 first-

Table 2. Age- and sex-adjusted incidence and adjusted HR for coronary heart disease according to the ACE genotype in the 2,125 subjects during the 19-year follow-up period

ACE genotype	Person-years at risk	No. of events	Age- and sex-adjusted incidence (per 1,000 PYs)	Age- and sex-adjusted		Multivariable-adjusted[a]		ACE genotype and interaction	Multivariable-adjusted[a]	
				HR (95% CI)	*p* value	HR (95% CI)	*p* value		HR (95% CI)	*p* value
II	14,890	68	5.8	1.00 (reference)		1.00 (reference)		II	1.00 (reference)	
ID	14,963	66	5.2	0.85 (0.61-1.19)	0.35	0.85 (0.60-1.20)	0.36	ID	0.69 (0.44-1.08)	0.10
DD	4,507	27	6.9	1.18 (0.76-1.85)	0.46	1.18 (0.75-1.85)	0.47	DD	0.60 (0.29-1.23)	0.16
								INT (ID x HC)	1.60 (0.80-3.19)	0.19
								INT (DD x HC)	3.33 (1.30-8.56)	0.01
II + ID	29,853	134	5.5	1.00 (reference)		1.00 (reference)		II + ID	1.00 (reference)	
DD	4,507	27	6.9	1.29 (0.85-1.95)	0.23	1.28 (0.85-1.95)	0.24	DD	0.72 (0.36-1.44)	0.36
								INT (DD x HC)	2.63 (1.10-6.32)	0.03
II	14,890	68	5.8	1.00 (reference)		1.00 (reference)		II	1.00 (reference)	
DD + ID	19,470	93	5.6	0.93 (0.68-1.27)	0.63	0.93 (0.67-1.28)	0.64	DD + ID	0.70 (0.46-1.07)	0.10
								INT (DD + ID x HC)	1.88 (0.99-3.58)	0.06
I	44,743	202	5.6	1.00 (reference)		1.00 (reference)		I	1.00 (reference)	
D	23,977	120	5.8	1.03 (0.82-1.29)	0.80	1.03 (0.82-1.30)	0.79	D	0.75 (0.54-1.03)	0.07
								INT (D x HC)	1.89 (1.20-2.99)	0.006

ACE, angiotensin I-converting enzyme; PYs, person-years; HR, hazard ratio; CI, confidence interval; HC, hypercholesterolemia; INT, interaction term
In the analysis of the I/D allele, the number of subjects and events was counted twice.
a) The risk estimates were adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, obesity, electrocardiogram abnormalities, smoking, alcohol intake and regular exercise.

ever CHD events (98 men and 63 women) were noted. As shown in **Table 2**, the age- and sex-adjusted incidence of CHD (per 1,000 person-years) was 5.8, 5.2 and 6.9 for the ACE genotypes II, ID and DD, respectively. There were no significant differences in the age- and sex-adjusted HRs for the development of CHD among the ACE genotypes. These associations remained substantially unchanged following adjustment for age, sex, ACE genotype, hypercholesterolemia, hypertension, diabetes, obesity, electrocardiogram abnormalities, smoking, alcohol intake and regular exercise. The same was true for the analysis of a genetic inheritance pattern of the recessive or dominant genotype as well as the analysis of the I/D allele. In addition, we investigated the interactions between the cardiovascular risk factors and the ACE genotype with respect to the development of CHD. The interaction terms between hypercholesterolemia and the ACE genotypes, except for the dominant genotype, were significant (**Table 2**). On the other hand, there was no evidence of a significant interaction regarding the extent of the effect of other cardiovascular risk factors, namely age, sex, hypertension, diabetes, obesity, smoking, alcohol intake and regular exercise, between the two ACE genotype subgroups (all *p* values for interaction >0.11).

Next, we compared the influence of the cardiovascular risk factors on the development of CHD among the subgroups of the ACE genotype (II + ID and DD), as individuals with the DD genotype have been reported to exhibit an increased plasma ACE activity[6]. When the current subjects were divided into four groups according to the ACE genotype and hypercholesterolemia, the cumulative incidence of CHD was found to be significantly higher in the subjects with the DD genotype and hypercholesterolemia than in those with the II + ID genotypes and non-hypercholesterolemia (**Fig. 2**). As shown in **Fig. 3**, after adjusting for the aforementioned confounding factors, hypercholesterolemia was found to be significantly associated with a 1.53-fold (95% CI 1.06-2.20) higher risk of incident CHD in the subjects with the II + ID genotypes, whereas this risk factor exerted much greater influence in the subjects with the DD genotype (HR 4.02, 95% CI 1.79-9.02; *p* for interaction = 0.03). When the serum total cholesterol levels were used as a continuous variable in the analysis, every 1 mmol/L increment in the serum total cholesterol level was identified to be associated with a 1.16-fold (95% CI, 0.98-1.36) higher risk of CHD in the subjects with the II + ID genotypes and a 1.78-fold (95% CI, 1.29-2.45) higher risk in those with the DD genotype (*p* for heterogeneity = 0.02). Similar findings were observed in the analysis of the I/D allele (**Supplementary Fig. 1**), whereas no significant interactions were observed in the analysis of the ID + DD/II genotypes (**Supplementary Fig. 2**).

We also performed a sensitivity analysis using the data for sudden cardiac death within 24, rather than one, hours after the onset of acute illness. Conse-

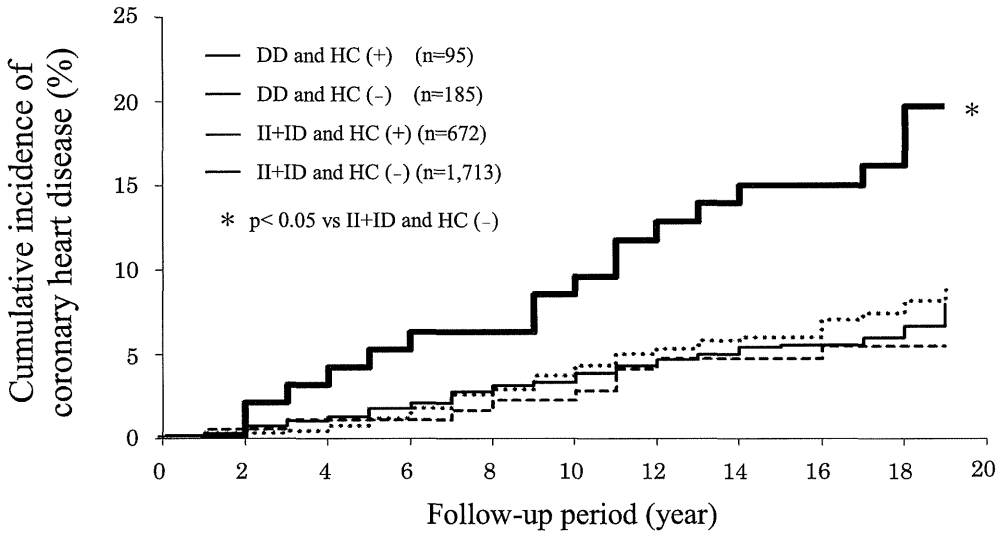

Fig. 2. Cumulative incidence of coronary heart disease according to the ACE genotype and cholesterol levels in the 2,125 subjects during the 19-year follow-up period.

ACE, angiotensin I-converting enzyme; HC, hypercholesterolemia
*$p < 0.05$ vs. II + ID and HC(−)

quently, the findings were not altered substantially (**Supplementary Table 1** and **Supplementary Fig. 3**).

Discussion

In the present study, there was no evidence of a significant association between the I/D polymorphism of the ACE gene and the risk of CHD. However, the magnitude of the effect of hypercholesterolemia on the risk of incident CHD was greater in the subjects with the DD genotype than those with the II + ID genotypes. To our knowledge, this is the first population-based prospective study to assess the interaction between the ACE polymorphism and hypercholesterolemia in patients with incident CHD.

Since the first report by Cambien *et al.* published in 1992[7], the DD genotype has been investigated as a potential CHD risk factor[8]. However, large cohort studies[9, 10] and subsequent meta-analyses have failed to confirm this association[28, 29]. In addition, both studies supporting these findings as well as questioning the veracity of the association have been published, resulting in uncertainty regarding the importance of this polymorphism. The present study also failed to confirm a significant association between the DD genotype of the ACE gene and an increased risk of incident CHD. Therefore, it may be said that there is no strong evidence to date to indicate that the DD genotype of the ACE gene is a significant risk factor for the development of CHD. This finding may reflect the relatively modest influence of the I/D polymorphism of the ACE gene itself on the risk of CHD.

In the present study, the observed differences in the magnitude of the association between hypercholesterolemia and the risk of CHD between the ACE genotypes were unexpected and may have been due to chance. However, we believe that these results represent a real difference, since similar heterogeneity of influence was observed in the analysis using the serum cholesterol level as a continuous variable. Several epidemiological studies have also demonstrated an interaction between the I/D polymorphism of the ACE gene and metabolic risk factors on the risk of CHD[11-14]. In addition, the results of a clinical trial conducted among 429 patients with coronary atherosclerotic lesions and hypercholesterolemia showed that the administration of cholesterol-lowering therapy with statins achieved a greater reduction in the plasma cholesterol levels and exhibited more significant preventive effects against the progression of coronary atherosclerosis in the subjects with the DD genotype than in those with the II and ID genotypes, suggesting that the I/D polymorphism of the ACE gene modifies the effectiveness of cholesterol-lowering therapy in ameliorating the risk of CHD[15]. On the other hand, recent findings concerning the influence of the ACE genotype with respect to the effects of cholesterol-lowering therapy on the risk of CHD are diverse[30, 31]. Therefore, further validation studies are needed to clarify this issue.

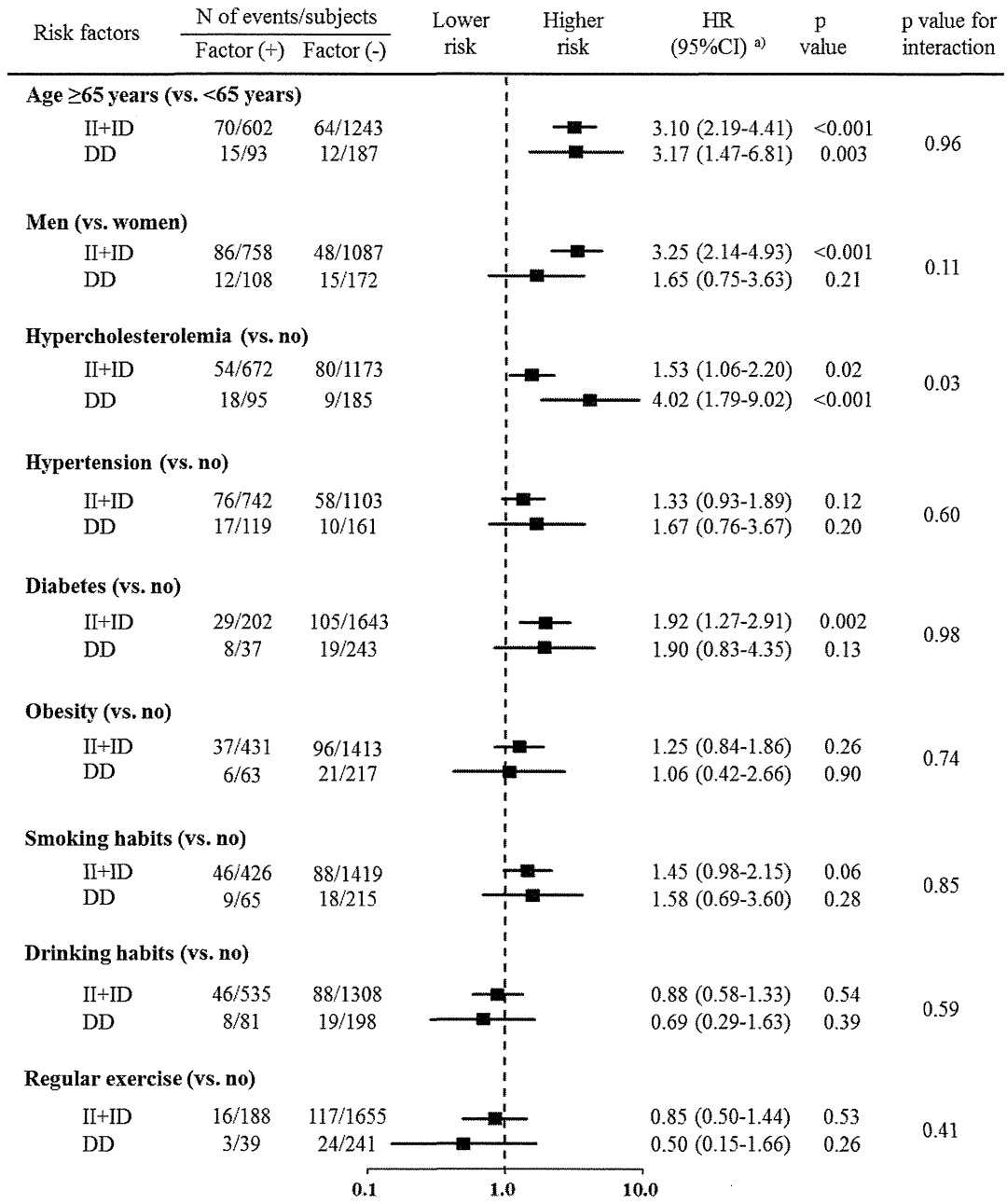

Fig. 3. Comparison of the effects of traditional cardiovascular risk factors on the development of coronary heart disease between the subgroups of the ACE DD/ID + II genotypes.

a) The risk estimates were adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, obesity, electrocardiogram abnormalities, smoking, alcohol intake and regular exercise. The variable relevant to the subgroup was excluded from each model.
ACE, angiotensin I-converting enzyme; CI, confidence interval

Existing evidence does not indicate a clear biologically plausible mechanism explaining the association observed in this study. However, several experimental studies have indicated that there is an interaction between the products of the ACE enzymatic activity (e.g., angiotensin II and bradykinin) and low-density lipoprotein (LDL) cholesterol peroxidation[32-35]. Under hyperlipidemic conditions, angiotensin II has been found to promote the oxidation of LDL cholesterol and upregulation of its receptor and lectin-like

oxidized LDL receptor-1 (LOX-1) in human coronary artery endothelial cells[32]. At the same time, oxidized LDL cholesterol increases the angiotensin II expression[33] and angiotensin II type 1 receptor expression[34] and decreases the vasodilator reactivity of bradykinin *in vitro*[35]. This vicious feedback cycle may contribute to the initiation and progression of atherosclerosis. Since ACE is a key factor in the regulation of vasoconstrictor angiotensin II and vasodilator bradykinin and the D polymorphism of the ACE gene increases the ACE activity in the plasma[6] and inflammatory cells[36], it is reasonable to suppose that the differences in the plasma and local tissue concentrations of the products of the ACE activity among ACE genotypes affect LDL cholesterol peroxidation, endothelial-cell LDL uptake[37], the foam cell activity[38] and thus the subsequent severity of coronary atherosclerosis[39]. Therefore, the D polymorphism may enhance the effect of hypercholesterolemia on the risk of incident CHD. The strengths of this study include the longitudinal population-based design, long duration of follow-up and accuracy of the diagnosis of incident CHD. However, some limitations should also be noted. First, there may have been selection bias in the study population. Compared with the 2,125 study subjects for whom genotype information was available, the 509 subjects excluded from the study for whom no DNA samples were available were younger (56 versus 60 years of age, $p<0.01$) and included a greater proportion of men (45.0% versus 40.8%, $p=0.02$). When adjustments were made for age and sex, the subjects without genomic information had a greater incidence of prevalent diabetes (17.1% versus 11.2%, $p=0.03$) and hypertension (49.8% versus 40.5%, $p<0.01$). However, no significant differences were observed between the two groups in terms of the other risk factors, including the serum total cholesterol levels (5.29 versus 5.36, $p=0.6$) and incidence of CHD (5.1 versus 5.7 per 1,000 person-years, $p=0.7$). Second, the present analysis lacked information on medications, such as statins and ACE inhibitors, that affect cholesterol metabolism and the ACE activity. Importantly, these medications were rarely used in Japan in the 1980's and the early 1990's, and this limitation thus does not appear to have the potential to alter our findings.

In conclusion, in this prospective population-based study, we found that the DD genotype increases the effects of hypercholesterolemia on the occurrence of CHD. This knowledge may lead to the application of non-invasive screening for individuals at high risk for CHD, an additional means of preventing the premature development of CHD, and development of targeted interventional strategies based on each subject's ACE genotype, thereby dramatically reducing both the incidence and lethality of this disease. The current findings indicate that strict and immediate management of hypercholesterolemia should be considered, especially in subjects with the DD genotype, as an effective means of preventing the development of CHD in Japan, where the prevalence of hypercholesterolemia has increased rapidly in recent years.

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Disclosures

There are no conflicts of interest to declare with respect to this study.

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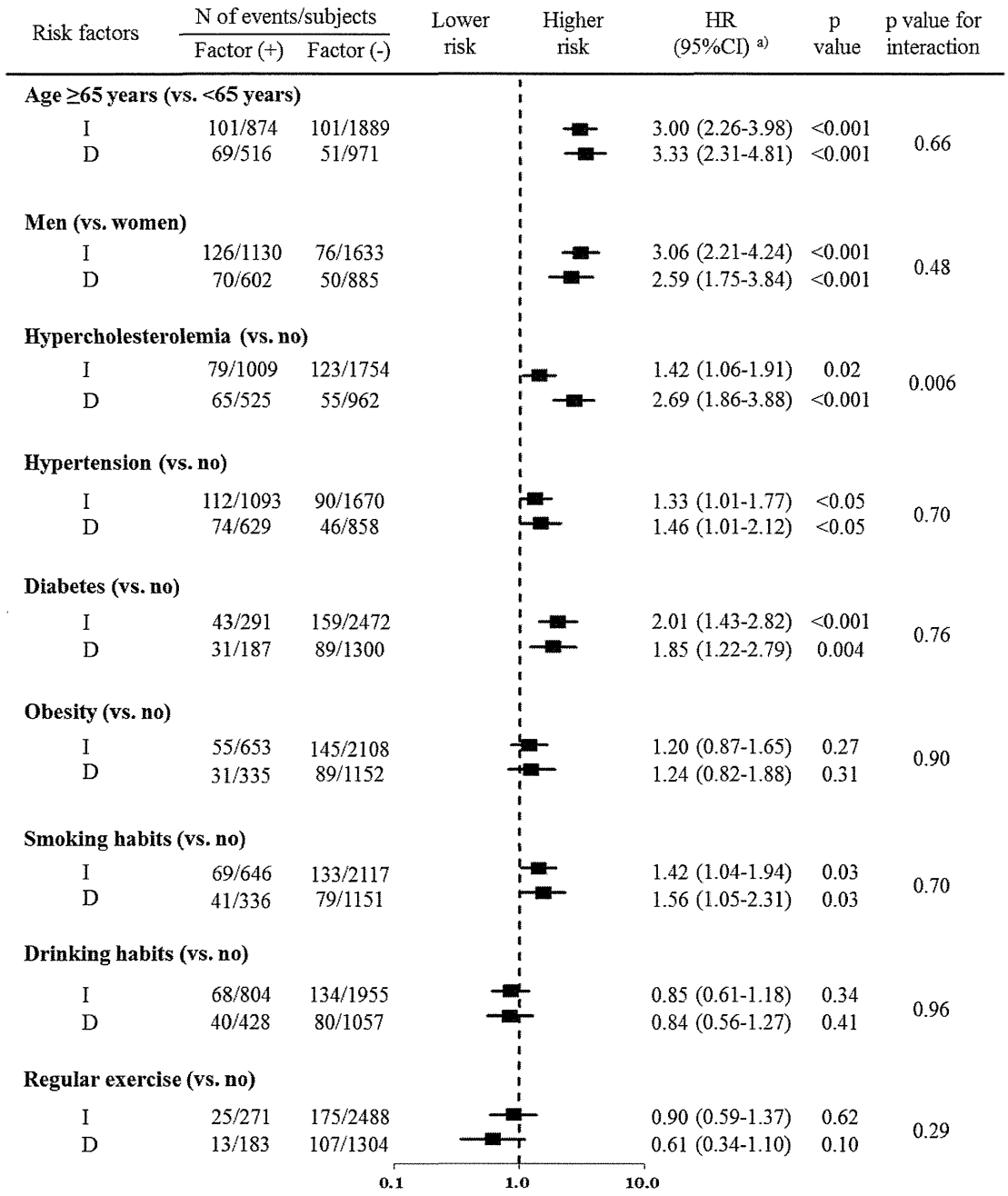
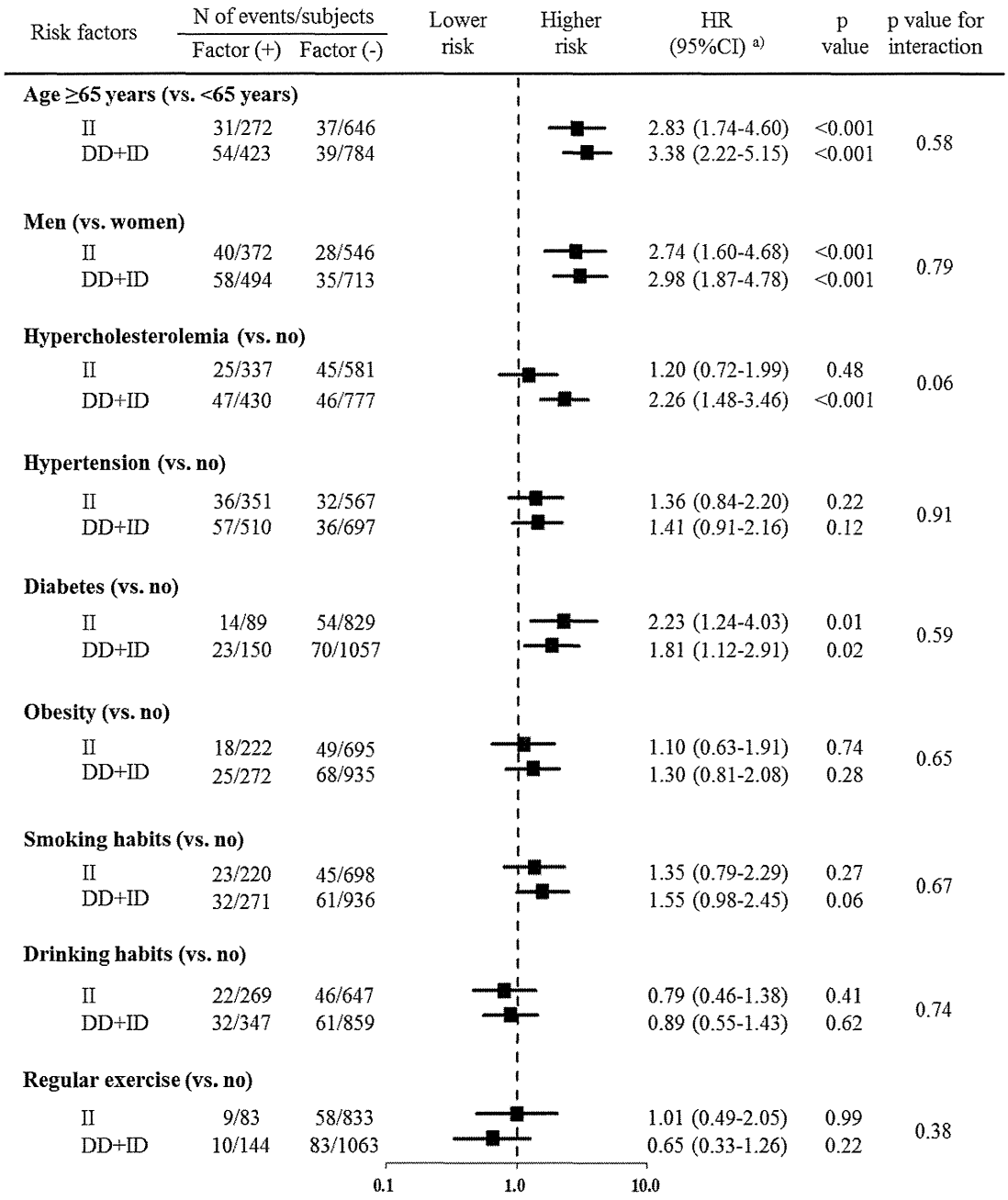


Supplementary Fig. 1.
Comparison of the effects of traditional cardiovascular risk factors on the development of coronary heart disease between the subgroups of the ACE D/I allele.

In the analysis of the I/D allele, the number of subjects and events was counted twice.
a) The risk estimates were adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, obesity, electrocardiogram abnormalities, smoking, alcohol intake and regular exercise. The variable relevant to the subgroup was excluded from each model.
ACE, angiotensin I-converting enzyme; CI, confidence interval



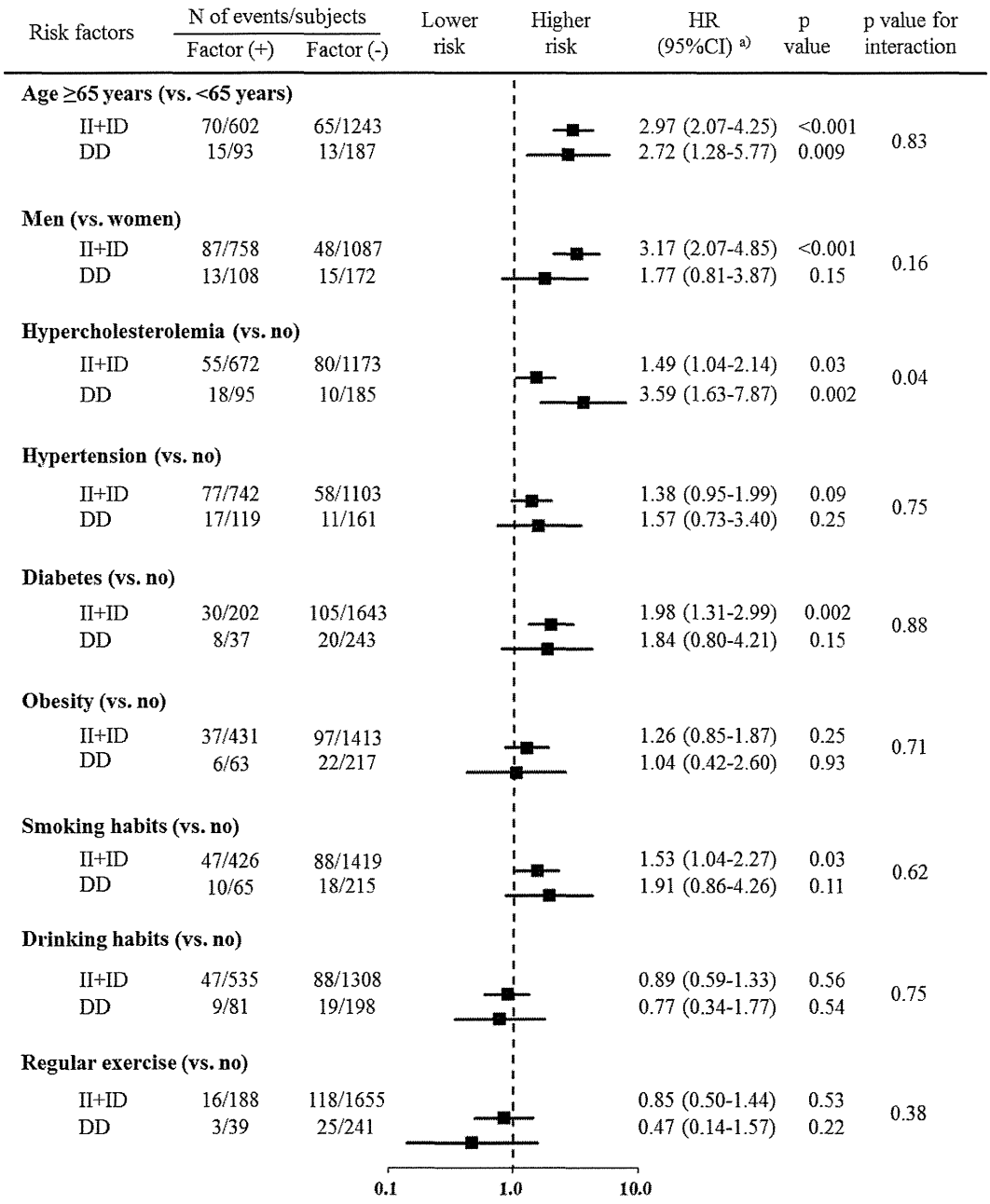
Supplementary Fig. 2.
Comparison of the effects of traditional cardiovascular risk factors on the development of coronary heart disease between the subgroups of the ACE DD + ID/II genotypes.

a) The risk estimates were adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, obesity, electrocardiogram abnormalities, smoking, alcohol intake and regular exercise. The variable relevant to the subgroup was excluded from each model.
ACE, angiotensin I-converting enzyme; CI, confidence interval

Supplementary Table 1.
Age- and sex-adjusted incidence and adjusted HR for coronary heart disease according to the ACE genotype in the 2,125 subjects during the 19-year follow-up period

ACE genotype	Person-years at risk	No. of events	Age- and sex-adjusted incidence (per 1,000 PYs)	Age- and sex-adjusted		Multivariable-adjusted[a]		ACE genotype and interaction	Multivariable-adjusted[a]	
				HR (95% CI)	*p* value	HR (95% CI)	*p* value		HR (95% CI)	*p* value
II	14,890	69	5.9	1.00 (reference)		1.00 (reference)		II	1.00 (reference)	
ID	14,963	66	5.2	0.84 (0.60-1.18)	0.31	0.83 (0.59-1.17)	0.29	ID	0.70 (0.45-1.10)	0.12
DD	4,507	28	7.1	1.21 (0.78-1.88)	0.39	1.18 (0.76-1.84)	0.46	DD	0.68 (0.34-1.36)	0.28
								INT (ID x HC)	1.50 (0.75-2.99)	0.25
								INT (DD x HC)	2.95 (1.18-7.37)	0.03
II + ID	29,853	135	5.5	1.00 (reference)		1.00 (reference)		II + ID	1.00 (reference)	
DD	4,507	28	7.1	1.33 (0.88-1.99)	0.17	1.30 (0.87-1.96)	0.21	DD	0.82 (0.42-1.58)	0.55
								INT (DD x HC)	2.40 (1.03-5.61)	0.04
II	14,890	69	5.9	1.00 (reference)		1.00 (reference)		II	1.00 (reference)	
DD + ID	19,470	94	5.6	0.92 (0.68-1.26)	0.62	0.91 (0.67-1.25)	0.57	DD + ID	0.70 (0.46-1.06)	0.09
								INT (DD + ID x HC)	1.84 (0.98-3.48)	0.06
I	44,743	204	5.6	1.00 (reference)		1.00 (reference)		I	1.00 (reference)	
D	23,977	122	5.9	1.04 (0.83-1.30)	0.75	1.03 (0.82-1.29)	0.81	D	0.79 (0.57-1.08)	0.13
								INT (D x HC)	1.79 (1.14-2.82)	0.02

ACE, angiotensin I-converting enzyme; PYs, person-years; HR, hazard ratio; CI, confidence interval; HC, hypercholesterolemia; INT, interaction term
In the analysis of the I/D allele, the number of subjects and events was counted twice.
a) The risk estimates were adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, obesity, electrocardiogram abnormalities, smoking, alcohol intake and regular exercise.



Supplementary Fig. 3.
Sensitivity analysis of the data for sudden cardiac death within 24 hours to compare the effects of traditional cardiovascular risk factors on the development of coronary heart disease between the subgroups of the ACE DD/ID + II genotypes.

a) The risk estimates were adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, obesity, electrocardiogram abnormalities, smoking, alcohol intake and regular exercise. The variable relevant to the subgroup was excluded from each model.
ACE, angiotensin I-converting enzyme; CI, confidence interval

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ORIGINAL ARTICLE
Epidemiology

Trends in the Incidence and Survival of Intracerebral Hemorrhage by its Location in a Japanese Community

– The Hisayama Study –

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Background: No previous population-based studies have examined secular trends in the incidence of intracerebral hemorrhage (ICH) by its location.

Methods and Results: We established 3 cohorts consisting of residents of Hisayama, Japan, aged ≥40 years without a history of stroke or myocardial infarction in 1961 (the first cohort, n=1,618), in 1974 (the second cohort, n=2,038), and in 1988 (the third cohort, n=2,637). Each cohort was followed for 13 years. The age- and sex-adjusted incidence of ICH significantly declined from the first to the second cohort and showed no further change in the third cohort. With regard to the ICH location, the incidence of putaminal hemorrhage decreased steadily, mainly in subjects aged 60–69 years, whereas the incidence of thalamic hemorrhage increased, especially in those aged ≥70 years. Both hypertension and alcohol intake were strong risk factors for ICH in the first cohort, but their influence declined with time. Blood pressure levels in hypertensive subjects decreased significantly, and the proportion of current drinkers decreased slightly over the study period.

Conclusions: Our findings suggest that the ICH incidence steeply declined from the 1960s to the 1970s in Japan as a result of the reduced influence of hypertension and alcohol intake, but that this decline has leveled off since then, probably because of the increased incidence of thalamic hemorrhage in the elderly in recent years. (*Circ J* 2014; **78:** 403–409)

Key Words: Epidemiology; Incidence; Intracerebral hemorrhage; Risk factors; Survival

Intracerebral hemorrhage (ICH) is the second most common type of stroke and has high morbidity and mortality.[1,2] Asian populations, especially Japanese, have been characterized by a higher incidence of ICH than other ethnic groups.[3–11] Brain imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) are useful tools for diagnosing ICH and its location, but their use was not widespread until the 1970s. Therefore, the most reliable population-based data regarding ICH incidence were established in the 1980s or later.[3] Some population-based studies have examined secular trends in the incidence and survival of ICH, but their results are inconsistent.[12–15] In addition, several studies have examined the distribution or incidence of ICH by its location,[5,8,16–20] but none has observed long-term trends in location-specific incidence in general populations.

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We have previously reported that the incidence of ICH declined steeply from the 1960s to the 1970s, but showed no further change in the 1980s and 1990s, based on data from the Hisayama Study, a population-based cohort study in Japan.[4] As the elderly population has been rapidly increasing in Japan, and the prevalence of cardiovascular risk factors has been changing over time, detailed analysis of the ICH incidence and survival by location is considered to be useful for reducing the

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Table 1. Mean (Standard Deviation) or Prevalence of Cardiovascular Risk Factors at Baseline Among 3 Cohorts of the Hisayama Study

	1st cohort (n=1,618)	2nd cohort (n=2,038)	3rd cohort (n=2,637)	P value for trend
Age, years	56 (11)	57 (12)	59 (12)	<0.001
Women, %	56.4	58.1	57.9	0.39
Hypertension, %	37.9	42.7	41.6	0.42
Antihypertensive agents, %	2.2	8.4	15.0	<0.001
Systolic BP, mmHg*	162.1 (20.6)	160.2 (20.1)	153.3 (18.2)	<0.001
Diastolic BP, mmHg*	89.7 (11.7)	86.2 (11.2)	82.2 (11.0)	<0.001
Hypercholesterolemia, %	5.1	17.3	35.9	<0.001
Total cholesterol, mmol/L	4.1 (1.0)	4.9 (0.9)	5.3 (1.1)	<0.001
Glucose intolerance, %	8.0	10.5	35.2	<0.001
Obesity, %	10.5	16.9	23.4	<0.001
Body mass index, kg/m^2	21.6 (2.7)	22.2 (3.1)	22.8 (3.2)	<0.001
Current drinker, %	34.9	30.1	30.5	0.006
Current smoker, %	42.7	36.9	25.0	<0.001

Values are given as the mean (standard deviation) or as percentage. *Mean systolic and diastolic BPs among hypertensive subjects in each examination.
BP, blood pressure.

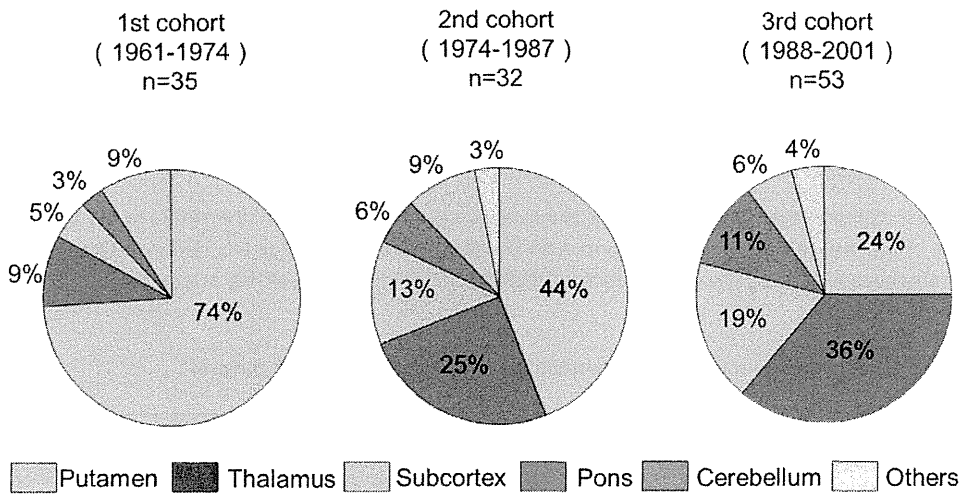

Figure 1. Distribution of hematoma location among patients with intracerebral hemorrhage in 3 cohorts of the Hisayama Study, with a 13-year follow-up for each cohort. Patients with no morphological brain evaluations were excluded from the analysis (8 for the first cohort, 1 for the second).

burden of ICH in the future. It was therefore our intent to examine long-term trends in location-specific incidence and survival of ICH in our population.

Methods

Study Population

The town of Hisayama is a suburban community adjacent to Fukuoka City, a metropolitan area in Kyushu, Japan. The population of the town has been stable over the past 50 years and was approximately 8,400 in 2010. Health examinations of the residents have been carried out annually since 1961. The study design has been previously described in detail.[4] Briefly, we established 3 study cohorts from Hisayama residents aged ≥40 years in 1961, 1974, and 1988 after the screening examinations. In 1961, a total of 1,658 subjects in that age group participated in the screening examination (participation rate, 90.1%). After the exclusion of 28 subjects with a history of stroke or myocardial infarction (MI) and 12 who died or moved out of town during the examination, 1,618 subjects were enrolled as the first cohort. Similarly, after excluding subjects with a history of stroke or MI, we established the second cohort, which consisted of 2,038 subjects from 2,135 participants (participation rate 81.2%), in 1974, and then the third cohort of 2,637 subjects from 2,742 participants (participation rate 80.9%) in 1988. Each cohort was followed based on the repeat health examinations or by mail or telephone for any subjects who did not undergo a regular examination or who moved out of town. We also established a daily monitoring system among the study team, local physicians, and members of the town's Health and Welfare

Table 2. Incidence (per 1000 Person-Years) of Intracerebral Hemorrhage and its Location Among 3 Cohorts of the Hisayama Study, With a 13-Year Follow-up for Each Cohort

	1st cohort (1961–1974) (19,681 PY)	2nd cohort (1974–1987) (25,398 PY)	3rd cohort (1988–2001) (32,854 PY)	P value for trend
Total intracerebral hemorrhage				
No. of events	43	33	53	
Incidence (crude)	2.18	1.30*	1.61	0.16
Incidence (adjusted)†	2.03	0.96*	1.14*	0.05
Putamen				
No. of events	26	14	13	
Incidence (crude)	1.32	0.55*	0.39*	<0.001
Incidence (adjusted)†	1.17	0.45*	0.40*	<0.001
Thalamus				
No. of events	3	8	19	
Incidence (crude)	0.15	0.31	0.58*	0.02
Incidence (adjusted)†	0.16	0.23	0.34	0.04
Subcortex				
No. of events	2	4	10	
Incidence (crude)	0.10	0.16	0.30	0.12
Incidence (adjusted)†	0.07	0.11	0.15	0.18
Pons				
No. of events	1	2	6	
Incidence (crude)	0.05	0.08	0.18	0.17
Incidence (adjusted)†	0.08	0.04	0.08	0.26
Cerebellum				
No. of events	3	3	3	
Incidence (crude)	0.15	0.12	0.09	0.51
Incidence (adjusted)†	0.16	0.08	0.04	0.34
Other determined locations				
No. of events	0	1	2	
Incidence (crude)	0	0.04	0.06	0.32
Incidence (adjusted)†	0	0.02	0.12	0.28
Undetermined location				
No. of events	8	1	0	
Incidence (crude)	0.41	0.04*	0	0.009
Incidence (adjusted)†	0.40	0.02*	0	0.008

*P<0.05 vs. the 1st cohort. †Adjusted for age and sex. There are no significant differences between the 2nd and the 3rd cohorts for all.
PY, person-years.

Office, through which we gathered information of new events of stroke, including suspected cases. When stroke occurred or was suspected, physicians in the study team, including the neurologist, examined the subject and evaluated his/her detailed clinical information. When the subjects died, autopsy examinations were performed at the Department of Pathology of Kyushu University. During the 13-year follow-up period for each cohort, autopsy examinations were performed on 358 subjects (81.9% of the deceased subjects) in the first cohort, 371 (85.9%) in the second, and 402 (76.1%) in the third. Only 2 subjects in the first cohort and 1 in the second were lost to follow-up.

Definition of ICH and its Location

Stroke was defined as a sudden onset of nonconvulsive and focal neurologic deficit persisting for >24 h and was further classified as ischemic stroke, ICH, subarachnoid hemorrhage, or undetermined type. The diagnosis of ICH was determined on the basis of clinical information, including brain imaging (CT and MRI), and autopsy findings. When a patient experienced recurrent stroke episodes, the first ICH event was analyzed. During the 13-year follow-up for each cohort, the first-ever ICH developed in 43 subjects in the first cohort, 33 in the second cohort, and 53 in the third cohort. Of these patients, brain imaging was conducted for 1 (2%) in the first cohort, 21 (64%) in the second cohort, and 53 (100%) in the third cohort, and autopsy was carried out for 35 (81%), 25 (76%), and 26 (49%) patients, respectively. Consequently, morphological examinations by brain imaging and/or autopsy were available for 35 (81%), 32 (97%), and 53 (100%) patients, respectively. For 9 patients (8 in the first cohort, 1 in the second) without a morphological examination, we diagnosed ICH based solely on clinical information, including rapid evolution of focal neurological signs or coma, signs of meningeal irritation, elevated blood pressure, headache, and frequently blood-stained cerebrospinal fluid. The location of the ICH was classified as the putamen, thalamus, subcortex, pons, cerebellum, other determined locations, or an undetermined location (for patients without a morphological examination). When a hematoma occupied ≥2 brain regions, the location was determined as a single region with the largest hematoma size.

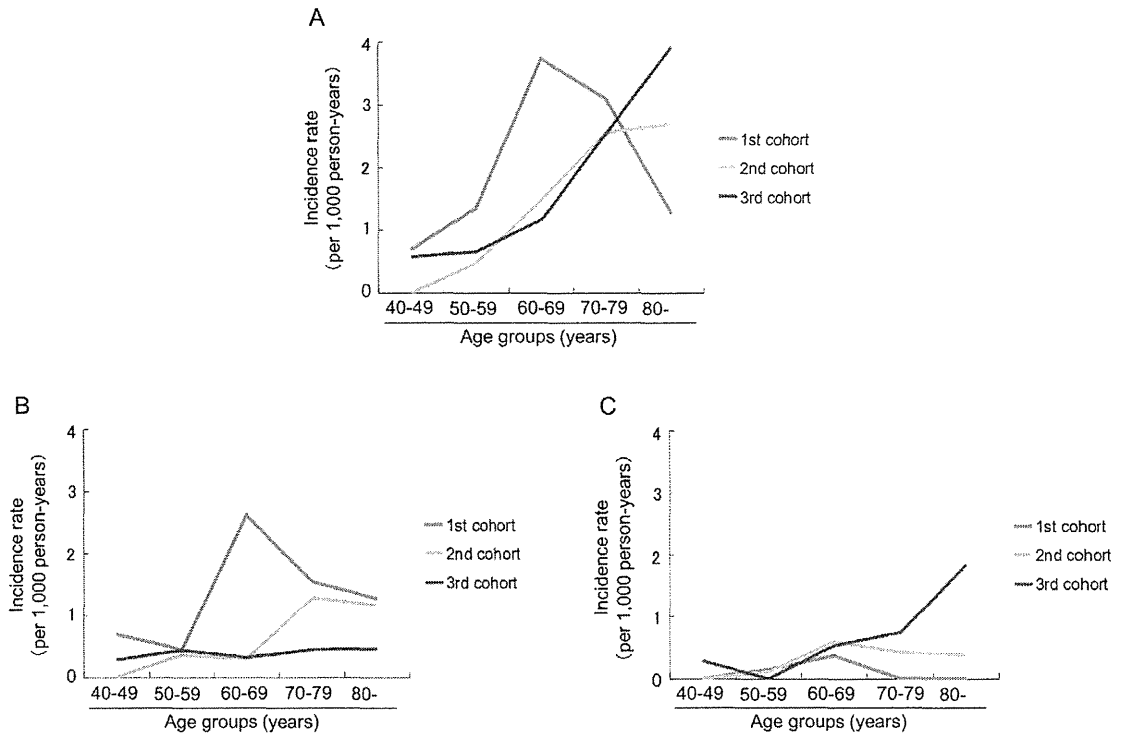

Figure 2. Age-specific incidence of intracerebral hemorrhage (ICH) and its location among 3 cohorts of the Hisayama Study, with a 13-year follow-up for each cohort. (A) Total ICH, (B) putaminal hemorrhage, (C) thalamic hemorrhage.

Risk Factors

Blood pressure (BP) was measured 3 times in a supine position at the baseline examination, and hypertension was defined as a mean BP ≥140/90 mmHg or use of antihypertensive agents. Glucose intolerance was defined by an oral glucose tolerance test in subjects with glycosuria in 1961, by fasting and postprandial glucose concentrations in 1974, and by a 75-g oral glucose tolerance test in 1988, in addition to medical history or treatment of diabetes. Serum cholesterol levels were measured by the modified Zak-Henly method in 1961, by the Zurkowski method in 1974, and by the enzymatic method in 1988. Hypercholesterolemia was defined as total cholesterol level ≥5.7 mmol/L (220 mg/dl). Body height and weight were measured in light clothing without shoes, and obesity was defined as a body mass index ≥25.0 kg/m^2. Information regarding antihypertensive treatment, alcohol intake, and smoking habits was obtained with the use of a standard questionnaire and was categorized as current habitual use or not.

Statistical Analysis

The SAS 9.3 (SAS Institute Inc, Cary, NC, USA) was used to perform all statistical analyses. The prevalences or mean values of risk factors were compared among the cohorts by the logistic regression or the linear regression model. The incidence of ICH was calculated by the person-year method and adjusted for age and sex by the direct method using the World Standard Population, and compared among the cohorts by Cox proportional hazards model. Subjects who developed ICH were also followed up for the next 5 years, and survival rates were estimated using Kaplan-Meier methods and tested with the log-rank test. The age- and sex-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) of risk factors for the development of ICH were estimated by the Cox proportional hazards model in each cohort, and the population attributable risk fraction (PAF) was calculated using a formula: PAF = PD(HR-1)/HR, where PD indicates the proportion of subjects exposed to a risk factor. Two-sided $P<0.05$ was considered statistically significant in all analyses.

Ethical Considerations

This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research, and written informed consent was given by the participants.

Results

Trends in Risk Factors

Our comparison of the prevalence and mean values of risk factors at the 3 baseline examinations is shown in Table 1. The prevalence of hypertension was stable among the cohorts, but the proportion of individuals using antihypertensive agents increased consistently with time. Consequently, among hypertensive subjects, mean BP levels significantly decreased from the first to the third cohort. The prevalence of glucose intolerance, hypercholesterolemia, and obesity increased progressively with time. The smoking rate declined linearly, and the drinking rate decreased slightly over the study period.

Trends in ICH Incidence by Location

The proportional distribution of hematoma location among ICH

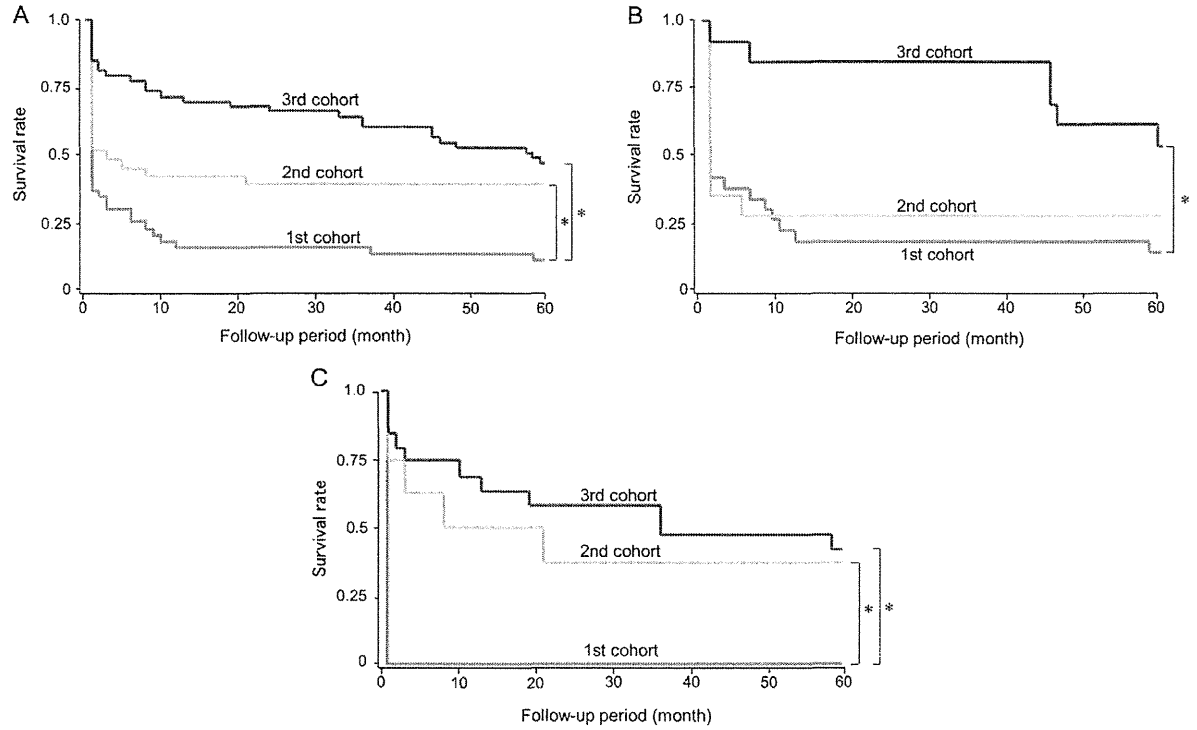

Figure 3. 5-year survival curves after intracerebral hemorrhage (ICH) and its location among 3 cohorts of the Hisayama Study. (A) Total ICH, (B) putaminal hemorrhage, (C) thalamic hemorrhage. *P<0.05.

Table 3. Age- and Sex-Adjusted Hazard Ratios (HR) of Cardiovascular Risk Factors for Development of Total Intracerebral Hemorrhage Among 3 Cohorts of the Hisayama Study

	1st cohort			2nd cohort			3rd cohort		
	HR (95% CI)	P value	PAF	HR (95% CI)	P value	PAF	HR (95% CI)	P value	PAF
Age, per 10 years	1.56 (1.18–2.05)	0.002		2.06 (1.51–2.81)	<0.001		1.89 (1.49–2.40)	<0.001	
Male	4.35 (2.19–8.67)	<0.001		1.82 (0.91–3.63)	0.09		1.52 (0.88–2.62)	0.13	
Hypertension	9.30 (4.03–21.5)	<0.001	0.75	2.38 (1.09–5.21)	0.03	0.40	2.89 (1.57–5.33)	<0.001	0.46
Alcohol intake	2.61 (1.13–6.02)	0.02	0.43	1.82 (0.76–4.40)	0.18	0.19	1.64 (0.82–3.28)	0.16	0.15
Glucose intolerance	1.29 (0.54–3.09)	0.47	0.03	2.08 (0.89–4.86)	0.09	0.11	1.30 (0.76–2.25)	0.34	0.10
Current smoker	1.01 (0.49–2.08)	0.95	0.01	1.05 (0.45–2.43)	0.92	0.02	1.13 (0.57–2.24)	0.73	0.03
Hypercholesterolemia	1.70 (0.52–5.53)	0.36	0.03	0.84 (0.32–2.22)	0.73	−0.03	0.79 (0.43–1.44)	0.44	−0.08
Obesity	0.26 (0.04–1.86)	0.18	−0.07	0.94 (0.32–2.74)	0.91	−0.01	0.88 (0.44–1.77)	0.73	−0.03

CI, confidence interval; PAF, population attributable risk fraction.

patients in each cohort is shown in Figure 1. The proportion of putaminal hemorrhage decreased steadily from the first to the third cohort. Otherwise, the proportions of thalamic, subcortical, and pontine hemorrhage increased with time.

Table 2 shows the trends in the crude as well as the age- and sex-standardized incidences of total ICH and those by location. The incidence of total ICH significantly declined from the first to the second cohort and showed no further change in the third cohort. The incidence of putaminal hemorrhage showed a similar decreasing pattern. Conversely, the incidence of thalamic hemorrhage increased significantly with time. The incidence of subcortical hemorrhage increased slightly, but was not significant.

Figure 2 demonstrates the trends in the age-specific incidences of total ICH, putaminal hemorrhage, and thalamic hemorrhage. The total ICH incidence showed a decreasing trend in the age group <80 years from the first to the second cohort, whereas the incidence in the age group aged ≥80 years increased constantly with time. The incidence of putaminal hemorrhage decreased with time mainly in subjects aged ≥60 years. In contrast, the incidence of thalamic hemorrhage increased, particularly among subjects aged ≥70 years.

Trends in Survival of ICH by Location

As shown in Figure 3, the 5-year survival rate after ICH improved steadily from the first to the third cohorts, mainly as a result of the improvement in the 1-month survival. There were comparable improvements in survival after putaminal and tha-

lamic hemorrhages.

Trends in the Effect of Risk Factors on ICH

The influence of risk factors on the development of total ICH was compared among the 3 cohorts (Table 3). In the first cohort, hypertension was the strongest risk factor for ICH (age- and sex-adjusted HR 9.30, 95% CI 4.03–21.5) and strongly contributed to its occurrence (PAF 75%). The influence of hypertension declined in the second and third cohorts, but was still a significant risk factor and made the largest contribution even in the third cohort (HR 2.89, 95% CI 1.57–5.33, PAF 46%). Alcohol intake was also a significant risk factor for ICH in the first cohort (HR 2.61, 95% CI 1.13–6.02), explaining 43% of ICH events. The effect of alcohol intake on the occurrence of ICH declined with time and was not significant in the second and third cohorts.

Discussion

To our knowledge, this is the first study to examine secular trends in the incidence and survival rates of ICH by its location. Among 3 cohorts established at different times in a Japanese community, the incidence of total ICH declined significantly, mainly from the first to the second cohort. The incidence of putaminal hemorrhage decreased with time, mainly in subjects aged ≥60 years, while an increasing trend in the incidence of thalamic hemorrhage was observed mainly in subjects aged ≥80 years. The 5-year survival rate after ICH improved significantly with time. Both hypertension and alcohol intake were strong risk factors for ICH in the first cohort, but their influence declined with time.

Although several community-based surveys of secular trends in the ICH incidence have been conducted, the findings have been inconsistent.[12–15] In a study from Australia, the ICH incidence decreased from 1989 to 2001.[12] A study in the United Kingdom found that the ICH incidence fell in the younger population, with no definite change in the older subjects between 1981 and 2006.[13] In contrast, the ICH incidence was stable in France (between 1985 and 2004)[14] and in the United States (between 1993 and 1999).[15] None of these studies observed the ICH incidence before the 1970s when brain imaging was not in practical use. In our present study, the ICH incidence decreased significantly from the 1960s to the 1970s, but the decline leveled off thereafter. Inconsistency in the findings from the studies may reflect different patterns in the prevalence of cardiovascular risk factors and ethnic diversity, as well as the different study periods.

Hypertension is the most powerful risk factor for ICH.[10,21] In our population, BP among hypertensive subjects decreased significantly with time as a result of the increment in the use of antihypertensive medication, though the prevalence of hypertension remained stable. As a result, the influence of hypertension on the development of ICH declined from the first to the second cohort. Alcohol intake was also a significant risk factor for ICH in the first cohort, but its influence decreased with time. We have previously demonstrated that alcohol intake and hypertension increase the risk of ICH in a synergistic manner.[22] The improvement in hypertension control might therefore have resulted in the reduced influence of alcohol intake. These changes in risk factors likely resulted in the decline in ICH incidence in our population.

Several studies have found that ICH in the deep brain, such as in the putamen or thalamus, occurs more frequently than in other locations.[5,8,16–19] Cross-sectional hospital-based studies have shown that the mean age of incident thalamic hemorrhage is higher than that of putaminal hemorrhage.[5,23] In the present study, the incidence of putaminal hemorrhage decreased steadily with time, mainly in the relatively young age group (60–69 years), whereas the incidence of thalamic hemorrhage increased, especially in the very old subjects (≥80 years). Although putaminal hemorrhage was the most common subtype of ICH, occurring mainly in the relatively young subjects with untreated hypertension in the 1960s, the improvement in hypertension control has reduced the risk of putaminal hemorrhage and subsequent premature death of hypertensive subjects in recent years. The prolonged longevity of hypertensive subjects might increase the probability of ICH in other locations, such as thalamic or subcortical hemorrhage, at a very old age. The precise reasons for these findings are not clear, but the influence of hypertension and aging on the development of ICH may be different among its locations.[23] Relatively mild but prolonged hypertension might cause degeneration of perforating arteries in the posterior circulation, resulting in thalamic hemorrhage. In our population, the decreasing trend in the ICH incidence has slowed since the 1980s, probably because of the increasing incidence of thalamic and subcortical hemorrhages in the elderly.

The 5-year survival rate of ICH improved significantly with time, mainly as a result of improvement in the first 1 month. Improvement in hypertension control before ICH onset and advances in the management of acute-phase ICH such as BP control and surgical procedures are likely to have contributed to this trend.

Study Limitations

Several should be discussed. First, the method of diagnosing ICH has changed remarkably with advances in the diagnostic techniques, and this change may have resulted in an inconsistency of ICH diagnosis and classification among cohorts. In this study, however, morphological evaluations by brain imaging and/or autopsy were performed for most of the ICH cases, even in the earlier cohorts. Therefore, the possibility of information bias in ICH diagnosis and classification was minimized. Moreover, our main conclusions did not change substantially when we excluded ICH events without morphological evaluation from the total ICH (data not shown). Second, because of the small number of events, we could not examine the influence of risk factors on each location. Third, the numbers of ICH events in our cohorts were relatively small, so we could not evaluate sex-specific trends in the incidence and survival of ICH by location. Fourth, we could not show trends in the volume of hematoma. Brain imaging such as CT and MRI is essential to determine hematoma volume, but it was conducted only for 2% of ICH patients in the first cohort and 64% in the second cohort. Finally, we were unable to investigate the influence of antithrombotic medications, which may increase the risk of ICH and affect its location.[24,25]

In conclusion, our findings suggest that the incidence of ICH steeply declined from the 1960s to the 1970s in Japan as a result of improved hypertension control, but this decline leveled off thereafter. The incidence of thalamic hemorrhage showed an increasing trend, especially in the elderly in recent years; in contrast, we observed a decreasing incidence for putaminal hemorrhage. Our findings indicate that strict management of hypertension is still important for the prevention of ICH, especially in the elderly Japanese population.

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