2.4. Selenium measurements

The selenium concentrations in serum samples were determined spectrofluorometrically by 2,3-diaminonaphthalene after digestion in nitric acid and perchloric acid [20]. Bovine liver and serum (SRM 1577b and 1582; National Institute of Standards and Technology, Gaithersburg, MD) were used as reference materials to validate the selenium measurements.

2.5. Detection of the selenium distribution

Instrumental configurations for separating and measuring the selenium distributions in serum are described and validated elsewhere [16]. Briefly, a high-performance liquid chromatography system (Integral 4000; Perkin-Elmer Japan, Yokohama, Japan) was equipped with 2 columns (AFpak heparin and Asahipak GS520HQ; Showa Denko, Tokyo, Japan) in tandem and coupled directly with an inductively coupled plasma–mass spectrometry (Elan 5000, Perkin-Elmer Japan).

2.6. Statistical analyses

In the present study, we conducted a nested case-control study to allow a case-control study within a cohort study [21]. The body mass index, total and HDL cholesterol, % HDL-C, and systolic and diastolic blood pressure of the cases and controls were compared using paired t tests. The number of current tobacco smokers and regular alcohol consumers in the stroke cases was compared with that among the controls using χ^2 tests.

Results of the total serum selenium and the selenium contents in GPx, albumin, and selenoprotein P were compared using paired t tests. A simple correlation analysis was performed to clarify the relationship among these proteins. A univariate logistic regression analysis was also performed separately to investigate their relationship with a response variable of stroke occurrence.

Table 1
Characteristics of stroke cases (n = 30) and matched controls (n = 30) in the Ohasama selenium-stroke study

Characteristics	Stroke cases	Controls	
Men/women [b]	14/16	14/16	NS
No. of tobacco smokers (%)	9 (30.0%)	6 (20.0%)	NS
No. of alcohol consumers (%)	6 (20.0%)	10 (33.3%)	NS
Age (y) [a, b]	70.2 ± 7.6	70.2 ± 7.6	NS
Total cholesterol (mg/dL) [a, b]	194.3 ± 28.8	195.4 ± 29.5	NS
HDL-C (mg/dL) [a]	47.3 ± 11.3	50.4 ± 16.2	NS
%HDL-C [a]	24.7 ± 6.3	26.0 ± 8.1	NS
Body mass index (kg/m^2) [a]	21.7 ± 2.8	23.0 ± 3.1	NS
Systolic blood pressure (mm Hg) [a]	135.3 ± 15.1	134.3 ± 16.3	NS
Diastolic blood pressure (mm Hg) [a]	74.3 ± 10.2	72.4 ± 9.9	NS

The cases and controls were compared with paired t tests and matched for sex, age, and total cholesterol of stroke cases. P less than .05 was considered as statistically significant. NS indicates not significant.

[a] Values are presented as means ± standard deviations.

[b] Matching variables.

Table 2
Total selenium concentrations and the distribution of selenium in serum in stroke cases (n = 30) and controls (n = 30) in the Ohasama selenium-stroke study

Selenium (μg/L)	Stroke cases [a]	Controls [a]	P value
Total selenium	105.2 ± 19.6	116.5 ± 16.6	.054
Selenium in GPx	17.9 ± 4.33	20.4 ± 5.31	.094
Selenium in albumin	31.1 ± 7.09	35.0 ± 7.11	.069
Selenium in selenoprotein P	54.5 ± 8.69	63.0 ± 9.18	.006

The cases and controls were compared with paired t tests. P less than .05 was considered as statistically significant.

[a] Values are presented as means ± standard deviations.

The selenium-containing proteins were used to form further explanatory sets with age, total and HDL cholesterol, systolic and diastolic blood pressure, and body mass index for a multivariate logistic regression analysis [22]. These statistical analyses were performed using the MacNAP version 4.0 [23], a statistical package for personal computers.

3. Results

Comparison of several characteristics of stroke cases and controls can be seen in Table 1. Data were obtained from health examinations conducted in 1992; the mean age and total cholesterol levels in the stroke cases and the controls were almost identical. The control subjects of the present study were matched with the stroke cases for sex, age, and total cholesterol. The mean HDL-C level and the mean % HDL-C were slightly lower in the stroke cases than in the controls, and the mean body mass index of the stroke cases was slightly lower than that of the controls; but these differences were not significant. There was also no difference in the rates of current tobacco smokers and regular alcohol users between the 2 groups.

The total serum selenium concentrations and the distribution of selenium content in GPx, albumin, and

Table 3
Correlation coefficients between serum selenium and selenium contents in GPx, albumin, and selenoprotein P in stroke cases (upper right, n = 30) and in controls (lower left, n = 30) in the Ohasama selenium-stroke study

Controls-cases	Total Se	Se in GPx	Se in albumin	Se in selenoprotein P
Total Se	–	0.701 †	0.644 †	0.787 †
Se in GPx	0.833 †	–	0.278	0.481 *
Se in albumin	0.777 †	0.634 †	–	0.09
Se in selenoprotein P	0.818 †	0.513 *	0.335	–

Pearson correlation coefficients were calculated among the total serum selenium and the selenium contents in GPx, albumin, and selenoprotein P. Se indicates selenium.

* P less than .05.

† P less than .01.

selenoprotein P are shown in Table 2. The total serum selenium was lower in the stroke cases than in the controls (105.2 vs 116.5 μg/L, $P = .054$). Selenium content in GPx and albumin also tended to be lower in the stroke cases than in the controls (17.9 vs 20.4 μg/L and 31.1 vs 35.0 μg/L, respectively). The selenium content of selenoprotein P was significantly lower ($P = .006$) in the stroke cases (54.5 μg/L) than in the controls (63.0 μg/L).

Simple correlation coefficients among selenium contents are shown in Table 3. The selenium content in GPx, albumin, and selenoprotein P positively contributed to the total selenium variations in the stroke cases and controls. Positive relationships between GPx and selenoprotein P were observed in both groups. However, selenium in albumin was not associated with the selenium in selenoprotein P in the controls, or with either GPx or selenoprotein P in the stroke cases.

Univariate logistic regression analyses of the total serum selenium and selenium contents in GPx, albumin, and selenoprotein P showed that the serum selenium level ($P = .031$) and selenoprotein P level ($P = .008$) were significant risk factors for subsequent stroke.

The results of the multivariate logistic regression analysis with the explanatory set for subsequent stroke occurrence are shown in Table 4. Among these variables, selenoprotein P (odds ratio = 0.28; 95% confidence interval [CI], 0.10-0.85) and HDL-C (odds ratio = 0.22; 95% CI, 0.05-0.85) were significantly associated with subsequent stroke; however, body mass index, which is commonly considered a risk factor for stroke, was not. Regression analysis showed that increased blood pressure was not a risk factor for subsequent stroke. However, this may be due to the exclusion of subjects with abnormal blood pressure in the present selected case-control study.

In summary, these results show that the serum selenoprotein P level contributes to the development of stroke. They further suggest that levels of selenoprotein P should also be measured in determining the selenium status.

Table 4
Standardized coefficients and odds ratios of selenium in the 3 selenium-containing proteins in serum and other characteristics for stroke in the Ohasama selenium-stroke study (n = 60)

Variable	Standardized coefficient	Odds ratio	95% CI
Se in GPx	−0.092	0.91	0.30-2.78
Se in albumin	0.192	1.21	0.44-3.37
Se in selenoprotein P	−1.233 *	0.28	0.10-0.85
Age	0.160	1.19	0.50-2.84
Total cholesterol	0.659	2.01	0.84-4.81
HDL-C	−1.483 *	0.22	0.05-0.85
Body mass index	−0.647	0.52	0.19-1.39
Systolic blood pressure	−0.087	0.88	0.27-2.85
Diastolic blood pressure	0.665	2.09	0.51-8.58

Standardized coefficients and odds ratios were analyzed with multivariate logistic regression analysis.
* P less than .05.

4. Discussion

We carefully designed our study to avoid the effects of other stroke risk factors such as hypertension, antihypertensive therapy, high cholesterol, and arrhythmia [24,25]. In this study, subjects were excluded if their blood pressure and HDL-C levels were outside the reference range. Subjects who were taking antihypertensive medication, had a history of stroke, or had atrial fibrillation were also excluded. Furthermore, we chose the control cases to match the sex, age, and level of total cholesterol of the stroke cases. After exclusions and adjustments, we found that depressed serum selenoprotein P levels may be used as a new indicator of stroke risk.

Results from multivariate logistic regression analysis showed that the association of selenoprotein P with stroke was statistically independent from the HDL-C level, which is a well-established protective factor for vascular diseases [26]. Bleys and coworkers [27] reported that serum selenium was positively associated with HDL-C and suggested that the effect of selenium is due to its having a positive effect on HDL-C. However, this phenomenon was not confirmed by subsequent studies [28,29].

Selenoproteins are defined as proteins containing selenium as selenocysteine, which is coded by UGA [30]. The result of the identification of UGA codons and the subsequent selenocysteine insertion RNA structures shows that the human selenoproteome consists of 25 selenoproteins [31]. Among them, selenoprotein P is an abundant extracellular protein that is rich in selenocysteine and functions uniquely in selenium homeostasis and oxidant defense [32]. Selenoprotein P was first isolated in 1977, when a selenium-containing protein that was different from GPx was identified in the plasma of rats [33]. The result was confirmed in 1982 [34], and the protein was then named *selenoprotein P*.

The mechanism of selenoprotein P protection in stroke may be explained in part by its function as an antioxidant [32]. Selenoprotein P is located on the walls of blood capillaries and anchored to the endothelium by heparin proteoglycans, which are located on the surface of capillary cells and have the same repeating disaccharide chain as heparin [35]. Subsequently, selenoprotein P has been shown to be associated with capillary endothelial cells in the brain, kidney, and liver; and it has been hypothesized that selenoprotein P has an antioxidant function on the capillary endothelium [32].

Furthermore, selenoprotein P is also the selenoprotein responsible for the distribution of selenium to the brain [36]. In stroke events, a dramatic increase in reactive oxygen species occurs. As part of a defense mechanism, selenium is reported to provide protection against reactive oxygen species through its selenium-dependent antioxidant enzymes, including GPx and thioredoxin reductase [37]. When the gene for selenoprotein P was disrupted, selenoprotein P knockout mice showed a dramatic reduction of selenium levels in the brain. The GPx and thioredoxin

reductase activities in the brain of the selenoprotein P knockout mice were significantly decreased [38]. Thus, although further study is needed, it is tempting to speculate that depression of selenoprotein P will disrupt selenium transport to the brain, decrease the protection of selenium-dependent antioxidant enzymes against reactive oxygen species, and finally, increase the risk of stroke.

The present study suggested that lower total serum selenium concentration may be associated with the development of stroke; moreover, results from multivariate logistic regression analysis showed that selenoprotein P was a significant risk factor in the development of stroke (odds ratio = 0.28; 95% CI, 0.10-0.85). These results support the hypothesis that the selenium status should be monitored not only by measuring the total selenium concentration in serum or plasma, but also by quantifying selenoproteins. However, the present study does have limitations, including the small subject number and lack of consideration of each subject's selenium intake and dietary habits; therefore, a more advanced cohort study with a larger number of subjects is needed.

In conclusion, despite the controversy surrounding the pathophysiologic role of selenium in the development of stroke, the results of this study showed that depressed serum levels of selenoprotein P were associated with a higher risk of stroke.

Acknowledgment

The authors would like to thank Chieko Inomata for technical assistance in the analytical procedures and Masako Sakai and Yuriko Takamura for data collection and processing. This work was financially supported by a Grant-in-Aid for Scientific Research C (06670396) from the Ministry of Education, Science, Sports, and Culture, Japan.

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Association of (Pro)renin Receptor Gene Polymorphism With Blood Pressure in Japanese Men: The Ohasama Study

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BACKGROUND

Recent studies have revealed that (pro)renin receptor ((P)RR), a newly identified member of the renin–angiotensin system (RAS), is associated with blood pressure regulation in animals. However, there is no information on (P)RR in humans. We investigated the association of *(P)RR* gene polymorphisms with blood pressure in a Japanese population.

METHODS

Subjects ($n = 1,112$) were recruited from participants in the Ohasama study, a Japanese cohort study. For the association study, we selected three polymorphisms: −782A>G (rs2968915), intervening sequence (IVS)5+169C>T (rs5918007), and +1513A>G (rs6609080). Because the *(P)RR* gene is on the X chromosome, men ($n = 357$) and women ($n = 755$) were analyzed separately.

RESULTS

In men, 24-h systolic blood pressure (SBP) and diastolic blood pressure (DBP) values, daytime SBP and DBP values, and nighttime SBP and DBP values were significantly higher in IVS5+169T allele carriers than C allele carriers. Multiple regression analysis showed that IVS5+169C>T was significantly and independently related to ambulatory blood pressure (ABP). IVS5+169C>T was not associated with casual blood pressure (CBP) in men. In women, there were no significant differences in blood pressure values among the three genotypes of IVS5+169C>T. This polymorphism had no significant association with any other clinical characteristic. −782A>G was weakly associated with ABP in men. +1513A>G was not associated with blood pressure values in either men or women.

CONCLUSIONS

We demonstrated for the first time that polymorphism of the *(P)RR* gene IVS5+169C>T is associated with ABP in Japanese men. This association suggests that (P)RR has a role in blood pressure regulation.

Am J Hypertens 2009; **22**:294-299 © 2009 American Journal of Hypertension, Ltd.

The renin–angiotensin system (RAS) plays an essential role in the regulation of blood pressure and electrolyte balance.[1–3] The RAS is also involved in cell growth, fibrosis, and inflammation in cardiovascular and renal tissues as locally produced and locally acting factors.[1–3] Therefore, the RAS is considered to be involved in the pathogenesis of hypertension and cardiovascular disease. The association of genetic polymorphisms of components of the RAS with hypertension or cardiovascular disease has been widely examined in the Ohasama study[4–8] and by others.[9,10]

Recently, (pro)renin receptor ((P)RR), a specific receptor for renin and prorenin,[11] was newly identified as a member of the RAS by Nguyen *et al.*[12,13] (P)RR is a 350 amino acid protein with a single transmembrane domain.[13] (P)RR has been reported to lead to nonproteolytic activation of prorenin and directly activate mitogen-activated protein kinases ERK1/2 independently from the RAS.[13,14] Several animal studies showed that (P)RR contributed to blood pressure regulation or development of end organ damage.[15–20] Burcklé *et al.* reported that elevated blood pressure and heart rate were observed in rats with overexpression of the human *(P)RR* gene.[15] Thus, (P)RR is also thought to be involved in hypertension and cardiovascular disease in humans.

The *(P)RR* gene is on chromosome Xp11.4 in humans.[13] It was reported that chromosome Xp11 was linked with diastolic

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Received 6 September 2008; first decision 3 October 2008; accepted 5 December 2008; advance online publication 8 January 2009. doi:10.1038/ajh.2008.357

blood pressure (DBP) in a genome wide association study.[21] However, there are no databases or reports showing an association between the *(P)RR* gene polymorphism and hypertension in humans. We investigated the association between *(P)RR* gene polymorphisms and blood pressure levels in a Japanese population.

METHODS

Design and study population. This study was performed as part of the Ohasama study and based on data obtained from subjects who participated in our blood pressure monitoring and genetic analysis project in a rural community of Ohasama, Iwate prefecture, Japan. The characteristics of this area and details of the study design are described elsewhere.[22,23] The study protocol, including the genetic analysis, was approved by the Institutional Review Board of Tohoku University School of Medicine and by the Department of Health of the Ohasama Town Government.

The population of Ohasama in 1998 was 7,202. Of those, 1,826 subjects gave written informed consent for blood pressure measurements and genetic analysis. All subjects were aged ≥40 years. Of the 1,826 subjects, 388 subjects were excluded because ambulatory blood pressure (ABP) values were not measured. Of the remaining 1,438 subjects, 326 subjects who lacked full data on clinical characteristics, blood pressure values, or information on genotypes were also excluded. As a result, 1,112 subjects (357 men and 755 women; mean age, 58.9 ± 10.1 years) were included in the study.

Blood pressure and biochemical measurements. Details of ABP monitoring have been described previously.[23,24] In brief, ABP was monitored every 30 min using a fully automatic device (ABPM 630; Nippon Colin, Komaki, Japan). Mean 24-h, daytime, and nighttime values for ABP were calculated for each participant. "Daytime" and "nighttime" were determined according to each participant's diary. We thus analyzed ABP data obtained during >6 h of daytime and >3 h of nighttime. The mean number of measurements was 44.4 ± 4.8.

Casual blood pressure (CBP), measured by public health nurses or technicians using an automatic device (HEM 907; Omron Healthcare, Kyoto, Japan), was measured twice consecutively with subjects in the sitting position, with a minimum 2-min rest between measurements. The mean of the two measured values was used for analysis.

Devices used to measure ABP or CBP met the criteria of the Association for the Advancement of Medical Instrumentation.[24–26] Hypertension was defined as 24-h systolic blood pressure (SBP) ≥135 mm Hg and/or 24-h DBP ≥80 mm Hg and/or use of antihypertensive medications.[27] Patients with secondary hypertension could be included in this study.

Biochemical parameters such as serum electrolytes and serum creatinine levels were measured with an autoanalyzer.

Genetic analysis. Genomic DNA samples were extracted from peripheral leukocytes of participants. In the first step of the study, we randomly selected 60 women from the 755 women, amplified the *(P)RR* gene by PCR and sequenced the PCR products. The promoter region and each exon, including an adjacent intron of the *(P)RR* gene, were amplified by PCR with primers summarized in **Supplementary Table S1** online. The PCR products were purified using a PCR purification kit (BIONEX, Seoul, Korea) and directly sequenced with an auto sequencer (ABI PRISM 3100; Applied Biosystems, Foster, CA). We calculated D′ and r^2 values to estimate the linkage-disequilibrium in pairwise combinations of each polymorphism.

In the second step, based on the results of the linkage-disequilibrium analyses on the 60 women, three single-nucleotide polymorphisms (SNPs) were genotyped in the total population (n = 1,112) containing the 60 women studied in the first step, as follows: −782A>G (rs2968915) in the promoter region, intervening sequence (IVS)5+169C>T (rs5918007) in intron5, and +1513A>G (rs6609080) in the 3′-untranslated region. −782A>G was genotyped by direct sequence method described above. IVS5+169C>T and +1513A>G were genotyped by PCR–restriction fragment length polymorphism method with FokI and BsmI, respectively. The region of genomic DNA including each SNP was amplified with primers summarized in **Supplementary Table S1** online. The PCR products were digested, analyzed by electrophoresis in 5% polyacrylamide gel, and visualized under UV light.

Statistical analysis. Statistical analysis was performed with the JMP 5.0.1 statistical software package (SAS Institute, Cary, NC). Because the *(P)RR* gene is on the X chromosome, men (n = 357) and women (n = 755) were analyzed separately. The Student t-test, χ^2-test, analysis of variance, and analysis of covariance were used where appropriate. The analysis of covariance model included the following traditional risk factors and covariates for blood pressure levels: age, body mass index (BMI), use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, current smoking, and current drinking. Multiple comparisons were performed with Tukey–Kramer's test. Multiple regression analysis was used to determine whether *(P)RR* gene polymorphisms predicted blood pressure values after adjustment for the traditional risk factors and covariates for blood pressure levels. Continuous values were expressed as mean ± s.d. Differences of $P < 0.05$ were considered statistically significant. Furthermore, we applied Bonferroni correction, because we conducted tests on three SNPs in two sexes against eight blood pressure traits (48 statistical tests). Differences of $P < 0.001$ (= 0.05/48) were considered statistically significant after Bonferroni correction.

RESULTS

Polymorphisms in the *(P)RR* gene

We identified 11 SNPs in the *(P)RR* gene in the 60 women sequenced (**Figure 1, Table 1**). −1024T>C and IVS5+1006G>A are new variants that have not been reported in the SNP database of the National Center for Biotechnology Information, but that showed a low frequency (1.7% for each). Other SNPs had already been registered, and were detected at a frequency

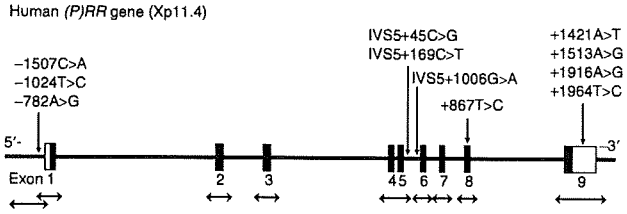

Figure 1 | Polymorphisms in the *(P)RR* gene in Japanese women ($n = 60$). White boxes and black boxes indicate the noncoding regions and coding regions, respectively. Two-headed arrows under the boxes indicate the regions sequenced. (+1) indicates the initiation site of mRNA transcription. IVS, intervening sequence. Reference sequence: NT_079573.

Table 1 | Polymorphism of *(pro)renin receptor* gene in Japanese women ($n = 60$)

No	Region	Polymorphism	NCBI SNP ID	Frequency (%)
1	Promoter	−1507C>A	rs2971599	86.7/13.3
2		−1024T>C	New	98.3/1.7
3		−782A>G	rs2968915	86.7/13.3
4	Intron5	IVS5+45C>G	rs3112299	88.3/11.7
5		IVS5+169C>T	rs5918007	88.3/11.7
6		IVS5+1006G>A	New	98.3/1.7
7	Exon8	+867T>C	rs5963811	73.3/26.7
8	Exon9 (3′-UTR)	+1421A>T	rs5963816	73.3/26.7
9		+1513A>G	rs6609080	73.3/26.7
10		+1916A>G	rs10536	73.3/26.7
11		+1964T>C	rs1060063	73.3/26.7

Frequency is shown as major allele frequency/minor allele frequency. (+1) indicates the mRNA transcription initiation site.
ID, identification number; IVS, intervening sequence; NCBI, National Center for Biotechnology Information; SNP, single-nucleotide polymorphism; UTR, untranslated region.

of >10%. Although four coding mutations (F30L, P43A, P90A, and A290P) have been registered in the National Center for Biotechnology Information database, no SNP had an amino acid substitution in these 60 Japanese women.

Linkage-disequilibrium analysis revealed that the *(P)RR* gene had three complete linkage-disequilibrium regions ($D' = 1.00$; $r^2 = 1.00$): −1507C>A and −782A>G; IVS5+45C>G and IVS5+169C>T; and +867T>C, +1421A>T, +1513A>G, +1916A>G, and +1964T>C. In addition, −1507C>A (and −782A>G) and IVS5+45C>G (and IVS5+169C>T) were in tight linkage-disequilibrium ($D' = 1.00$; $r^2 = 0.86$), and IVS5+45C>G (and IVS5+169C>T) and +867T>C (+1421A>T, +1513A>G, +1916A>G, and +1964T>C) were in weak linkage-disequilibrium ($D' = 1.00$; $r^2 = 0.05$). Based on the linkage-disequilibrium analyses, we selected −782A>G, IVS5+169C>T, or +1513A>G from each complete linkage-disequilibrium region for the association study.

The genotype frequencies of each SNP in the total population are shown in **Supplementary Table S2** online. The genotype frequencies of each SNP were consistent with the Hardy–Weinberg equilibrium in women (as the *(P)RR* gene is located on the X chromosome, genotype data were omitted in men), and there were no significant differences in allele frequencies between men and women.

Table 2 | Clinical characteristics and blood pressure values of the study population

	Men ($n = 357$)	Women ($n = 755$)	*P* value
Clinical characteristics			
Age, years	61.1 ± 9.6	57.8 ± 10.2	<0.001
Body mass index, kg/m^2	23.3 ± 2.8	23.9 ± 3.2	0.01
Antihypertensive medications, %	36.7	27.7	0.002
Hypertension, %	51.0	35.6	<0.001
Cardiovascular disease, %	13.7	6.1	<0.001
Ambulatory blood pressure			
24-h systolic, mm Hg	125.8 ± 12.3	120.5 ± 12.8	<0.001
24-h diastolic, mm Hg	74.4 ± 7.2	70.4 ± 7.5	<0.001
Casual blood pressure			
Systolic, mm Hg	136.6 ± 17.5	130.0 ± 16.8	<0.001
Diastolic, mm Hg	76.8 ± 10.7	73.4 ± 9.6	<0.001

Continuous values are expressed as mean ± s.d.

Association study

All clinical characteristics, including age, BMI, use of antihypertensive medications, prevalence of hypertension, history of previous cardiovascular disease, and blood pressure values were significantly different between men and women (**Table 2**).

IVS5+169C>T was significantly associated with ABP in men (**Table 3**). The blood pressure values in IVS5+169T allele carriers were significantly higher than in C allele carriers (24-h SBP: $P < 0.001$, 24-h DBP: $P < 0.001$, daytime SBP: $P = 0.004$, daytime DBP: $P = 0.006$, nighttime SBP: $P < 0.001$, nighttime DBP: $P < 0.001$). After the Bonferroni correction, the associations with 24-h SBP, 24-h DBP, nighttime SBP, and nighttime DBP remained significant. Although adjustment applied for age, BMI, use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, current smoking, and current drinking, the associations with nighttime SBP ($P < 0.001$) and nighttime DBP ($P < 0.001$) remained significant, but the associations with 24-h SBP ($P = 0.006$), 24-h DBP ($P = 0.003$), daytime SBP ($P = 0.02$) and daytime DBP ($P = 0.02$) were borderline significance. On the other hand, no significant differences were observed in CBP between the two alleles of IVS5+169C>T. A higher prevalence of hypertensive subjects was observed in subjects with the T allele (61.7%) than in those with the C allele (49.4%), but the difference was not significant ($P = 0.1$). There were no significant differences in clinical characteristics and biochemical parameters such as age, BMI, use of antihypertensive medications, history of previous cardiovascular disease, Na, K, and serum creatinine between the two alleles of IVS5+169C>T. In women, there were no significant differences in blood pressure and prevalence of hypertension (**Supplementary Table S3B**

Table 3 | Clinical characteristics and blood pressure values according to the alleles of IVS5+169C>T in men

	C (n = 310)	T (n = 47)	P value	Adjusted P value
Clinical characteristics				
Age, years	60.7 ± 9.6	63.4 ± 9.5	0.07	
Body mass index, kg/m^2	23.3 ± 2.8	23.3 ± 2.6	1	
Antihypertensive medications, %	35.2	46.8	0.1	
Hypertension, %	49.4	61.7	0.1	
Cardiovascular disease, %	13.9	12.8	0.8	
Ambulatory blood pressure				
24-h systolic, mm Hg	125.0 ± 11.8	131.4 ± 14.6	<0.001*	0.006
24-h diastolic, mm Hg	73.9 ± 6.9	77.7 ± 8.3	<0.001*	0.003
Daytime systolic, mm Hg	130.5 ± 12.6	136.5 ± 16.2	0.004	0.02
Daytime diastolic, mm Hg	78.0 ± 7.5	81.4 ± 9.2	0.006	0.02
Nighttime systolic, mm Hg	114.4 ± 12.8	121.5 ± 14.6	<0.001*	<0.001*
Nighttime diastolic, mm Hg	66.1 ± 7.1	70.4 ± 7.7	<0.001*	<0.001*
Ambulatory blood pressure				
Systolic, mm Hg	136.4 ± 17.0	138.0 ± 20.4	0.5	
Diastolic, mm Hg	76.9 ± 10.6	75.8 ± 11.5	0.5	

Continuous values are expressed as mean ± s.d. Adjusted P values: adjusted for age, body mass index, use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, current smoking, and current drinking.
IVS, intervening sequence.
*Differences of $P < 0.001$ (= 0.05/48) were considered statistically significant after Bonferroni correction.

Table 4 | Multiple regression analysis of nighttime ambulatory systolic blood pressure in men

Factors	β	P value
Age (per year)	0.699	<0.001
Body mass index (per kg/m^2)	0.789	<0.001
Antihypertensive medications (never = 0, present = 1)	3.865	0.01
Diabetes mellitus (never = 0, present = 1)	0.049	0.9
Cardiovascular disease (never = 0, present = 1)	3.392	0.1
Smoking status (never or ex = 0, current = 1)	1.145	0.4
Alcohol intake (never or ex = 0, current = 1)	−1.567	0.3
IVS5+169C>T (C allele carriers = 0, T allele carriers = 1)	5.754	<0.001

IVS, intervening sequence.

online) among the three genotypes of IVS5+169C>T. Although we categorized the genotypes in dominant model (CC+CT vs. TT) or recessive model (CC vs. CT+TT), no significant differences were observed in those parameters between the two groups. −782A>G was also associated with ABP in men, but P values were higher than those of IVS5+169C>T (24-h SBP: P = 0.02, 24-h DBP: P = 0.03, daytime SBP: P = 0.04, daytime DBP: P = 0.08, nighttime SBP: P = 0.01, nighttime DBP: P = 0.01; **Supplementary Table S3A** online). +1513A>G was not associated with blood pressure in men or women (**Supplementary Table S3C** online).

Multiple regression analysis of nighttime SBP in men showed that IVS5+169C>T was significantly and independently related to ABP (β = 5.754, $P < 0.001$) after adjustment for traditional covariates including age, BMI, use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, current smoking, and current drinking (**Table 4**). Moreover, the same relation was observed after multiple regression analysis of nighttime DBP (β = 3.822, $P < 0.001$). Whereas IVS5+169C>T was borderline significance in multiple regression analysis for ABP of 24-h SBP (β = 5.023, P = 0.006), 24-h DBP (β = 3.272, P = 0.003), daytime SBP (β = 4.510, P = 0.02), and daytime DBP (β = 2.864, P = 0.02).

Finally, we reconstructed haplotypes from the three SNPs (−782A>G, IVS5+169C>T, and +1513A>G) to examine the association of haplotypes with hypertensive parameters such as blood pressure values and prevalence of hypertension. No significant associations were observed in men or women.

DISCUSSION

This study has shown for the first time the potential importance of the *(P)RR* gene in blood pressure regulation in humans. In men, the T allele of IVS5+169C>T in the *(P)RR* gene was associated with a significantly higher ABP than the C allele. The blood pressure difference of 6.4 mm Hg in 24-h SBP is higher than values found in previous reports on the association between blood pressure and polymorphisms of hypertensive genes such as angiotensin-converting enzyme gene I/D (3.4 mm Hg difference in SBP)[28] and angiotensinogen gene M235T (1.4 mm Hg difference in SBP).[29] The association between −782A>G and ABP was also observed. Because −782A>G was in tight linkage-disequilibrium with IVS5+169C>T, the association of IVS5+169C>T with ABP affects that of −782A>G. Thus, the IVS5+169C>T locus seems to be the major relevant determinant of blood pressure value.

Moreover, IVS5+169C>T was more closely and strongly related to nighttime blood pressure values than 24-h and daytime blood pressure values. It is not clear why the IVS5+169C>T was more closely related to nighttime blood pressure than daytime blood pressure. The (P)RR is abundantly present in brain. Shan *et al.* have shown that (P)RR blockade by handle region peptide significantly inhibited the renin-induced decrease in neuronal activity.[30] Therefore, it is possible that the (P)RR in the brain may have a certain role in the relation more closely to nighttime blood pressure than daytime blood pressure. Several studies have shown that nighttime blood pressure is superior to daytime blood pressure in predicting target organ damage and prognosis.[31–33] Therefore, IVS5+169T allele carriers who have elevated nighttime blood pressure may have an increased risk for cardiovascular events or progression to end organ damage.

In contrast to ABP, IVS5+169C>T was not associated with CBP. CBP usually does not reflect basal blood pressure, but is susceptible to physical or psychological stress and environmental factors. Therefore, ABP may reflect basal blood pressure better than CBP, and is an indicator of a patient's "true"

blood pressure.[34] These findings suggest that IVS5+169C>T may affect basal blood pressure regulation and be related to hypertensive organ damage.

In contrast to men, no significant association was observed in women between IVS5+169C>T and blood pressure. Several possible mechanisms may explain this gender-specific association. First, the random expression of chromosome X, which is called the "Lyon hypothesis,"[35] may be related to this gender-specific effect. Second, sex hormones such as testosterone and estrogen affect (P)RR.[36] In addition, gender-specific effects of polymorphisms of RAS components were reported previously.[37,38] Therefore, the effect of (P)RR on blood pressure may also be influenced by sex hormones. Third, large differences in clinical characteristics between the men and women in this study may have affected the gender-specific association.

The biological mechanism by which the IVS5+169C>T polymorphism is associated with blood pressure is unclear. An effect on alternative splicing of the primary mRNA transcript to generate a different protein may be involved. It was reported that a silent mutation in exon4 of the human *(P)RR* gene (c321C>T, p.D107D) enhanced the expression of (P)RR that lacked exon4, and this mutant receptor could bind to renin and increase renin catalytic activity, similar to the wild-type receptor but resulting in a modest and reproducible impairment of ERK1/2 activation.[39] In our laboratory, splice variants lacking exon4 or both exon4 and exon5 were also observed (data not shown). As IVS5+169C>T and IVS5+45C>G were close to the exon5 and intron5 boundary, these SNPs might affect splicing activity to induce alternative splicing in the region near exon4 and exon5. However, there are no data as of yet to support the hypothesis that the variant in question affects the process leading to such alternative forms of the (P)RR.

This study has some limitations. Schefe *et al.* demonstrated the downregulation of (P)RR by renin and prorenin.[40,41] Susic *et al.* recently showed that dietary sodium may reduce the contribution of (P)RR to hypertensive cardiac hypertrophy, although handle region peptide has a benefit in either SHR fed a normal salt diet or SHR fed a high-salt diet.[42] However, we could not evaluate the influence of these biological parameters on the association of (P)RR polymorphism with blood pressure in this study, because they were not measured in most participants in this study. There are no functional studies to substantiate why IVS5+169C>T might raise blood pressure, nor data on why the association should be sex-specific. Furthermore, the results might be affected by complex and relative interactions in the balance between genes and environment. Therefore, another replication study with a more sophisticated study design, including a larger sample size, different ethnic groups, longitudinal surveillance, and functional analysis, should help clarify the role of *(P)RR* gene polymorphisms in blood pressure regulation.

To our knowledge, this is the first report to show the association of (P)RR with blood pressure in humans. Only the association between IVS+169C>T and nighttime SBP and DBP in men remained significant after Bonferroni correction. Whether the associations will prove to stand up to further genetic studies remains to be seen. These will be required in different settings before we reach a final conclusion. Nevertheless, we believe that the study is a good start and may encourage research by others.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ajh

Acknowledgments: We are grateful to the residents in Ohasama Town, all related investigators and study staff, and staff members of the Ohasama Town Government, Ohasama Hospital and Iwate Prefectural Stroke Registry for their valuable support on this project. This study was performed in part to use an equipment in Biomedical Research Core of Tohoku University Graduate School of Medicine. This study was supported in part by Grants for Scientific Research (15790293, 16590433, 17790381, 18390192, 18590587, 19590929, and 19790423) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; Grant-in-Aid (H17-Kenkou-007, H18-Junkankitou[Seishuu]-Ippan-012, and H20-Junkankitou[Seishuu]-Ippan-009, 013) from the Ministry of Health, Labor and Welfare, Health and Labor Sciences Research Grants, Japan; Grant-in-Aid for Japan Society for the Promotion of Science (JSPS) fellows (16.54041, 18.54042, 19.7152, 20.7198, 20.7477, and 20.54043); Health Science Research Grants and Medical Technology Evaluation Research Grants from the Ministry of Health, Labor and Welfare, Japan; Japan Atherosclerosis Prevention Fund; Uehara Memorial Foundation; Takeda Medical Research Foundation; National Cardiovascular Research Grants; and Biomedical Innovation Grants.

Disclosure: The authors declared no conflict of interest.

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サプリメント摂取者の人口学的特性及び生活習慣に関する研究-大迫研究

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医薬品相互作用研究　Vol. 33 No. 1 2009

医薬品相互作用研究
一 般 論 文
33 (1) 7-13 (2009)

サプリメント摂取者の人口学的特性及び生活習慣に関する研究-大迫研究

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Demographic and Lifestyle Characteristics of Supplement Users - the Ohasama study

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(Received July 7, 2009 ; Accepted August 5, 2009)

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OBJECTIVE: The number of supplement users is increasing in Japan. The objective of this study was to clarify demographic and lifestyle characteristics of supplement users in a Japanese general population.
METHODS: By using a self-administered questionnaire, we collected data on demographic and lifestyle characteristics including information on the use of supplements among 4,227 residents aged 35 years or more {mean age (SD), 59 (14) years, 54 % women} in a general population of Ohasama town, Japan. The subjects were classified into 3 groups according to the status of supplement use: no supplement users, daily supplement users and occasional supplement users.
RESULTS: Of the 4,227 participants, 1,107 subjects (26 %) were supplement users {daily users: 278 (7 %), occasional users: 829 (19 %)}. The proportion of women was significantly higher among supplement users. In women, supplement users were characterized by significantly older, lower levels of daily activity and self-rated health, higher level of education, and higher proportions of those who prefer to eat outside and had a habit of drinking. Supplement users in women also had a higher proportion of those who had a tendency of extroversion (i.e., sociability and cheerfulness) among younger women and those of neuroticism (i.e., anxiety and emotional instability) among older women. In men, supplement users had significantly higher proportions of those with a previous history, a habit of regularly taking breakfast, and a tendency of neuroticism.
CONCLUSION: Approximately one-forth of residents used supplements in a rural area population in Japan. Supplement use was associated with several factors broadly characterized by health consciousness and conversely non-health consciousness. It is suggested that supplements might be used for healthcare by the health conscious individuals and for alternative to neglect their healthcare by the non-health conscious individuals. Our data underscore the importance of clarifying the background characteristics of supplement users to better conduct medication teaching in community settings.

Keywords: supplement, lifestyle, general population

はじめに

近年消費者の健康に対する関心の高まりなどを受けて、健康食品やサプリメントの消費が急速に増大している[1,2]。2006年から2008年に行われた老化に関する長期縦断疫学調査(NILS-LSA)第5次調査によれば、40歳以上の女性65%、男性53%がサプリメントを摂取していることが報告されている[2]。これまでにサプリメント摂取の関連要因について検討した先行研究では、米国人において、サプリメント摂取と生活習慣、社会的要因との間に関連があるといった報告[3]や、日本人において、栄養補助食品および健康食品摂取者が、主観的健康度不良、主観的体力レベル低値、ストレス認知度高値、運動実施者および規則的な食事と関連があるという報告[4]、また日本人高齢者において、ビタミンサプリメントと健康行動が関連している、との報告[5]がなされている。

本研究の目的は、大迫研究のデータを用い、一般地域住民におけるサプリメント摂取者の特性及び生活習慣について検討することである。

方　法

1)対象

本研究は、1986年より開始された岩手県花巻市大迫町における高血圧・循環器疾患に関する長期コホート研究の一環である[6]。大迫町は盛岡市の南東20km、仙台市の北100 kmに位置し、北上山地に囲まれた農村である。青壮年層の多くは盛岡市内あるいは同町内に勤務しつつ果樹栽培を営む兼業農家に属する。1998年当時の大迫町の人口は7,202名であり、このうち、1998年に実施された「生活習慣と健康に関するアンケート調査」に回答した、35歳以上の岩手県花巻市大迫町の地域住民4,227名(平均年齢59歳、女性54%)を対象とした。

本研究の実施は東北大学医学部倫理委員会および大迫町より承認を受けている。

2)評価項目

サプリメント摂取の評価に関しては、1998年に実施された「生活習慣と健康に関するアンケート調査」の調査項目より、「ビタミンの入っている錠剤などをのみますか。」の設問において、「1.毎日のようにのむ」、「2.ときどきのむ」と回答した者をサプリメント摂取者と定義し、「3.のまない」を非摂取者とした。なお、本研究と同時期に同様の対象者において行われたアンケート調査[7]において、ビタミン剤の摂取の把握が、75%の感度を持って、ビタミン以外のサプリメント摂取の把握につながることが報告されているため、本研究においては、「ビタミンの入っている錠剤などをのみますか。」への回答に基づいてサプリメント摂取を定義し、解析することとした。

また、自記式質問票には、生活習慣、既往歴、社会的要因、運動機能、パーソナリティなどの項目が含まれており、それらを調査項目とした。さらに、身体機能はMedical Outcomes Studyの身体機能スコアにより分類し[8]、0-1点を虚弱群、2-3点を体力不足群、4-6点を体力充足群と定義した。パーソナリティの評価は、EYSENCK PERSONALITY QUESTIONNAIRE-REVISED (EPQ-R) 日本語版により判定した[8]。

3)統計解析

解析には、SAS解析ソフト(Version 9.1, SAS Institute Inc., Cary, NC, USA)を用い、単変量解析(Studentのt-検定、χ^2検定、Fisher's exact probability test)および多変量解析(ロジスティック回帰分析)を適宜使用した。

また、数値は平均値±標準偏差および百分率で表し、

統計学的有意水準は5％未満とした。

結　果

1）対象者の背景

対象者の背景を表１に示す。全対象者4,227名のうち、2,296名が女性、1,931名が男性であり、平均年齢は59.1歳であった。サプリメントをときどき摂取する者は全体の19％、毎日摂取する者は7％であった。男性に比べ、女性においてサプリメントを摂取する割合が高かった。

また、サプリメント摂取率を性･年齢別に検討したところ(図1)、女性において、55-75歳の年齢群でサプリメント摂取の割合が高率であった。一方、男性においては、特に傾向は認められなかった。

		全対象者	女性	男性
N		4227	2296	1931
年齢 (歳)		59.1±13.7	60.4±13.9	57.5±13.4
Body Mass Index 25 kg/m²以上 (%)		27.5	29.3	25.5
喫煙歴 (%)		37.0	7.6	71.9
飲酒歴 (%)		52.6	28.1	81.8
身体機能* (%)	体力充足群(4-6点)	82.3	76.7	89.0
	体力不足群(2-3点)	8.4	10.7	5.7
	虚弱群(0-1点)	9.3	12.6	5.3
主観的健康度不良 (%)		30.9	32.3	29.4
学歴高校卒業以上 (%)		34.7	32.4	37.5
朝食毎日摂取 (%)		91.2	92.9	89.2
外食週1回以上 (%)		23.2	17.3	30.3
家庭血圧計保有 (%)		75.8	77.3	74.0
高血圧症既往 (%)		33.3	32.9	33.7
高脂血症既往 (%)		12.2	13.1	11.1
糖尿病既往 (%)		9.0	8.2	9.9
脳卒中既往 (%)		3.3	2.5	4.3
心臓病既往 (%)		7.1	6.5	7.7
肝臓病既往 (%)		4.3	3.4	5.4
胃･十二指腸潰瘍既往 (%)		11.8	8.0	16.3
結核･肋膜炎既往 (%)		2.5	2.0	3.1
がん既往 (%)		2.3	3.1	1.4
腹部手術 (%)		38.4	44.1	31.5
便秘薬週2回以上服用 (%)		11.3	15.5	6.3
輸血歴 (%)		9.1	9.9	8.2
難聴既往 (%)		5.0	4.5	5.6
関節炎既往 (%)		7.3	10.2	3.9
骨粗しょう症既往 (%)		4.4	7.4	0.9
サプリメント摂取 (%)	ときどき摂取	19.6	20.6	18.4
	毎日摂取	6.6	7.1	6.0

*Medical Outcomes Studyの身体機能スコアにより分類

表1. 対象者の背景

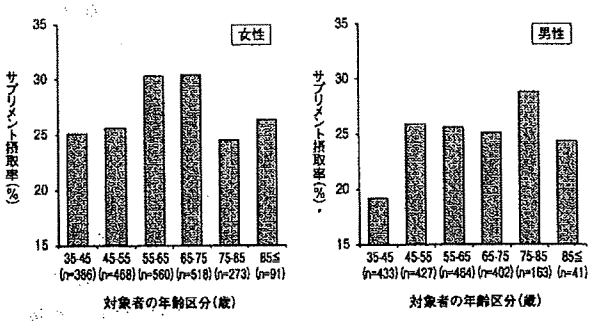

図1. 性・年齢別に検討したサプリメント摂取率

サプリメント摂取に関する設問において、「1.毎日のようにのむ」、「2.ときどきのむ」と回答した者をサプリメント摂取者と定義し、「3.のまない」を非摂取者とした。

2）サプリメント摂取の規定因子

サプリメント摂取と各種因子との関連について、単変量解析を実施した(表2・表3)。

女性において(表2)、サプリメントを毎日ないしときどき摂取する者は、高齢(P = 0.005)であり、また特に中壮年者において摂取率が高かった(P = 0.0002)。また、飲酒歴があり(P = 0.02)、身体機能が悪く(P = 0.001)、主観的健康度が不良であり(P = 0.01)、学歴が高く(P = 0.03)、また外食を週に1回以上とる者の割合が高率であった(P = 0.0002)。さらにサプリメント摂取と関連する疾病既往歴を調べたところ、サプリメントを毎日摂取またはときどき摂取する者は、摂取しない者と比べて、高脂血症、心臓病、肝臓病、胃・十二指腸潰瘍、結核、がん、難聴、関節炎、骨粗しょう症の既往の割合が高率であった(all P < 0.05)。また、腹部手術に伴うと考えられる便秘薬の服用の割合も高かった(P = 0.02)。一方、サプリメントをときどき摂取する者は、毎日摂取する者及び摂取しない者と比べて、高血圧症既往、糖尿病既往の割合が低率であった(all P < 0.05)。

同様に男性において(表3)、サプリメントを摂取する者は、主観的健康度不良であり(P = 0.01)、朝食を毎日摂取する者の割合が高率であった(P = 0.02)。疾病既往歴に関しては、サプリメントを毎日摂取する者で、ときどき摂取する者･摂取しない者と比べ、便秘薬の服用、および輸血歴を有する者の割合が高率であった。また、サプリメント摂取をときどき摂取する者は、毎日摂取する者及び摂取しない者と比べて、腹部手術の既往者の割合が高率であった(all P < 0.05)。

次に、単変量解析でサプリメント摂取と関連が認められた因子と年齢を補正項目とし、サプリメント摂取に対するオッズ比をロジスティック回帰分析で解析した。疾病既往ありの定義は、単変量解析でサプリメント摂取との関連が認められた既往病及び腹部手術の既往のうち、一つ以上ある者を疾病既往ありとした。

女性において、サプリメントの摂取は、高齢(中壮年vs若中年, P < 0.0001)、飲酒歴あり(P = 0.01)、体力不足(P = 0.004)、主観的健康度不良(P = 0.03)、学歴高校卒業以上(P = 0.001)、週1回以上の外食習慣(P = 0.0002)と有意に関連していた(表4)。さらに年齢で層別化し、同様の解析を行うと、若中年の女性において、飲酒歴(P = 0.02)が、中壮年の女性において、学歴高校卒業以上(P = 0.007)、および外食習慣(P = 0.0005)が、60歳以上の女性において、体力不足(P = 0.001)、学歴高校卒業以上(P = 0.004)が、それぞれサプリメント摂取と有意に関連していた(表5)。

男性において、サプリメントの摂取は、疾病既往あり(P = 0.008)、朝食の毎日摂取(P = 0.01)と有意に関連していた。年齢で層別化し、同様の解析を行うと、若中

		サプリメント摂取			P
		毎日摂取	ときどき摂取	摂取せず	
N		163	473	1660	
年齢 (歳)		63.7 ± 14.3	59.7 ± 13.1	60.3 ± 14.0	0.005
年齢群 (%)	若中年者(35-54歳)	28.2	36.2	38.4	
	中壮年者(55-69歳)	34.4	41.7	33.6	0.0002
	高齢者(70歳以上)	37.4	22.2	28.1	
Body Mass Index 25 kg/m²以上 (%)		30.1	29.0	29.3	0.97
喫煙歴 (%)		8.0	7.8	7.5	0.95
飲酒歴 (%)		28.8	33.0	26.6	0.02
身体機能*(%)	体力充足群(4-6点)	66.9	75.7	78.0	
	体力不足群(2-3点)	16.0	13.7	9.3	0.001
	虚弱群(0-1点)	17.2	10.6	12.7	
主観的健康度不良 (%)		40.5	35.1	30.7	0.01
学歴高校卒業以上 (%)		33.1	37.4	30.8	0.03
朝食毎日摂取 (%)		92.6	92.8	92.9	0.99
外食週1回以上 (%)		25.2	21.4	15.4	0.0002
家庭血圧計保有 (%)		79.8	79.1	76.6	0.4
高血圧症既往 (%)		37.4	27.7	34.0	0.02
高脂血症既往 (%)		15.3	16.1	12.0	0.045
糖尿病既往 (%)		9.2	5.3	9.0	0.03
脳卒中既往 (%)		3.1	1.9	2.6	0.6
心臓病既往 (%)		13.5	6.3	5.9	0.0009
肝臓病既往 (%)		6.1	4.2	2.9	0.05
胃・十二指腸潰瘍既往 (%)		15.3	7.8	7.3	0.001
結核・肋膜炎既往 (%)		3.7	3.4	1.5	0.008
がん既往 (%)		7.4	2.8	2.8	0.006
腹部手術 (%)		52.2	44.6	43.2	0.09
便秘薬週2回以上服用 (%)		22.7	16.5	14.5	0.02
輸血歴 (%)		12.3	9.7	9.8	0.6
難聴既往 (%)		12.3	3.4	4.1	<0.0001
関節炎既往 (%)		17.8	11.4	9.1	0.001
骨粗しょう症既往 (%)		13.5	7.8	6.7	0.006

*Medical Outcomes Studyの身体機能スコアにより分類

表2. サプリメント摂取の関連要因(女性)

		サプリメント摂取			P
		毎日摂取	ときどき摂取	摂取せず	
N		115	356	1460	
年齢 (歳)		59.8 ± 13.7	58.1 ± 12.6	57.2 ± 1.4	0.08
年齢群 (%)	若中年者(35-54歳)	38.3	42.1	45.7	
	中壮年者(55-69歳)	37.4	38.8	34.1	0.3
	高齢者(70歳以上)	24.4	19.1	20.2	
Body Mass Index 25 kg/m²以上 (%)		16.5	24.4	26.4	0.056
喫煙歴 (%)		71.3	68.3	72.9	0.2
飲酒歴 (%)		81.7	80.1	82.3	0.6
身体機能* (%)	体力充足群(4-6点)	81.7	89.9	89.3	
	体力不足群(2-3点)	9.6	5.6	5.4	0.1
	虚弱群(0-1点)	8.7	4.5	5.3	
主観的健康度不良 (%)		41.7	28.7	28.6	0.01
学歴高校卒業以上 (%)		32.2	34.3	38.7	0.1
朝食毎日摂取 (%)		94.8	91.9	88.1	0.02
外食週1回以上 (%)		29.6	33.4	29.6	0.4
家庭血圧計保有 (%)		73.9	75.8	73.6	0.7
高血圧症既往 (%)		37.4	32.0	33.8	0.6
高脂血症既往 (%)		11.3	12.9	10.7	0.5
糖尿病既往 (%)		8.7	9.0	10.2	0.7
脳卒中既往 (%)		6.1	4.5	4.0	0.6
心臓病既往 (%)		9.6	7.9	7.5	0.7
肝臓病既往 (%)		8.7	6.2	5.0	0.2
胃・十二指腸潰瘍既往 (%)		18.3	17.1	16.0	0.7
結核・肋膜炎既往 (%)		5.2	3.1	3.0	0.4
がん既往 (%)		3.5	0.6	1.4	0.06
腹部手術 (%)		28.7	38.5	30.0	0.007
便秘薬週2回以上服用 (%)		12.2	6.2	5.9	0.03
輸血歴 (%)		13.9	9.8	7.3	0.02
難聴既往 (%)		6.1	5.1	5.7	0.8
関節炎既往 (%)		6.1	3.9	3.7	0.4
骨粗しょう症既往 (%)		0.0	1.1	0.9	0.7

*Medical Outcomes Studyの身体機能スコアにより分類

表3. サプリメント摂取の関連要因(男性)

		全女性 (N = 2298)			若中年女性 (N = 854)			中壮年女性 (N = 810)			高齢女性 (N = 632)		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
年齢	10歳ごと	–	–	–	1.08	0.79-1.49	0.6	1.15	0.79-1.67	0.5	0.97	0.69-1.36	0.9
	中壮年vs.若中年	1.71	1.33-2.21	<0.0001	–	–	–	–	–	–	–	–	–
	高齢vs.若中年	1.26	0.92-1.73	0.1	–	–	–	–	–	–	–	–	–
飲酒歴	ありvs.なし	1.32	1.06-1.66	0.01	1.48	1.07-2.04	0.02	1.38	0.93-2.03	0.1	1.001	0.57-1.76	0.996
身体機能*	体力不足群vs.体力充足群	1.59	1.16-2.18	0.004	0.95	0.37-2.43	0.9	1.26	0.77-2.09	0.4	2.24	1.37-3.66	0.001
	虚弱群vs.体力充足群	1.11	0.79-1.55	0.6	0.77	0.21-2.87	0.7	1.20	0.64-2.25	0.6	1.40	0.84-2.33	0.2
主観的健康度	不良vs.良好	1.26	1.02-1.56	0.03	1.40	0.96-2.05	0.08	1.27	0.90-1.80	0.2	1.07	0.71-1.60	0.7
学歴	高校卒業以上vs.中学卒業以下	1.46	1.16-1.85	0.001	1.09	0.76-1.56	0.6	1.72	1.16-2.53	0.007	2.27	1.31-3.92	0.004
疾病既往**	ありvs.なし	1.14	0.92-1.40	0.2	0.95	0.69-1.33	0.8	1.30	0.91-1.86	0.1	1.13	0.71-1.82	0.6
外食頻度	週1以上vs.週1未満	1.59	1.24-2.02	0.0002	1.31	0.93-1.85	0.1	2.13	1.39-3.27	0.0005	1.60	0.85-3.03	0.1

OR: オッズ比、95%CI: 95%信頼区間
*Medical Outcomes Studyの身体機能スコアにより分類
**疾病既往:高脂血症、心臓病、肝臓病、胃・十二指腸潰瘍、結核・肋膜炎、がん、腹部手術、便秘薬服用、輸血、関節炎、骨粗しょう症のうち、一つ以上の既往がある者

表4. サプリメント摂取の規定因子(女性)

		全男性 (N = 1931)			若中年男性 (N = 861)			中壮年男性 (N = 679)			高齢男性 (N = 391)		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
年齢	10歳ごと	–	–	–	1.21	0.88-1.67	0.3	1.01	0.67-1.52	0.97	1.001	0.63-1.60	0.996
	中壮年vs.若中年	1.10	0.85-1.42	0.5	–	–	–	–	–	–	–	–	–
	高齢vs.若中年	0.95	0.68-1.32	0.8	–	–	–	–	–	–	–	–	–
身体機能*	体力不足群vs.体力充足群	1.08	0.68-1.71	0.8	1.81	0.53-6.25	0.3	0.92	0.41-2.07	0.8	1.16	0.58-2.33	0.7
	虚弱群vs.体力充足群	0.90	0.54-1.49	0.7	0.25	0.02-3.14	0.3	0.94	0.36-2.44	0.9	1.13	0.56-2.28	0.7
主観的健康度	不良vs.良好	1.14	0.90-1.46	0.3	1.62	1.13-2.34	0.009	0.86	0.56-1.32	0.5	0.85	0.48-1.48	0.6
学歴	高校卒業以上vs.中学卒業以下	0.86	0.67-1.10	0.2	0.73	0.51-1.05	0.09	0.70	0.45-1.09	0.1	2.80	1.47-5.33	0.002
疾病既往**	ありvs.なし	1.34	1.08-1.67	0.008	1.08	0.76-1.52	0.7	1.43	1.002-2.03	0.049	1.86	1.13-3.05	0.01
朝食毎日摂取	摂取vs.非摂取	1.86	1.15-2.99	0.01	1.84	1.07-3.19	0.03	1.88	0.62-5.66	0.3	1.19	0.10-14.28	0.9

OR: オッズ比、95%CI: 95%信頼区間
*Medical Outcomes Studyの身体機能スコアにより分類
**疾病既往:腹部手術、便秘薬服用、輸血のうち、一つ以上の既往がある者

表5. サプリメント摂取の規定因子(男性)

		全体			若中年者			中壮年者			高齢者		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
女性	N	1604			702			516			386		
	非協調性	0.99	0.92-1.06	0.7	0.98	0.88-1.09	0.7	0.95	0.83-1.08	0.4	1.09	0.94-1.26	0.2
	外向性傾向	1.04	1.00-1.08	0.04	1.04	0.99-1.10	0.1	1.07	1.00-1.15	0.06	0.99	0.91-1.08	0.8
	神経症傾向	1.04	1.01-1.08	0.03	1.03	0.97-1.09	0.3	1.05	0.99-1.12	0.1	1.08	1.00-1.17	0.0502
男性	N	1448			735			464			249		
	非協調性	1.02	0.95-1.09	0.6	1.00	0.91-1.10	0.99	1.04	0.92-1.17	0.5	1.06	0.89-1.27	0.5
	外向性傾向	1.01	0.97-1.05	0.8	1.02	0.96-1.08	0.5	0.98	0.92-1.05	0.6	1.02	0.92-1.13	0.8
	神経症傾向	1.04	1.00-1.08	0.04	1.03	0.98-1.09	0.3	1.06	0.98-1.13	0.1	1.01	0.91-1.12	0.8

OR: オッズ比、95%CI: 95%信頼区間
補正項目:(女性)年齢、飲酒歴、身体機能*、主観的健康度、学歴高校卒業以上、疾病既往**、週1回以上の外食
(男性)年齢、身体機能*、主観的健康度、学歴高校卒業以上、疾病既往***、朝食毎日摂取
*Medical Outcomes Studyの身体機能スコアにより分類
**疾病既往(女性):高脂血症、心臓病、肝臓病、胃・十二指腸潰瘍、結核・肋膜炎、がん、腹部手術、便秘薬服用、難聴、関節炎、骨粗しょう症のうち、一つ以上の既往がある者
***疾病既往(男性):腹部手術、便秘薬服用、輸血のうち、一つ以上の既往がある者

表6. サプリメント摂取とパーソナリティ

年の男性において、主観的健康度不良 (P = 0.009)、および朝食の毎日摂取 (P = 0.03) が、中壮年の男性において、疾病既往 (P = 0.049) が、高齢の男性において、学歴高校卒業以上 (P = 0.002)、および疾病既往 (P = 0.001) がサプリメント摂取と有意に関連した。

3) サプリメント摂取とパーソナリティ

対象者のうち、パーソナリティの項目に回答が得られた者 (女性1,604名、男性1,448名) において、サプリメント摂取とパーソナリティに関する検討を行った (表6)。補正項目には前述の多変量解析と同様、単変量解析で有意な関連が認められた項目を用いた。

女性において、外向性傾向 (P = 0.04) および神経症傾向 (P = 0.03) がサプリメント摂取と有意に関連していた。また有意ではなかったものの、女性全体、特に若壮年女性において、外向性傾向が (P = 0.06)、また高齢女性において神経症傾向 (P = 0.0502) がサプリメント摂取と関連している傾向が認められた。

同様に男性においてサプリメント摂取とパーソナリティについて解析したところ、神経症傾向とサプリメント摂取との間に有意な関連が認められた (P = 0.04)。

考　察

本地域住民の、およそ26%がサプリメントを摂取していた。サプリメント摂取の関連因子は、性別・年齢によって異なっていた。また、本研究はサプリメント摂取とパーソナリティとの関連をみた初めての研究であり、男女とも神経症傾向がサプリメント摂取と関連することを明らかにした。また女性において、外向性傾向もサプリメント摂取と関連する傾向が認められた。

1) サプリメントの利用状況

本研究において、全対象者の26%が、サプリメント (ビタミンなど) を毎日またはときどき摂取していた。また、女性は男性よりもサプリメントを摂取している割合が高率であった。

2001年度に15歳以上を対象に行われた国民栄養調査では、ビタミン・ミネラルのサプリメント摂取率は、女性24%、男性17%であった[10]。また、老化に関する長期縦断疫学調査 (NILS-LSA) における、ビタミン・ミネラルのサプリメント摂取率は、1997年から2000年に行われた第1次調査において29% (女性32%、男性27%)[11]、2006年から2008年に行われた第5次調査において30% (女性38%、男性22%) であった[2]。アメリカでは、1999年から2000年に行われた健康調査 (NHANESⅢ) において、35% (女性38%、男性32%) の者でビタミン・ミネラルのサプリメントを摂取していたことが報告されている[12]。本研究の利用状況は、アメリカの調査と比較すると低率であるが、国内の他の研究とはほぼ一致した結果であった。また、女性の摂取率が高いことも他の研究と同様の結果であった。

2) サプリメント摂取の関連因子

サプリメント摂取と関連する因子について、先行研究ではサプリメント利用者は高齢、教育歴が高い、Body Mass Indexが適当、喫煙率が低い、適度な飲酒習慣であるなど、生活習慣に多くの特徴がみられることが報告されているが、一貫していない因子もある[3,7,12,13]。

本邦における調査としては、2000年に27都道府県の1万3,500名を対象として実施された、栄養補助食品および健康食品の摂取状況に関する質問票調査がある (回収率88%)。その結果、栄養補助食品および健康食品摂取者は、主観的健康度不良、主観的体力レベル低値、ストレス認知度高値、運動実施者および規則的な食事と関連があり、健康意識の高い者において、栄養補助食品および健康食品の摂取率が高い傾向が認められた[4]。一方、2006年に東北6県の消費者を対象にランダム・デジット・ダイヤリング法により実施された調査では、健康食品を「よく利用する」「たまに利用する」と回答した者の食習慣の特徴は、「朝食の欠食傾向」のみであった[14]。このように、食生活に関する本邦の報告は一致していない。

本研究では、サプリメント摂取との関連因子が、性・年齢により異なっていた。男性では、すべての年代で不良な健康状態を示す因子と関連しており、さらに若中年者では朝食習慣と関連が認められ、健康に対し関心の高い者がサプリメントを摂取している傾向がうかがえる。一方、女性においては、高齢者では男性と同様不良な健康状態を示す因子が関連しているが、若年者から壮年者では、飲酒歴、外食習慣がサプリメント摂取と関連しており、健康に対する関心が高いとは言い難い。さらに、男女ともに、神経症傾向とサプリメント摂取が関連していたが、若中壮年の女性では、外向性傾向も関連していた。よって、性・年齢によって、サプリメント摂取の要因が異なっており、高齢女性および男性においては、健康に対する関心・意識の高い者でサプリメントを摂取していたのに対し、若年から壮年の女性に関しては、逆に健康意識が低くその代わりとしてサプリメントに依存している可能性が考えられる。しかしながら、サプリメント摂取が、「健康意識の向上」につながるのか、それとも「サプリメントに頼る健康意識の低下」の現れであるか、どちらの解釈が正しいかについて、本研究結果のみから明らかにすることはできない。

3）臨床への応用

サプリメントの問題点として、医薬品とは異なり、成分が必ずしも一定ではなく、その効果やリスクの個人差は大きい点が考えられる。一方で、患者は医薬品の副作用には敏感であるが、サプリメントに関しては副作用を有することに対する認識が薄いことが知られている。また、サプリメントによる栄養素の過剰摂取、栄養のアンバランスも懸念される。さらに、医薬品との相互作用による中毒・健康被害の危険性についての報告もされている。2006年に東北6県の消費者を対象に行なった調査[14]では、以前に摂っていた健康食品をやめたことがある者が、25%にも達した。その理由は、「効果がない」が約半数と最も多いほか、「かえって身体の調子が悪くなった」も一割ほど認められた。健康食品の有効性や副作用などの安全性の問題が、市民の実感としても一部裏付けられた形と考えられる。そもそもサプリメントに効果はあるのか、安全なのか、使用法は適切か、食生活自体には問題はないのか、あらためて検討する必要がある。このような現状において、副作用・相互作用などのリスクを少しでも減らすために、患者のサプリメント利用状況を把握することは重要なことである。しかしながら、患者は自分のサプリメント摂取を医療者に言いたがらない傾向が見受けられる。実際、大阪府内科医会の調査[16]において、通院患者の約6割が健康食品の使用経験があるが、約9割が主治医に黙って使用していたことが報告されている。本研究によって示されたサプリメント摂取の関連因子があてはまる者に対して、服薬指導時に慎重に聴きとり・確認を行なうなどのアプローチを行うことにより、患者のサプリメント摂取状況の把握につながると考えられる。医療者は患者の医薬品の使用状況を十分に聞き取った上で的確な情報提供と指導を行う必要がある。

2005年に東京都により実施された、医療関係者へのアンケートおよびヒアリング調査[15]によると、医師も薬剤師も半数以上がサプリメント・健康食品に対する関心を持っている一方で、知識の蓄積や情報入手の試みにあらわされるような、“サプリメント・健康食品に対する積極的な取り組み”は、一般的に医師よりも薬剤師において高いことが報告されている。この背景として、薬剤師のほうがより頻繁に患者から相談を受け、また利用状況をできるだけ確認していることがあげられる。医療関係者の中で、薬剤師には製品情報や副作用・相互作用に関する情報の収集・提供の役割がとりわけ期待されている。今やサプリメント・健康食品は医療の現場においてもその存在を無視することのできないものとなっており、薬剤師、医師、看護師、栄養士などがそれぞれの立場の役割を担い、かつ連携していくことがより一層求められている。

4）本研究の限界

本研究は1998年に施行されたアンケート調査データに基づいており、現在の状況とは異なっている可能性が考えられる。しかしながら、本研究のサプリメント摂取率は、他の比較的最近の先行研究[1,2]における値と比較を行なっても同程度であった。また、関連する因子も共通しているものが多い[3,4,7,12,13]。したがって、以前のデータではあるものの、現在の臨床現場にも十分活かすことができると考えられる。また、ビタミン剤の摂取状況の把握が、それ以外のサプリメントの摂取状況を強く反映することが報告されているが[7]、本研究でのサプリメント摂取に関する質問項目では、ビタミン以外のサプリメントまで調査できていない可能性は明らかである。現在市場には多種多様なサプリメントが溢れており、今後、ビタミンに限定せずさまざまなサプリメントに関して広範かつ詳細に調査していく必要がある。さらに、本研究対象者は東北地方の農村地域に在住しており、日本の一般地域住民としての一般化は難しい可能性がある。大迫町は農村地域であり、サプリメント・健康食品も配置販売(置き薬)業者から購入することも多く、ドラッグストアにてサプリメントを購入する都市部とは異なる点が多くある。しかしながら、本研究結果は、日本の農村地域におけるサプリメント利用状況に関わる様々な要因を詳細に検討したデータとして、一定の価値を有するものと考えられる。

結 論

本地域住民の26%がサプリメントを摂取しており、またサプリメント摂取の関連要因は、性・年齢により異なることが明らかとなった。加えて、高い健康意識・低い健康意識に関連する要因が、それぞれサプリメント摂取と関連していることが示された。これより、健康意識が高い者がサプリメントを摂取している可能性、および逆に健康意識による行動を怠ることの代替手段としてサプリメントに頼る傾向を有する者がサプリメントを摂取している可能性の、二つの可能性の存在が示された。

以上より、本研究において明らかとなったサプリメント摂取者の特性を用いてサプリメント摂取者を把握し、また適切なアプローチを行うことにより、薬局や店舗販売業(薬店)などの臨床現場において薬剤管理指導業務等を効果的に実施し得ることが期待されると考えられる。

謝 辞

本研究の実施にあたり、御協力くださいました大迫保健福祉センターの関係者の皆様、御回答くださいました住民の皆様に深く感謝致します。

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Relationship Between Blood Pressure Category and Incidence of Stroke and Myocardial Infarction in an Urban Japanese Population With and Without Chronic Kidney Disease

The Suita Study

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Background and Purpose—Chronic kidney disease (CKD) is increasingly recognized as an independent risk factor for stroke and myocardial infarction (MI). Few studies, however, have examined the relationship between blood pressure (BP) category and these diseases in subjects with and without CKD.

Methods—We studied 5494 Japanese individuals (ages 30 to 79, without stroke or MI at baseline) who completed a baseline survey and received follow-up through December 2005. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease study equation modified by the Japanese coefficient. CKD was defined as an estimated GFR <60 mL/min/1.73m^2. BP categories were defined by the European Society of Hypertension and European Society of Cardiology 2007 criteria.

Results—In 64 395 person-years of follow-up, we documented 346 incidences of cardiovascular diseases (CVD; 213 strokes and 133 MI events). Compared with the GFR (≥90 mL/min/1.73m^2) group, the hazard ratios (95% confidential intervals) for stroke were 1.9 (1.3 to 3.0) in the GFR 50 to 59 mL/min/1.73m^2 group and 2.2 (1.2 to 4.1) in the GFR <50 mL/min/1.73m^2 group. Results for cerebral infarction were similar. Compared with the optimal BP subjects without CKD, the normal BP, high-normal BP, and hypertensive subjects without CKD showed increased risks of CVD and stroke; however the impact of each BP category on CVD (*P* for interaction: 0.04 in men, 0.49 in women) and stroke (0.03 in men, 0.90 in women) was more evident in men with CKD.

Conclusions—CKD may increase the association of BP and CVD in a Japanese urban population. (***Stroke*. 2009;40:2674-2679.)**

Key Words: chronic kidney disease ■ blood pressure category ■ stroke ■ myocardial infarction ■ epidemiology ■ prospective studies ■ general population

Recently, chronic kidney disease (CKD) has become a major public health problem and a risk factor for all-causes mortality, stroke, and myocardial infarction (MI).[1] In end-stage renal disease, the cardiovascular disease (CVD) mortality rate is more than 10 times as high as that in the general population.[2] In asymptomatic general populations or outpatients, a severely or moderately decreased glomerular filtration rate (GFR) has been shown by most but not all studies to be an independent risk factor for stroke and MI.[1] However, in low-risk or general populations, the relationship between levels of kidney function and clinical outcomes has not been as clear. Some studies have demonstrated no association between CKD and CVD,[3,4] whereas others have shown CKD as an independent risk factor for CVD.[5–8] These inconsistencies may be attributable to differences between the selected study populations as well as the severity of the CKD.

The frequency of hypertension is relatively higher in Japanese than in Western countries.[9] Hypertension is one of the major risk factors for both CVD and CKD. Recently, a larger prospective study has indicated that CKD increased the association between blood pressure (BP) categories and CVD, although the relevant data were gathered from 10 rural areas with different methods

Received February 19, 2009; final revision received March 25, 2009; accepted April 14, 2009.

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***Stroke* is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.109.550707**

for the measurement of creatinine.[10] A few studies in general population have demonstrated a stronger association between BP and CVD in subjects with CKD.[5,10] We examined the association between BP category and incidence of stroke and MI subjects with and without CKD in a Japanese urban population.

Methods

Study Subjects

Suita city is located adjacent to Osaka city, which is the second largest metropolitan area in Japan. The Suita Study,[11–13] an epidemiological study of cerebrovascular and cardiovascular diseases, was based on a random sampling of 12 200 Japanese urban residents. As a baseline, participants (aged 30 to 79 years) were randomly selected from the municipality population registry and stratified into groups by sex and age in 10-year increments in 1989. Of these, 6485 people underwent regular health checkups between September 1989 and March 1994.

Cohort members in the study population were excluded from these analyses if they had a past or present history of CVD at baseline (n=208), were missing data (n=170), attended health checkups after April 1994 (n=79), or failed to complete the follow-up health surveys or questionnaires after the baseline examination (n=534). After applying these exclusions, a total of 5494 participants aged 30 to 79 years old were selected. Informed consent was obtained from all participants. This study was approved by the Institutional Review Board of the National Cardiovascular Center.

Measurement of Blood Pressure and Covariates

Well-trained physicians measured BP 3 times using a mercury column sphygmomanometer, an appropriate-size cuff, and a standard protocol. Before the initial BP reading was obtained, participants were seated at rest for at least 5 minutes. First, systolic blood pressure (SBP) was measured for the purpose of obtaining approximate SBP levels. SBP and diastolic blood pressures (DBP) were taken as the average of the second and third measurements, which were recorded more than 1 minute apart.

At the time of the baseline examination, subjects were classified into 1 of the 5 BP categories based on the European Society of Hypertension and European Society of Cardiology (ESH-ESC) 2007 criteria[14]: optimal (SBP <120 mm Hg and DBP <80 mm Hg), normal (SBP 120 to 129 mm Hg or DBP 80 to 84 mm Hg), high-normal BP (SBP 130 to 139 mm Hg or DBP 85 to 89 mm Hg), and hypertensive (SBP ≥140 mm Hg or DBP ≥90 mm Hg). Antihypertensive drug users were classified according to their BP levels at the baseline survey. If the SBP and DBP readings for a subject were in different categories, the subjects were categorized into the higher of the two BP categories.

At the baseline examination, we performed routine blood tests that included serum total cholesterol, HDL cholesterol, and glucose levels. Physicians or nurses administered questionnaires covering personal habits and present illness. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Hypercholesterolemia was defined as total cholesterol levels ≥5.7 mmol/L or current use of antihyperlipidemic medications. Diabetes was defined as a fasting plasma glucose level ≥7.0 mmol/L, a nonfasting plasma glucose level ≥11.0 mmol/L, or current use of antidiabetic medications.

Definition of CKD

Serum creatinine (Cre) was measured by noncompensated kinetic Jaffé methods. The glomerular filtration rate (GFR) of each participant was calculated from the Cre value and the age, using the MDRD equation modified by the Japanese coefficient (0.881), as follows[15]:

$$\text{GFR (ml/min/1.73 m}^2\text{)} = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \text{ (for men)}$$

$$\text{and GFR (ml/min/1.73 m}^2\text{)} = 0.881 \times 186 \times \text{age}^{-0.203} \times \text{Cre}^{-1.154} \times 0.742 \text{ (for women).}$$

CKD was defined as an estimated GFR <60 mL/min/1.73m^2.

Confirmation of Stroke and MI and End Point Determination

The confirmation of stroke and MI in the Suita Study has been described elsewhere.[11–13] In brief, the 5 hospitals in this area, where acute stroke and MI patients were admitted, were all capable of performing computed tomographic scans or MRI. Medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline data. Strokes were defined according to the U.S. National Survey of Stroke criteria.[16] For each stroke subtype (ie, cerebral infarction [thrombotic or embolic infarction], intracerebral hemorrhage, and subarachnoid hemorrhage), a definite diagnosis was established based on examination of computed tomographic scans, magnetic resonance images, or autopsies. Definite and probable MIs were defined according to the criteria set out by the MONICA project.[17] Sudden deaths of unknown origin were deaths that occurred within 24 hours from the onset of symptoms, and were also classified as MI. In this study CVD was defined as stroke or MI.

To detect MI and stroke occurrences, each participant's health status was checked at clinical visits to the National Cardiovascular Center every 2 years. Yearly questionnaires by mail or telephone were also completed for all participants. In addition, to complete our surveillance for fatal strokes and MIs, we conducted a systematic search for death certificates. All the data (health check-ups, questionnaires, and death certificates) were checked against medical records to confirm the incidence of CVD. We identified possible strokes or MIs using data from (1) the health examination and questionnaires from the stroke and MI registries without informed consent for medical records survey; and (2) death certificates bearing a diagnosis of probable stroke or MI without registration of CVD incidence.

The end points of the current follow-up study were (1) date of the first MI or stroke event (2); date of death (3); date of leaving Suita; and (4) December 31, 2005 (censored).

Statistical Analysis

Analyses of variances and χ^2 tests were used to compare mean values and frequencies. The Cox proportional-hazard ratios (HRs) were fitted to the GFR categories and CKD after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors at the baseline survey: namely, present illness of hypertension, hypercholesterolemia and diabetes, smoking status (never, quit, and current smoker), and drinking status (never, quit, and current drinker). The Cox proportional HRs were fitted to the combination of the BP categories and CKD (positive or negative) after adjusting for sex and age in 5-year increments as stratified variables and other potential confounding factors including an interactive term for CKD and BP categories. The fit of the proportional hazards model was evaluated by examining discrete regression models and by permitting the proportionality assumption to vary with time, and assessments of nonlinearity involving associations with blood pressure and GFR categories were made. The probability values for the model of interaction between CVD incidence and log (person year) were 0.38 in men and 0.81 in women. Proportionality was also checked by log-log survival plot.

To express the impact of CKD on CVD occurrence in the participants, we estimated the population attributable fraction (PAF, %). PAF was estimated as follows:

$$Pe \times (HR-1)/HR,$$

in which Pe is the proportion of incident cases in CKD, and HR is the multiple-adjusted hazard ratio.[18] All statistical analyses were conducted using the SAS statistical package software (release version 8.2, SAS Institute Inc).

Results

Figure 1 shows that the frequency of CKD increases with age in both men and women. At the baseline survey, both men and women with CKD (8.9% for men and 11.3% for women)