longitudinal observational studies in Japan.[14] Our aims were to assess the impact of a reduction in GFR on the development of CVD in the general population and to examine the association of blood pressure with the risk of CVD in individuals with a reduced GFR.

Methods

Study Population

The rationale, study design, and methods of the JALS-ECC have been described elsewhere.[14] In brief, cohort studies were eligible for inclusion in this project if they satisfied the following criteria: (1) Japanese population; (2) prospective cohort study; (3) at least 3000 person-years of follow-up; (4) date of birth, sex, height, weight, blood pressure, and serum total cholesterol recorded at baseline; and (5) date of death or the age at death recorded during a follow-up. Quality control of the collected cohort data were performed at the JALS Coordinating Center. The individual records of 66 691 participants in 21 cohort studies were included in the present project, with 82.7% of the participants from 17 community-based cohorts and 17.3% from 4 work-site–based cohorts. Permission to submit each collection of cohort data to the JALS Coordinating Center was obtained from the relevant institutional review boards for ethical issues.

Of the 21 cohort studies, 11 cohorts were excluded from the present analysis for the following reasons: 4 were work-site–based cohorts, 3 did not include creatinine data, 3 lacked many values for relevant variables, and 1 included no event data for either stroke or myocardial infarction. From the remaining 10 cohorts, we excluded participants less than 40 years of age and those 90 years of age or older, those with unavailable examination data at baseline or unavailable event data, those with a history of CVD, and those with an estimated GFR <15 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}. For the analysis regarding CVD and stroke, the Niigata cohort was excluded, because information on stroke events was unavailable. A final total of 23 033 participants were enrolled in the CVD analysis, 23 084 in the stroke analysis, 30 657 in the myocardial infarction analysis, and 31 374 in the all-cause death analysis. The average follow-up period was 7.4 years.

Risk Factors

The JALS Coordinating Center requested individual participant data from the collaborating investigators. Serum creatinine was measured by Jaffe's method in 8 cohorts, by the enzymatic method in 1 cohort, and by both methods in 1 cohort. Serum creatinine values measured by the enzymatic method were corrected for Japanese subjects by the addition of 18.3 μmol/L.[15] GFR was estimated with the 4-variable Modification of Diet in Renal Disease study equation.[3] In accordance with the National Kidney Foundation Kidney Disease Outcomes Quality Initiative guidelines,[16] GFR levels were classified in the following ranges: $\geq$90, 60 to 89, and $<$60 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}. Blood pressure was measured by a standard sphygmomanometer in all cohorts. Mean values were used in several cohorts that measured 2 or more blood pressure values. Blood pressure levels at baseline were classified into 4 categories (normal, prehypertension, stage 1 hypertension, and stage 2 hypertension) according to the criteria of the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[17] Diabetes was defined as a fasting blood glucose level of $\geq$7.0 mmol/L, a casual blood glucose level of $\geq$11.1 mmol/L, current use of insulin or oral medication for diabetes, and/or a history of diabetes. Serum total cholesterol was determined enzymatically. Information on smoking habit was obtained through a standard questionnaire and classified as current habitual use or lack thereof.

End Points

In each cohort, vital status and the development of CVD were ascertained during follow-up by use of death certificates, hospital medical records, and/or questionnaire surveys. All outcomes were classified according to the International Classification of Diseases, 9th Revision (ICD-9). All events were recoded by coordinating center staff members. CVD was defined as the development of either stroke or myocardial infarction. Stroke was defined as an acute disturbance of focal neurological function with symptoms that lasted $>$24 hours or death caused by a stroke event (ICD-9 codes 430, 431, 433, 434, or 436). Myocardial infarction included both fatal and nonfatal myocardial infarction, which was diagnosed by use of an appropriate clinical history supported by ECG changes and/or elevations of cardiac enzymes or other biochemical markers of myocardial injury (ICD-9 410). Only the first event of the relevant outcome type was included in each analysis.

Statistical Analysis

The SAS software package for Windows, release 9.13 (SAS Institute, Inc, Cary, NC) was used to perform all statistical analyses. The incidence rate of each outcome for the GFR subgroups was calculated by the person-year method and adjusted for the age and sex distribution of the overall population enrolled in the CVD analysis by the direct method, in which the subgroups and study population were subdivided into the same set of age groups (defined by decade) and the age- and sex-specific incidence rates were calculated within each subgroup.[18] The hazard ratios (HRs) and their 95% CIs for the development of events were estimated with the Cox proportional hazards regression model. The cohort effect was adjusted as a fixed effect by taking the study as a strata variable, assuming only proportional hazards within each study and not between studies.[18] Heterogeneity across cohorts was examined with the Cochran Q test and the I^2 statistic.[18] The risks of events according to blood pressure levels were also estimated with the Cox regression model. Trends in relationship between blood pressure levels and the risk of events were assessed by fitting models with a linear term for blood pressure categories according to kidney function status, and the heterogeneity of these relationships between kidney function status subgroups was estimated by the addition of an interaction term of a linear term for blood pressure levels and kidney function status to the relevant model. $P<0.05$ was considered statistically significant in all analyses.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

The characteristics of the 10 cohorts examined in the present study are shown in Table 1. Among all subjects, the mean age was 57.6 years, and the proportion of men was 38.0%. The mean value of serum creatinine was 78.6 μmol/L, and the frequency of GFR $<$60 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2} was 8.2%. During the average follow-up period of 7.4 years, a total of 727 subjects experienced CVD, 592 had strokes, 180 had myocardial infarctions, and a total of 2104 died.

Table 2 shows the baseline characteristics of the 23 033 subjects enrolled in the CVD analysis by sex. Their mean ages were 56.9 years for men and 58.2 years for women, and the frequency of GFR $<$60 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2} was 5.2% for men and 10.1% for women. The frequencies of normal blood pressure, prehypertension, stage 1 hypertension, and stage 2 hypertension were 19.6%, 41.8%, 26.0%, and 12.6% for men and 24.3%, 41.0%, 24.3%, and 10.4% for women, respectively. Similar findings were observed in subjects enrolled in the analyses of stroke, myocardial infarction, and all-cause death.

The age- and sex-adjusted incidences of CVD and stroke increased with declining GFR levels in the overall population (Table 3); the differences were statistically significant between subjects with a GFR $\geq$90 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2} and

Table 1. Characteristics of Included Cohort Studies

Regions	Cohort Name	No. of Population	Mean Age, y	Men, %	Mean sCr, μmol/L*	GFR <60, %†	Mean SBP/DBP, mm Hg	Mean Fasting Blood Glucose, mmol/L	Mean Total Cholesterol, mmol/L	Current Smoking, %	Follow-Up, y		No. of Events			
											Start-End	Mean	CVD	Stroke	MI	Death
Hokkaido	Tanno/Soubetsu	2066	60.1	43.9	89.6	19.4	133/78	5.1	5.0	25.9	1991–1999	5.5	120	93	27	136
Akita 2	Ikawa	2595	56.1	43.6	76.0	2.6	135/81	6.6	4.9	28.5	1985–1999	10.7	44	41	3	146
Ibaraki	Kyowa	4479	54.8	42.8	76.9	5.3	137/82	6.9	5.0	30.4	1985–1999	10.1	168	128	51	350
Niigata	Tokamachi	8480	58.0	33.1	79.6	7.7	127/73	NA	5.1	18.8	1993–2003	7.8	NA	NA	29	400
Osaka	Yao Minami-takayasu	3855	54.0	34.8	78.7	6.7	132/80	6.0	5.2	27.2	1985–1998	9.6	79	62	18	191
Shiga 1	Shigaraki	2934	56.6	41.1	81.3	10.5	132/78	6.0	5.0	29.4	1992–2001	7.3	82	69	13	260
Hiroshima	Hiroshima	2222	72.1	28.7	84.0	23.8	136/78	6.2	5.6	15.4	1992–2000	3.6	73	63	12	350
Ehime	Ohzu	5300	59.5	33.9	76.9	6.2	130/76	5.3	5.3	15.2	1996–2003	5.5	99	89	10	184
Fukuoka 1	Hisayama	757	60.8	39.5	83.1	9.5	133/78	5.4	5.4	21.1	1990–2000	9.9	57	45	14	86
Kumamoto	...	2465	47.0	70.0	65.4	0.2	127/80	5.7	5.4	46.4	1999–2003	4.2	5	2	3	1
Total	...	35 153	57.6	38.0	78.6	8.2	131/78	6.0	5.2	24.5	1985–2003	7.4	727	592	180	2104

sCr indicates serum creatinine; SBP/DBP, systolic or diastolic blood pressure; MI, myocardial infarction; and NA, not available.

*Serum creatinine was measured by Jaffe's method in 8 cohorts, by enzymatic method in the Ehime cohort, and by either method in the Niigata cohort. The values of serum creatinine measured by the enzymatic method were corrected by the addition of 18.3 μmol/L.

†GFR (unit: mL · min^{-1} · 1.73 m^{-2}) was estimated by the Modification of Diet in Renal Disease formula.

those with a GFR <90 mL · min^{-1} · 1.73 m^{-2} (all $P<0.01$). Subjects with a GFR <60 mL · min^{-1} · 1.73 m^{-2} showed a significantly higher age- and sex-adjusted incidence of myocardial infarction and all-cause mortality than those with a GFR ≥90 mL · min^{-1} · 1.73 m^{-2} ($P<0.001$). The age-adjusted incidences of CVD, stroke, and all-cause mortality were significantly higher in subjects with a GFR <60 mL · min^{-1} · 1.73 m^{-2} than in those with a GFR ≥90 mL · min^{-1} · 1.73 m^{-2} in both sexes (all $P<0.05$).

The risks of CVD, stroke, myocardial infarction, and all-cause death increased progressively with declining GFR levels in the overall population after adjustment for age and sex (Table 4). Even after adjustment for potential confounding factors, specifically age, sex, cohort, systolic blood pressure, diabetes, serum total cholesterol, body mass index, and current smoking status, the risks of CVD, myocardial infarction, and all-cause death were significantly higher in subjects with a GFR <60 mL · min^{-1} · 1.73 m^{-2} than in the overall population. There was no evidence of heterogeneity in these associations among study cohorts (all P for heterogeneity >0.6; Q=2.46, I^2=0% for CVD; Q=4.06, I^2=0% for stroke; Q=3.75, I^2=0% for myocardial infarction; and Q=1.14, I^2=0% for all-cause death). Subjects with a GFR <60 mL · min^{-1} · 1.73 m^{-2} had a significantly greater risk of myocardial infarction and death in men and of CVD, stroke, and death in women.

Table 2. Baseline Characteristics of the Study Population by Sex

Risk Factors	Men (n=9574)	Women (n=13 459)
Age, y	56.9 (11.1)	58.2 (11.4)
Serum creatinine, μmol/L	87.3 (16.7)	71.6 (13.6)
GFR, mL · min^{-1} · 1.73 m^{-2}	87.3 (20.2)	81.0 (19.1)
GFR levels (mL · min^{-1} · 1.73 m^{-2}), %		
≥90	39.0	27.1
60–89	55.9	62.8
<60	5.2	10.1
Systolic blood pressure, mm Hg	133.6 (19.1)	132.3 (19.8)
Diastolic blood pressure, mm Hg	81.1 (11.5)	77.9 (11.0)
Blood pressure levels, %		
Normal	19.6	24.3
Prehypertension	41.8	41.0
Stage 1 hypertension	26.0	24.3
Stage 2 hypertension	12.6	10.4
Diabetes, %	9.5	5.2
Serum total cholesterol, mmol/L	5.0 (0.9)	5.4 (1.0)
Body mass index, kg/m^2	23.2 (3.0)	23.2 (3.3)
Current smoking, %	56.2	7.1

Values are means (SD) or frequencies.

The Figure shows the log-linear relationship between blood pressure levels at baseline and the hazard of CVD, stroke, and all-cause death regardless of kidney function status after adjustment for potential confounding factors (all P for trend <0.01). There was no evidence of heterogeneity of the patterns in the association of blood pressure levels with the risk of outcomes between subgroups of kidney function status (all P for heterogeneity >0.7). The age- and sex-adjusted HR of myocardial infarction increased in a log-linear fashion with increasing blood pressure levels in the normal, prehypertension, stage 1 hypertension, and stage 2 hypertension groups in subjects with a GFR ≥60 mL · min^{-1} · 1.73 m^{-2} (HR 0.56 [95% CI 0.33 to 0.95], 1.00 [reference], 1.60 [1.08 to 2.37], and 1.75 [1.06 to 2.87]; P for trend 0.03) and in those with a GFR <60 mL · min^{-1} · 1.73 m^{-2} (0.19 [0.02 to 1.47], 1.00 [reference], 1.72 [0.80 to 3.70], and 2.36 [1.02 to 5.44]; P for trend 0.04). The number of myocardial infarctions in subjects with normal blood pressure levels was too small to assess reliably for multivariate-adjusted analysis.

We also performed sensitivity analyses to assess the risk of CVD according to GFR levels estimated by the MDRD formula corrected according to the Japanese coefficient of 0.881.[15] The correction shifted the GFR distribution to a

Table 3. Incidence Rate of CVD According to Kidney Function Status

GFR Levels, mL · min^{-1} · 1.73 m^{-2}	Overall				Men				Women			
	No. of Events	No. of Participants	PY at Risk	Incidence Rate per 1000 PY (95% CI)*	No. of Events	No. of Participants	PY at Risk	Incidence Rate per 1000 PY (95% CI)*	No. of Events	No. of Participants	PY at Risk	Incidence Rate per 1000 PY (95% CI)*
CVD												
GFR ≥90	105	7199	51 203	2.9 (2.1–3.6)	78	3672	23 964	4.4 (3.3–5.6)	27	3527	27 239	1.8 (0.9–2.8)
GFR 60–89	489	13 967	104 334	4.3 (3.9–4.7)†	245	5404	39 794	5.5 (4.8–6.2)	244	8563	64 540	3.5 (3.1–3.9)†
GFR <60	133	1867	12 013	6.5 (5.0–8.0)‡	49	498	3018	9.1 (5.7–12.5)‡	84	1369	8995	4.7 (3.6–5.8)‡
Stroke												
GFR ≥90	84	7206	51 315	2.2 (1.6–2.8)	61	3676	24 033	3.5 (2.5–4.5)	23	3530	27 281	1.4 (0.6–2.1)
GFR 60–89	404	14 003	104 808	3.5 (3.2–3.9)†	192	5433	40 160	4.2 (3.6–4.8)	212	8570	64 648	3.0 (2.6–3.4)†
GFR <60	104	1875	12 092	5.0 (3.7–6.4)‡	33	501	3048	6.6 (3.5–9.7)§	71	1374	9044	4.0 (3.0–5.0)‡
Myocardial infarction												
GFR ≥90	25	8350	60 807	0.6 (0.2–0.9)	21	4179	28 164	0.9 (0.4–1.4)	4	4171	32 643	0.4 (−0.2–0.9)
GFR 60–89	116	19 786	151 527	0.7 (0.6–0.8)	72	7345	54 855	1.1 (0.9–1.4)	44	12 441	96 672	0.4 (0.3–0.5)
GFR <60	39	2521	16 926	1.4 (0.9–1.9)‡	21	643	4039	2.4 (1.3–3.6)‡	18	1878	12 887	0.7 (0.4–1.1)
All-cause death												
GFR ≥90	289	8445	62 754	7.6 (6.4–8.7)	217	4225	29 119	11.4 (9.7–13.1)	72	4220	33 635	5.1 (3.5–6.6)
GFR 60–89	1388	20 280	161 168	7.0 (6.7–7.4)	809	7529	58 344	10.4 (9.7–11.1)	579	12 751	102 824	4.8 (4.4–5.2)
GFR <60	427	2649	18 935	12.9 (10.2–15.5)‡	184	681	4540	21.3 (14.9–27.7)‡	243	1968	14 395	7.3 (5.9–8.6)‡

PY indicates person-years.

*Incidence rates were adjusted for age by the direct standardized method. Overall results were additionally adjusted for sex.

†$P<0.01$, ‡$P<0.001$, §$P<0.05$ vs GFR ≥90 mL · min^{-1} · 1.73 m^{-2}.

lower level. Consequently, more participants (21%) were assigned to the group whose GFR was <60 mL · min^{-1} · 1.73 m^{-2}, and the age- and sex-adjusted risk of CVD among these subjects relative to those with a GFR ≥90 mL · min^{-1} · 1.73 m^{-2} was attenuated by 85% (95% CI 32% to 160%), although it was still significant. Similarly, a log-linear relationship between blood pressure levels and the risk of CVD was still observed in the subgroup whose GFR was <60 mL · min^{-1} · 1.73 m^{-2}, even after correction with the Japanese coefficient (Data Supplement Figure).

Discussion

In the present study, we demonstrated a clear association between reduced GFR and high risk of CVD. To the best of our knowledge, this is the first overview of this issue in a Japanese community-based longitudinal study. Furthermore, the relationship between blood pressure levels at baseline and CVD risk was found to be strong and continuous, regardless of kidney function status.

There have been few studies showing the association of reduced GFR with an increased risk of CVD or mortality in the general Japanese population.[6–8] The findings of the Hisayama study revealed that a GFR <60 mL · min^{-1} · 1.73 m^{-2} was a significant risk factor for the development of coronary heart disease in men and of CVD and stroke in women.[6] In a large cohort study conducted by Irie et al,[7] reduced GFR was strongly associated with mortality due to CVD or stroke. A report from NIPPON DATA 90 also showed an association between a GFR <30 mL · min^{-1} · 1.73 m^{-2} and a high risk of cardiovascular death.[8] In the present study, we demonstrated a clear association between reduced GFR and the risks of CVD, stroke, myocardial infarction, and death in an overview of 10 Japanese cohort studies. These results, therefore, highlight the importance of taking kidney function status into consideration in trying to reduce the burden of CVD in the general Japanese population.

There are several possible explanations for the association of reduced GFR with CVD.[3] First, reduced GFR is associated with a high prevalence of traditional CVD risk factors, such as aging, hypertension, diabetes, smoking habits, and dyslipidemia.[19] In the present study, reduced GFR was found to be a significant risk factor for the development of stroke after adjustment for demographic factors, but not after adjustment for potential traditional CVD risk factors, which suggests that an accumulation of traditional CVD risk factors in individuals with reduced GFR increases the risk of stroke. In contrast, the risks of CVD, myocardial infarction, and all-cause death in individuals with reduced GFR were also attenuated, although still significant, after adjustment for traditional CVD risk factors. Reduced GFR has been shown to be associated with increased levels of novel CVD risk factors, such as inflammation, asymmetric dimethylarginine, oxidative stress, and thrombogenic factors.[19,20] Second, reduced GFR may be a marker of vascular disease; it is well recognized that renal arteriosclerosis and glomerular sclerosis are closely related to systemic atherosclerosis.[21]

In the present study, reduced GFR was associated with a high risk of stroke in men after adjustment for demographic factors but not after adjustment for potential confounding factors; however, this relationship was still observed in women even after adjustment for confounding factors. This sex difference may be a consequence of the effects of residual confounding factors, specifically, hypercoagulable states[22] or gonadal steroids,[23] in women. Furthermore, the lack of a significant association between reduced GFR and a high risk of myocardial infarction is probably due to the relatively small number of events.

Table 4. Effects of Kidney Function on Development of CVD

	Age- and Sex-Adjusted*		Multivariate-Adjusted†	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Overall				
CVD				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.41 (1.13–1.75)	0.002	1.24 (0.98–1.58)	0.07
GFR <60	2.26 (1.71–2.99)	<0.001	1.57 (1.14–2.15)	0.005
Stroke				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.40 (1.10–1.79)	0.007	1.24 (0.95–1.61)	0.11
GFR <60	2.06 (1.51–2.81)	<0.001	1.41 (0.99–2.00)	0.06
Myocardial infarction				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.32 (0.84–2.08)	0.22	1.26 (0.77–2.05)	0.35
GFR <60	3.35 (1.94–5.79)	<0.001	2.37 (1.29–4.34)	0.005
All-cause death				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.01 (0.88–1.15)	0.94	1.10 (0.96–1.27)	0.17
GFR <60	1.70 (1.44–2.00)	<0.001	1.65 (1.38–1.97)	<0.001
Men				
CVD				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.21 (0.93–1.58)	0.16	1.01 (0.75–1.35)	0.95
GFR <60	2.13 (1.45–3.11)	<0.001	1.47 (0.94–2.29)	0.09
Stroke				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.17 (0.86–1.57)	0.32	0.99 (0.71–1.38)	0.95
GFR <60	1.69 (1.08–2.65)	0.02	1.10 (0.64–1.89)	0.72
Myocardial infarction				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.25 (0.75–2.07)	0.39	1.05 (0.61–1.81)	0.85
GFR <60	3.95 (2.07–7.55)	<0.001	2.56 (1.24–5.27)	0.01
All-cause death				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	0.97 (0.83–1.14)	0.72	1.06 (0.90–1.25)	0.48
GFR <60	1.75 (1.42–2.16)	<0.001	1.73 (1.37–2.17)	<0.001
Women				
CVD				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.93 (1.28–2.92)	0.002	1.81 (1.17–2.79)	0.008
GFR <60	2.84 (1.79–4.52)	<0.001	1.97 (1.19–3.29)	0.009
Stroke				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	2.01 (1.28–3.14)	0.002	1.81 (1.14–2.89)	0.01
GFR <60	2.89 (1.75–4.79)	<0.001	1.98 (1.15–3.42)	0.01
Myocardial infarction				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.60 (0.55–4.60)	0.39	2.14 (0.63–7.24)	0.22
GFR <60	2.93 (0.93–9.23)	0.07	2.79 (0.74–10.56)	0.13
All-cause death				
GFR ≥90	1.00 (Reference)		1.00 (Reference)	
GFR 60–89	1.13 (0.87–1.47)	0.35	1.23 (0.94–1.62)	0.13
GFR <60	1.79 (1.34–2.38)	<0.001	1.68 (1.24–2.30)	<0.001

GFR was measured in mL · min^{-1} · 1.73 m^{-2}.

*Sex was removed from model for the analysis stratified by sex.

†Estimates were adjusted for age, sex, cohort, systolic blood pressure, diabetes, serum total cholesterol, body mass index, and current smoking status. Sex was removed from model for the analyses stratified by sex.

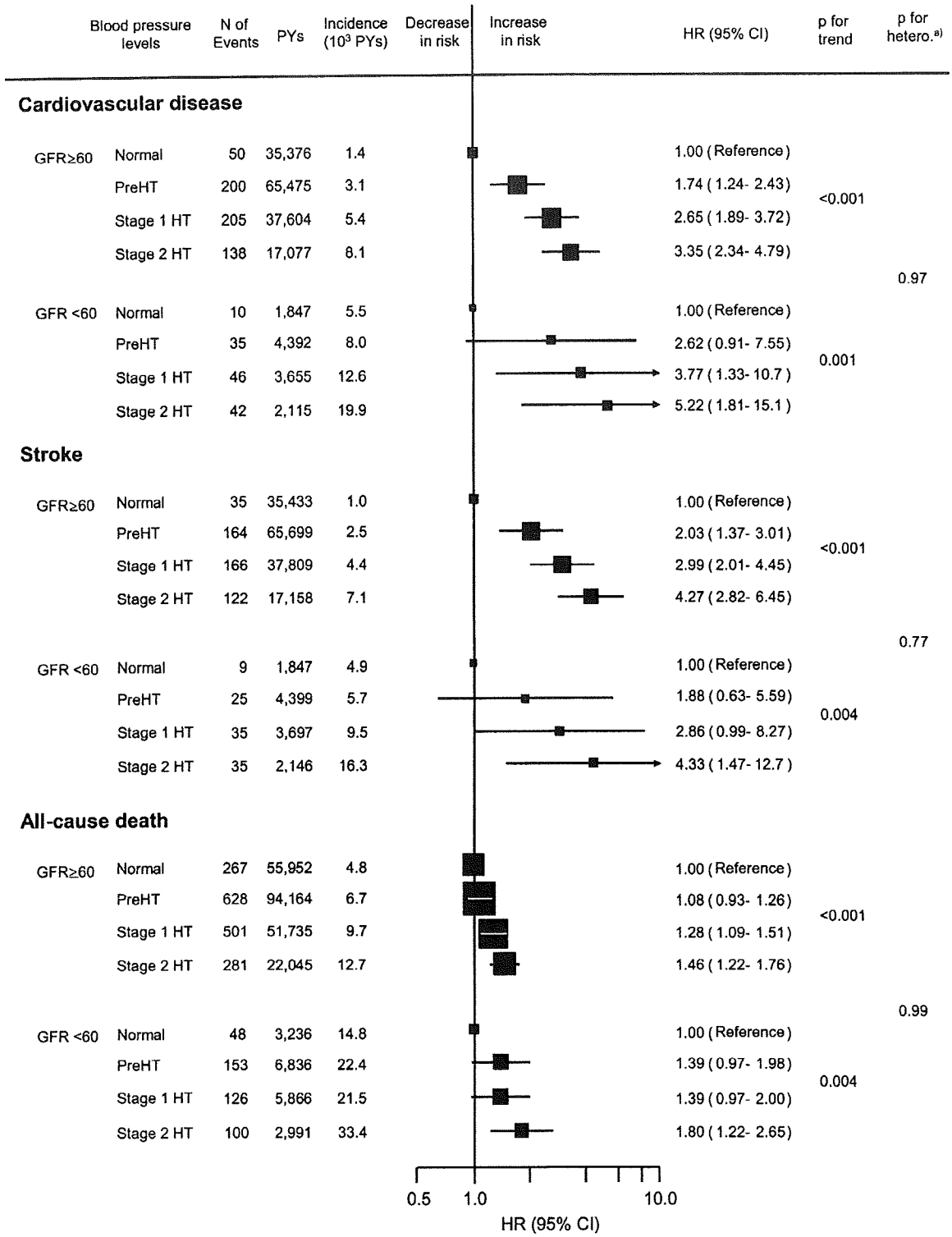

Figure. Effects of blood pressure levels on the development of CVD according to kidney function status. Estimates were adjusted for age, sex, cohort, diabetes, serum total cholesterol, body mass index, and current smoking status. Solid boxes represent estimates on the risk of outcomes for each blood pressure level. Areas of the boxes are proportional to the number of events. "*P* for trend" tested the log-linear relationship between blood pressure levels at baseline and the risk of outcomes by kidney function status. "*P* for hetero." tested the heterogeneity of the association of blood pressure levels with the risk of outcomes between kidney function status subgroups. HT indicates hypertension; PYs, person-years.

In the present study, we demonstrated a clear log-linear association between blood pressure levels and the risks of CVD, stroke, and all-cause death, regardless of kidney function status. These findings are consistent with the results of other studies conducted in the general population.[9,10] Recent publications of prospective cohort data suggest, however, that individuals with a reduced GFR and a systolic blood pressure below 120 mm Hg may be at increased risk of stroke or death.[12,13] Other post hoc analyses of trials conducted on individuals with coronary heart disease[24] and with diabetic nephropathy[25] suggest an increased risk of coronary events at the lower achieved blood pressures. In the present study, however, no evidence of an increased risk of myocardial infarction was observed at the lower blood pressure level. One possible explanation for the J-curve association observed in the previous studies may be the phenomenon of reverse causality,[26] in which extensive vascular disease or subclinical cardiac dysfunction is associated with lower blood pressure levels and reduced GFR and is associated independently with a relatively high risk of CVD, rather than with any adverse effects of low blood pressure itself.

Several limitations of the present study should be noted. First, the generalizability of our findings to some populations at high risk for CVD may be limited. The participants excluded from the analysis due to missing baseline examination data or event data were likely to have a higher cardiovascular risk, because they were older (mean 63 years), had higher blood pressure levels (mean 138/80 mm Hg), and had a greater prevalence of diabetes (8.7%) than the study population. This bias has the potential to alter our findings, which may therefore be conservative. Second, the present GFR estimates, which were made with a simplified prediction equation, may not be sufficiently correct, which possibly could lead to a certain number of misclassifications of estimated kidney function status. Such misclassifications would weaken the association found in the present study, biasing the results toward the null hypothesis. Third, we were unable to obtain information regarding the use of antihypertensive drugs, medication compliance, or blood pressure control during the follow-up period. The lack of this information may reduce the accuracy of our findings to some extent. Fourth, the applicability of the present results to populations with severe kidney dysfunction is limited, because very few of our subjects (0.1%) had a GFR <30 mL $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}. Moreover, the absence of data on proteinuria in the present study makes it impossible to assess the effects of the earliest stages of kidney disease on the risk of CVD. Finally, creatinine measurement was conducted locally rather than at a central laboratory, which introduces a certain amount of variability that may reduce the reliability of the results.

In conclusion, the present findings suggest that a reduced GFR is associated significantly with a high risk of CVD in the general Japanese population. Furthermore, we observed a continuous relationship between blood pressure levels at baseline and the risk of CVD, regardless of kidney function status. The optimization of blood pressure control in individuals with kidney dysfunction is therefore likely to substantially reduce the burden of CVD in the general population.

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Disclosures

None.

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CLINICAL PERSPECTIVE

There have been several studies reporting a strong association between reduced kidney function and cardiovascular risk. The findings, however, have been inconsistent in Asian populations, and there has been no attempt to date to review the evidence. Hence, we conducted an overview of individual participant data from Japanese community-based cohort studies to reliably assess the impact of reduced kidney function on cardiovascular risk in the general Japanese population. Our findings suggest a clear association between reduced kidney function and a 57% greater risk of cardiovascular disease in the Japanese population, as well as a log-linear relationship between blood pressure levels and cardiovascular risk in individuals with reduced kidney function. The optimization of blood pressure control in individuals with reduced kidney function is therefore likely to substantially reduce the burden of cardiovascular disease in the general population. Given that the prevalence of reduced kidney function is ≈10% in the general population, we believe that these novel findings are significant in the areas of clinical and public health.

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Original Article

Incidence of Hypertension in Individuals with Abdominal Obesity in a Rural Japanese Population: The Tanno and Sobetsu Study

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Although abdominal obesity (AO) assessed by waist circumference (WC) is an important component of the metabolic syndrome (MetS), the usefulness of AO as a predictor of hypertension (HT) is not known. In this study, we investigated the incidence of HT in residents of two rural communities in Japan. The subjects were 187 men and 209 women selected from 712 residents who had undergone medical examinations in the towns of Tanno and Sobetsu, Hokkaido, in 1994 and 2002. Participants with HT in 1994 were excluded. Participants with AO were determined according to the WC criteria in the Japanese definition of MetS (≥85 cm for men, ≥90 cm for women). The participants were divided into two groups: a non-AO group and an AO group. We compared the incidence of HT between the two groups and found a significantly higher incidence in the AO group. The results of logistic regression analysis showed that the relative risk of developing HT in individuals with AO was 2.33 (p=0.017; 95% confidence interval [CI], 1.17–4.63) and that the risk per 1-cm increase in WC from 1994 to 2002 was 1.06 (p=0.003; 95% CI, 1.02–1.10), both adjusted for several confounding factors. The results of this study suggest that, to prevent HT in Japanese, it is important to manage abdominal obesity and to monitor WC in individuals with or without abdominal obesity. (*Hypertens Res* 2008; 31: 1385–1390)

Key Words: **abdominal obesity, hypertension, waist circumference, metabolic syndrome, community-based survey**

Introduction

In 2005, the Japanese Society of Internal Medicine and eight related scientific societies jointly announced new Japanese diagnostic criteria for the metabolic syndrome (MetS) (*1*). The new criteria include abdominal obesity as defined by waist circumference (WC).

The Ministry of Health, Labour and Welfare started a new program of health examinations in Japan in April 2008 (Health Service Bureau, Ministry of Health, Labour and Welfare: Standard program of medical examination and health guidance (fixed version). http://www.mhlw.go.jp/bunya/kenkou/seikatsu/index.html [accessed February 7, 2008; in Japanese]). This program adopts the Japanese diagnostic criteria for MetS in order to identify individuals at high risk for lifestyle-related and atherosclerotic diseases. Although the WC criterion will also be used to identify high-risk individuals in the new system, the usefulness of the criterion's definition of abdominal obesity as a predictor of hypertension (HT) is not known.

In this study, we investigated the incidence of HT in resi-

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dents of two rural communities in Japan to determine the relationship between HT and abdominal obesity.

Methods

Of the 1,525 residents who were aged 30 years or older when they received medical examinations in the towns of Tanno and Sobetsu, Hokkaido, in 1994, 712 also underwent medical examinations in 2002. We excluded the following individuals from those 712 residents: 14 individuals without data on blood pressure (BP) or WC, 140 individuals who were defined as having HT (systolic BP [SBP]≥140 mmHg and/or diastolic BP [DBP]≥90 mmHg) without medication, 146 individuals who were on medication for HT, and 16 individuals who had received medical treatment for coronary heart disease or cerebral vascular disease. The remaining 396 individuals were participants in this analysis. We received written informed consent from all participants.

WC, body mass index (BMI), SBP, DBP, fasting plasma glucose (FPG), total cholesterol (T.chol), triglyceride (TG), and HDL cholesterol (HDL-C) were measured in each subject. Blood samples were collected early every morning when the subjects felt hungry, at least 10 h after they had last eaten.

Participants with abdominal obesity were determined according to the new Japanese diagnostic criteria for MetS (*1*). Abdominal obesity is defined as WC≥85 cm for men and ≥90 cm for women.

The participants were divided into two groups: an abdominal obesity (AO) group and a non-AO group. The measured items were compared between the groups. We also compared the incidence of HT between the groups for subjects who were newly determined as having HT (subjects with SBP≥140 mmHg and/or DBP≥90 mmHg or subjects who were on medication for HT) on the basis of the 2002 medical examination data. Moreover, we estimated and compared the relative risk of developing HT between the groups.

SPSS Ver.12.0J (SPSS, Chicago, USA) was used for statistical analysis. All numerical values are expressed as means±SD. The unpaired *t*-test and the χ^2 test were used for the examination of intergroup differences and for frequency comparison, respectively. Multiple logistic regression analysis was used to estimate the relative risk of HT. The relative risk was adjusted for age, sex, and high-normal BP (SBP≥130 mmHg and/or DBP≥85 mmHg) in 1994, smoking (yes/no), FPG, and T.chol. In the same model, we assessed the effect of an increase in WC on the development of HT by using ΔWC (=WC [cm] in 2002 − WC [cm] in 1994). The significance level in all analyses was set at $p<0.05$.

This study was approved by the Ethics Committee of Sapporo Medical University.

Results

Table 1 shows the characteristics of the subjects in the non-AO and AO groups in 1994. Age, percentage of men, BMI, SBP, DBP, TC, TG, and FPG were higher in the AO group than in the non-AO group. HDL-C was significantly lower in the AO group than in the non-AO group.

Table 1. Basal Characteristics in the Non-AO Group and the AO Group in 1994

	Non-AO group (n=312)	AO group (n=84)
Age	57.2±9.3	59.5±8.8*
Men/women	112/200	75/9#
BMI (kg/m^2)	22.4±2.3	25.5±3.0*
SBP (mmHg)	121.3±10.5	126.3±9.5*
DBP (mmHg)	73.5±6.9	77.4±6.6*
T.chol (mg/dL)	188.4±30.1	193.8±29.0*
TG (mg/dL)	110.1±68.5	159.8±82.1*
HDL-C (mg/dL)	58.1±13.8	48.6±12.2*
FPG (mg/dL)	92.1±11.7	105.1±27.8*

Age, percentage of men, BMI, SBP, DBP, TC, TG, and FPG were higher in the AO group than in the non-AO group. HDL-C was significantly lower in the AO group than in the non-AO group. *$p<0.05$, unpaired *t*-test, #$p<0.05$ χ^2 test. AO, abdominal obesity; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; T.chol, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; FPG, fasting plasma glucose.

In the 1994 data, there are significant positive correlations between WC and SBP and between WC and DBP in both men and women. There are also significant positive correlations between WC in 1994 and SBP in 2002 and between WC in 1994 and DBP in 2002 in both men and women (Fig. 1).

Figure 2 shows the percentage of HT in 2002 in each 1994 WC category. The higher the WC category, the higher the incidence of HT in both men and women. *p* for the trend was significant in both men and women.

The results of 10–11 years of follow-up are shown in Fig. 3. There were 312 individuals in the non-AO group and 84 in the AO group. Of the 312 individuals in the non-AO group, 177 remained in the non-AO category in 2002, but the remaining 79 individuals were changed to the AO category in 2002. Sixty-nine of the 84 individuals in the AO group remained in the AO category in 2002, but the remaining 15 individuals changed to the non-AO category. We divided the participants into these four groups (non-AO to non-AO, non-AO to AO, AO to non-AO and AO to AO) and compared the incidence of HT among them.

Figure 4 shows the incidences of HT in the four groups. The incidence was higher in the non-AO to AO group than in the non-AO to non-AO group (45.6% *vs.* 31.8%, $p=0.019$). It was also higher in the AO to AO group than in the AO to non-AO group (58.0% *vs.* 26.7%, $p=0.027$). There was no significant difference in the incidence of HT between the non-AO to non-AO group and the AO to non-AO group ($p=0.782$), or between the non-AO to AO group and the AO

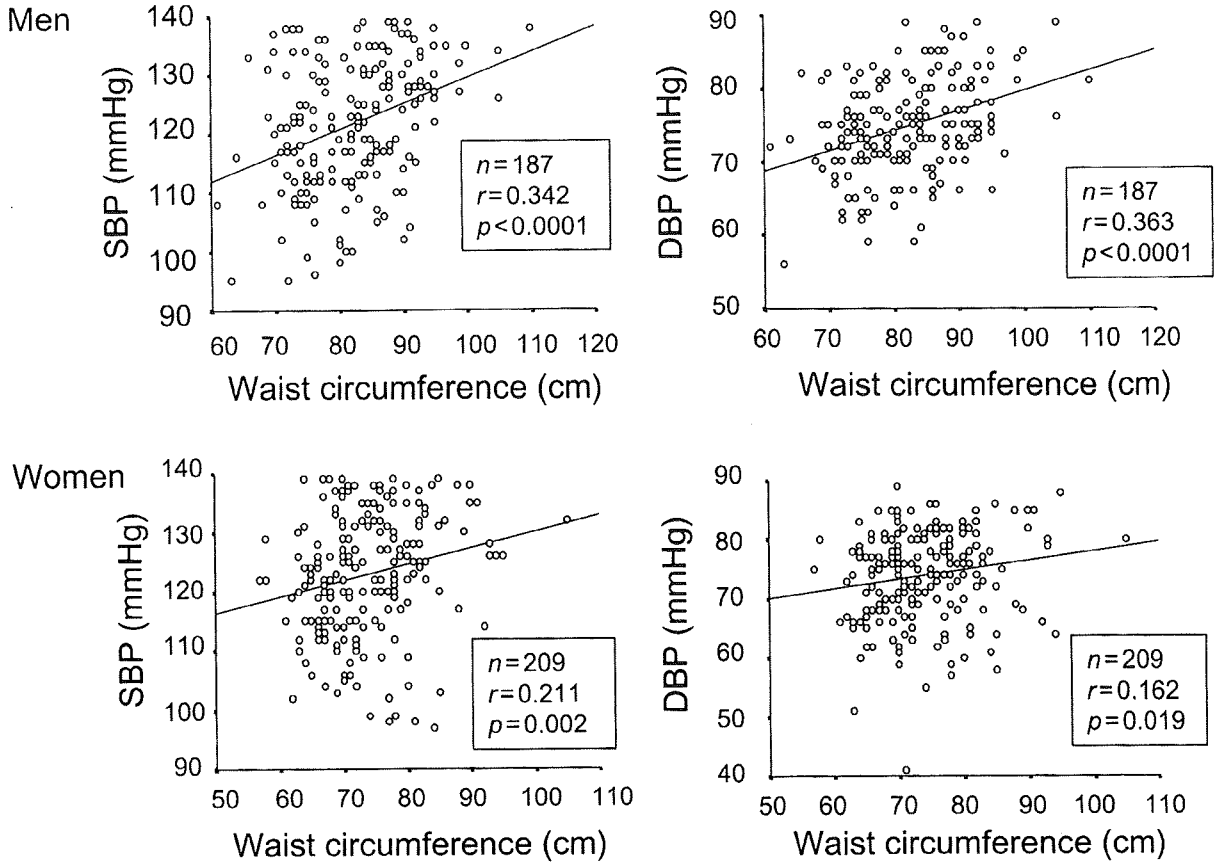

Fig. 1. *Correlations of waist circumference in 1994 with SBP and DBP in 2002. Upper: for men; lower: for women. Graphs on the left are relationships between waist circumference and SBP, and graphs on the right are relationships between waist circumference and DBP. Waist circumference shows significant positive correlations with SBP and DBP in both men and women.*

to AO group (p=0.142).

Table 2 shows the results of multiple logistic regression analysis. The relative risk of developing HT in individuals with AO was 2.33 (p=0.016; 95% confidence interval [CI], 1.17–4.63), and the risk per 1-cm increase in WC from 1994 to 2002 was 1.06 (p=0.003; 95% CI, 1.02–1.10), both adjusted for age, sex, high-normal BP in 1994, smoking (yes/no), FPG, and T.chol. When we additionally adjusted for BMI≥25 (yes/no) in the logistic regression model, the significance of AO disappeared (data not shown).

Discussion

The main findings of this study are 1) the incidence of HT was higher in the AO group than in the non-AO group, 2) increased WC, which may indicate the accumulation of visceral fat, increased the incidence of HT, 3) AO assessed by WC was significantly related to the development of HT (relative risk of HT: 2.33), 4) increasing WC was significantly related to the development of HT after adjustment for 1994 AO.

The Japanese Society of Internal Medicine and eight related scientific societies in Japan jointly announced new Japanese diagnostic criteria for MetS in April 2005 (*1*). According to the new criteria, the definition of MetS must include abdominal obesity, because the accumulation of visceral fat in individuals with MetS is considered to be important for the mechanism underlying the accumulation of risk factors for cardiovascular disease. Accumulation of visceral fat leads to insulin resistance and disorder of adipocytokines, and these factors in turn lead to high BP *via* mechanisms such as an increase in reabsorption of sodium in the renal tubule, hyperactivity of the sympathetic nervous system, proliferation of vascular smooth muscle cells and development of atherosclerosis. The results of this study show that abdominal obesity is significantly related to the development of HT and that an increase in WC, which may indicate the accumulation of visceral fat, is a risk factor for the development of HT.

It is well known that obesity is significantly related to HT, and many reports show relationships between BP levels and

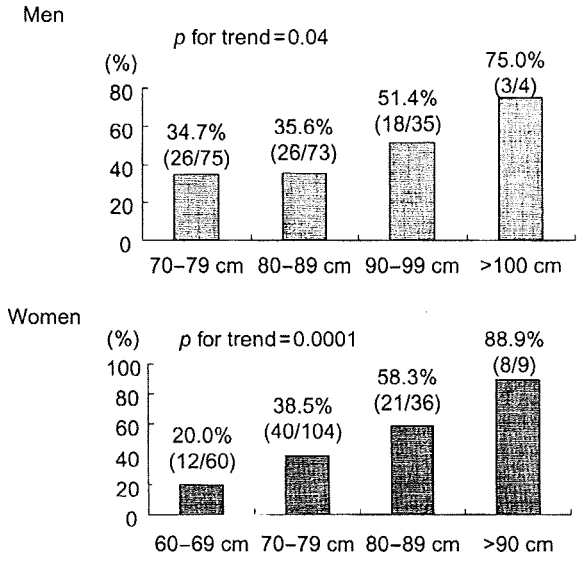

Fig. 2. *Percentage of hypertension (HT) in 2002 in each 1994 waist circumference (WC) category. The higher the WC category, the higher the incidence of HT in both men and women. p for the trend is significant in both men and women.*

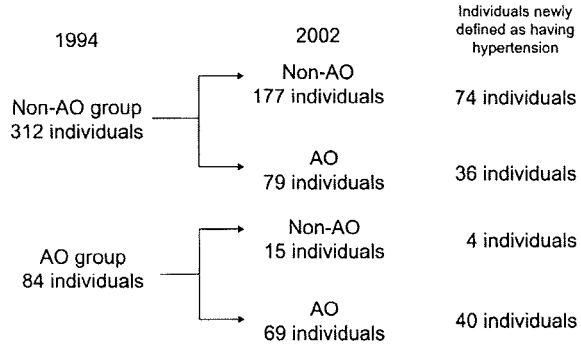

Fig. 3. *Follow-up results. There were 312 individuals in the non-AO group and 84 in the AO group. Of the 312 individuals in the non-AO group, 177 remained in the non-AO category in 2002, but the remaining 79 individuals changed to the AO category in 2002. Sixty-nine of the 84 individuals in the AO group remained in the AO category in 2002, but the remaining 15 individuals changed to the non-AO category in 2002. AO, abdominal obesity. Hypertension (HT): SBP≥140 mmHg and/or DBP≥90 mmHg and/or receiving medication for HT.*

various anthropometric parameters (*2–12*). We also have reported a strong correlation between obesity assessed by BMI and the development of HT according to our cohort data (*13*), as well as a correlation between ultrasound-assessed visceral fat accumulation and BP levels (*14*).

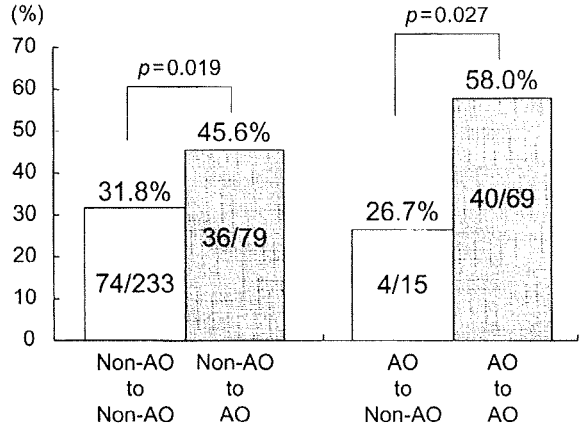

Fig. 4. *Incidences of hypertension (HT) in participants in the four groups. The incidence of HT is higher in the non-AO to AO group than in the non-AO to non-AO group (45.6% vs. 31.8%, p=0.019). The incidence of HT is also higher in the AO to AO group than in the AO to non-AO group (58.0% vs. 26.7%, p=0.027). There is no significant difference in the incidence of HT between the non-AO to non-AO group and the AO to non-AO group (p=0.782), or between the non-AO to AO group and the AO to AO group (p=0.142). AO, abdominal obesity.*

It is also known that a reduction in body weight leads to a decrease in BP levels (*15–20*). In the present study, no significant difference was found between the incidences of HT in the non-AO to non-AO group and the AO to non-AO group. Although this study was not interventional, the results suggest that weight reduction is effective for the prevention of HT. These results suggest that, to prevent hypertension, lifestyle modification is important for individuals with AO as well as for individuals with high-normal BP.

There are grounds for controversy about the current Japanese cutoff points for abdominal obesity (85 cm for men and 90 cm for women). The International Diabetes Federation (IDF) recommends that Asian cutoff points (90 cm for men and 80 cm for women) should be used for diagnosing MetS in Japanese people (The IDF consensus worldwide definition of the metabolic syndrome. http://www.idf.org/webdata/docs/MetS_def_update2006.pdf [accessed February 7, 2008]). In the present study, the prevalence of abdominal obesity was significantly lower in women than in men. According to Fig. 1, the incidence of HT in women increased continuously with the increase of WC. We tried to plot the receiver operator characteristic (ROC) curves for WC to predict the development of HT in men and women separately. The areas under the curves were 0.560 for men and 0.684 for women. According to the ROC curves, the cutoff levels yielding the maximal sensitivity plus specificity for predicting the development of

Table 2. Relative Risks for Hypertension (HT) in Individuals with Abdominal Obesity (AO)

	Wald	*p*	Relative risk	95% CI
Age	11.28	0.001	1.05	1.02–1.08
Sex	1.07	0.301	1.47	0.71–3.02
High normal category in 1994 (yes/no)*	54.42	<0.0001	6.33	3.84–10.43
Smoking	0.78	0.379	1.34	0.70–2.56
FPG	0.22	0.64	0.99	0.98–1.01
T.chol	0.68	0.41	0.99	0.98–1.01
Abdominal obesity in 1994 (yes/no)#	5.78	0.016	2.33	1.17–4.63
ΔWaist circumference (cm)§	8.59	0.003	1.06	1.02–1.10

The relative risk for development of HT in individuals with AO was 2.33 (p=0.016; 95% CI, 1.17–4.63) and the risk for HT in individuals with increase in waist circumference of 1 cm from 1994 to 2002 was 1.06 (p=0.003; 95% CI, 1.02–1.10), both adjusted for age, sex, high normal category of blood pressure in 1994 (yes/no), smoking, FPG and T.chol. *High normal category of blood pressure, SBP≥130 mmHg and/or DBP≥85 mmHg. #Abdominal obesity, waist circumference ≥85cm for men and ≥90cm for women. §ΔWaist circumference=(waist circumference in 2002) − (waist circumference in 1994). CI, confidence interval; FPG, fasting plasma glucose; T.chol, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

HT were 84 cm for men and 74 cm for women. These results suggest that the current cutoff point in men is acceptable but that a lower cutoff point is appropriate to identify women at high risk for HT. Further studies are needed to establish appropriate cutoff points of WC in the Japanese population.

Despite this controversy, in the present study we used the current Japanese WC cutoff points because the Ministry of Health, Labour and Welfare started a new program of health examinations in Japan in April 2008 (Health Service Bureau, Ministry of Health, Labour and Welfare: Standard program of medical examination and health guidance [fixed version]). The Japanese WC criterion is used to identify high-risk individuals in the new program. Therefore, an accumulation of evidence using the current WC cutoff points is important for medical staff who will be involved in the new health examination program, such as doctors in clinics, public health nurses, and senior nutritionists in local governments. The results of this study showed the usefulness of the current WC cutoff points for identifying individuals at high risk for HT. The results also indicated the possibility that many individuals, especially women, who are at high risk for HT will be missed if attention is given to only abdominal obesity defined by the current cutoff points.

In conclusion, our results suggest that, to prevent HT in Japanese, it is important to manage abdominal obesity and to monitor waist circumference in individuals with or without abdominal obesity.

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Pioglitazone treatment stimulates circulating CD34-positive cells in type 2 diabetes patients

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ABSTRACT

Circulating bone marrow derived immature cells, including CD34-positive ($CD34^+$) cells, contribute to maintenance of the vasculature, not only as a pool of endothelial progenitor cells (EPCs), but also as a source of growth/angiogenesis factor. We hypothesized that the thiazolidineone compound pioglitazone could stimulate the circulating $CD34^+$ cells in diabetic patients. Thirty-four patients with type 2 diabetes received 15–30 mg pioglitazone for 24 weeks. The number of circulating $CD34^+$ cells significantly increased at 12 and continued this effect for 24 weeks (1.08 ± 0.39, 1.34 ± 0.34 and 1.32 ± 0.28 cells/μl at 0, 12 and 24 weeks, respectively). The change of $CD34^+$ cell levels ($\Delta CD34^+$ cells) between 0 and 12 weeks was significantly correlated with the change of high sensitive C reactive protein levels (Δhs-CRP) and change in adiponectin levels (Δadiponectin) ($r = -0.412$, $r = 0.359$, respectively). Our study demonstrated that pioglitazone treatment increased circulating $CD34^+$ cells, suggesting that this effect may at least partly contribute to the anti-atherosclerotic action of pioglitazone.

1. Introduction

Endothelial dysfunction plays a pivotal role in the progression of the atherosclerosis. Circulating EPCs contribute to the maintenance of vascular homeostasis and repair. They also play an important role in the maintenance of vascular endothelial function [1,2]. In diabetic patients, both a decrease in number and function of circulating EPCs are reported, suggesting that circulating EPCs participate in diabetic vascular complications [3].

Recent studies have identified circulating bone marrow derived immature cells, including $CD34^+$ cells, contribute to maintenance of the vasculature, not only as a pool of EPCs, but also as a source of growth/angiogenesis factor [4]. In fact, one recent report indicates that circulating $CD34^+$ cells are more strongly correlated with cardiovascular risk than circulating $CD34^+$/kinase insert domain receptor $(KDR)^+$ cells generally regarded as EPCs [5]. We have also reported that circulating $CD34^+$ cell levels are associated with cerebral infarction [6]. These findings indicate that persistent stimulation of $CD34^+$ cells may be a useful method to repair endothelial injury and microcirculation, and to suppress the progression of atherosclerotic disease at least theoretically. Recent experimental and clinical studies demonstrate that thiazolidinediones, peroxisome-proliferator-activated receptor γ (PPARγ) agonists, has the effects on the prevention of atherosclerosis including the maintenance of vascular endothelial function [7–9]. Therefore, we hypothesized that the thiazolidineone

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compound pioglitazone could stimulate the circulating CD34+ cells in diabetic patients.

2. Methods

2.1. Study subjects

All subjects gave a written informed consent. The study was approved by the local ethics committee. Thirty-four patients with type 2 diabetes (age 60 ± 10, M/F; 18/16, HbA1c 9.3 ± 1.4%) received 15 or 30 mg pioglitazone for 24 weeks (15 mg; 31 patients, 30 mg; 3 patients). Other medications for diabetes, hypertension and hyperlipidemia were unchanged throughout the study. Insulin was given to 9 patients. Sulfonylurea was given to 15 patients. Biguanide was given to 21 patients. Alpha glucosidase inhibitor was given to 10 patients. Angiotensin converting enzyme inhibitor and/or angiotensin receptor blocker was given to 21 patients. Statin was given to 18 patients. Sixteen patients afflicted with cardiovascular diseases (CVD). Eighteen patients afflicted with nephropathy, 14 patients afflicted with retinopathy, and 15 patients afflicted with neuropathy.

2.2. Measurement of CD34+ cells

Three milliliters of heparinized peripheral blood were obtained after 12-h fasting and measured CD34+ cells. The precise number of circulating CD34+ cells was quantified as we described previously [10]. We evaluated circulating CD34+ cells with Stem-Kit™ (BeckmanCoulter, Marseille, France) according to manufacturers' protocols. These protocols are based on International Society of Hematotherapy and Graft Engineering (ISHAGE) Guidelines [11], and are frequently used for quantification of CD34+ cells mobilized into peripheral blood. To increase the reproducibility of CD34+ cell counts, the protocol of Stem-Kit was modified as follows: the blood sample volume, antibodies and lysing solution were doubled. After adding 30 μl of internal control (Stem count: BeckmanCoulter), samples were centrifuged for 5 min at 450 × *g* and 3860 μl of supernatant was removed carefully with a pipet. Samples were analyzed by Coulter CYTOMICS™ FC500 & XL-system II software (BeckmanCoulter) for 6 min each.

2.3. Other laboratory analysis

Blood samples were taken after 12-h fasting to measure adiponectin and, high sensitive C-reactive protein (hs-CRP) concentrations. Serum adiponectin and concentration was measured by enzyme-linked immunosorbent assay (SRL, Tokyo, Japan). Serum hs-CRP concentration was measured by latex nephelometry method (SRL, Tokyo, Japan). We also measured HbA1c, total cholesterol, HDL cholesterol and triglyceride levels.

2.4. Statistical analysis

Data was expressed using the mean ± S.D. The Student's t-test was used to compare parameter changes over time. The strength of correlation between variables was performed using Spearmann's correlation coefficient.

3. Results

3.1. Effects of pioglitazone on glucose and lipid metabolism

Treatment of pioglitazone significantly decreased HBA1c levels (9.3 ± 1.4, 7.4 ± 1.2 and 7.5 ± 1.7% at 0, 12 and 24 weeks, respectively). Systemic blood pressure levels did not change throughout the study period. BMI did not change throughout the study period (26.8 ± 3.2, 27.5 ± 3.0 and 27.9 ± 3.3 at 0, 12 and 24 weeks, respectively). Total cholesterol and triglyceride levels did not change throughout the study, whereas HDL cholesterol levels significantly increased at 12 and 24 weeks (1.08 ± 0.39, 1.34 ± 0.34 and 1.32 ± 0.28 mmol/l at 0, 12 and 24 weeks, respectively).

3.2. Effects of pioglitazone on adiponectin and inflammatory marker

The inflammatory marker, hs-CRP significantly decreased at 12 and 24 weeks (1518 ± 2350, 840 ± 975, and 838 ± 904 ng/ml at 0, 12, and 24 weeks, respectively). Serum adiponectin levels significantly increased at 12 and 24 weeks (5.0 ± 2.2, 13.5 ± 6.7 and 13.8 ± 8.4 μg/ml at 0, 12 and 24 weeks, respectively). The change in adiponectin levels between 0 and 12 weeks (Δadiponectin) of 30 mg pioglitazone was significantly larger than 15 mg of pioglitazone (15 mg; 7.9 ± 4.7 vs. 30 mg; 19.6 ± 2.5, $p < 0.05$), whereas there was no significant difference in the change in hs-CRP levels (Δhs-CRP) between 15 mg and 30 mg of pioglitazone (15 mg; 267 ± 322 vs. 30 mg; 480 ± 1883).

3.3. Effects of pioglitazone on circulating CD34+ cell level

The number of circulating CD34+ cells significantly increased at 12 and 24 weeks (0.90 ± 0.48, 1.10 ± 0.50, and 1.10 ± 0.57 cells/μl at 0, 12, and 24 weeks, respectively (Fig. 1). This effect was found in both patients with CVD and without CVD (patients with CVD; 0.81 ± 0.51, 1.05 ± 0.46 and 1.04 ± 0.50 cells/μl at 0, 12 and 24 weeks, respectively, $n = 16$, patients without CVD; 0.98 ± 0.41,

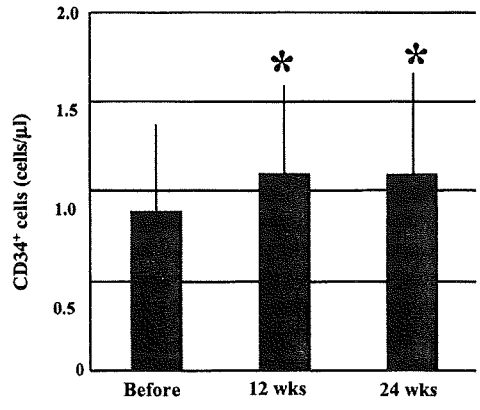

Fig. 1 – CD34+ cell level at 0, 12 and 24 weeks, *$p < 0.05$ vs. 0 week.

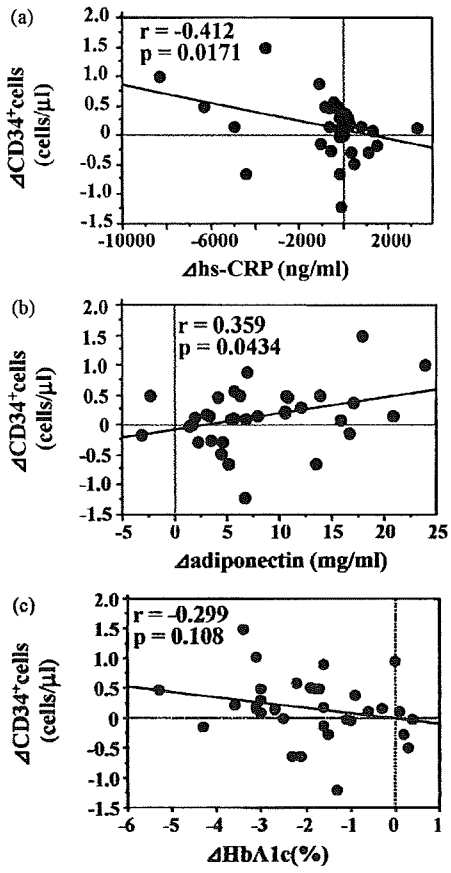

Fig. 2 – Correlation between ΔCD34⁺ cells and ΔhsCRP ($r = -0.412$, $p = 0.017$) (a), correlation between ΔCD34⁺ cells and Δadiponectin ($r = 0.359$, $p = 0.043$) (b), and correlation between ΔCD34⁺ cells and ΔHbA1c ($r = -0.299$, $p = 0.108$) (c).

1.15 ± 0.57 and 1.15 ± 0.65 cells/μl at 0, 12 and 24 weeks, respectively, $n = 18$). There was no significant difference in the change in CD34⁺ cell level (ΔCD34⁺ cells) between 15 mg and 30 mg of pioglitazone (15 mg; 0.07 ± 1.01 vs. 30 mg; 0.14 ± 0.32).

3.4. Factors involved in the stimulation of CD34⁺ cells

We next investigated which factors were correlated with the stimulation of CD34⁺ cells. ΔCD34⁺ cells were significantly correlated with Δhs-CRP in univariate analysis ($r = -0.412$, $p = 0.017$) (Fig. 2a). Further, Δadiponectin correlated with Δ CD34⁺ cells ($r = 0.359$, $p = 0.043$) (Fig. 2b). On the other hand, change in HbA1c levels (ΔHbA1c) ($r = -0.299$, $p = 0.108$) (Fig. 2c), change in HDL-C levels (ΔHDL-C) ($r = 0.253$, $p = 0.168$) and change in triglyceride levels (Δtriglycerides) ($r = 0.0072$, $p = 0.969$), were not significantly correlated to Δ CD34⁺ cells.

4. Discussion

Accumulating evidence shows that PPARγ agonists have anti-atherosclerotic actions other than their blood glucose level reduction effects [7,9]. One recent report showed that pioglitazone treatment could stimulate circulating EPCs in patients with coronary artery disease and normal glucose tolerance [12]. In this study, we demonstrated that pioglitazone treatment also increased circulating CD34⁺ cells and this effect continued for 24 weeks in type 2 diabetic patients. We studied the effects of pioglitazone on the stimulation of CD34⁺ cells but not CD34⁺/KDR⁺ cells regarded as EPCs. However, these circulating CD34⁺ cells have the capacity to participate in neovascularization of ischemic tissue. Indeed, their administration enhances the repair of ischemic tissue in ischemic stroke model [13] and improves myocardial circulation in myocardial infarction model [14]. Clinically, circulating CD34⁺ cell levels were reported to be correlated with cerebral blood flow in hypoperfusion area [6] and formation of collateral vessels in stroke patients [15]. These reports suggest that CD34⁺ cells may play a role in the maintenance of microcirculation. One recent clinical trial, PROactive Study, demonstrated that pioglitazone treatment could prevent cardiovascular events including stroke in type 2 diabetic patients [16]. Taken together, it is suggested that the stimulation of CD34⁺ cells may partly contribute to the preventive effects of pioglitazone on cardiovascular diseases. Our study also demonstrated that pioglitazone treatment increased circulating CD34⁺ cells in type 2 diabetic patients irrespective of with or without CVD, suggesting that pioglitazone treatment may be useful for primary prevention as well as secondary prevention of diabetic macroangiopathy.

It has been reported that the number of circulating EPCs is inversely correlated with HbA1c levels [3]. Since pioglitazone treatment significantly decreased HbA1c levels and this study did not have control group, we could not exclude the possibility that the stimulation of CD34⁺ cells was associated with the improvement of glycemic control. However, the results of this study suggest that pioglitazone may be capable of stimulating circulating CD34⁺ cells independently of glycemic control because ΔCD34⁺ cells was not positively correlated with ΔHbA1c at levels that achieved statistical significance.

Adipocyte derived factors and inflammation participate in atherogenesis of type 2 diabetic patients. Accumulating evidence show that adiponectin, one of adipocyte derived factors, has anti-atherogenic properties, and hypoadionectinemia was reported to be associated with endothelial dysfunction [17]. Pioglitazone treatment decreased hs-CRP levels and increased serum adiponectin levels in metabolic syndrome subjects [8], suggesting that these effects contribute to the anti-atherosclerotic action of pioglitazone. In this study, we also demonstrated that pioglitazone treatment decreased hs-CRP levels and increased serum adiponectin levels in type 2 diabetes patients. Interestingly, ΔCD34⁺ cells were significantly correlated with Δhs-CRP and Δadiponectin. An in vitro study showed that CRP impaired EPC migration and function [18]. In clinical study, it has been reported that circulating EPCs were inversely correlated to serum interleukin 6 levels [19]. These reports suggested that chronic inflammation may be involved in the regulation of EPCs. One recent clinical study showed that circulating EPCs were positively correlated to serum adiponectin levels in patients with coronary artery disease [20]. Another report showed that

adiponectin treatment increased EPC number and migration [12]. Taken together, it is suggested that the inhibitory effects on chronic inflammation and the effect on adiponectin regulation of pioglitazone may be directly or indirectly involved in the increase of $CD34^+$ cells. However, further study is necessary to delineate this hypothesis.

In conclusion, our study demonstrated that pioglitazone treatment increased circulating $CD34^+$ cells, suggesting that this effect may at least partly contribute to the anti-atherosclerotic action of pioglitazone.

Conflict of interest

There are no conflicts of interest.

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Mutation Site Dependent Variability of Cardiac Events in Japanese LQT2 Form of Congenital Long-QT Syndrome

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Background In the LQT2 form of long QT syndrome (LQTS), mutation sites are reported to correlate with clinical phenotypes in Caucasians, but the relationship in Asian patients remains unknown. The present study was designed to determine whether the location of *KCNH2* mutations would influence the arrhythmic risk in LQT2 patients.

Methods and Results In 118 genetically-confirmed LQT2 patients (69 families, 62 *KCNH2* mutations), the ECG parameters, Schwartz scores, and the incidence of cardiac events, defined as syncope, aborted cardiac arrest, and sudden cardiac death, were evaluated. To examine the effect of mutation sites, the participants were divided accordingly: pore (n=56) and non-pore (n=62) groups. The corrected QT_{end} interval was significantly greater in the pore than in the non-pore group (QTc; 522±63 ms vs 490±49 ms, p=0.002). In this study, the clinical course of each of the probands did not differ according to the mutation sites, whereas non-probands carrying the pore site mutation experienced their first cardiac events at significantly younger age than those with the non-pore site mutation (log-rank, p=0.0005).

Conclusions In a Japanese LQT2 cohort, family members with the pore site mutation were at higher arrhythmic risk than those with the non-pore site mutation. (*Circ J* 2008; 72: 694–699)

Key Words: Arrhythmia; Long-QT syndrome; QTc interval; Risk factors; Torsade de pointes

The long QT syndrome (LQTS) is an inherited arrhythmogenic disease of the structurally normal heart that may cause sudden death. LQTS is characterized by an abnormality in myocardial repolarization that leads to prolongation of the QT interval, morphological changes in T waves and torsades-de-pointes (Tdp) type of ventricular tachycardia on surface ECGs[1,2] To date, 8 distinct genes responsible for LQTS have been identified, including those of Andersen (LQT7) and Timothy (LQT8) syndromes: on chromosome 11q15.5 (*KCNQ1*; LQT1), 7q35–36 (*KCNH2*; LQT2), 3p21 (*SCN5A*; LQT3), 4q25–27 (*ANKB*; LQT4), 21q22 (*KCNE1*; LQT5), 21q22 (*KCNE2*; LQT6), 17q23 (*KCNJ2*; LQT7) and 12p13.3 (*CACN1C*; LQT8)[3–10]

Moss et al[11] extensively examined the relationships between the site of mutation and clinical phenotype in approximately 44 different LQT2-related *KCNH2* mutations. They reported that subjects with causative mutations in the pore region (n=38, amino acid residues 550 through 650) had more severe clinical manifestations and experienced a higher frequency (74% vs 35%; p<0.001) of arrhythmia-related cardiac events occurring at younger age than did subjects with non-pore mutations (n=166).

In LQT1, based on the United States portion of the International LQTS Registry (n=425), the Netherlands' LQTS Registry (n=93), and the Japanese LQTS Registry (n=82), 600 patients with *KCNQ1* mutations were classified into 2 groups of patients with transmembrane and C-terminus mutations and their clinical phenotypes were examined[12] That study found that patients with transmembrane mutations were at increasing risk for cardiac events (hazard ratio, 2.06; p<0.001). Shimizu et al also studied the mutation site-dependent differences in 95 LQT1 patients from a multicenter Japanese population and also found that patients with transmembrane mutations were at higher risk of cardiac events and had longer QTc and $T_{peak\text{-}end}$ intervals[13]

In Japanese LQT2 patients, mutation site dependency is unclear, although this has been reported in Caucasian patients. Therefore, in the present study we aimed to compare the genotype and phenotype relationship, according to the classification adopted by Moss et al[11] in 118 Japanese LQT2 patients who were genetically identified in the 3 genetic centers in Japan and had no other mutations in LQTS-related genes (except LQT4 and 8).

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Table 1 ***KCNH2*** **Mutations by Location, Amino-Acid Coding, Type of Mutation, and Reported Functional Effects**

	No. of families	No. of subjects	Position	Exon	Type of mutation	Functional effect in expression studies
Pore regions						
A561T	1	1	S5	7	Missense	Trafficking defect (22)
A561V	1	1	S5	7	Missense	Dominant negative (23)
W563C*	1	1	Pore	7	Missense	
W563G*	1	2	Pore	7	Missense	
C566F*	1	1	Pore	7	Missense	
G572S	2	4	Pore	7	Missense	
M574V*	1	3	Pore	7	Missense	
R582L	1	2	Pore	7	Missense	
R582C	1	1	Pore	7	Missense	
G584C*	1	2	Pore	7	Missense	
G590V*	1	3	Pore	7	Missense	
I593V*	1	1	Pore	7	missense	
K595N*	1	2	Pore	7	Missense	
K595E*	1	1	Pore	7	Missense	
G601S	2	5	Pore	7	Missense	Trafficking defect (22, 24)
G604S	2	2	Pore	7	Missense	
S606P*	1	1	Pore	7	Missense	
T613M	2	3	Pore	7	Missense	Dominant negative (25)
A614V	4	6	Pore	7	Missense	Dominant negative (26)
T623I	1	1	Pore	7	Missense	Trafficking defect (22)
G628S	1	2	Pore	7	Missense	Trafficking defect (22)
N629K	1	1	Pore	7	Missense	Dominant negative (27)
N633S	1	1	Pore	7	Missense	
K638del	1	1	S6	7	Deletion	
F640del*	1	1	S6	7	Deletion	
S641F	1	3	S6	7	Missense	
V644F	1	4	S6	7	Missense	
Subtotal	34	56				
Non-pore regions						
N-terminal regions						
V41A*	1	1	N-term	2	Missense	
Y43D*	1	3	N-term	2	Missense	
E50fs+10X*	1	1	N-term	2	Deletion/frameshift	
G53S*	1	1	N-term	2	Missense	
82-84insIAQ	1	1	N-term	2	Insertion	
F106L*	1	1	N-term	3	Missense	
D111V*	1	1	N-term	3	Missense	
V115M*	1	1	N-term	3	Missense	
P151fs+179X	1	1	N-term	3	Insertion/frameshift	
G187-A190del*	1	3	N-term	4	Deletion	
R312-S318del*	1	2	N-term	5	Deletion	
S320L	1	1	N-term	5	Missense	
P334L	1	1	N-term	5	Missense	
K364fs+3X*	1	3	N-term	5	Insertion/deletion/frameshift	
K386fs+3X*	1	4	N-term	5	Insertion/frameshift	
Transmembrane domains other than pore regions						
Q391X	1	2	S1	6	Nonsense	
F471fs+50X*	1	1	S1-S2	6	Deletion/frameshift	
I489F*	1	1	S1-S2	6	Missense	
A490T	1	1	S1-S2	6	Missense	Current density↓ (28)
H492Y*	1	2	S1-S2	6	Missense	
W497X*	1	3	S3	6	Nonsense	
D501N	1	1	S3	6	Missense	
R534C	1	2	S4	7	Missense	Trafficking defect (22)
C-terminal region						
Q738X*	1	2	C-term	9	Nonsense	
G745-G749del, Fins/fs+56X*	1	1	C-term	9	Insertion/deletion/frameshift	
R752W	1	2	C-term	9	Missense	Trafficking defect (22)
S818L	1	1	C-term	10	Missense	Reduced I_{Kr} current (29)
P846T*	1	1	C-term	10	Missense	
W853fs+14X*	1	2	C-term	10	Deletion/frameshift	
R863X	1	2	C-term	10	Nonsense	
L911fs+6X*	1	3	C-term	12	Deletion/frameshift	
R912fs+63X*	1	2	C-term	12	Insertion/frameshift	
S1029fs+23X*	1	3	C-term	13	Deletion/frameshift	
P1034fs+23X*	1	3	C-term	13	Insertion/frameshift	
A1144T*	1	2	C-term	15	Missense	
Subtotal	35	62				

**Novel mutation.*
del, deletion; ins, insertion; fs, first amino acid affected by a frameshift (number after fs is number of amino acids before termination); term, terminus.

Table 2 Clinical Characteristics of Pore and Non-Pore Mutations

	Pore (n=56)	Non-pore (n=62)	p value
Demographics			
Female gender (%)	33 (59%)	42 (68%)	0.344
Proband (%)	33 (59%)	34 (55%)	0.712
Age (years) at baseline ECG (range)	31±18 (7–74)	31±16 (2–71)	0.920
Age (years) at first event (range)	16±10 (5–48)	20±13 (5–71)	0.203
Diagnosis			
Schwartz score	5.3±1.6	4.5±1.8	0.017
Schwartz score ≥4 (%)	47 (84%)	41 (66%)	0.034
ECG measurements			
Heart rate (beats/min)	65±13	64±15	0.537
RR (ms)	953±188	975±186	0.510
QT_{end} (ms)	505±79	482±69	0.089
QT_{peak} (ms)	377±67	382±65	0.650
$T_{peak-end}$ (ms)	129±55	99±41	0.001
Corrected QT_{end} (ms)	522±63	490±49	0.002
Corrected QT_{peak} (ms)	389±62	388±47	0.927
Corrected $T_{peak-end}$ (ms)	134±52	101±42	<0.001
Torsade de pointes (%)	17 (30%)	18 (29%)	1.000
T-wave alternans (%)	7 (13%)	4 (7%)	0.346
Notched T wave (%)	43 (77%)	32 (52%)	0.007
Cardiac events			
All cardiac events (%)	38 (68%)	32 (52%)	0.092
Syncope (%)	36 (64%)	32 (52%)	0.194
Aborted cardiac arrest/SCD (%)	6 (11%)	2 (3%)	0.145
Therapy			
β-blocker therapy	26 (53%)	21 (36%)	0.117
Pacemaker (%)	1 (2%)	0	1.000
Sympathectomy (%)	0	0	1.000
Defibrillator (%)	1(2%)	2 (3%)	1.000

Data are mean value±SD or number (%) of subjects.
ECG, electrocardiography; SCD, sudden cardiac death.

Methods

Study Population

The study population consisted of 118 patients from 69 unrelated Japanese LQT2 families enrolled from 3 institutes in Japan: National Cardiovascular Center, Kyoto University Graduate School of Medicine and Shiga University of Medical Science. The *KCNH2* mutations were confirmed in all patients by using standard genetic tests[14–17] Screening for mutations in *KCNQ1*, *SCN5A*, *KCNE1*, *KCNE2*, and *KCNJ2* was also conducted, and patients with compound mutations of *KCNH2* and/or additional mutations in these LQTS-related genes were excluded from the analysis. Symptomatic patients were defined as *KCNH2* mutation carriers who experienced at least 1 episode of syncope (ie, complete loss of consciousness, or cardiac arrest requiring cardiac resuscitation), while asymptomatic patients were those without these events. Follow-up was censored at age 50 years to avoid the influence of coronary artery disease on cardiac events.

Genetic Analysis and Characterization

Genomic DNA was isolated from venous blood by use of the QIAamp DNA blood midikit (Qiagen, Hilden, Germany). The protocol for genetic analysis was approved by the institutional ethics committee and was performed under its guidelines. Established primer settings were used to amplify the entire coding regions of the known LQTS genes from genomic DNA[14–17] Denaturing high-performance liquid chromatography (DHPLC) was used for screening. For aberrant conformers, direct sequencing techniques were performed as described elsewhere[13] PCR products were denatured at 95°C for 5 min then analyzed by DHPLC. PCR fragments presenting abnormal signals in the DHPLC analysis were subsequently sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides in an ABI 3130 genetic analyzer (PE Applied Biosystems).

The pore region of the *KCNH2* channel was defined as the area extending from S5 to the mid-portion of S6 involving amino acid residues 550 through 650, according to a previous report[11] The non-pore region included the N-terminus region, transmembrane domains other than the pore region and the C-terminus region.

Clinical Characterization

Routine demographic data and basal 12-lead ECGs were obtained for all subjects at the time of enrollment in each institute and there was at least yearly follow-up contact. All ECGs were taken before or without β-blocker medication. The ECG parameters measured from the basal recordings were the RR, QT_{end}, QT_{peak} and $T_{peak-end}$ ($QT_{end}-QT_{peak}$) intervals. The latter is thought to reflect the transmural dispersion of ventricular repolarization (TDR)[18–20] The rate-dependent QT intervals were corrected for heart rate by Bazett's method[21] The QT_{peak} was defined as the time interval between QRS onset and the peak of the positive T wave or the nadir of the negative T wave. $T_{peak-end}$ was then obtained by calculating QT_{end} minus QT_{peak}.

These parameters were measured manually in lead Vs averaged from 2 or 3 consecutive beats. Bifid T waves other than U waves were included in the QT measurements. If ECG recordings were obtained during a cardiac event, the patients were requested to undergo the examination again after improving. Measurements were performed by 3 investigators who were completely unaware of the patient's clinical and genetic status. There were no significant differences in the measured data between the investigators, and the