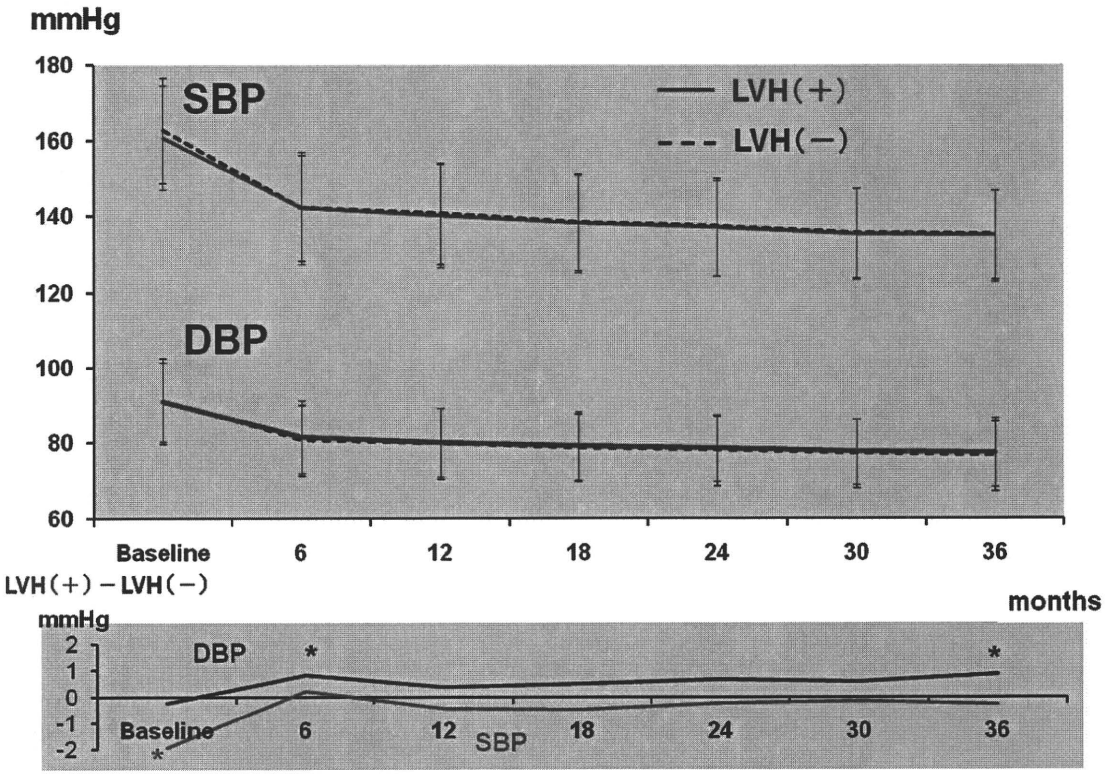

Figure 1. Changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) during the follow-up period. Mean SBP and DBP measured in 2 groups and differences between means. *P<0.05. *P<0.05 LVH (–) vs LVH (+). LVH, left ventricular hypertrophy.

t-test between the 2 groups. Frequency analysis was performed by the χ^2 test.

When we evaluated the time-course of eGFR in patients with and without LVH, we performed a mixed-effect linear regression (PROC MIXED in SAS version 9.1) to account for non-independence of the same participants, and adjusted for baseline eGFR and possible baseline confounders (allocated drug, age, sex, body mass index, SBP, DBP, history of previous antihypertensive treatment, severe hypertension, type 2 diabetes, history of cerebrovascular disease, history of ischemic heart disease, renal dysfunction, and history of vascular disease). Further, we also evaluated the time-course of eGFR in patients with and without CKD, adjusted for possible baseline confounders (allocated drug, age, sex, body mass index, SBP, DBP, history of previous antihypertensive treatment, severe hypertension, type 2 diabetes, history of cerebrovascular disease, history of ischemic heart disease, LVH, and history of vascular disease). We examined the association of the LVH and CKD with the time-course of the eGFR after adjusting for baseline eGFR and possible baseline confounders (allocated drug, age, sex, body mass index, SBP, DBP, history of previous antihypertensive treatment, severe hypertension, type 2 diabetes, history of cerebrovascular disease, history of ischemic heart disease, and history of vascular disease).

Boneferroni correction for multiple comparisons was applied to each analysis. All statistical tests were 2-sided with an α level of 0.05, and were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC, USA).

Results

Changes in BP

BP was strictly controlled to a level lower than 140/80 mmHg in both groups (**Figure 1**). Mean SBP/DBP was 160.7/90.9 mmHg at baseline and 134.9/77.4 mmHg after 3 years in patients with LVH, and 162.7/91.1 mmHg at baseline and 135.2/76.5 mmHg after 3 years in those without LVH. During the follow-up period, there were slight but statistically significant differences in SBP and DBP between the 2 groups (**Figure 1**).

Association of LVH With Changes in Renal Function

In both groups, the adjusted eGFRs were increased under strict BP control during the follow-up period, from 63.6 to 65.1 $ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ in patients with LVH, and from 63.6 to 68.5 $ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ in those without LVH. This improvement in eGFR was significantly greater in patients without LVH than in those with LVH (P=0.004, **Figure 2**).

Although the adjusted eGFR did not significantly change in patients without CKD, it significantly increased from 53.7 to 60.4 $ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ in those with CKD (P<0.001, **Figure 3**).

When we evaluated the adjusted eGFR among CKD patients with or without LVH, the adjusted eGFR increased from 52.7 to 60.5 $ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ in patients without LVH, but from 53.1 to 57.2 $ml \cdot min^{-1} \cdot 1.73\,m^{-2}$ in those with LVH. Thus, the improvement of eGFR was significantly greater in CKD patients without LVH than in those with LVH (P<0.001,

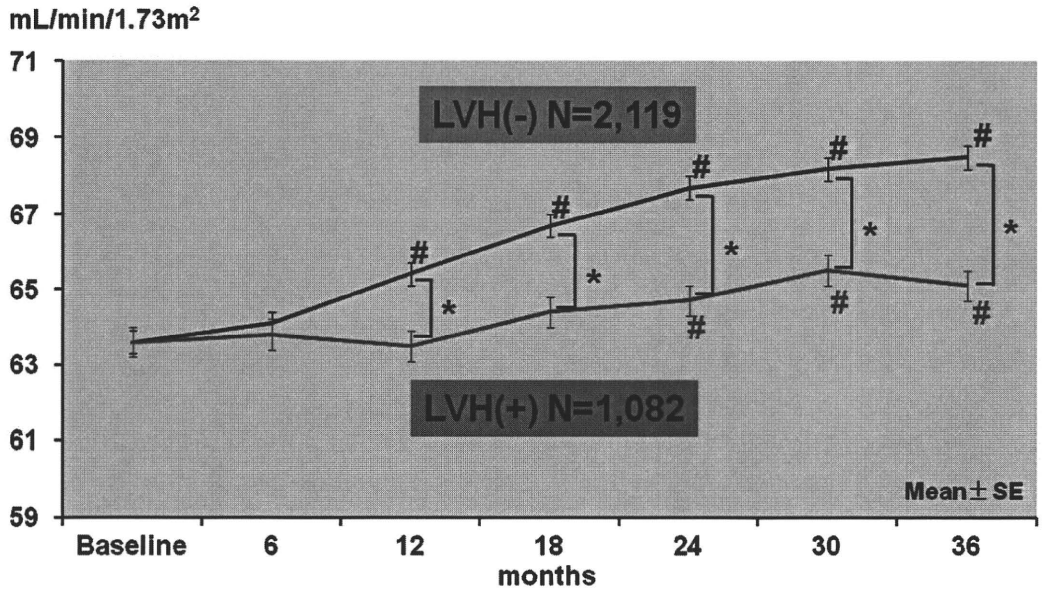

Figure 2. Changes in adjusted estimated glomerular filtration rate (eGFR) in patients with and without left ventricular hypertrophy (LVH). #P<0.05 vs eGFR at enrollment, *P<0.05 vs LVH (–).

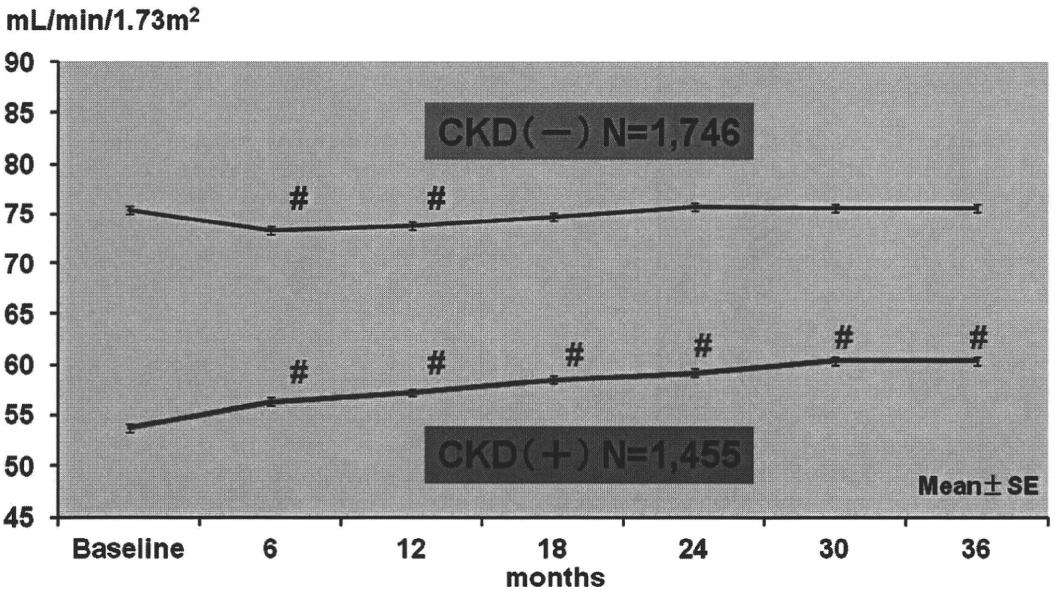

Figure 3. Changes in adjusted estimated glomerular filtration rate (eGFR) in patients with and without chronic kidney disease (CKD). #P<0.05 vs eGFR at enrollment.

Figure 4). Further, the adjusted eGFR changed from 76.5 to 75.4 ml·min^{-1}·1.73 m^{-2} in patients without CKD but with LVH, and from 76.5 to 77.5 ml·min^{-1}·1.73 m^{-2} in those without either CKD or LVH. Although the adjusted eGFR did not change notably among the patients without CKD, the final adjusted eGFR was significantly higher in patients without LVH than in those with LVH (P=0.048, **Figure 4**).

When we evaluated the effects of LVH to the time course of eGFR, CV disease were adjusted in our statistical analysis. However, because the participants with heterogeneous CV disease were included in the present study, changes in the eGFR in patients with or without CV disease at baseline were investigated. Then, the complication of LVH deteriorated the improvement of the time-course of the eGFR in patients with or without CV disease at baseline (data not shown).

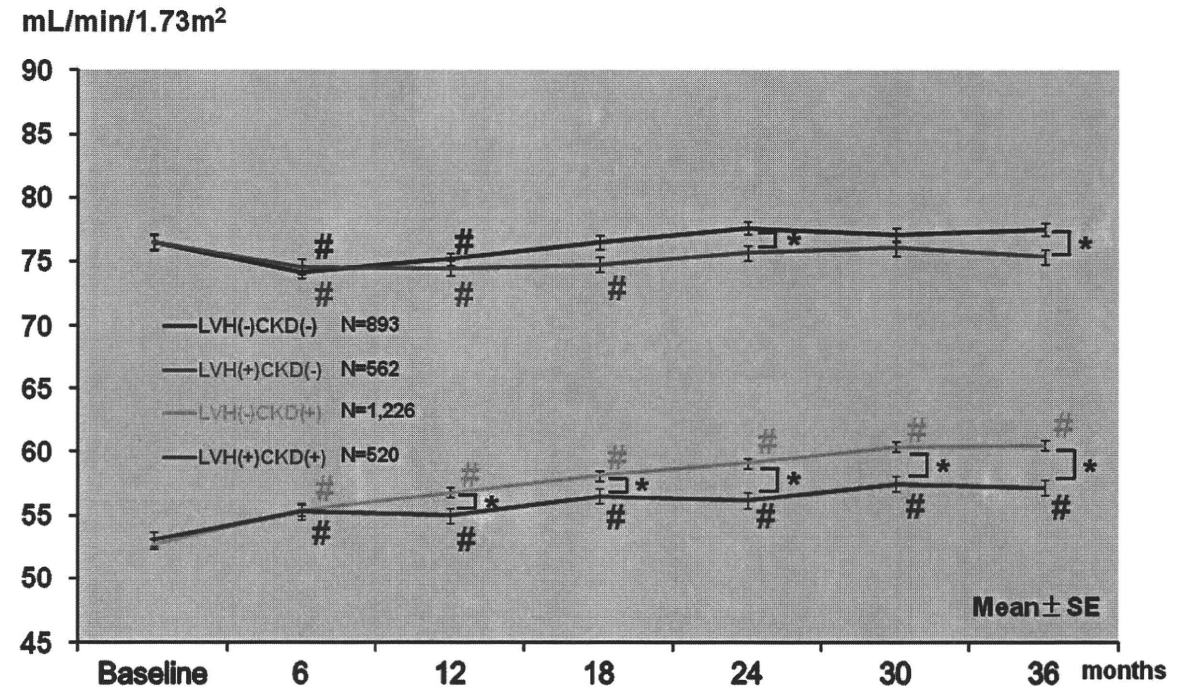

Figure 4. Changes in adjusted estimated glomerular filtration rate (eGFR) in patients with and without left ventricular hypertrophy (LVH) and in patients with and without chronic kidney disease (CKD). #P<0.05 vs eGFR at enrollment, *P<0.05 vs LVH (–).

Discussion

Because hypertension affects both the heart and kidney, and dysfunction of one negatively affects the other, CV and renal disease frequently coexist. This interaction, referred to as 'cardiorenal syndrome', was originally defined as characterized by the aggravation of renal function due to heart failure.[16] The 2 major clinical presentations of heart disease in patients with CKD are coronary artery disease and LVH.[17] Many studies have sought to understand the pathophysiology of kidney–heart interaction by investigating the impact of renal function on LVH, but few have investigated from the opposite point of view.[8,9] In the present study, conducted as a subanalysis of the CASE-J trial, we found that renal function improved under strict BP control, and that its improvement in high-risk hypertensive patients was greater in patients without LVH than in those with LVH.

LVH is an adaptive response that reduces LV wall stress against volume and pressure overload.[18,19] Although originally considered a compensatory and beneficial response to overload, large population studies have provided much evidence that LVH confers an increased risk for CV events.[3,4,20] The mechanism of the close association between LVH and the time-course of renal function is not clear. In the present analysis, achieved DBP levels in patients with LVH were slightly but significantly higher than those without LVH. But, given that the difference in achieved BP levels was small (less than 1 mmHg) and moreover the significant difference of the achieved DBP level was observed only at 2 points (6 months and 36 months), this would not have influenced the difference in the time-course of renal function between the 2 groups.

In this regard, Ito et al recently proposed the 'strain vessel' hypothesis.[21] According to the hypothesis, cerebrovascular events occur most frequently in the area of small perforating arteries that are exposed to high pressure and have to maintain strong vascular tone in order to provide large pressure gradients from the parent vessels to the capillaries. In the kidneys, the glomerular afferent arterioles of the juxtamedullary nephrons are analogous to the perforating arteries. Hypertensive vascular damage occurs first and more severely in the juxtamedullary glomeruli. On this basis, albuminuria might be an early sign of vascular damage imposed on 'strain vessels' such as perforating arteries and juxtamedullary afferent arterioles. Supporting this 'strain vessel' hypothesis, we previously reported that proteinuria is a strong risk factor for CV events.[22] In the heart, LVH might be analogous to renal damage because longstanding exposure to high BP leads to LVH.[23] We previously reported that LVH was strongly associated with the risk of cerebrovascular events (adjusted HR: 2.38; 95%CI: 1.62–3.48; P<0.001).[10] Furthermore, higher urinary albumin excretion has been observed in patients with LVH,[24,25] suggesting that cardiac and glomerular vascular damage might occur concurrently. These findings are consistent with the idea that LVH is analogous to albuminuria. However, de Andrade et al reported that the impairment of volume-sensitive cardiopulmonary reflex control of renal sympathetic nerve activity in spontaneously hypertensive rats correlates better with the magnitude of LVH than the level of arterial pressure.[26] Thus, LVH might have partial direct effects on the time-course of renal function. When we previously evaluated the association of changes in LV mass with time-course of serum creatinine concentrations as another subanalysis of the CASE-J trial, the protection against LVH during antihypertensive treatment might be related to the preservation of renal function.[27] Early detection of LVH

Table 2. The Dose of Each Antihypertensive Treatment in Patients With or Without LVH During the Follow-up Period

	0.5 year	1 year	1.5 year	2 year	2.5 year	3 year
Patients without LVH						
Candesartan (number)	1,068	1,051	1,038	1,028	1,030	1,021
Mean dose (mg)	7.4	7.6	7.8	7.9	8.1	8.2
Amlodipine (number)	1,044	1,018	1,012	1,008	1,004	997
Mean dose (mg)	5.2	5.4	5.4	5.4	5.5	5.5
Patients with LVH						
Candesartan (number)	534	521	511	507	508	506
Mean dose (mg)	7.8	8.1	8.4	8.6	8.7	8.8
Amlodipine (number)	545	536	533	529	529	523
Mean dose (mg)	5.5	5.6	5.8	5.9	6.0	6.1

LVH, left ventricular hypertrophy.

and aggressive BP control might contribute to the prevention of deterioration in renal function.[9,27,28]

In general, the expected decline in eGFR is approximately $1\ ml \cdot min^{-1} \cdot year^{-1}$.[1] In the present analysis, we were surprised to observe an improvement in renal function in CKD patients with or without LVH under strict BP control. To our knowledge, this current study is the first to report an improvement of renal function with antihypertensive treatment. In chronic hypertension, the small arteries of the kidneys, including the afferent arterioles, undergo a number of pathological changes that alter renal autoregulation.[29] The initial response of renal function to a decrease in BP is therefore a decrease in GFR. In this regard, a subanalysis of the REIN study demonstrated the ability of long-term ACE inhibition to effectively prevent progression to end-stage renal disease via the substantial healing of tubular injury as a result of a decrease in urinary protein overload.[30]

Several limitations of the present study warrant mention. First, because the analysis was post-hoc, this cohort should not be regarded as an ordinary epidemiological cohort. Second, hypertensive patients with any of several high-risk factors were enrolled, including LVH, so our evaluation of data for patients with and without LVH required adjustment by respective baseline characteristics owing to their statistical differences. Third, the definition of LVH was based on ECG or echocardiographic criteria. Because echocardiography was only performed when feasible, only a small number of patients underwent the procedure. Fourth, we cannot exclude the possibility that the dose of each allocated drug and the number of concomitant antihypertensive drugs might affect the results in this study. The dose of each antihypertensive treatment in patients with or without LVH during the follow-up period was shown in **Table 2**. In addition, the incidence of the patients with LVH who received concomitant antihypertensive drugs was larger than that of patients without LVH (23.3% vs 17.1% in diuretics, 25.0% vs 16.8% in β-blockers, and 24.8% vs 19.4% in α-blockers; $P<0.001$, respectively). However, because the additional antihypertensive treatments were considered as intermediate variables between LVH and CV events, we did not conduct the statistical adjustment for these factors. Finally, the mean follow-up period of 3.2 years might have been too short to evaluate changes in renal function. The CASE-J trial was extended for 3 years from 2006 as an observational study named CASE-J Ex,[31] which might resolve this issue in the near future.

Conclusion

In the present subanalysis, we found that renal function in high-risk hypertensive patients improved under strict BP control irrespective of the presence or the absence of LVH. However, the improvement in eGFR was significantly greater in patients without LVH than in those with LVH, particularly among those with CKD. LVH appears to have an adverse impact on the improvement of renal function despite strict BP control. Since early detection and prevention of LVH allows the prevention of deterioration in renal function is still not clear, additional investigation is needed in a future study.

Source of Funding

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Disclosures

K. Ueshima, M. Mukoyama, T. Ogihara, T. Saruta, and K. Nakao have received honoraria for lectures from Takeda Pharmaceutical Co Ltd and Pfizer Japan Inc.

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ORIGINAL ARTICLE

Role of diabetes and obesity in outcomes of the candesartan antihypertensive survival evaluation in Japan (CASE-J) trial

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The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial assessed cardiovascular outcomes in high-risk hypertensive patients receiving either candesartan or amlodipine. The aim of this study was to examine the role of pre-existing diabetes or obesity on these outcomes as a sub-analysis of the trial. We examined the influence of pre-existing diabetes on cardiovascular morbidity and mortality using a multivariate Cox regression model. The cardiovascular morbidity and mortality of candesartan and amlodipine were compared between subgroups with or without pre-existing diabetes or by body mass index (BMI) category, and new-onset diabetes was compared by BMI category. Pre-existing diabetes greatly increased the cardiovascular mortality and morbidity, regardless of the allocated drugs. Furthermore, all-cause mortality was significantly higher with amlodipine than with candesartan among patients with BMI $\geqslant$27.5 kg m^{-2} (adjusted hazard ratio (HR)=0.32; range=0.13–0.75; P=0.009). New-onset diabetes occurred significantly less frequently with candesartan than with amlodipine, with an adjusted HR of 0.66 (P=0.043). Furthermore, the increase in new-onset diabetes was dependent on BMI among patients receiving amlodipine, whereas no such dependency was observed for candesartan (interaction P=0.016). In conclusion, preexisting diabetes increased the risk of experiencing a cardiovascular event among high-risk Japanese hypertensive patients. Candesartan treatment may suppress all-cause death and reduce the incidence of new-onset diabetes in patients with obesity.
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Keywords: cardiovascular diseases; diabetes; obesity; randomized controlled trial

INTRODUCTION

Hypertension is often associated with insulin resistance, and hypertensive patients tend to develop type 2 diabetes mellitus (DM), which increases the risk of cardiovascular (CV) events in these patients. Hypertension, insulin resistance, obesity and dyslipidemia frequently occur together in a single individual, and such clustering is recognized as metabolic syndrome. With the prevalence of obesity increasing worldwide, even in Asian nations,[1] researchers have sought to develop treatment options capable of comprehensively addressing these risk factors in hypertensive patients.

As preexisting DM and obesity are thought to be related to the renin–angiotensin system (RAS), considerable interest has been focused on the difference between the treatment effects of angiotensin II receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACEIs) and calcium channel blockers (CCBs) with regard to treating hypertensive patients with DM or obesity. Several large randomized clinical trials have found the therapeutic benefits from ARBs,[2] CCBs[3,4] and ACEIs[5] to be superior to beta blocker-based treatments or placebos with regard to reducing mortality and CV events in these patients. The Blood Pressure Lowering Treatment Trialists' Collaboration (BPLTTC) conducted an individual patient-based meta-analysis and concluded that all of the major classes of blood pressure (BP)-lowering agents are capable of producing substantial reductions in short- to medium-term risk associated with the leading causes of death and CV events in patients with diabetes, although small differences in the effects of regimens on macrovascular events cannot be excluded.[6] However, none of the previous trials have analyzed the relationship between obesity and antihypertensive treatment effects on cardiovascular outcomes.

With regard to new-onset DM, the ARB, valsartan, has been shown to suppress incidence of new-onset DM more effectively than the CCB, amlodipine.[7] We recently reported the principal results of the Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) study, a prospective, multi-center, open-label randomized controlled trial with blinded assessment of end points, which was designed to evaluate the efficacy of candesartan and amlodipine in reducing the

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incidence of CV morbidity and mortality in a Japanese population with high-risk hypertension.[8] Although results ultimately showed no significant difference in incidence of CV events between the two treatments,[9] of particular note among the findings was the fact that the ARB, candesartan, prevented new-onset DM more effectively than amlodipine, thereby raising the possibility that candesartan's treatment effect may be affected by obesity condition, given present knowledge regarding the mechanism of ARB action.

To clarify the role of preexisting DM and obesity in the findings of the CASE-J trial, we evaluated the influence of diabetic status and body mass index (BMI) on trial outcomes and the interaction between these factors and allocated treatments. Preliminary data have been described previously,[9] and here we present a detailed *post-hoc* analysis.

METHODS

Study design

As the rationale and complete design of the CASE-J trial have been previously published,[8] relevant details are briefly described below. For the trial, written informed consents were obtained from all patients before enrollment, and the trial protocol was approved by the ethics committee of Kyoto University and undertaken in accordance with the Declaration of Helsinki Principles. The data and safety monitoring board made periodic recommendations to the steering committee regarding the ethical aspects of trial continuation by evaluating each occurrence of a possible adverse event.

The CASE-J trial enrolled eligible Japanese hypertensive patients with, at least, one high-risk factor. High-risk factors in the CASE-J trial were as follows: severe hypertension that is systolic BP (SBP) $\geqslant$180 mm Hg or diastolic BP (DBP) $\geqslant$110 mm Hg on two consecutive visits; type 2 DM (fasting blood glucose $\geqslant$126 mg per 100 ml, casual blood glucose $\geqslant$200 mg per 100 ml, hemoglobin A1c (HbA1c) $\geqslant$6.5%, 2-h blood glucose on 75 g oral glucose tolerance test $\geqslant$200 mg per 100 ml, or currently receiving treatment with a hypoglycemic agent); history of cerebral hemorrhage, cerebral infarction or transient ischemic attack occurring more than 6 months before screening; left ventricular hypertrophy on either echocardiography or electrocardiogram, angina pectoris or history of myocardial infarction occurring more than 6 months before screening; proteinuria or serum creatinine (sCr) $\geqslant$1.3 mg per 100 ml; and symptoms of arteriosclerotic peripheral artery obstruction.

After randomization, patients were allocated to receive either candesartan by oral administration at 4–8 mg day^{-1}, increasing up to 12 mg day^{-1} as necessary (or 2 mg day^{-1}, increasing up to 8 mg day^{-1} as necessary in patients with renal impairment) or amlodipine by oral administration at 2.5–5 mg day^{-1}, increasing up to 10 mg day^{-1} as necessary. Targets for BP control were determined according to practice guidelines developed by the Japanese Society of Hypertension (JSH),[10] as reported previously.[8]

Outcome measures

Outcome measures evaluated in this analysis were CV event, non-renal CV event, all-cause death, CV death and new-onset DM. In the original CASE-J trial, CV event was the primary end point, which is the first fatal or non-fatal CV event and represented a composite of the following: sudden unexpected death that happened within 24 h without external causes; cerebrovascular events including stroke or transient ischemic attack; cardiac events including heart failure, angina pectoris or acute myocardial infarction; renal events, including sCr $\geqslant$4.0 mg per 100 ml, doubling of sCr (although sCr $\leqslant$2.0 mg per 100 ml in any context was not regarded as an event) and end-stage renal disease; and vascular events, including dissecting aortic aneurysm or arteriosclerotic occlusion of a peripheral artery. As renal pathology in diabetic patients is affected by glycemic control, we also evaluated the incidence of non-renal CV events that excluded renal events from primary CV events. All-cause death and new-onset diabetes were the secondary and pre-specified end point in the CASE-J trial. Event evaluation for CV event and all-cause death was independently performed by the event evaluation committee members, who were blinded to the assigned treatment groups and assessed events according to the protocol criteria. Cases of new-onset DM were defined as patients who were reported to have developed DM as an adverse event or who were on anti-diabetic agents during the course of the study as reported in the case report form.

Statistical methods

Baseline characteristics were expressed as mean ± s.d. or proportions, and between-groups using the χ^2 test or *t*-test. Analyses were divided into two parts: we first evaluated the influence of preexisting DM at baseline on each end point as a prognostic factor analysis. Here, we conducted multivariate Cox regression analysis with adjustment for allocated drugs, BMI, age, sex, hyperlipidemia, smoking history, high-risk factors in the CASE-J trial (severe hypertension, cerebrovascular history, cardiac complications, renal dysfunction and vascular disease) and antihypertensive drug use at baseline.

We then conducted a comparison with regard to allocated drugs (candesartan *vs.* amlodipine) across subgroups for baseline presence or absence of preexisting DM and the BMI category ($<$22.0, $\geqslant$22.0–25.0, $\geqslant$25.0–$<$27.5 and

Table 1a Baseline characteristics in patients with and without diabetes[a]

	DM (+) at baseline			*DM (−) at baseline*		
	Candesartan (N=1011)	*Amlodipine (N=1007)*	*P-value*	*Candesartan (N=1343)*	*Amlodipine (N=1342)*	*P-value*
Age (years)	63.9 ± 9.5	64.1 ± 9.9	0.660	63.6 ± 11.2	63.8 ± 11.7	0.684
BMI (kg m^{-2})	25.1 ± 3.9	25.1 ± 3.6	0.739	24.2 ± 3.5	24.0 ± 3.5	0.099
Female (%)	445 (44.0)	447 (44.4)	0.866	647 (48.2)	567 (42.3)	0.002
Severe hypertension[b] (%)	58 (5.7)	64 (6.4)	0.560	396 (29.5)	429 (32.0)	0.164
Cerebrovascular history[b] (%)	70 (6.9)	59 (5.9)	0.328	178 (13.3)	166 (12.4)	0.493
Cardiac complications[b] (%)	291 (28.8)	313 (31.1)	0.260	716 (53.3)	710 (52.9)	0.833
Renal dysfunction[b] (%)	228 (22.6)	216 (21.5)	0.550	344 (25.6)	327 (24.4)	0.455
Vascular disease (%)	11 (1.1)	5 (0.5)	0.134	18 (1.3)	19 (1.4)	0.867
Antihypertensive drug use (%)	712 (70.4)	686 (68.1)	0.262	900 (67.0)	867 (64.6)	0.188
Current or smoking history (%)	319 (31.6)	319 (31.7)	0.952	386 (28.7)	474 (35.3)	<0.001
Hyperlipidemia (%)	460 (45.5)	440 (43.7)	0.415	608 (45.3)	570 (42.5)	0.144
SBP (mm Hg)	159.8 ± 12.9	160.0 ± 12.5	0.690	164.5 ± 14.7	165.6 ± 14.9	0.054
DBP (mm Hg)	88.3 ± 9.9	88.3 ± 10.3	0.943	94.0 ± 11.2	94.5 ± 11.4	0.249

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; SBP, systolic blood pressure.
[a]Data are shown as the mean ± s.d. or *n* (%) in each category.
[b]Severe hypertension (blood pressure $\geqslant$180 or $\geqslant$110 mm Hg), cerebrovascular event history (history of stroke or transient ischemic attack), cardiac complication (left ventricular hypertrophy, angina pectoris or history of myocardial infarction), renal dysfunction (proteinuria or serum creatinine $\geqslant$1.3 mg per 100 ml).

Table 1b Baseline characteristics in patients with BMI category[a]

	<22.0 kg m^{-2}			22.0–<25.0 kg m^{-2}			25.0–<27.5 kg m^{-2}			≥27.5 kg m^{-2}		
	Candesartan (N=561)	*Amlodipine (N=565)*	P-value	*Candesartan (N=813)*	*Amlodipine (N=853)*	P-value	*Candesartan (N=536)*	*Amlodipine (N=527)*	P-value	*Candesartan (N=444)*	*Amlodipine (N=404)*	P-value
Age (years)	67.1 ± 9.9	66.4 ± 10.6	0.257	63.8 ± 9.6	64.1 ± 9.7	0.419	63.5 ± 10.1	63.7 ± 10.1	0.704	59.9 ± 11.9	60.4 ± 11.8	0.551
BMI (kg m^{-2})	20.3 ± 1.3	20.2 ± 1.4	0.384	23.5 ± 0.9	23.6 ± 0.9	0.820	26.1 ± 0.7	26.1 ± 0.7	0.490	30.2 ± 2.8	30.2 ± 2.8	0.875
Female (%)	299 (53.3)	267 (47.3)	0.043	337 (41.5)	342 (40.1)	0.573	250 (46.1)	205 (38.9)	0.011	206 (46.4)	200 (49.5)	0.365
Severe hypertension[b] (%)	125 (22.3)	130 (23.0)	0.771	144 (17.7)	173 (20.3)	0.182	104 (19.4)	108 (20.5)	0.656	81 (18.2)	82 (20.3)	0.448
Preexisting DM (%)	195 (34.8)	186 (32.9)	0.514	355 (43.7)	360 (42.2)	0.547	236 (44.0)	235 (44.6)	0.854	225 (50.7)	226 (55.9)	0.125
Cerebrovascular history[b] (%)	72 (12.8)	66 (11.7)	0.555	85 (10.5)	84 (9.8)	0.623	60 (11.2)	47 (9.3)	0.308	31 (7.0)	27 (6.7)	0.863
Cardiac complications[b] (%)	238 (42.4)	260 (46.0)	0.225	372 (45.8)	385 (45.1)	0.799	216 (40.3)	231 (43.8)	0.243	181 (40.8)	147 (36.4)	0.191
Renal dysfunction[b] (%)	140 (25.0)	137 (24.3)	0.783	182 (22.4)	175 (20.5)	0.352	130 (24.3)	130 (24.7)	0.875	120 (27.0)	101 (25.0)	0.502
Vascular disease (%)	13 (2.3)	9 (1.5)	0.380	9 (1.1)	9 (0.9)	0.733	3 (0.6)	5 (1.0)	0.463	4 (0.9)	2 (0.5)	0.481
Antihypertensive drug use (%)	377 (67.2)	378 (66.9)	0.915	540 (66.4)	536 (62.8)	0.126	385 (71.8)	374 (71.0)	0.756	310 (69.8)	265 (65.6)	0.188
Current or smoking history (%)	156 (27.8)	201 (35.6)	0.005	254 (31.2)	286 (33.5)	0.319	155 (28.9)	183 (34.7)	0.042	140 (31.5)	123 (30.5)	0.733
Hyperlipidemia (%)	196 (34.9)	192 (34.0)	0.736	364 (44.8)	371 (43.5)	0.599	268 (50.0)	240 (45.5)	0.146	240 (54.1)	207 (51.2)	0.412
SBP (mm Hg)	164.5 ± 13.8	165.3 ± 14.0	0.348	162.1 ± 13.8	163.0 ± 14.0	0.193	162.1 ± 14.3	162.3 ± 14.5	0.763	161.9 ± 14.7	162.0 ± 14.2	0.413
DBP (mm Hg)	90.4 ± 11.3	91.7 ± 11.1	0.106	91.3 ± 10.6	91.5 ± 11.5	0.748	91.6 ± 11.3	91.7 ± 11.5	0.841	93.1 ± 11.0	92.8 ± 11.0	0.725

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DM, diabetes mellitus; SBP, systolic blood pressure.
[a]Data are shown as the mean ± s.d. or *n* (%) in each category.
[b]Severe hypertension (blood pressure ≥180 or ≥110 mm Hg), cerebrovascular event history (history of stroke or transient ischemic attack), cardiac complication (left ventricular hypertrophy, angina pectoris or history of myocardial infarction), renal dysfunction (proteinuria or serum creatinine ≥1.3 mg per 100 ml).

Table 2 Adjusted HRs of prognostic factors for primary CV events

Baseline characteristics	*HR*[a]	95% *CI*	P-*value*
DM (present)	2.58	1.99–3.33	<.001
Allocated drugs (candesartan)	0.99	0.78–1.26	0.922
Age	1.37	1.19–1.57	<0.001
BMI	1.16	0.98–1.37	0.092
Sex (female)	0.89	0.67–1.18	0.411
Severe hypertension (yes)	1.10	0.74–1.65	0.632
Cerebrovascular history (yes)	2.04	1.47–2.83	<0.001
Cardiac complications (yes)	2.21	1.72–2.84	<0.001
Renal dysfunction (yes)	2.93	2.30–3.74	<0.001
Vascular disease, yes	1.68	0.69–4.10	0.255
Antihypertensive drug use at baseline (yes)	1.42	1.05–1.91	0.024
Current or former smoker (yes)	1.02	0.77–1.36	0.871
Hyperlipidemia (yes)	0.89	0.69–1.15	0.368

Abbreviations: BMI, body mass index; CI, confidence interval; CV, cardiovascular; DM, diabetes mellitus; HR, hazard ratio.
[a]The HR value was adjusted for all baseline variables listed in Table 2 entering in the multivariate Cox regression model.

≥27.5 kg m^{-2}). The cutoff points of the BMI category (22 kg m^{-2} as normal BMI for the Japanese; 25 kg m^{-2} as upper limit of normal BMI range; and 27.5 kg m^{-2} as the mid-point of the overweight BMI range) were pre-specified before analyzing the data. In the comparison between candesartan and amlodipine, hazard ratios (HRs), 95% confidence intervals (CIs) and *P*-values of the Wald test were also calculated with adjustment for age, sex, smoking history, hyperlipidemia, severe hypertension, cerebrovascular history, cardiac complications, renal dysfunction and antihypertensive drug use at baseline in the multivariate Cox regression model. Interactions of HRs between allocated drugs and the baseline variables of preexisting DM status and BMI (as continuous variable) were also evaluated in the multivariate Cox regression model with interaction terms. All statistical tests were two-sided and performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Baseline characteristics and medication adherence

Of the 4703 patients that enrolled in the CASE-J trial, 2018 (42. 9%) patients had DM at baseline and 2685 (57.1%) did not. Table 1a summarizes the baseline characteristics by diabetic status at baseline and allocated drug. With regard to between-group comparisons, background characteristics were well balanced between both treatment groups except in regards to sex ratio and smoking history in patients without DM at baseline. The baseline characteristics of the BMI category were shown in Table 1b. There were some statistical differences in sex and current smoking history between two groups in the two BMI categories (<22.0 and ≥25.0–<27.5 kg m^{-2}, respectively), whereas there was no statistical difference in the BMI ≥27.5 kg m^{-2} category.

Percentages of patients who took more than 80% of the allocated drug and details regarding the distribution of additional drugs during the follow-up period have already been reported in the main results of the CASE-J trial.[9] Regarding drug doses, 59.4% of patients in the candesartan group were taking 8 mg at last follow-up, whereas 23.5% were taking 12 mg and 13.8% were taking 4 mg. In the amlodipine group, 71.6% were taking 5 mg at last follow-up, whereas 14.9% were taking 10 mg and 9.5% were taking 2.5 mg. Mean doses in the candesartan and amlodipine groups were 8.3 and 5.6 mg, respectively.

Association of diabetic status at baseline with the CASE-J trial outcome

Over 3.3 ± 0.8 mean years of follow-up, primary CV events occurred in 103 (3.8%) patients without DM at baseline (11.8 per 1000 patient-years) and in 165 (8.2%) with DM at baseline (25.5 per

Table 3 Hazard ratios for each event in patients with and without diabetes

	DM (+) at baseline				*DM (−) at baseline*				*DM (+) vs. DM (−)*		
Events	*At risk*	*Events*	*Rate*[a]	*95% CI*	*At risk*	*Events*	*Rate*	*95% CI*	*HR*[b]	*95% CI*	P-*value*
Primary CV events	2018	165	25.5	21.8–29.7	2685	103	11.8	9.7–14.3	2.58	2.00–3.33	<0.001
Cerebrovascular	2018	54	8.2	6.1–10.7	2685	57	6.5	4.9–8.4	1.49	1.01–2.19	0.044
Cardiac	2018	67	10.2	7.9–12.9	2685	23	2.6	1.7–3.9	4.99	3.05–8.16	<0.0001
Renal	2018	38	5.7	4.0–7.8	2685	8.0	0.9	0.4–1. 8	7.44	3.47–16.22	<0.0001
Non-renal CV events	2018	138	21.2	17.8–25.1	2685	96	11.0	8.9–13.4	2.34	1.78–3.08	<0.001
All-cause deaths	2018	89	13.3	10.7–16.4	2685	70	7.9	6.2–10.0	2.04	1.46–2.87	<0.001
CV deaths	2018	26	3.9	2.5–5.7	2685	21	2.4	1.5–3.6	1.93	1.03–3.61	0.041

Abbreviations: CI, confidence interval; CV, cardiovascular; DM, diabetes mellitus; HR, hazard ratio.
[a]Rate is expressed as incidence per 1000 patient-years.
[b]HR value was adjusted for the potential confounders (allocated drugs, age, body mass index, sex, severe hypertension, cerebrovascular history, cardiac complications, renal dysfunction, antihypertensive drug use at baseline, smoking history and hyperlipidemia).

Table 4 Comparisons between candesartan and amlodipine for each event for subgroups of diabetes status at baseline

	Candesartan				*Amlodipine*				*Comparison of treatment groups*			*Interaction test*
	At risk	*Events*	*Rate*[a]	*95% CI*	*At risk*	*Events*	*Rate*[a]	*95% CI*	*HR*[b]	*95% CI*	P-*value*	P-*value*
Primary CV events												
DM (−)	1343	54	12.5	9.4–16.3	1342	49	11.2	8.3–14.8	1.06	0.72–1.58	0.744	0.565
DM (+)	1011	80	24.8	19.6–30.8	1007	85	26.3	21.0–32.5	0.92	0.67–1.24	0.568	
Cerebrovascular events												
DM (−)	1343	31	7.1	4.8–10.1	1342	26	5.9	3.9–8.7	1.13	0.67–1.90	0.661	0.820
DM (+)	1011	30	9.1	6.1–13.0	1007	24	7.2	4.6–10.8	1.22	0.71–2.10	0.466	
Cardiac events												
DM (−)	1343	12	2.7	1.4–4.8	1342	11	2.5	1.2–4.5	1.12	0.49–2.57	0.785	0.619
DM (+)	1011	31	9.4	6.4–13.4	1007	36	10.9	7.7–15.1	0.87	0.54–1.40	0.562	
Renal events												
DM (−)	1343	2	0.5	0.1–1.6	1342	6	1.4	0.5–3.0	0.31	0.06–1.57	0.158	0.329
DM (+)	1011	17	5.1	3.0–8.2	1007	21	6.3	3.9–9.7	0.72	0.38–1.37	0.320	
Non-renal CV events												
DM (−)	1343	52	12.0	9.0–15.7	1342	44	10.0	7.3–13.5	1.16	0.77–1.73	0.484	0.525
DM (+)	1011	68	21.0	16.3–26.6	1007	70	21.5	16.7–27.1	0.97	0.70–1.36	0.879	
All-cause deaths												
DM (−)	1343	33	7.5	5.1–10.5	1342	37	8.4	5.9–11.5	0.96	0.60–1.54	0.853	0.775
DM (+)	1011	40	11.9	8.5–16.3	1007	49	14.7	10.8–19.4	0.84	0.55–1.27	0.407	
CV deaths												
DM (−)	1343	11	2.5	1.2–4.5	1342	10	2.3	1.1–4.1	1.13	0.48–2.68	0.783	0.818
DM (+)	1011	11	3.3	1.6–5.9	1007	15	4.9	2.5–7.4	0.78	0.36–1.71	0.537	

Abbreviations: CI, confidence interval; CV, cardiovascular; DM, diabetes mellitus; HR, hazard ratio.
[a]Rate is expressed as incidence per 1000 patient-years.
[b]HR value was adjusted for the potential confounders (age, body mass index, sex, severe hypertension, cerebrovascular history, cardiac complications, renal dysfunction, antihypertensive drug use at baseline, smoking history and hyperlipidemia).

1000 patient-years). Multivariate Cox regression analysis revealed that DM was an independent predictor of primary CV events (adjusted HR=2.58, 95% CI=1.99–3.33, $P<0.0001$; Table 2), as were aging, cerebrovascular history, cardiac complications, renal dysfunction and antihypertensive drug use at baseline. In addition, DM was significantly associated with risk of each CV event and all-cause death (Table 3).

We then examined the effects of candesartan- and amlodipine-based regimens on the incidences of each event and all-cause deaths among subgroups of diabetic status at baseline, with analysis revealing no significant differences in event incidence between the two treatment regimens, regardless of diabetic status at baseline (Table 4). New-onset DM occurred in 38 (2.8%) patients receiving candesartan-based regimens (8.7 per 1000 patient-years) and in 59 (4.4%) receiving

Table 5 Comparisons between candesartan and amlodipine for each event for BMI category at baseline

	Candesartan				Amlodipine				Comparison of treatment groups				Interaction test
	At risk	Events	Rate[a]	95% CI	At risk	Events	Rate[a]	95% CI	HR[b]	95% CI		P-value	P-value
Primary CV events													
BMI <22	561	37	20.9	14.7–28.7	565	33	18.1	12.5–25.4	1.13	0.70	1.81	0.623	0.904
BMI ⩾22–<25	813	31	11.6	7.9–16.5	853	48	17.4	12.8–23.0	0.66	0.42	1.04	0.075	
BMI ⩾25–<27.5	536	37	21.6	15.2–29.8	527	28	16.4	10.8–23.7	1.37	0.83	2.25	0.214	
BMI ⩾27.5	444	29	20.6	13.8–29.6	404	25	19.1	12.3–28.1	1.09	0.63	1.87	0.761	
Non-renal CV events													
BMI <22	561	34	19.1	13.2–26.7	565	25	13.6	8.8–20.1	1.38	0.82	2.33	0.221	0.763
BMI ⩾22–<25	813	28	10.5	7.2–15.5	853	41	14.8	10.6–20.1	0.71	0.44	1.16	0.169	
BMI ⩾25–<27.5	536	33	19.2	12.8–26.5	527	24	14.0	9.0–20.8	1.48	0.87	2.52	0.148	
BMI ⩾27.5	444	25	17.7	11.4–26.1	404	24	18.3	11.7–27.2	0.98	0.55	1.73	0.937	
All-cause deaths													
BMI <22	561	29	15.9	10.6–22.8	565	30	16.1	10.9–23.0	1.00	0.60	1.67	0.997	0.102
BMI ⩾22–<25	813	26	9.6	6.3–14.0	853	25	8.8	5.7–12.9	1.11	0.64	1.92	0.722	
BMI ⩾25–<27.5	536	11	6.3	3.1–11.2	527	11	6.4	3.2–11.4	1.08	0.46	2.52	0.854	
BMI ⩾27.5	444	7	4.8	1.9–9.9	404	20	15.0	9.1–23.1	0.32	0.13	0.75	0.009	
CV deaths													
BMI <22	561	5	2.7	0.9–6.4	565	5	2.7	0.9–6.3	1.03	0.29	3.58	0.969	0.170
BMI ⩾22–<25	813	9	3.3	1.5–6.3	853	7	2.5	1.0–5.1	1.44	0.53	3.89	0.474	
BMI ⩾25–<27.5	536	5	2.8	0.9–6.6	527	5	2.9	0.9–6.8	1.02	0.29	3.65	0.977	
BMI ⩾27.5	444	3	2.0	0.4–6.0	404	8	6.0	2.6–11.8	0.34	0.09	1.28	0.110	
New-onset diabetes													
BMI <22	366	7	5.9	2.4–12.1	379	7	5.7	2.3–11.8	1.09	0.38	3.13	0.868	0.016
BMI ⩾22–<25	458	14	9.4	5.1–15.8	493	23	14.1	9.0–21.2	0.67	0.34	1.32	0.250	
BMI ⩾25–<27.5	300	11	11.3	5.6–20.2	292	16	17.2	9.9–28.0	0.64	0.29	1.39	0.258	
BMI ⩾27.5	219	6	8.5	3.1–18.5	178	13	23.4	12.5–40.0	0.35	0.13	0.94	0.036	

Abbreviations: BMI, body mass index; CI, confidence interval; CV, cardiovascular; HR, hazard ratio.
[a]Rate is expressed as incidence per 1000 patient-years.
[b]HR value was adjusted for the potential confounders (age, diabetes mellitus, sex, severe hypertension, cerebrovascular history, cardiac complications, renal dysfunction, antihypertensive drug use at baseline, smoking history and hyperlipidemia).

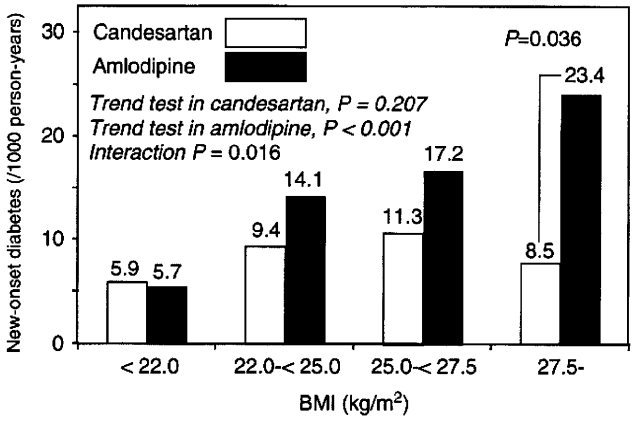

Figure 1 Relationship between new-onset diabetes and body mass index (BMI) at baseline.

amlodipine-based regimens (13.6 per 1000 patient-years). Multivariate Cox regression analysis also revealed that candesartan suppressed the incidence of new-onset DM to significantly greater degree than amlodipine (adjusted HR=0.66, 95% CI=0.43–0.99, P=0.043). This adjusted HR was comparable with the unadjusted one reported previously.[9]

Association of BMI at baseline with the CASE-J trial outcomes

The actual number and incidence rate of primary CV events, non-renal CV events, and all-cause death in each BMI category are shown in Table 5. No significant difference in incidence of primary and non-renal CV events was noted between the two treatment regimens in any of the four BMI categories.

With regard to all-cause death, incidence in both regimens was most frequent in the lowest BMI category ($<22.0\,kg\,m^{-2}$). Incidence was similar between treatment regimen groups at BMI $<22.0\,kg\,m^{-2}$; $22.0 \leqslant BMI < 25.0\,kg\,m^{-2}$; and $25.0 \leqslant BMI < 27.5\,kg\,m^{-2}$. However, a statistically significant difference was noted in the incidence among patients in the BMI $\geqslant 27.5\,kg\,m^{-2}$ category, with an adjusted HR of 0.32 (95% CI=0.13–0.75, P=0.009). Furthermore, in this category, deaths from both CV (three in the candesartan group and eight in the amlodipine group) and non-CV causes (four in the candesartan group and 12 in the amlodipine group) were more frequent in the amlodipine group than in the candesartan group, although the number of events was small.

Table 5 describes the number of patients with new-onset DM and the adjusted HRs in each category stratified according to BMI at study enrollment. In BMI subgroup analyses, candesartan significantly suppressed new-onset DM in the BMI $\geqslant 27.5\,kg\,m^{-2}$ category (adjusted HR=0.35, 95% CI=0.13–0.94, P=0.036) compared with amlodipine. Figure 1 clearly shows that the increase in new-onset DM was dependent on BMI in the amlodipine group, whereas no such dependency was observed in the candesartan group, highlighting the statistically significant interaction between BMI and treatment group (interaction P=0.016).

DISCUSSION

In this analysis, we noted that DM was a strong risk factor for CV events among high-risk Japanese hypertensive patients, in addition to all-cause death, and other outcomes of the CASE-J trial. Furthermore, we showed that the ARB, candesartan, exerted a favorable effect in suppressing new-onset DM among patients with elevated BMI. However, no difference in occurrence of primary CV events was noted between candesartan and amlodipine groups regardless of diabetic status and BMI, albeit these findings had insufficient statistical power.

Previous studies that showed a decrease in the new-onset DM with antihypertensive treatment did not report the influence of BMI on the outcome.[5,7] In our analysis, the incidence of new-onset DM was lower in patients with relatively low BMI than in those with relatively high BMI in the amlodipine group, suggesting that onset of DM is affected by the degree of patient obesity in this group. However, no such dependency was observed in patients receiving candesartan. When we conducted the additional *post-hoc* analysis with quintile cutoff points of the BMI category (<21.6, $\geqslant 21.6$–23.4, $\geqslant 23.4$–<25.1, $\geqslant 25.1$–<27.2 and $\geqslant 27.2\,kg\,m^{-2}$), the similar dependency was observed compared with that based on the pre-specified cutoff points of the BMI category (data not shown). These results suggested that candesartan reduced the new-onset DM by preventing the metabolically deleterious effects of increased adiposity in high-risk hypertensive patients.

The average BMI of participants in the CASE-J trial was approximately $25\,kg\,m^{-2}$ among patients diabetic at enrollment and $24\,kg\,m^{-2}$ among non-diabetic patients. As reported previously,[9] patients with a BMI greater than or equal to $27.5\,kg\,m^{-2}$, who were receiving candesartan had a significantly lower risk of all-cause death than those receiving amlodipine, whereas incidence of all-cause death was similar between the two treatment groups among patients occupying lower BMI strata. A U-shaped association between all-cause death and BMI in a Japanese population has been previously reported,[11] and this same relationship was also observed between all-cause death and BMI among patients receiving amlodipine in this study. In contrast, no increase in all-cause death associated with increased BMI was observed for patients receiving candesartan (Table 5); results we interpret to indicate that candesartan treatment reduced the incidence of all-cause death among patients whose BMI was in the highest category, who might otherwise have suffered increased mortality.

The improvement in the insulin resistance observed under antihypertensive treatment has been attributed in part to the direct effect of reduced blood pressure on endothelial function. However, candesartan[12] and enalapril,[13] both suppressed the development of DM in patients with congestive heart failure without hypertension, suggesting that the effect of RAS suppression on the development of DM is not necessarily a direct result of lowered BP. As angiotensinogen is produced by adipose tissue,[14] and angiotensin II has a role in increasing insulin resistance through its effects on adipocyte function,[15] the suppression of new-onset DM by candesartan may well depend on the state of adiposity and therefore be more profound in obese patients, as was observed in the present analysis. Hypertension and impaired glucose homeostasis associated with obesity can be considered a reflection of the pathophysiological process of metabolic syndrome.[16–18] The suppression of both hypertension and glucose intolerance induced by candesartan in this study supports the hypothesis that RAS does indeed have a role in obesity and the development of metabolic syndrome, and results from this and previous studies will aid in development of treatment strategies for pathological conditions associated with obesity, such as metabolic syndrome.

Several limitations to this analysis warrant mention. First, our study was conducted as a *post-hoc* analysis, and therefore there was also a risk that our findings might occur by chance. Second, the number of patients in each subgroup may not have been sufficient to allow for a thorough examination of the relationship between DM (or BMI) and trial outcomes. Third, patients were not vigorously tested for the presence of DM at the end, and diagnosis of new-onset DM relied solely on the attending clinician's decision to prescribe anti-diabetic medications or a report of DM in the adverse event form. According to the National Diabetes Survey in 2002, 69.6% of patients diagnosed as diabetic underwent either drug or insulin treatment.[19] Therefore, although the number of new-onset DM as defined in this study may have underestimated the overall incidence of DM, it is highly probable that a considerable portion of new-onset DM cases were included in the results. Last, we cannot accurately claim that the decrease in new-onset DM observed in this study directly affected the overall CV morbidity and mortality of non-diabetic patients. The recent CASE-J Ex study[20] may provide insights into these and other questions.

In conclusion, this analysis showed that DM increased CV risk among high-risk Japanese hypertensive patients. Candesartan treatment may produce significant suppression of all-cause death and reduced new-onset DM in patients with obesity. Results from this analysis will likely be of long-term benefit to obese hypertensive patients.

CONFLICT OF INTEREST

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REVIEW ARTICLE

Searching for novel intercellular signal-transducing molecules in the kidney and their clinical application

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Abstract In this review, isolation and characterization of several kidney-derived molecules are described, namely carbonic anhydrase XIV, cysteine-rich protein 61, and kidney–liver-specific immunoglobulin-like protein. Features of neutrophil gelatinase-associated lipocalin (LCN2 or human neutrophil lipocalin) as a kidney differentiation inducer and renal injury biomarker and also as an iron-carrier protein are also summarized. Furthermore, the concepts of forest fire theory and the biology of siderophore-binding proteins are discussed.

Keywords Acute kidney injury · Chronic kidney disease · Iron chelation · Screening

Introduction

In general, intercellular signal-transducing molecules, especially secreted proteins, have substantial advantages for application in diagnosis, treatment, and elucidation of the pathophysiology of renal disorders, since it is considerably easy to measure their blood or urinary concentrations and to administer related recombinant proteins to humans. For instance, in cardiology, atrial and brain natriuretic peptides (ANP and BNP) are highly accumulated in the circulation of patients with heart failure, allowing quantitative evaluation of cardiac disorders, and injection of recombinant ANP or BNP can improve heart failure [1, 2].

Carbonic anhydrase XIV

As a first trial of searching for novel intercellular signal-transducing molecules in the kidney, using the signal sequence trap method, we screened a complementary DNA (cDNA) library constructed from normal mouse kidney [3, 4]. In this method, random cDNA fragments are ligated with a reporter cDNA encoding a type 1 transmembrane protein lacking its own signal sequence. When the reporter (interleukin-2 receptor alpha chain, in our case) is fused with a stretch of hydrophobic amino acid residues which can work as a signal sequence, live COS-7 cells expressing the fusion protein are detected by immunofluorescence recognizing the extracellular portion of the reporter. Otherwise, a fusion protein without signal sequence remains intracellular, and COS-7 cells become immunonegative. We screened 10,000 cDNA clones; 60 were positive (allowing overlaps), and their amino acid sequences were determined. Approximately 50% of positive clones had some sort of name and 25% had functional data in the year 1999, although these numbers should have increased by now. Positive clones were categorized into 6 major classes (Table 1) [4–6]. Unexpectedly, transmembrane domains of channels and transporters functioned as signal sequences in the screening. One clone encoded a putative signal sequence followed by a portion of carbonic anhydrase (CA)-like domain [4]. Full-length cDNA was isolated by polymerase chain reaction (PCR)-based rapid amplification of 3′ cDNA end (RACE) method. The entire

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Table 1 Representative molecules isolated from normal mouse kidney by signal sequence trap

Hormone and secretory protein
Uroguanylin, uromodulin, osteopontin, osteonectin (SPARC)
Enzyme
Carbonic anhydrase XIV [4], kidney-derived aspartic protease-like protein (KAP) [5], hepatocyte growth factor activator, gamma-glutamyltransferase, *N*-acetylglucosaminyltransferase III
Receptor
Folate receptor 1, signal sequence receptor alpha chain
Adhesion molecule
Kidney-specific cadherin
Channel
Aquaporin 2
Transporter
Renal-specific transporter (RST) [6], dibasic and neutral amino acid transporter, sodium-dependent phosphate transporter (NaPi-7)

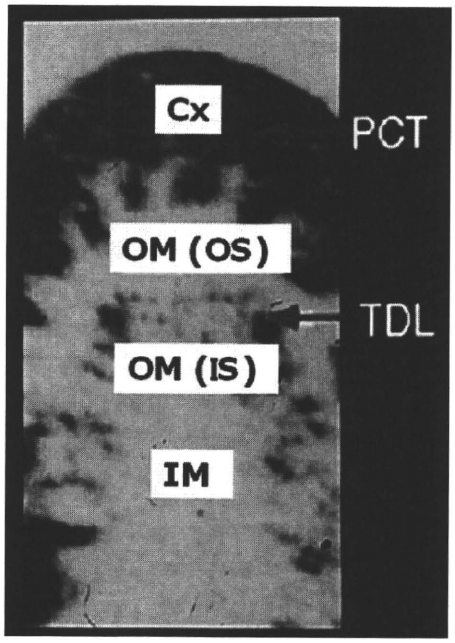

Fig. 1 Intrarenal localization of carbonic anhydrase (CA) XIV messenger RNA (mRNA) expression [4]. *Cx* cortex, *OM* outer medulla, *IM* inner medulla, *OS* outer stripe, *IS* inner stripe, *PCT* proximal convoluted tubule, *TAL* thick ascending limb of Henle, *TDL* thin descending limb of Henle

cDNA encoded a novel transmembrane protein with active CA activity, which was termed CA XIV. Expression of the gene was abundant in kidney, heart, and muscle. By in situ hybridization, it was expressed in the proximal convoluted tubules and thin descending limb of Henle, a pattern differing from those of CA II or CA IV (Fig. 1) [7].

Kidney–liver-specific immunoglobulin-like protein

Another interesting clone we identified by signal sequence trap encoded an immunoglobulin-like V-type domain. It was specifically expressed in the kidney and liver and was thus named kidney–liver-specific immunoglobulin-like protein (KLIG) [8]. KLIG was expressed in the proximal convoluted tubules, and its expression was highly induced in ureteral obstruction and 5/6 nephrectomy (Fig. 2). KLIG had 32% amino acid identity with monkey hepatitis A virus cellular receptor-1 and 19% identity with mouse CD 28 (a T-cell costimulatory molecule). We were not able to identify its function at all, and we learned that, to discover new and important molecules, we should try other approaches which are more based upon biological phenomena. We deposited the nucleotide and deduced amino acid sequences of KLIG into GenBank in 1997 (accession no. AB009015) and reported its expression in 1998 [8]. Now it appears that mouse KLIG (recently renamed as T-cell immunoglobulin and mucin domain containing 2, Timd2 or TIM-2) is one of the family of molecules of rat kidney injury molecule-1 (KIM-1 or TIM-1) with 60% amino acid identity. Importantly, Ichimura and Nagata et al. independently reported that KIM-1/TIM-1 and TIM-4 are receptors for an eat-me signal (phosphatidyl serine) expressed specifically on the surface of apoptotic cells [9–11].

Cysteine-rich protein 61

Thy-1 glomerulonephritis (GN) is a rat model of glomerular disease which is reversible, to some extent. Sawai et al. hypothesized that podocytes may secrete factors which help resolution of glomerular lesions in Thy-1 GN. Using suppressive subtractive hybridization method, we screened cDNAs which are more abundantly expressed in immortalized mouse podocytes than in normal whole kidneys, and are upregulated during recovery phase of Thy-1 GN. Cysteine-rich protein 61 (Cyr61) was one such molecule [12]. We also found that supernatant of Cyr61-overexpressing COS-7 cells has activity to inhibit platelet-derived growth factor (PDGF)-BB-induced migration of mesangial cells. We could not demonstrate but proposed that Cyr61 might be a podocyte-derived mesangial repellent factor avoiding excessive influx of mesangial cells from the vascular pole into glomeruli in GN [12]. Recently, it was elegantly shown that vascular endothelial growth factor (VEGF)-A secreted by podocytes in adult mice can flow back against glomerular filtration and act upon endothelial cells to maintain normal architecture of glomeruli [13].

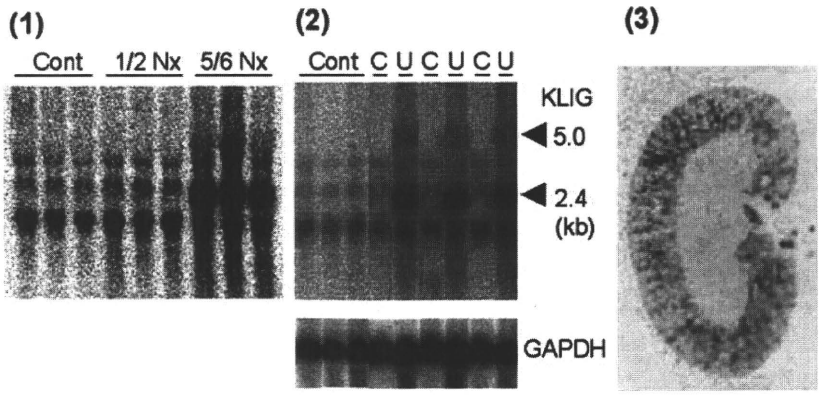

Fig. 2 Upregulation of renal KLIG (or TIM-2) mRNA expression in mouse kidney injury. (*1*) The 5.0- and 2.4-kb transcript of KLIG mRNA was increased at 48 h after 5/6 nephrectomy (*5/6 Nx*), as compared with control (*Cont*) and heminephrectomy (*1/2 Nx*). (*2*) KLIG expression was induced at 7 days after unilateral ureteral obstruction (*U*), as compared with control (*Cont*) and contralateral kidney (*C*). (*3*) KLIG mRNA was expressed in proximal convoluted tubules in normal kidney by in situ hybridization

Neutrophil gelatinase-associated lipocalin in kidney development and acute kidney injury

The mammalian kidney develops through complicated interaction between ureteric bud and metanephric mesenchyme [14]. Barasch et al. established an organ culture system which allows conversion of rat metanephric mesenchyme into nephrons (mesenchymal–epithelial transition, MET) by conditioned media from cultured mouse ureteric bud cells. Leukemia inhibitory factor (LIF) was the first secreted factor purified at the protein level to have such inductive activity [15]. Nephrogenetic activity in mammals is highly redundant and protected, therefore mice lacking gp130 (the common signal transducer for LIF and other interleukin-6 superfamily molecules) exhibit only 50% reduction in nephron numbers. Yang et al. [16] isolated and characterized the second nephron inducer, and found neutrophil gelatinase-associated lipocalin (Ngal). About the same time, Goetz et al. [17] reported that human Ngal protein expressed in *E. coli* is bound to enterochelin, a siderophore synthesized by *E. coli*. Siderophores are iron-binding, organic chemicals produced by bacteria, fungi, and plants to enable efficient collection of iron from the environment [18, 19].

Devarajan et al. revealed that Ngal is one of the most highly upregulated molecules in mouse models of renal ischemia–reperfusion injury [20, 21] and cisplatin nephropathy [20]. Mori et al. [22] reported that Ngal protein accumulates in kidney cortex, blood, and urine in human cases of various types of acute kidney injury (AKI). Mori et al. [22] also elucidated that recombinant Ngal protein has renoprotective activity, which is enhanced by the presence of bacterial siderophore (enterochelin). Mishra and Wagener et al. reported that blood and urinary Ngal levels are elevated within a few hours after cardiopulmonary bypass surgery in children and adults who are

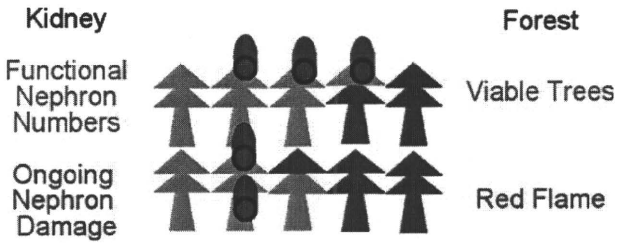

Fig. 3 Forest fire theory for worsening renal function [25]. To evaluate renal injury, we should examine not only functional nephron numbers (results of previous injury, *green*) but also ongoing active damage (*red*). Monitoring of urinary Ngal levels may be helpful for the latter purpose

going to develop AKI, defined by 50% elevation in serum creatinine level [23, 24]. To understand the clear discrepancy between the time course of serum creatinine and blood or urinary Ngal levels, we have proposed that Ngal, at least in part, reflects ongoing kidney injury, whereas serum creatinine, cystatin C, and glomerular filtration rate are indicators of past injury, based on the concept of the "forest fire theory" [25] (Fig. 3). Importantly, Nickolas et al. [26] examined urinary Ngal levels in emergency-department patients at their admission and found that urinary Ngal is very useful in early diagnosis of AKI triggered by various causes, including renal ischemia and nephrotoxins. They also reported that prerenal azotemia (or dehydration) alone does not cause significant elevation in urine Ngal levels.

Ngal in chronic kidney disease

Kuwabara and Kasahara et al. reported that urinary Ngal levels are elevated in mouse models of diabetes mellitus and hypertensive patients having either obesity or diabetes

[27, 28]. Ngal levels were markedly decreased by treatment with angiotensin II receptor blocker. By using labeled Ngal, we have elucidated that reduction of reabsorption efficiency at proximal tubules plays a major role in the appearance of Ngal in the urine of mice having diabetes [27]. A similar observation was reported for albumin excretion in diabetic rats, suggesting that tubular dysfunction may generally play an important role in the elevation of various urinary biomarkers during kidney damage [29, 30]. Urinary Ngal excretion is also increased in patients with nephrotic syndrome or tubulointerstitial nephritis, and it is sharply decreased by steroids and immunosuppressants [27]. These findings suggest that urinary Ngal may be useful for monitoring of disease activity and treatment efficacy in chronic kidney disease (CKD).

Blood or urine Ngal levels are positively correlated to serum creatinine and urinary protein levels [31]. Circulating Ngal levels are elevated not only by kidney injury but also by bacterial infection [32], inflammatory disorders [33], obesity [34], and cancers [35]. Concerning the sources of urinary Ngal during kidney damage, increased synthesis in the kidney, lung, and liver, release from circulating and tissue-infiltrating leukocytes, and impairment of tubular reabsorption all likely make significant contribution [25, 27]. At first glance, lack of disease or tissue specificity may appear a critical weak point of Ngal as a kidney biomarker, but we believe that this merely reflects the fact that the site of synthesis, regulatory mechanism, and metabolic pathway are best characterized for Ngal among various new kidney biomarkers [36].

Biological activity of Ngal

Recombinant mouse Ngal protein expressed in *E. coli* has activities to induce embryonic kidney (metanephric mesenchyme) differentiation (or MET) [16] and kidney protection against ischemic injury [22]. These activities are potentiated in the presence of enterochelin (*E. coli*-derived siderophore) and Fe^{3+} ion [22, 37]. Ngal is expressed in various cancers and the surrounding inflammatory epithelia. Hanai et al. [38] overexpressed Ngal in mammary-gland-derived H-ras-transformed epithelial cells by adenoviral infection or by cDNA transfection and found that Ngal suppresses the degradation of an epithelial marker E-cadherin and inhibits the invasive and metastatic features of the transformed cells (MET). These activities were reproduced by treatment of cells with Ngal–enterochelin–iron complex. The above are Ngal's actions exerted as an iron donor. On the other hand, Lee, Miharada, and Devireddy et al. reported that iron-free Ngal induces apoptosis of microglia, erythroid progenitors, and pro-B cells [39–41]. As demonstrated by Ngal-knockout mice, capturing of pathogen-derived siderophores by Ngal is a crucial component of innate immunity against *E. coli*, *Mycobacterium tuberculosis*, and *Klebsiella pneumoniae* [42–44]. Thus, Ngal is a new prototype iron-binding protein in living organisms whose iron-donating and iron-chelating activities depend on small chemicals which are called siderophores, opening the research field of the biology of siderophore-binding proteins.

Conclusions

Ngal is a unique protein possessing iron-carrying activity and diagnostic and therapeutic utilities for kidney injury, attracting attention from researchers and clinicians in a wide variety of fields of biology.

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REVIEW

Translational research of C-type natriuretic peptide (CNP) into skeletal dysplasias

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Abstract. By using transgenic and knockout mice, we have elucidated that C-type natriuretic peptide (CNP) is a potent stimulator of endochondral bone growth. In humans, loss-of-function mutations in the gene coding for guanylyl cyclase-B (GC-B), the specific receptor for CNP, have been proved to be the cause of acromesomelic dysplasia, type Maroteaux, one form of human skeletal dysplasias. Following these results, we have started to translate the stimulatory effect of CNP on endochondral bone growth into the therapy for patients with skeletal dysplasias. We have shown that targeted overexpression of CNP in cartilage or systemic administration of CNP reverses the impaired skeletal growth of mice model of achondroplasia, the most common form of human skeletal dysplasias.

Key words: C-type natriuretic peptide (CNP), Guanylyl cyclase-B (GC-B), Skeletal dysplasia, Achondroplasia, Translational research

THE NATRIURETIC peptide family consists of three structurally related peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) [1]. The biological actions of natriuretic peptides are mediated by activation of two subtypes of membranous guanylyl cyclase (GC), GC-A and GC-B, followed by intracellular accumulation of cyclic GMP (cGMP) [2]. The rank order of potency to induce cGMP production via GC-A is ANP ≥ BNP >> CNP, while that via GC-B is CNP > ANP ≥ BNP [3]. Therefore, ANP and BNP serve as endogenous ligands for GC-A, whereas CNP is specific for GC-B. A third natriuretic peptide receptor with no intracellular guanylyl cyclase domain, dubbed the clearance receptor (C-receptor), is thought to be engaged in the receptor-mediated degradation of natriuretic peptides [2]. The ANP, BNP/GC-A system plays a pivotal role in the regulation of cardiovascular homeostasis, as demonstrated by their augmentation in various pathophysiological states such as heart failure [4-8], myocardial infarction [9, 10], cardiac hypertrophy [11, 12], and hypertension [13-15]. In fact, ANP and BNP are cardiac hormones secreted primarily by the atrium and ventricle of the heart, respectively [8, 15], with strong diuretic, natriuretic, and vasodilatory activities [4, 5, 8]. ANP and BNP are used in the treatment of heart failure [16, 17] and serve as sensitive biochemical markers for heart failure and cardiac hypertrophy [6-8].

CNP, the third member of natriuretic peptide family, was first purified from porcine brain [18]. While CNP is the primary natriuretic peptide in the human brain [19], it is also produced by vascular endothelial cells [20-22] and macrophages [23], and is thought to act as an autocrine/paracrine regulator and as a neuropeptide [19]. Furthermore, analysis of genetically engineered mice of the CNP/GC-B system revealed that CNP and GC-B play a pivotal role in the regulation of endochondral bone growth.

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I. The growth promoting effect of the CNP/GC-B system on endochondral bone growth

I-1. Skeletal phenotypes of genetically engineered mice of the CNP/GC-B system

We generated mice with a targeted disruption of the CNP gene (*Nppc*); the resultant CNP-KO mice ex-

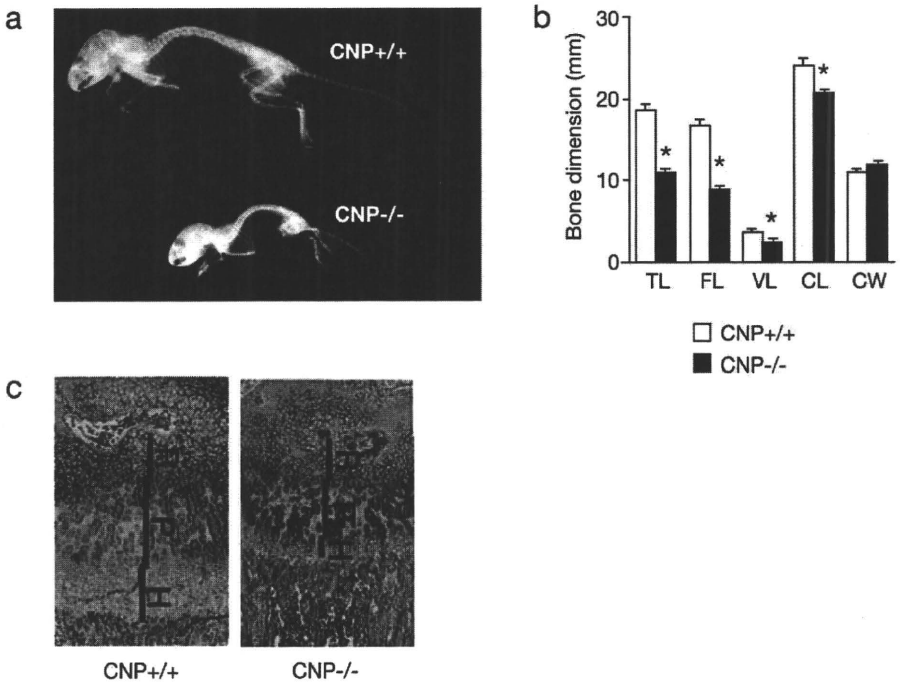

Fig. 1 Impaired skeletal growth observed in CNP-KO mouse. a. Soft x-ray picture of CNP-KO mouse (CNP-/-) compared by that of wild-type mouse (CNP+/+). b. The dimension of each bone from wild-type (CNP+/+) or CNP-KO (CNP-/-) mouse at the age of 10 weeks. TL: tibial length, FL: femoral length, VL: fifth lumber vertebral length, CL: naso-occipital length of the calvarium, CW: maximal interparietal distance of the calvarium. *, $P < 0.05$ *vs.* wild-type mouse. c. Histological analysis of the tibial growth plates from 7-day-old wild-type (CNP+/+) and CNP-KO (CNP-/-) mice. R: resting chondrocyte zone, P: proliferative chondrocyte zone, H: hypertrophic chondrocyte zone.

hibited markedly short stature due to impaired bone growth (Fig. 1) [24]. Mammalian bones are formed through two different mechanisms, endochondral ossification and membranous ossification. Most mammalian bones are formed through endochondral ossification, a process during which chondrocytes in the growth plate undergo proliferation, hypertrophy, cell death, and osteoblastic replacement [25]. The short stature phenotype of CNP-KO mice resulted from impaired bone growth through endochondral ossification [24]. Histological analysis of the growth plate of CNP-KO mice revealed that every chondrocyte layer of the growth plate is narrower in CNP-KO mice than in wild-type mice. Furthermore, mice depleted with the GC-B gene (*Npr2*) exhibit the same short stature phenotype as observed in CNP-KO mice [26], demonstrating that the CNP/GC-B system is a physiologically important stimulator of endochondral bone growth. On the contrary, cartilage specific CNP-transgenic mice under the control of type II collagen promoter (col2-CNP-Tg mice) exhibited prominent overgrowth of bones formed through endochondral ossification (Fig. 2) [27]. In contrast to CNP- or GC-B-KO mice, every chondrocyte layer of the growth plate of col2-CNP-Tg mice was wider than that of wild-type mice. Collectively, the CNP/GC-B system is a potent stimulator of endochondral bone growth.

I-2. The role of other molecules related to the CNP/GC-B system on endochondral bone growth (Fig. 3)

cGMP-dependent protein kinase (cGK) has been identified as a molecule activated downstream of the natriuretic peptide family and guanylyl cyclase system [28]. Mice depleted of one subtype of the cGK gene, cGKII (cGKII-KO mice), exhibit a short stature phenotype secondary to impaired endochondral bone growth [29], similar to that observed in CNP-KO mice [24]. We demonstrated that cGKII affected endochondral bone growth by functioning downstream of the CNP/GC-B system by showing that the impaired endochondral bone growth observed in cGKII-KO mice could not be rescued by targeted overexpression of

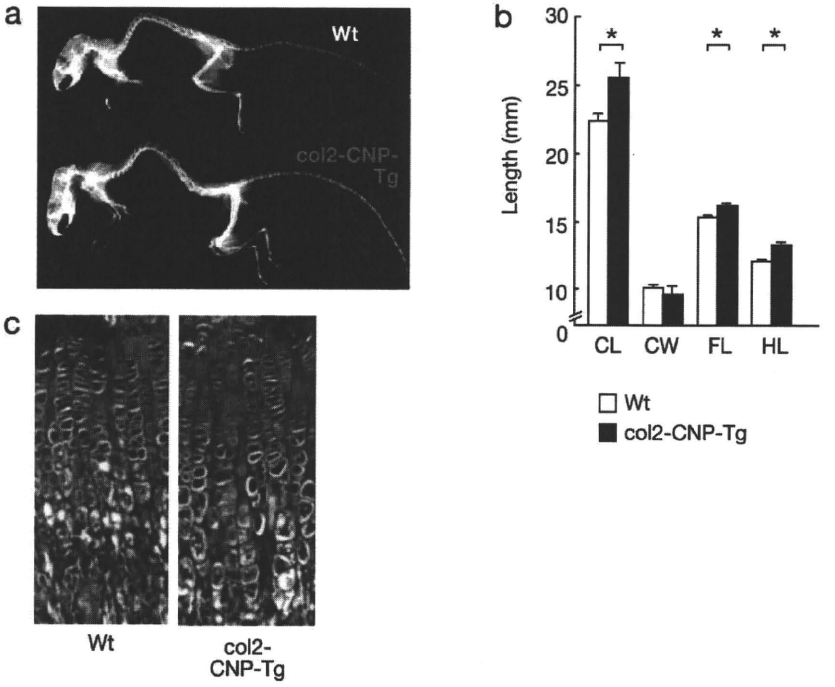

Fig. 2 Skeletal phenotype of col2-CNP-Tg mouse. a. Soft x-ray picture of wild-type (Wt) and col2-CNP-Tg mice. b. The length of each bone from wild-type (Wt) or col2-CNP-Tg mouse. CL: naso-occipital length of the calvarium, CW: maximal interparietal distance of the calvarium. FL: femoral length, HL: Humeral length. *, $P < 0.05$ *vs.* wild-type mouse. c. Histological analysis of the tibial growth plates from 7-day-old wild-type and CNP-KO (CNP-/-) mice.

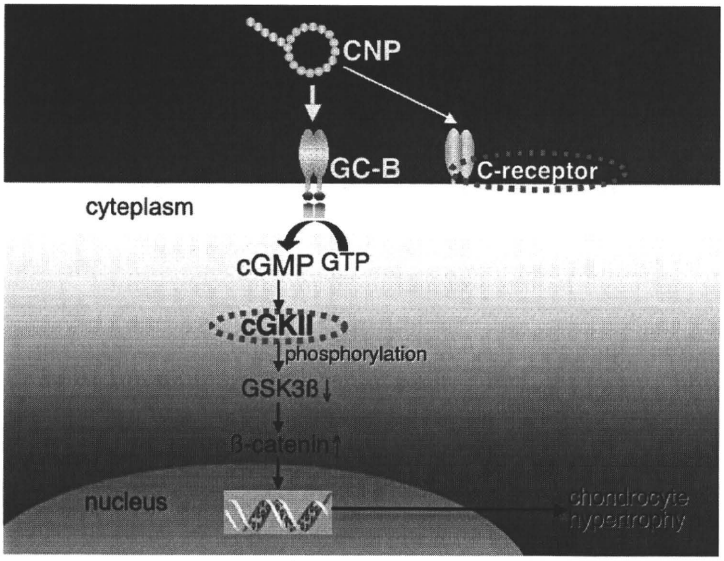

Fig. 3 Schematic representation of the pathway of the CNP/GC-B system.

CNP in the growth plate cartilage [30]. cGKII is reported to phosphorylate and inactivate GSK3β as the downstream molecule, resulting in the increased accumulation and transactivation function of β-catenin followed by hypertrophic differentiation of the growth plate chondrocyte [31].

As previously mentioned, C-receptor is thought to be engaged in the clearance of natriuretic peptide ligands, and mice depleted with C-receptor exhibit skeletal overgrowth phenotype like col2-CNP-Tg mice