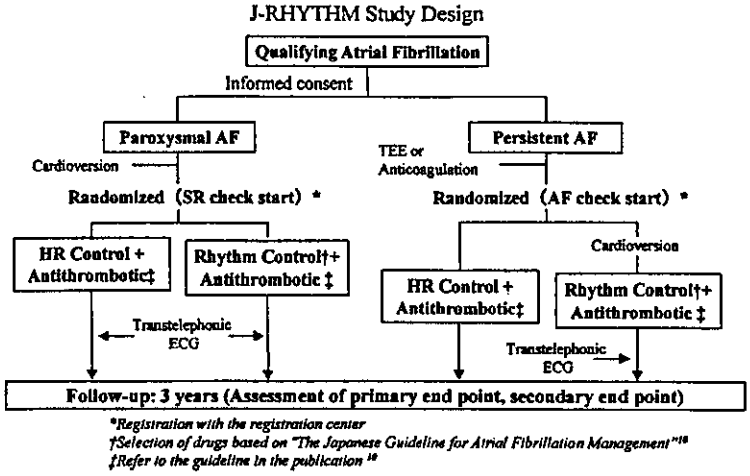

Fig 1. Diagram of the J-RHYTHM study design. Patients with paroxysmal atrial fibrillation (AF) are randomly assigned to treatment groups after sinus rhythm (SR) has been confirmed.

tic strategy. In the present study, for the first time, patients' will to move to the other strategy is also included as an end-point representing patients' disablement under the assigned strategy, because it should be an important factor in clinical decision making and because, as the earlier studies reported, there is no significant difference in mortality between the 2 treatment options[4,5] The most likely causes of patient disablement are uncontrollable symptoms, hesitation in repeating cardioversion, or anxiety about the adverse effects of drugs without any life-threatening consequences, and could not be avoided without movement from one assigned strategy to the other.

The composite secondary end-point of this study is patient QOL scores and the efficacy and safety of drugs required in the AF treatment. In contrast with the AFFIRM and RACE studies[1,4–6] our study will analyze AF-specific QOL as assessed by an original questionnaire and general health-related QOL[9] (vide infra).

Secondary Objective

The secondary objective of the present study is to verify the usefulness of 'The Japanese Guideline for Atrial Fibrillation Management' produced by the Japanese Circulation Society![10]

Study Design

J-RHYTHM is a randomized multicenter comparative study of paroxysmal and persistent AF under treatment by the rhythm control strategy and by the rate control strategy. Paroxysmal AF is defined as AF in which spontaneous conversion to sinus rhythm is expected within less than 48 h of onset, and persistent AF is defined as AF that persists for 48 h or more, and less than 1 year after onset.

After giving informed consent, the patients will be separated and randomly assigned to one of 2 treatment groups: the rate control group, to be treated by heart rate control in combination with antithrombotic therapy, and the rhythm control group, to be treated by rhythm control with antiarrhythmic drugs in combination with antithrombotic therapy. At the time of randomization, investigators will confirm the presence of sinus rhythm in patients with paroxysmal AF (sinus rhythm check start), so that electrical or pharmacological cardioversion can be performed before treatment if necessary, and the presence of AF will be confirmed in patients with persistent AF (AF check start). If they are assigned to the rhythm control group, electrical cardioversion will be performed to recover sinus rhythm and in the event of unsuccessful cardioversion, the patients will be treated by the rate control strategy (Fig 1).

The exclusion criteria are as follows.

(1) Persistent AF lasting 1 year or longer, and permanent AF.
(2) Initial episode of paroxysmal AF.
(3) AF that has occurred within 1 month of the onset of myocardial infarction.
(4) Transient AF associated with cardiac surgery.
(5) Requirement of continuous treatment with β-blockers and Ca antagonists, excluding dihydropyridines, that affect the heart rate.
(6) AF with a history of 2 or more electrical cardioversions.
(7) Contraindication for anticoagulation therapy.
(8) Pregnancy or possibility of pregnancy, and breast feeding.
(9) Judgment by attending physician that patient participation would be inappropriate.

Each patient will read and sign the informed consent form approved by the institution where he or she will be participating in the study.

Baseline Tests

Before randomization, the patients will undergo clinical assessment, which will include patient history with quantification of AF duration, frequency, and predisposing factors, and a physical examination. Specified cardiac tests including electrocardiography, chest X-ray, and echocardiography will be also performed.

The QOL of the patients will be evaluated by a questionnaire comprising general health-related and also AF-specific questions? The general health-related questions are based on a publication commissioned by the Japanese Ministry of Health and Welfare, which covers similar aspects of QOL as the SF-36![11] However, we find that AF affects QOL in specific ways that might not be addressed by general health-related questions and thus should be evaluated by a disease-specific QOL assessment. Therefore, the relevant section of the questionnaire has been written by the Committee on the Arrhythmia-Related QOL of the Japanese Society of Electrocardiology and comprises 26 questions concerning frequency and severity of symptoms, limitation of daily and special activities, and anxiety related to AF itself and its treatment?

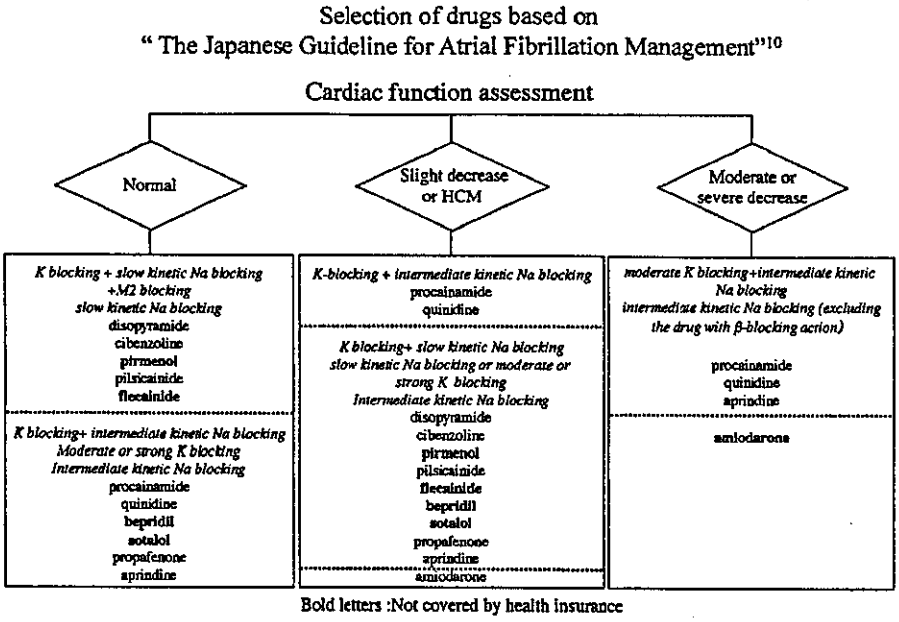

Fig 2. Selection of antiarrhythmic drugs in the rhythm control group based on 'The Japanese Guidelines for Atrial Fibrillation Management' (reproduced with permission from *Jpn Circ J* 2001; **65**(Suppl V): 931–979).

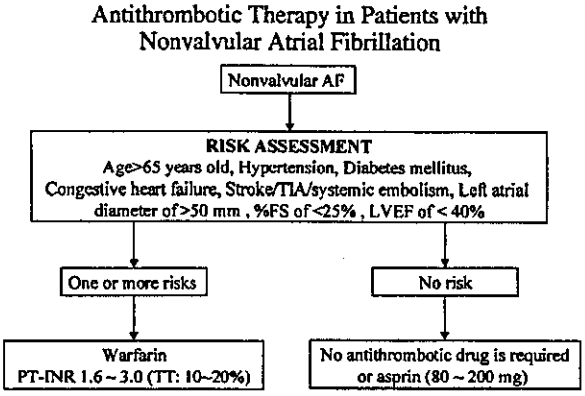

Fig 3. The antithrombotic strategy employed in the J-RHYTHM study is a modification of that used in the AFFIRM study[1,4]

Interventions

Rhythm Control Group The antiarrhythmic drugs will be selected on the basis of 'The Japanese Guideline for Atrial Fibrillation Management' (Fig 2) according to the attending physician's assessment of the patient's cardiac function.[10] Patients with persistent AF who are assigned to the rhythm control group will receive electrical cardioversion with prior administration of antiarrhythmic drugs. If pharmacological cardioversion is successful, the same drugs will be continued thereafter, but if the follow-up examination shows that the selected drugs are ineffective or adverse, either pharmacological or electrical cardioversion will be performed as required before changing the drug regimen. The drug dosages will be determined by the attending physician with consideration of the patient's history including age, renal and hepatic function, and underlying heart diseases.

Heart Rate Control Group As in the AFFIRM study, the therapeutic purpose in this group is the control of the heart rate itself rather than of the doses of drugs administered.[1,4] The target heart rate is 60–80 beats/min at rest. Patients will receive digitalis, Ca antagonists (excluding bepridil), or β-blockers as required, selected on the basis of the patient's clinical background. The selection of drugs and their dosages will be adjusted as necessary.

Antithrombotic Therapy In the present study, different antithrombotic treatment strategies will be used for patients with nonvalvular AF and those with valvular AF. Patients with nonvalvular AF will receive a modified form of the treatment used in the AFFIRM study[1,4] (Fig 3), and patients with valvular AF will be treated according to the published guidelines.[10] Patients with nonvalvular AF will be assessed for the risk of stroke using the following factors: age ≥65 years, hypertension, diabetes mellitus, congestive heart failure, history of stroke/transient ischemic attack/systemic embolism, left atrial diameter >50 mm, fractional shortening <25%, or ejection fraction <40%. In patients with one or more of these factors, warfarin will be administered to maintain a PT-INR (prothrombin time-international normalized ratio) between 1.6 and 3.0. In patients without risk factors, either no antithrombotic treatment or aspirin at a dose of 80–200 mg/day will be administered. The antithrombotic treatment in the J-RHYTHM study differs substantially from that in the AFFIRM study:[1,4] patients with one or more risks will receive continued anticoagulation therapy even if sinus rhythm appears to be maintained by rhythm control. Another difference from the AFFIRM study is maintenance of low intensity (PT-INR 1.6–2.0) anticoagulation therapy possible because of the bleeding tendency in Japanese patients undergoing warfarin treatment.[12] Anticoagulation therapy for 3 weeks before defibrillation of persistent AF that has continued for 48 h or more, will be mandatory in this study if transesophageal echocardiography is unavailable.[13]

Patient Follow-up

After randomization, every effort will be made to maintain the patients' original group assignments. Heart rhythm

(presence or absence of AF, duration and frequency of AF episodes, classification as paroxysmal or persistent AF), electrocardiographic findings, blood pressure, heart rate, cardiac function, and QOL will be assessed at 1, 3, and 6 months after initiation of treatment and every 6 months thereafter. In selected institutions, transtelephonic electrocardiograms will be recorded daily and if any symptoms occur during the first 1 month after the initiation of treatment. All cardiac and extracardiac events, including the primary end-point and any drug-induced adverse events, will be investigated during the 3-year follow-up period.

Data Analyses and Sample Size

The primary analysis will be an unadjusted intention-to-treat comparison between groups of time to any part of the composite primary end-point using the Kaplan-Meier method and log-lank test. Secondary end-point questionnaire results will be collected for each group, and absolute values for each question will be used to calculate the mean value, SD, number of cases, and median value (as required) per question per group. The inter-group difference will be assayed at each measurement point question by unpaired Student's t-test or Mann-Whitney's U test. Differences between groups and over time in the absolute values of measurement points will be investigated by repeated ANOVA. Items that cannot be appropriately assessed and analyzed by absolute values will be subjected to separate analysis by the most appropriate method. Patient background factors and other observation items will be aggregated by group, and any inter-group differences will be analyzed by methods corresponding to the nature of the data.

The target number of cases (2,600) has been established on the basis of our estimate of the primary end-point incidence (the projected incidence of rate control-related events during the study period is estimated as 15% and the expected event decrease rate in the rhythm control group as 30%) and with reference to the measurement method established by Freedman et al.[14,15]

Expected Implications

The J-RHYTHM study will emphasize both mortality and physical/psychological disablement of all types of AF. The study design makes the best use of the AFFIRM and RACE results[4,5] and focuses on the safety of antiarrhythmics in response to the anxiety about drug-induced adverse effects that is frequently seen in Japanese patients. Attention to these points of emphasis is expected to improve patient mortality. Moreover, the J-RHYTHM study moves beyond the previous trials to improve the QOL of AF patients, and thus will support optimal medical care for AF patients in the clinical settings. Simultaneously, the study will generate the first large database of Japanese AF patients, which can be used for subgroup analyses that could provide further information to improve AF therapy.

Acknowledgments

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ORIGINAL ARTICLE

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Interim evidence of the renoprotective effect of the angiotensin II receptor antagonist losartan versus the calcium channel blocker amlodipine in patients with chronic kidney disease and hypertension: a report of the Japanese Losartan Therapy Intended for Global Renal Protection in Hypertensive Patients (JLIGHT) Study

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Abstract

Background. Insufficiency of renal function and high blood pressure influence each other and eventually result in life-threatening endstage renal disease. It has been proposed that proteinuria per se is a determinant of the progression of chronic kidney disease (CKD). The therapeutic strategy for patients with proteinuric CKD and hypertension should therefore be targeted with a view not merely toward blood pressure reduction but also toward renoprotection.

Methods. We examined the effect of the angiotensin $(AT)_1$ receptor antagonist losartan and the calcium channel blocker amlodipine, throughout a period of 12 months, on reduction of blood pressure and renoprotection. This was done by assessing amounts of urinary protein excretion, serum creatinine (SCr), and creatinine clearance (CCr) in patients with hypertension (systolic blood pressure [SBP] ≧ 140 mmHg or diastolic blood pressure [DBP] ≧ 90 mmHg) and CKD (male, body weight [BW] ≧ 60 kg: 1.5 ≦ SCr < 3.0 mg/dl; female or male BW < 60 kg: 1.3 ≦ SCr < 3.0 mg/dl), manifesting proteinuria of 0.5 g or more/day. Losartan was administered once daily at doses of 25 to 100 mg/day, and amlodipine was given once daily at 2.5 to 5 mg/day. No

antihypertensive combination therapy was allowed during the first 3-month period.

Results. A 3-month interim analysis revealed that, despite there being no difference in blood pressure between the two groups, there was a significant reduction in 24-h urinary protein excretion in the losartan group ($n = 43$), but there was no change in the amlodipine group ($n = 43$). Analysis of stratified subgroups with proteinuria of 2g or more/day and less than 2g/day showed that losartan lowered proteinuria by approximately 24% in both subgroups, while amlodipine lowered proteinuria by 10%, but only in the subgroup of less than 2g/day (NS). SCr and CCr did not change throughout the period of 3 months in either group. No severe or fatal adverse event was experienced in either group during the study period.

Conclusions. Losartan appeared to be efficacious for renoprotection in patients with proteinuric CKD and hypertension, with the mechanism being independent of its antihypertensive action.

Key words Losartan · Amlodipine · Proteinuria · Kidney · Creatinine · Angiotensin · Hypertension

Introduction

Evolution of research of the renin angiotensin system (RAS) has provided a great deal of evidence covering fields from molecular biology to clinical medicine. Based on experimental and clinical evidence of the effects of angiotensin II on cardiovascular physiology, hypertension and related cardiovascular diseases have been the most important targets of research. The crucial roles of RAS in the pathophysiology of such diseases and the therapeutic benefits of pharmacological intervention in the RAS have now been extensively documented.[1,2] On the other hand, although the direct actions of angiotensin II in hemodynamic and nonhemodynamic aspects of renal physiology are well established, and there is evidence of the close relationship between high blood pressure and renal disease, the role of angiotensin II in the pathophysiology of many types of renal disease is still not clearly explained, because the features of these diseases are complex and evidence of how and to what extent the RAS is involved is still limited.

Renal failure, or renal insufficiency, is known to be a life-threatening disease, especially when the disease shows acute or chronic progression. In this disease, proteinuria per se plays a key role in the progression,[3–6] eventually leading to endstage renal disease (ESRD). Diabetic nephropathy is considered to be responsible for many causative diseases of ESRD worldwide; however, nondiabetic chronic renal diseases also lead to ESRD.

Renal insufficiency is, in a large majority of cases, accompanied by high blood pressure. High blood pressure is a factor leading to renal injury, and conversely, renal insufficiency can cause hypertension. Thus, the two critical factors act synergistically to cause deterioration of the kidney disease toward the terminal stage.

Compelling arguments have been made that aggressive control of blood pressure is important to prevent the progression of kidney disease to ESRD.[7,8] Based on this concept, antihypertensive agents have been widely used to treat patients with kidney disease and hypertension. It has been conjectured that antihypertensive agents improve systemic and renal hemodynamics, and prevent glomerular protein leakage through a reduction of high filtration pressure.

However, whether the blood-pressure lowering effect of antihypertensive agents shows parallelism with the renoprotective effect is still controversial. In this view, current evidence of the involvement of angiotensin II in the pathophysiology of renal disease has led to an interest in comparing the effect of intervention in the RAS with the effect of other conventional antihypertensive agents. Although several clinical trials comparing the effects of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor antagonists (AIIAs) with β-blockers or calcium channel blockers (CCBs) in patients with renal disease and hypertension have been reported,[8–10] only limited information has been available about the potential renoprotective efficacy of AIIAs in Japanese patients with chronic kidney disease (CKD) and hypertension. The present clinical trial therefore aimed to elucidate the effect of the AIIA losartan on renoprotection, comparing it with the effect of the dihydropyridine CCB amlodipine in Japanese patients with CKD manifesting proteinuria and hypertension. The study protocol was designed to pursue the effect of losartan and amlodipine for 12 months, with interim analysis at 3 months and follow-up analysis at 12 months. We herein report the result of the interim analysis at 3 months, because the amelioration of proteinuria was achieved by losartan at this point of time.

Methods

Patients

Patients, men and women, aged ≧20 to <75 years, who had CKD and hypertension were eligible for the study, if they satisfied the following criteria during the pretreatment screening period of 8 weeks:

(a) CKD; serum creatinine (SCr) levels were $1.5 \leqq SCr < 3.0$ mg/dl in men of body weight (BW) 60kg or more, and $1.3 \leqq SCr < 3.0$ mg/dl in females or males of BW less than 60kg.
(b) Hypertension; systolic (SBP) and diastolic (DBP) blood pressures in a sitting position measured at least two times at their visits to clinics were SBP, 140mmHg or more or DBP, 90mmHg or more.
(c) Proteinuria; urinary protein excretion was 0.5g or more/day.

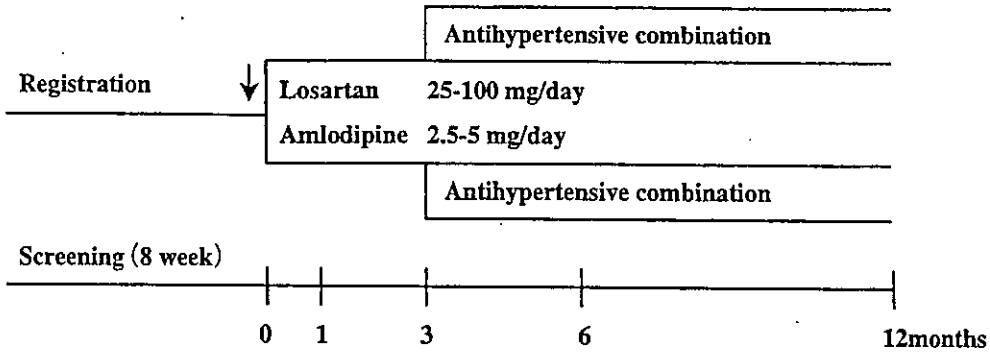


Fig. 1. Study design for treatment of patients with proteinuric chronic kidney disease (CKD) and hypertension. Antihypertensive combination therapy was allowed after the first 3-month period, if necessary (see text)

Study design and clinical endpoints

The overview of the study design is shown in Fig. 1. This study was a randomized parallel-group open-labeled trial with the two drugs. The randomization method was modified by dynamic balancing for SCr, by 24-h urinary protein excretion measured at the time of registration, and by allocating patients with or without diabetic nephropathy so that patients were allocated to the two groups to avoid significant differences. After the screening period, patients in the two groups received either losartan 25 mg as a starting dose, up to 100 mg once daily, or amlodipine 2.5 mg as a starting dose, up to 5 mg once daily. However, if the patients' compliance was considered to be fair to receive higher doses, either 50 mg of losartan or 5 mg of amlodipine was adopted as a starting dose. During the first 3 months, the effects of blood pressure were targeted at SBP less than 130 mmHg and DBP less than 85 mmHg, and patients were not allowed combination therapy with any other antihypertensive agents. However, after 3 months, if the blood pressure did not reach SBP less than 130 mmHg and DBP less than 85 mmHg, antihypertensive combination therapy with α-blockers, β-blockers, α/β-blockers, diuretics (except for potassium-sparing diuretics), and other CCBs was considered to be adopted. Guidance was given to patients to maintain their usual diet, especially for those under dietary restrictions. The study protocol was reviewed and approved by the Institutional Review Boards of 35 affiliated institutions at which patients' enrollment was established for this study. Written informed consents were obtained from the enrolled patients. Exclusion criteria were as follows:

(a) Diastolic blood pressure (DBP, ≧120 mmHg)
(b) Renovascular hypertension and endocrine hypertension
(c) Blood pressure control treatment with antihypertensive agent(s)
(d) Any patients in whom antianxiety drugs could not be discontinued
(e) Pregnancy, possibility of pregnancy, and in a period of lactation
(f) Patients that the chief investigator judged not to be eligible.

Assay parameters

Blood pressure, with the patient in a sitting position, was measured at patients' visit to the clinic.

A 24-h urine collection was performed from 8:00 AM of one day before to 8:00 AM of the day of the clinic visit, to obtain the 24-h urine volume, urinary protein excretion, and the urinary creatinine level, as well as the amount of sodium excretion. The creatinine clearance (CCr) was calculated by the following formula: $CCr = Ucr \times V / SCr \times 1.73/A$, where CCr is creatinine clearance (ml/min); UCr is urinary creatinine (mg/dl), V is urine volume (ml/min); SCr is serum creatinine (mg/dl); and A is body surface area.

Urinary protein, UCr, and SCr levels were determined by a standard method at each center. The magnitude of renal impairment was expressed by the reciprocal of SCr (1/SCr).

Protein and sodium chloride (NaCl) intakes were estimated by measurements of urea nitrogen plus protein concentrations, and NaCl concentrations in the collected urine, respectively, by the following formulas:

Protein intake (g/day)[11]
= [urinary urea nitrogen (g/day) + 0.031(g) × BW (kg)] × 6.25 + urinary protein excretion (g/day)
NaCl intake (g/day)
= urinary sodium excretion (mEq/day)/17.

Statistics

All values were expressed as means ± SD. The baseline characteristics of the enrolled patients were tested for comparability between the losartan group and the amlodipine group, using unpaired *t*-test or Fisher's exact test. The difference in SBP and DBP changes between the losartan group and the amlodipine group was tested by a repeated-measures analysis of covariance with treatment effect, period effect, and center effect. Changes in urinary protein excretion, SCr, and CCr within each group were analyzed by paired *t*-test. Unpaired *t*-test was used to compare the percent changes of urinary protein excretion, SCr, and CCr between the losartan group and the amlodipine group. *P* values of less than 0.05 were considered statistically significant.

Table 1. Baseline characteristics of patients enrolled in the study

	Losartan group (n = 47)	Amlodipine group (n = 46)	P value
Age (years)	56.0 ± 14.3	57.4 ± 11.7	NS[a]
Male/female	25/22	35/11	$P < 0.05$[b]
BMI (kg/m^2)	24.1 ± 3.9	22.8 ± 3.4	NS[a]
Systolic BP (mmHg)	155.4 ± 10.7	156.1 ± 14.4	NS[a]
Diastolic BP (mmHg)	92.8 ± 8.6	93.6 ± 7.9	NS[a]
Serum creatinine (mg/dl)	2.01 ± 0.51	1.99 ± 0.51	NS[a]
Urinary protein (g/day)	2.64 ± 2.61	2.79 ± 3.72	NS[a]
Serum albumin (g/dl)	3.78 ± 0.47[c]	3.78 ± 0.49[c]	NS[a]
Diagnoses (no. of patients)			
Chronic glomerulonephritis (IgA nephropathy)	27 (8)	30 (12)	
Diabetic nephropathy	7	6	
Hypertensive nephrosclerosis	9	8	
Tubulointerstitial nephritis	2	0	
Polycystic kidney desease	1	0	
Preeclampsia	1	0	
Renal amyloidosis	0	1	
Castleman's disease	0	1	

Mean ± SD
BMI, body mass index; BP, blood pressure; NS, not significant
[a]Unpaired t-text
[b]Fisher's exact test
[c]n = 44 in each group

Results

A total of 93 patients were enrolled for the present study during the period from December 1999 to September 2001 – 47 in the losartan group and 46 in the amlodipine group. Table 1 shows the baseline characteristics of patients enrolled in the study. The characteristics of the two randomized groups were similar. The dietary compliance assessment by measurements of 24-h urinary urea nitrogen plus protein and sodium showed that there was no significant difference in total protein and NaCl intake between the two groups, nor were there any differences in the values between the baseline and the 3-month values (protein intake [g/day], losartan, 53.71 ± 17.35 [baseline], 52.76 ± 15.69 [3 months]; amlodipine, 53.30 ± 16.48 [baseline], 55.64 ± 20.23 [3 months]; NaCl (g/day), losartan, 7.85 ± 3.37 [baseline], 7.69 ± 3.62 [3 months]; amlodipine, 9.62 ± 4.91 [baseline], 9.16 ± 5.08 [3 months]). At the time point of the 3-month analysis, 43 patients in the losartan group and 43 in the amlodipine group were available for analysis of the measured parameters and their corresponding statistics. Of the total of 93 patients recruited for the study, 86 patients (43 patients each for the losartan group and the amlodipine group) were available for measurement of the urinary protein endpoint.

The blood-pressure-lowering effect, for both SBP and DBP, was similar with losartan and amlodipine. Figure 2 shows changes in SBP and DBP, during treatment for 3 months with losartan and amlodipine. In the losartan group, SBP was reduced from 155.4 ± 10.7 mmHg at baseline to 141.0 ± 16.2 mmHg at 3 months (−8.9 ± 8.7%), and DBP was reduced from 92.8 ± 8.6 mmHg at baseline to 84.3 ± 8.6 mmHg at 3 months (−9.1 ± 9.4 %). In the amlodipine group, the reduction in SBP was from 156.1 ± 14.4 mmHg at baseline to 137.6 ± 13.9 mmHg at 3 months (−11.1 ± 8.8%), and DBP was reduced from 93.6 ± 7.9 mmHg at baseline to 83.1 ± 8.1 mmHg at 3 months (−11.3 ± 9.2 %).

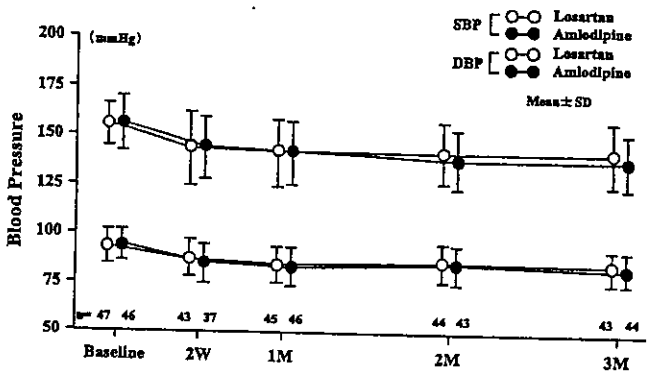


Fig. 2. Systolic blood pressure (*SBP*) and diastolic blood pressure (*DBP*) changes during 3 months in groups treated with losartan and amlodipine. *W*, weeks; *M*, months

However, proteinuria was reduced more with losartan than with amlodipine. As shown in Fig. 3a, the urinary protein excretion in a large number of individuals in the losartan group showed a rightward decline, resulting in a significant decrease in the average protein excretion from the baseline to the 3 months, while in the amlodipine group individuals showed dispersion. As a result, as shown in Fig. 3b, the mean percent change of urinary protein excretion from the baseline was evident in the losartan group, but there was no statistically significant change in the amlodipine group.

In order to examine whether the severity of proteinuria affected the result of treatment with losartan and

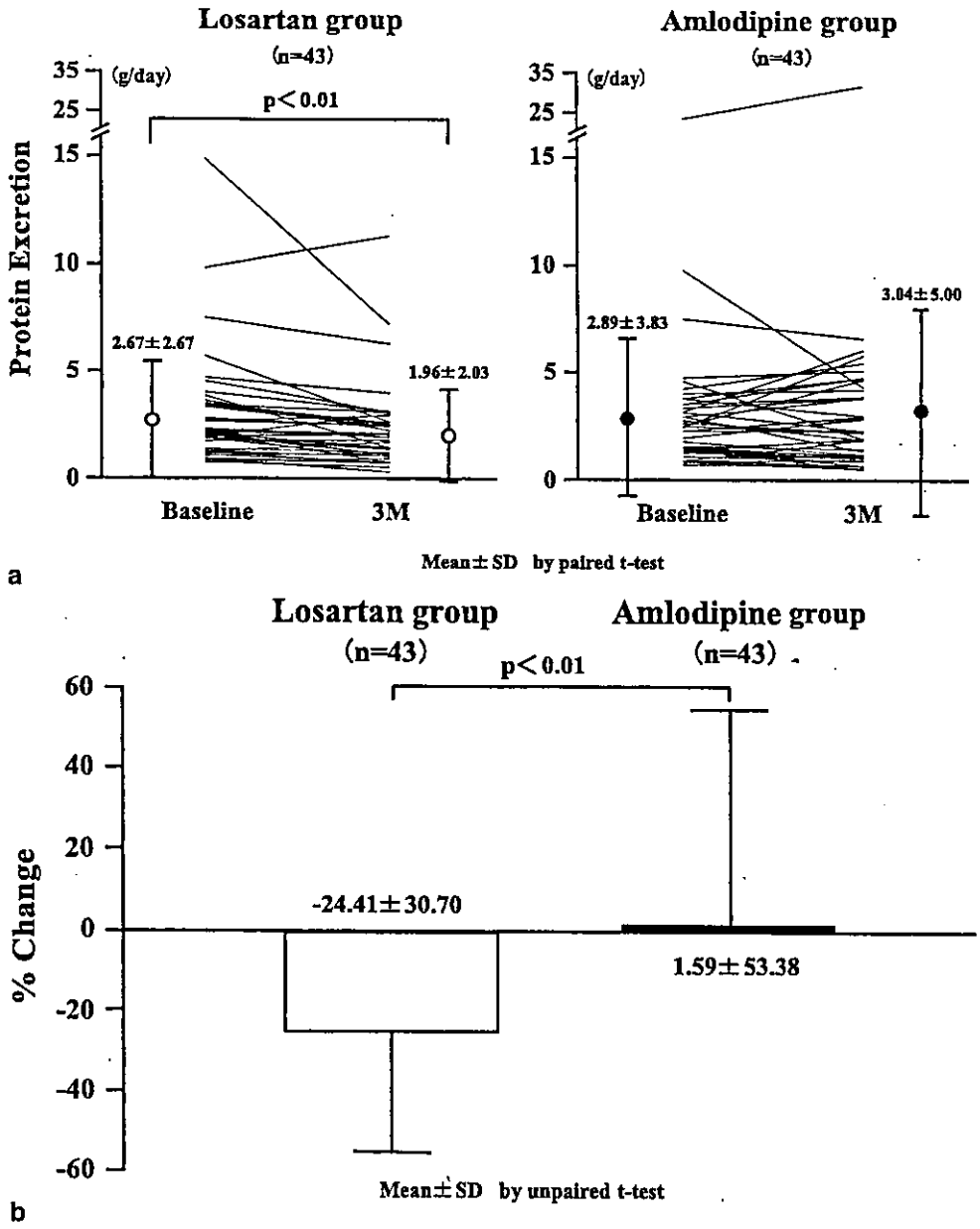


Fig. 3a,b. Changes in 24-h urinary protein excretion from baseline to 3 months after initiation of treatment with losartan and amlodipine. **a** Values for protein excretion in individuals in the respective groups. **b** Percent changes compared with the respective baseline values

amlodipine, we stratified patients into two subgroups, those with proteinuria less than 1 g/day and those with 1 g or more /day at baseline. In these subgroups, the change in urinary protein excretion from baseline to 3 months was not statistically significant between the losartan group and the amlodipine group. We next stratified patients with levels of less than 2 g/day and 2 g or more/day at baseline. As shown in Fig. 4, the reduction in urinary protein excretion at 3 months was evident in the losartan subgroups of both less than 2 g/day and 2 g or more/day, while at 3 months, amlodipine did not significantly reduce urinary protein excretion in either subgroup of less than 2 g/day or 2 g or more/day. The percent change in urinary protein excretion showed a significant difference between the losartan group and the amlodipine group of 2 g or more/day, but there was no significant difference between the subgroups of less than 2 g/day.

By diagnosis, 26 patients in the losartan group and 28 in the amlodipine group had chronic glomerulonephritis, and only 6 in each group had diabetic nephropathy. Analysis of the proteinuria subgroups of the 6 patients with diabetic nephropathy showed that there was a slight decrease in urinary protein excretion at the 3 months in both the losartan and amlodipine groups, but there was no significant difference between the two groups (data not shown). Analysis of the proteinuria subgroups in the patients with chronic glomerulonephritis showed that proteinuria was significantly inhibited in the losartan group during the 3 months, while there was no change in the urinary protein excretion in the amlodipine group, resulting in a significant difference between the two groups in terms of the parameter of percent change (Fig. 5).

Figure 6 illustrates changes in SCr and 1/SCr as a function of time elapsed. There was no significant difference in

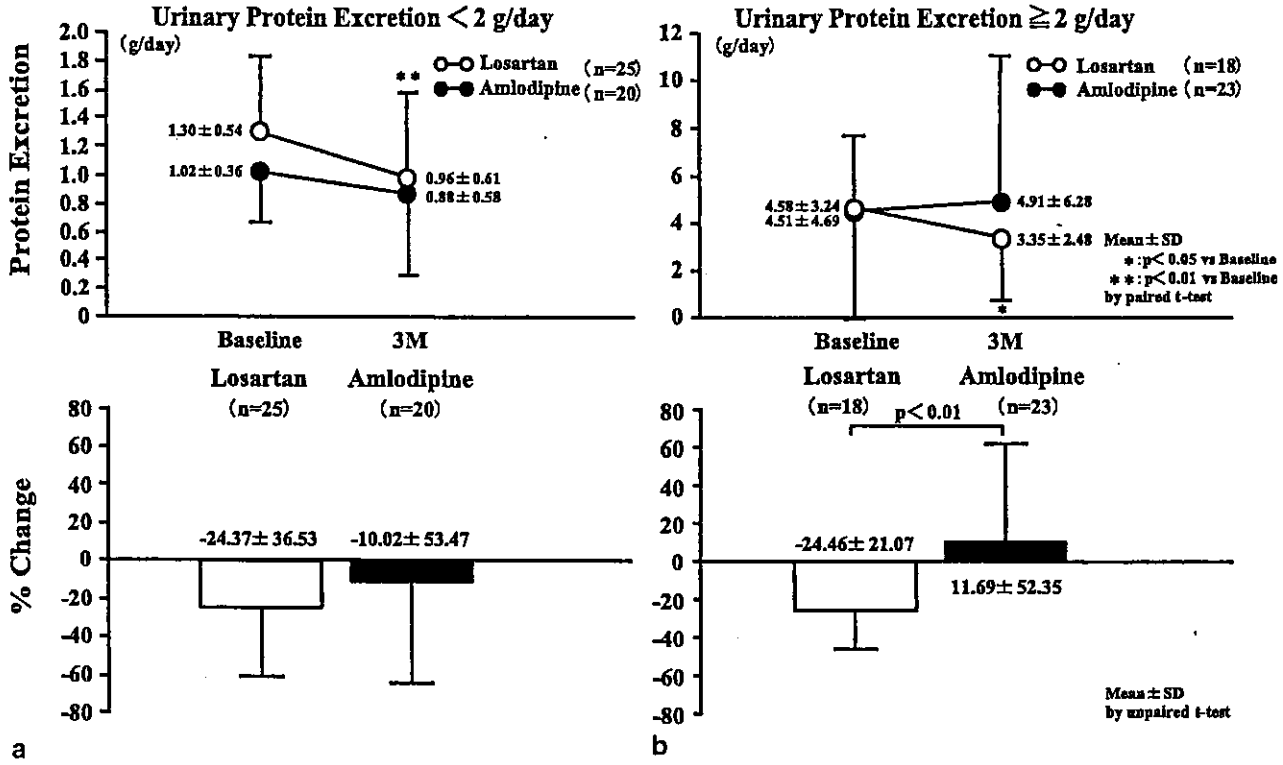
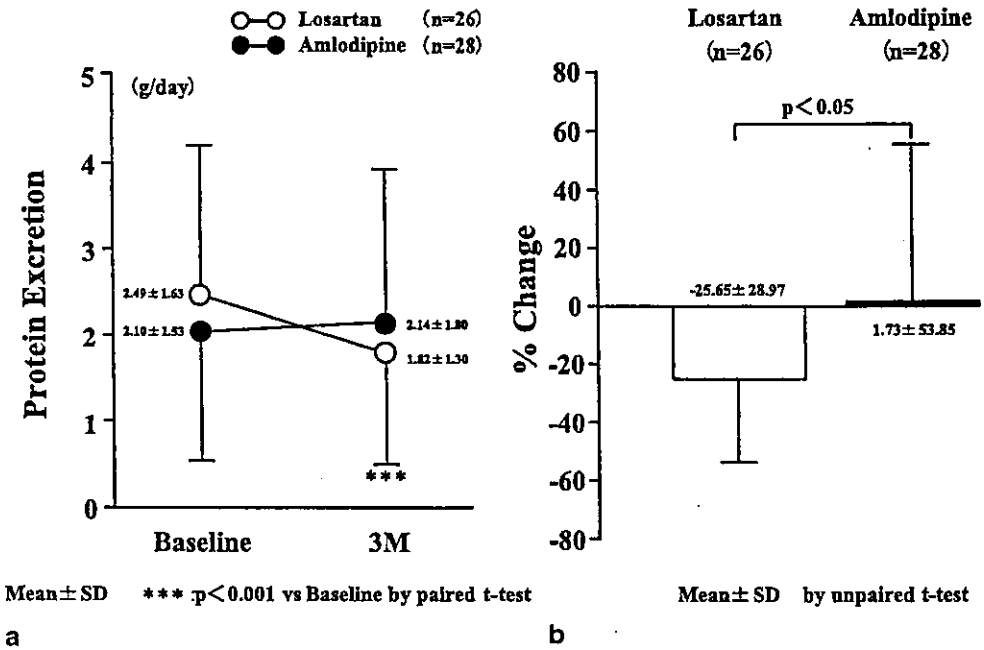


Fig. 4a,b. Changes in 24-h urinary protein excretion from baseline to 3 months after initiation of treatment in the losartan and amlodipine subgroups, showing baseline urinary protein excretion of less than 2 g/day (a) and 2 g or more/day (b). *Upper panels* Mean values of urinary protein excretion. $*P < 0.05$ vs baseline; $**P < 0.01$ vs baseline. *Lower panels*, Percent changes compared with respective baselines values

Fig. 5a,b. Changes in 24-h urinary protein excretion in patients with chronic glomerulonephritis from baseline to 3 months after initiation of treatment with losartan and amlodipine. Note that there was no statistically significant difference in baseline protein excretion between the two groups. **a** Mean values of urinary protein excretion. $***P < 0.001$ vs baseline. **b** Percent change in protein excretion compared with the baseline values of the respective groups



the values of SCr and 1/SCr between the two groups, although SCr slightly increased from the baseline to the 3 months in both groups. Also, CCr showed no significant difference between the two groups, either for the values or for percent change (Fig. 7).

Adverse experiences that were considered by the investigators to be possibly related to the study were reported for increases in aspartate aminotransferase (AST; GOT) (n = 2), alanine aminotransferase (ALT; GPT) (n = 1), and γ-guanosine triphosphate (GTP) (n = 4). These experiences

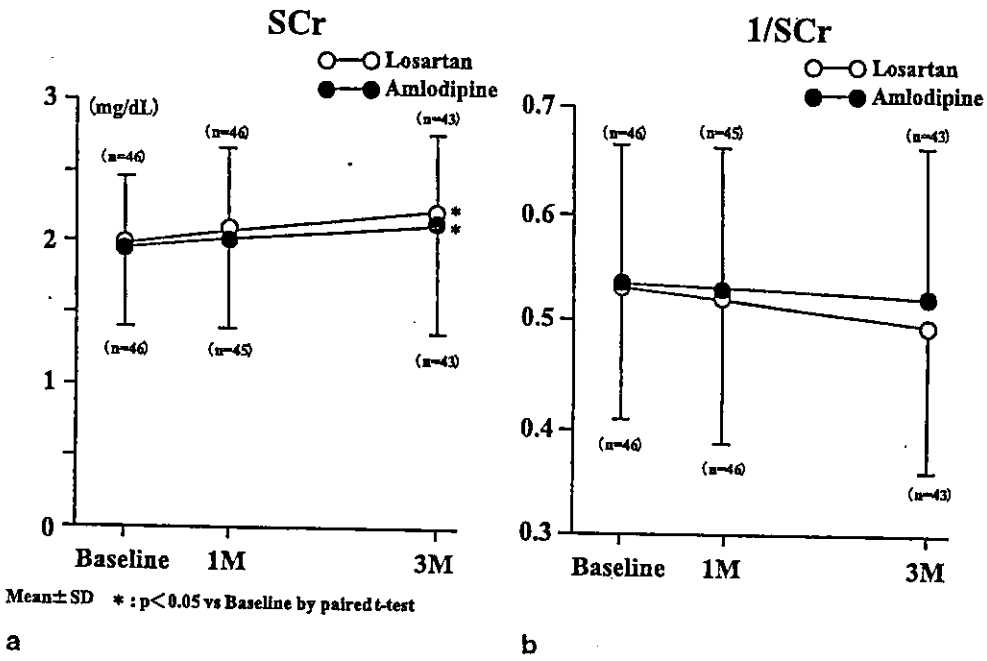


Fig. 6a,b. Changes in serum creatinine levels during 3 months in the losartan and amlodipine groups. *$P < 0.05$ vs baseline. **a** Serum creatinine levels expressed as mg/dl (*SCr*). **b** Values expressed as reciprocal of serum creatinine (*1/SCr*)

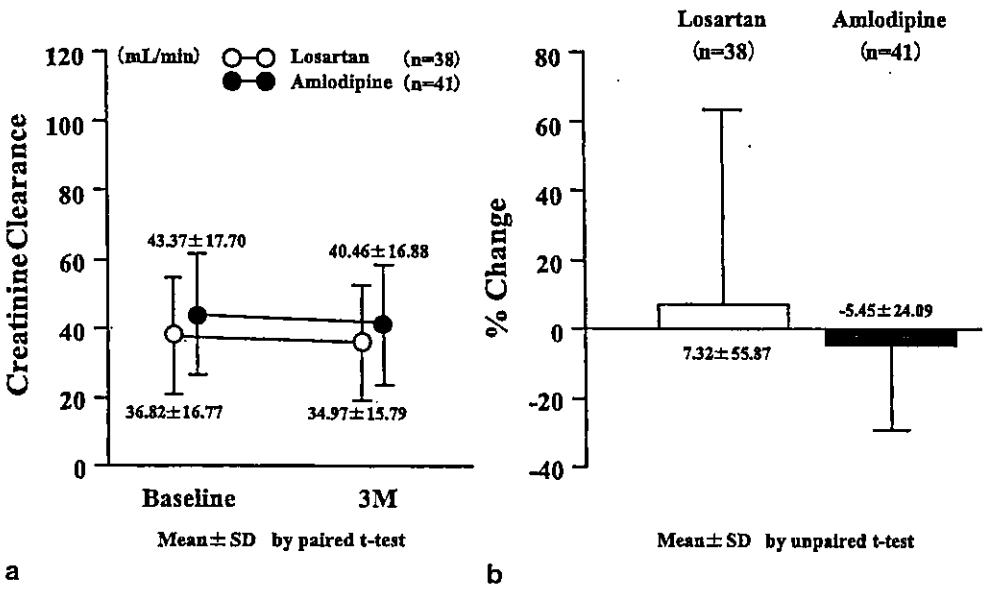


Fig. 7a,b. Changes in creatinine clearance from baseline to the 3 months after initiation of treatment with losartan and amlodipine. **a** Creatinine clearance, in ml/min (CCr). **b** Percent changes in CCr compared with the baseline values of the respective groups

were mild and the incidence was almost the same in the losartan group and the amlodipine group. Hyperkalemia was reported in three patients in the losartan group (5.1, 6.1, and 6.9 mEq/l at 3 months) and in two patients in the amlodipine group (5.9 and 5.2 mEq/l at 3 months). Dizziness ($n = 2$) and transient ischemic attack ($n = 1$) in the losartan group, and an increase in serum uric acid ($n = 2$) in the amlodipine group were reported. No severe or fatal adverse events were observed in either of the groups during the period of 3 months.

Discussion

The present report, although it is the result of the 3-month interim analysis of a total 12-month clinical trial, provides the first evidence that the AIIA losartan is more effective than the CCB amlodipine to ameliorate proteinuria in Japanese patients with proteinuric CKD and hypertension. The difference in the urinary protein-sparing effect between the two drugs was more prominent in patients whose baseline proteinuria was 2 g or more/day. It is noteworthy that the two drugs exerted the same magnitude of systolic and diastolic blood-pressure-lowering effects. Of the 47 patients in the losartan group, 22 were females, while in the amlodipine

group, 11 of the 46 patients were females. It was considered that, in females, a sex hormone such as estrogen per se might act for renal protection through its anti-atherosclerotic effect and other cardiovascular effects; however, a large majority of female patients in the losartan group were over 50 years old (54–59 years, 4; in their 60s, 9; in her 70s, 1), suggesting the presence of menopause. Although there was a possibility that estrogen level in these patients was not negligible, estrogen was not considered to play a protective role against glomerular sclerosis and mesangial proliferation, which are major structural changes associated with CKD-related proteinuria. Thus, it was unlikely that the larger number of female patients in the losartan group gave a bias to the result. Notwithstanding this, we anticipate that, in the final analysis at 12 months, we will pursue the results of female patients separately from those of male patients.

There is controversy regarding the comparative evaluation of various antihypertensive agents such as AIIAs, ACEIs, CCBs, and β-blockers with respect to their potential renoprotective efficacy. Among these agents, the effects of ACEIs as renal protective agents have been widely documented.[12] The renoprotective effect of ACEIs is not exerted solely in diabetic nephropathy, as nondiabetic chronic renal failure was demonstrated to be capable of being treated with ACEIs.[13–16] Many antihypertensive agents have been considered to be effective in patients with renal impairment, most of which were for patients with diabetic nephropathy, given with the expectation of reducing systemic or intraglomerular high blood pressure. In 1998, a result for the clinical trial of the United Kingdom Prospective Diabetes Study Group (UKPDS)[8] was published, which concluded that, in 1148 hypertensive patients with type 2 diabetes, the blood-pressure-lowering effect with captopril and atenolol was similar, and the two drugs also exerted similar effects in reducing the risk of macrovascular and microvascular complications related to this type of diabetes. However, there is a question as to whether all antihypertensive agents exert similar clinicals effect only by reducing systemic or intraglomerular blood pressure.

The RAS is now well understood to be involved in the pathogenesis of renal impairment independently of its vasoconstrictive actions, inducing disturbance of glomerular and tubular functions. The direct actions of angiotensin II in the kidney include an increase in tubular sodium reabsorption and an influence on glomerular filtration rate (GFR), but morphopathological changes such as accumulation of extracellular matrix and mesangial cell proliferation and hypertrophy[17,18] are of more importance for the pathogenesis of renal impairment. Therefore, the question has arisen as to whether pharmacological intervention in the RAS confers an additional benefit beyond the lowering of blood pressure. Based on this view, clinical studies to test the therapeutic effects of drugs that interfere with the actions of angiotensin II, in terms of the direct endpoints of renal function, such as serum creatinine (SCr), creatinine clearance (CCr), and proteinuria, and the ultimate goal, ESRD, have been conducted in patients with diabetic nephropathy. In 2001, the results of two studies to evaluate the clinical effects of AIIA in patients with type 2 diabetes were published, one of which evaluated losartan,[19] and the other, irbesartan.[20] The study with losartan clearly demonstrated that the drug treatment reduced the incidence of the doubling of the SCr level and decreased the amount of urinary protein excretion, and reduced the incidence of ESRD. The study with irbesartan also showed a similar effect for renoprotection by reducing the incidence of severe proteinuria. These two studies clearly demonstrated the advantage of angiotensin II receptor blockade in patients with type 2 diabetes, but no clinical evidence has yet been provided for the effect of angiotensin II receptor blockade in patients with nondiabetic renal failure and related kidney diseases in Japanese. In addition, comparative clinical evidence of the effects of AIIAs and other widely used antihypertensive agents is necessary for the selection of appropriate drugs to treat patients with such kidney diseases.

The African-American Study of Kidney Disease and Hypertension (AASK), a randomized double-blind controlled trial,[9] aimed to compare the effects of the ACEI ramipril with the CCB amlodipine and the β-blocker metoprolol on hypertensive renal disease progression in African-Americans. This study was stopped prematurely because interim analysis showed a slower decline in glomerular filtration rate and a reduced rate of clinical endpoints with ramipril than with amlodipine. However, the results of this study are, in its concept, in accordance with the results of our present study, because intervention in the RAS is better than calcium channel blockade with amlodipine, although our study employed direct angiotensin II receptor blockade with losartan, while the AASK employed ACE inhibition with ramipril.

In our present study, urinary protein excretion was significantly inhibited by losartan in patients with chronic glomerulonephritis, which is very common in Japan (losartan, $n = 26$; amlodipine, $n = 28$). Chronic glomerulonephritis involves many factors in its etiology, and the complicated proteinuria is not solely a result of hyperfiltration by the glomeruli. Rather, remodeling of the glomerulus, including impairment of permeability of the glomerular basement membrane, must be considered. Thus, our present result is of particular interest in considering the direct actions of angiotensin II on the structure and function of the glomerulus in the pathophysiology of the progression of proteinuria in this disease. There was no significant difference in urinary protein excretion between losartan- and amlodipine-treated patients in the subgroup with diabetic nephropathy; however, because only six patients were analyzed in this interim report, we cannot conclude that the effect of losartan and amlodipine is similar for the prevention of proteinuria in diabetic nephropathy.

Throughout the 3 months, there was no change in CCr in either the losartan or the amlodipine group. Andersen et al.[21] conducted a randomized double-blind crossover clinical trial to evaluate the effect of losartan and the ACEI enalapril in patients with type 1 diabetic nephropathy, for 2 months, and reported that intervention in the RAS reduced urinary protein excretion without changing the GFR. Together with our present study, these results suggest

that short-term treatment with losartan is probably effective to ameliorate proteinuria without influencing GFR, although the mechanism of action, including how the drug acts on renal functions or affects the microstructure of the glomerulus during a 2- to 3-month period remains to be elucidated. Our trial will be continued to obtain the 12-month follow-up analysis, and we anticipate that CCr will become different between the two drug-treated groups, reflecting the degree of reduction in urinary protein excretion. Likewise, it may be possible that longterm treatment of the patients with losartan has beneficial effects on renal function beyond the effect to reduce proteinuria.

The mechanism and mode of action of losartan and amlodipine to explain their exertion of different effects on renoprotection are not thoroughly explained, and are controversial. It seems to be true that the actions of angiotensin II to reduce renal function are mediated by angiotensin $(AT)_1$ receptors. Calcium channel blockers act to dilate the microvasculature, improving regional circulation by regulating voltage-dependent calcium channels. The blockade of angiotensin II receptors results in a reduction in renal perfusion pressure, in addition to dilation of the efferent arterioles to a greater extent than afferent arterioles because of their different manner of constriction in response to angiotensin II;[22] thereby, AIIAs reduce the glomerular filtration pressure to some extent. On the other hand, angiotensin II does not act solely to constrict macrovascular and microvascular trees, but has a variety of cellular actions. A number of reports describe the roles of angotensin II through angiotensin $(AT)_1$ receptors to produce extracellular matrix, as well as to stimulate the proliferation and/or hypertrophy of many types of cells, via the direct stimulation of mitogen-activated protein kinase (MAPK), transforming growth factor (TGF-β), nuclear factor (NF-κB), the induction of protooncogenes, and so on.[17,18,23] Thus, although there is still no confirmatory theory, the wider biological functions of angiotensin II may explain the diversity of the renoprotective activity of the two drugs (losartan and anlodipine) without a dependence on their blood-pressure-lowering efficacy. The precise mechanism of action of these drugs should be further investigated. While we were performing the study, Nakao et al.[24] studied the effects of combination therapy and monotherapy with losartan and the ACEI trandolapril in patients with nondiabetic renal disease. In their recently published result, they demonstrated that losartan as well as trandolapril effectively lowered urinary protein excretion, although the combination of these two drugs exerted a more favorable effect on proteinura than either drug alone. Our present result is consistent with their results in terms of the effect of losartan in ameliorating proteinuria in patients with nondiabetic CKD.

In conclusion, by the 3-month interim analysis of the total 12-month treatment of Japanese patients with proteinuric CKD and hypertension, losartan reduced proteinuria more effectively than amlodipine, although the blood-pressure-lowering effect was not different between the two drug-treated groups. The superiority of losartan was more evident in patients whose baseline urinary protein excretion was 2 g or more /day. Thus, losartan is effective not only for patients with hypertension but also for patients with CKD manifesting proteinuria and hypertension.

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Genetic variations of matrix metalloproteinase-1 and -3 promoter regions and their associations with susceptibility to myocardial infarction in Japanese

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Abstract

Matrix metalloproteinases (MMPs) are involved in plaque rupture, which is the main pathological cause of myocardial infarction (MI). Recently, several genetic studies have demonstrated that MMP-1 1G/2G polymorphism and MMP-3 5A/6A polymorphism modify each transcriptional activity in allele specific manners. Within this context, we conducted case–control studies to examine whether these genetic polymorphisms are associated with susceptibility to MI. Two groups comprising patients with MI (group-1 164 patients, group-2 302 patients) were compared with control group comprising 335 patients without cardiovascular diseases. The MMP-3 5A allele was more frequent in patients with MI than in the control subjects ($P=0.018$ MI group-1, $P=0.0059$ MI group-2), whereas there was no disease association for MMP-1 genotypes. Logistic regression analyses revealed that MMP-3 5A/6A polymorphism was associated with susceptibility to MI [odds ratio(OR) (95% confidential interval) 1.67 (1.02–2.74); $P=0.042$, MI group-1; 1.61 (1.12–2.23); $P=0.0095$, MI group-2]. Other important findings were that there was strong linkage disequilibrium between these polymorphisms, which are located closely on chromosome 11q.22, and that the 5A–1G haplotype was a genetic risk factor for MI (OR 1.97 $P=0.0082$, MI group-1 OR 1.51 $P=0.017$, MI group-2). Taken together, the present findings suggest that genetic variations in these MMP genes and especially their haplotype may be useful genetic markers for determining susceptibility to MI in Japanese.

Keywords: Matrix metalloproteinase-1; Matrix metalloproteinase-3; Polymorphism; Myocardial infarction; Association

1. Introduction

Myocardial infarction (MI) is one of the major causes of death all over the world. Because MI frequently occurs suddenly without any preceding clinical symptoms, the prediction of MI is clinically of great importance. The principal element of the pathogenesis of MI is the rupture of coronary atherosclerotic plaques rather than gradual progression of atherosclerosis to complete occlusion [1–3]. Identification of factors concerned with plaque rupture should therefore enable us to predict the risk of MI.

Matrix metalloproteinases (MMPs) are proteases that degrade collagens and other matrix proteins. MMPs have been shown to play central roles in plaque rupture by degrading the fibrous caps of atheromas [4–7], and contribute to the occurrence of MI. The MMP family consists of more than 15 enzymes, each of which has unique substrates. Matrix metalloproteinase-1 (MMP-1) mainly degrades type 1 collagen, the most abundant extracellular matrix protein. Matrix metalloproteinase-3 (MMP-3, also called stromelysin-1) meanwhile has a broad range of substrates, such as gelatin, fibronectin, vitronectin, laminin, and type IV collagen.

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Recently, genetic polymorphisms of promoter regions of MMP-1 and MMP-3 have been identified [8,9]. The insertion of a guanine (G) at the −1607 bp in the MMP-1 promoter region creates the sequence 5′-GGA-3′, which is the core consensus binding sequence of transcriptional factor ets-1, and MMP-1 promoter with 2G allele has higher transcriptional activity than that with 1G allele in melanoma cells [8]. On the other hand, there is a polymorphism at the −1171 bp in the promoter region of MMP-3 with one allele having a run of six adenosines (6A) and another five adenosines (5A). This MMP-3 5A allele has higher transcriptional activity than the 6A allele in fetal foreskin fibroblasts [9].

These findings led us to hypothesize that these genetic polymorphisms, which modify the transcriptional activities of MMPs, could be genetic predictors for MI. In the present study, we compared the distributions of the MMP-1 −1607 1G/2G polymorphism and MMP-3 −1171 5A/6A polymorphism between patients with MI and control subjects in order to determine whether these polymorphic genetic markers are clinically useful for predicting MI in Japanese.

2. Subjects and methods

2.1. Subjects

Written informed consent was obtained from every subject after a full explanation of the study, which was approved by the institutional Ethics Committees. This case–control study included two groups of MI (MI group-1 and MI group-2) and one control group. MI group-1 included 164 patients who consecutively admitted to the University of Tokyo Hospital (146 males and 18 females; 42–88 years old; mean age 66.6 ± 8.3years). MI group-2 included 302 patients who consecutively admitted to the Cardiovascular Institute in Tokyo (257 males and 45 females; 30–95 years old; mean age 63.7 ± 10.0 years). Three-hundred and thirty-five control subjects (212 males and 123 females; 36–91 years old; mean age 67.8 ± 9.5 years) were recruited consecutively from the Institute for Adult Diseases Asahi Life Foundation, which is located in the same area as the University of Tokyo Hospital and the Cardiovascular Institute. All subjects were of Japanese ancestry and were not first- or second-degree relatives. All female participants were postmenopausal.

Prior myocardial infarction was verified by an episode of persistent ST elevation or depression in electrocardiography and raised serum cardiac markers. The control subjects were consecutively enrolled based on having normal ECG pattern and no medical history of coronary artery disease or stroke in the monthly follow-up or health check-up program at the outpatient clinic of the Institute for Adult Diseases Asahi Life Foundation Hospital, Tokyo. The subjects who had two or more coronary risk factors (diabetes mellitus, hypertension, hypercholesterolemia, and habit of smoking) or had history of chest pain were further evaluated in the Master or Treadmill exercise test. The subjects who showed ECG abnormality in the additional testing were eliminated in advance.

At the time of enrolment, relevant data on past medical history, family history of CAD, and smoking habits were obtained from all study participants. Hypertension and diabetes mellitus were diagnosed according to their respective World Health Organization criteria. Hypercholesterolemia was diagnosed when total plasma cholesterol levels were >220 mg/dl or when a subject was already being treated with lipid-lowering medication. Fasting venous blood samples were drawn for biochemical and genetic analyses.

As malignancies and aortic aneurysm are known to be associated with MMP polymorphisms, we excluded the patients with these diseases from the present study.

2.2. Genetic analysis

Venous blood samples were collected in tubes containing Na_2EDTA and applied to genomic DNA extracting columns (Genomix kit, Talent) according to the manufacturer's protocol. Genotyping was accomplished using PCR followed by melting curve analysis with specific fluorescent hybridization probes in a Light Cycler™ System (Roche) [10]. To detect the MMP-1 −1607 1G/2G genotype, the 292-bp fragment was amplified by primer extension reaction (PCR) using the sense and anti-sense primers, 5′-GTGTTC-TTTGGTCTCTGCCG-3′ and 5′-CCCACCTTTCCCACTG-TATCA-3′, respectively. The PCR condition was as follows,

Table 1
Baseline characteristics of the study populations

	Control	MI	
	(n=335)	Group-1 (n=164)	Group-2 (n=302)
Age>65 years	226 (67.5%)	98 (59.8%)	152 (50.3%)†
Sex (male)	212 (63.3%)	146 (89.0%)†	257 (85.1%)†
Smoke	118 (35.2%)	117 (71.3%)†	N.a.
BMI>25 kg/m	103 (30.7%)	56 (34.1%)	N.a.
Hypertension	238 (71.0%)	131 (79.9%)*	104 (34.4%)†
Hypercholesterolemia	141 (42.1%)	140 (85.4%)†	85 (28.1%)†
Diabetes mellitus	67 (20.7%)	81 (49.3%)†	79 (26.2%)

BMI indicates body mass index. *, and †, $P<0.05$ and $P<0.0001$, respectively (compared with the control group). N.a. indicates not available.

Table 2
MMP-3 genotype distributions and allele frequencies

	MMP-3 genotype			Allele frequency	P
	6A/6A	6A/5A	5A/5A	of 5A	value
Control ($n=335$)	257 (76.7%)	76 (22.7%)	2 (0.6%)	0.12	
MI group-1 ($n=164$)	110 (67.1%)	50 (30.5%)	4 (2.4%)	0.18	0.018
MI group-2 ($n=302$)	208 (68.8%)	82 (27.2%)	12 (4.0%)	0.18	0.0059

MI indicates myocardial infarction. The P values represent the statistical significance of the comparison of allele frequencies (compared with the control group).

95 °C for 15 s, 53 °C for 15 s, and 72 °C for 12 s (40 cycles). Following PCR, melting curve analysis was performed using a FITC-labeled anchor probe (5′TACCCTCTTGAACTCACATGTTATGCCACTTAGATGAGGAAATTGTAGTT-3′) and a Light Cycler Red 640-labeled mutation probe (5′-AATAATTAGAAAGGATATGACTTATCTCAAATCA–3′). As for the MMP-3 – 1171 5A/6A genotype, the sense and antisense primers for PCR were 5′-TAGAAGGAATTAGAGCTGCC-3′ and 5′-CTCAACCT CTCAAAGTGCTA-3′, respectively. Forty cycles (95 °C for 15 s, 52 °C for 15 s, 72 °C for 12 s) were used to amplify a 282-bp product. Following PCR, melting curve analysis was performed using a FITC-labeled anchor probe (5′-CCTCATATCAATGTGGCCAAATATTTTCCCTGTATTTCAATCAGGACAAG-3′) and a Light Cycler Red 640-labeled mutation probe (5′-CATGGTTTTT CCCCCCAT).

2.3. Statistical analysis

Comparisons of genotype distributions and allele frequencies were assessed using the χ^2 test with 2 and 1 degree of freedom with Hardy–Weinberg predictions. Continuous variables were analyzed by univariate analysis with the Mann–Whitney rank-sum test. Multiple logistic regression analysis was performed to eliminate confounding influences. Independent variables were coded with dummy scores; for instance, 0 (absence) and 1 (presence) were assigned for male sex, age (>65 years old), smoking status, obesity (body mass index>25), hypertension, diabetes mellitus, and hypercholesterolemia. For the bi-allelic polymorphic markers of MMP-1 and MMP-3, the three inheritance models were evaluated by assigning dummy scores to the individual genotypes; for instance, 1G/1G=0, 1G/2G=1, 2G/2G=2 for MMP-1 polymorphism in the additive inheritance model.Linkage disequilibrium coefficients (D') between the gene polymorphisms were calculated as previously described [11,12]. Haplotype analyses were performed with the EM algorithm to estimate individual haplotypes [13]. Statistical analysis was performed with SAS (statistical analysis system). A two-tailed value of $P<0.05$ was considered significant unless otherwise indicated. For multiple testing, we applied Bonferroni's correction and $P<0.025$ was considered significant because we analyzed two genetic polymorphisms. Values are expressed as the mean ± S.D.

3. Results

3.1. Characteristics of the study populations

The baseline characteristics of the patients and control subjects are shown in Table 1. Conventional risk factors such as sex (male), smoking, hypertension, hypercholesterolemia, and diabetes mellitus were significantly more frequent in the MI group-1 than the control group. The information of either the smoking status or body mass index of MI group-2 was not available. The patients in MI group-2 were younger and the proportion of males was more frequent in MI group-2 than in the control group. Other risk factors such as hypertension and hyperlipidemia were more frequent in the control subjects.

3.2. Association of MMP-3 5A/6A polymorphism with susceptibility to MI

The genotype distributions of the MMP-3 5A/6A polymorphism in the control and the two MI groups are shown in Table 2. The genotype distributions satisfied Hardy–Weinberg equilibrium. The 5A allele frequency was higher in the MI group-1 than the control group ($P=0.018$). In the additive inheritance model, the genotype distributions were also different between the control group and the MI group-1 ($P=0.028$). Multiple logistic regression analysis revealed the 5A allele tended to be an independent genetic risk factor for the occurrence of MI [relative risk (95% CI) 1.67 (1.02–2.74); $P=0.042$ (additive inheritance model)].

Table 3
MMP-1 genotype distributions and allele frequencies

	MMP-1 genotype			Allele frequency	P
	1G/1G	1G/2G	2G/2G	of 1G	value
Control ($n=335$)	31 (9.3%)	144 (43.0%)	160 (47.7%)	0.31	
MI group-1 ($n=164$)	23 (14.0%)	74 (45.1%)	67 (40.9%)	0.37	N.s.
MI group-2 ($n=302$)	32 (10.6%)	133 (44.0%)	137 (45.4%)	0.33	N.s.

MI indicates myocardial infarction. The P values represent the statistical significance of the comparison of allele frequencies (compared with the control group).

Table 4
Distributions of genotypes of MMP-1 and MMP-3 polymorphism

		MMP-1 genotype								
		Control			MI (group-1)			MI (group-2)		
		1G/1G	1G/2G	2G/2G	1G/1G	1G/2G	2G/2G	1G/1G	1G/2G	2G/2G
MMP-3	6A/6A	13	90	154	4	40	66	8	72	128
genotype	6A/5A	17	53	6	15	34	1	15	58	9
	5A/5A	1	1	0	4	0	0	9	3	0

MI, myocardial infarction.

Because these findings indicated that MMP-3 5A/6A polymorphism might be a useful genetic marker for MI, we recruited another 302 patients of MI (MI group-2) from another institute in the same area of Tokyo and made cross validation to confirm this tendency. The results showed that the frequency of the 5A allele was also significantly more frequent in the MI group-2 than in the control group ($P=0.0059$). In the additive inheritance model, the genotype distributions were also different between the control group and the MI group-2 ($P=0.0044$). Multiple logistic regression analysis also revealed that the 5A allele was an significantly independent risk factor for the occurrence of MI [relative risk (95% CI) 1.61 (1.12–2.23); $P=0.0095$ (additive inheritance model)]. According to these findings, we concluded that the 5A allele is a useful genetic marker for the susceptibility to MI in Japanese.

3.3. Lack of association between MMP-1 promoter 1G/2G polymorphism and MI

The genotype distributions of the MMP-1 1G/2G polymorphism in the control group and the two MI groups are shown in Table 3. The genotype distributions satisfied the criteria for Hardy–Weinberg equilibrium. The allele frequency of the 1G was not different between the MI group-1 and the control group ($P>0.05$). Also, there were no significant differences in the genotype distributions of these three groups among the three inheritance models. This lack of association between MMP-1 1G/2G polymorphism and the susceptibility to MI was also confirmed by the comparison between the MI group-2 and the control group as shown in Table 3.

3.4. Linkage disequilibrium between the MMP-1 and the MMP-3 promoter polymorphisms and haplotype analysis

Table 4 presents the combined genotype distributions of the MMP-1 1G/2G and MMP-3 5A/6A polymorphism in the control and two MI groups. Strong linkage disequilibrium was observed between the MMP-1 1G and MMP-3 5A alleles in each group. The pairwise linkage disequilibrium coefficients of the control, MI group-1 and MI group-2 were $D'=0.84$, $D'=0.97$ and $D'=0.80$, respectively ($P<0.001$, respectively). Next, we tested whether haplotypes in the two genes were associated with disease susceptibility. Table 5 represents expected haplotype frequencies of the control group and the two MI groups. The 5A–1G haplotype frequency was larger both in the MI group-1 and MI group-2 (odds ratio 1.97 $P=0.0058$; MI group-1 odds ratio 1.51 $P=0.017$; MI group-2) than in the control group.

Table 5
Expected haplotype frequencies

Haplotype	Population		Haplotype frequency	*P* value
6A/1G	Control	($n=670$)	133 (19.9%)	
	MI group-1	($n=328$)	61 (18.6%)	N.s.
	MI group-2	($n=604$)	103 (17.1%)	N.s.
	Control	($n=670$)	457 (68.2%)	
6A/2G				
	MI group-1	($n=328$)	209 (63.7%)	N.s.
	MI group-2	($n=604$)	395 (65.4%)	N.s.
	Control	($n=670$)	73 (10.9%)	
5A/1G				
	MI group-1	($n=328$)	57 (17.4%)	0.0058
	MI group-2	($n=604$)	94 (15.6%)	0.017
	Control	($n=670$)	7 (1.0%)	
5A/2G				
	MI group-1	($n=328$)	1 (0.3%)	N.s.
	MI group-2	($n=604$)	12 (2.0%)	N.s.

P value represents the statistical significance of the difference of haplotype frequencies between the control group and the MI groups.

4. Discussion

In the present study, we demonstrated that the 5A allele of MMP-3 promoter 5A/6A polymorphism was a significant genetic risk factor for MI independent of conventional coronary risk factors in Japanese. In addition, we found that there was strong linkage disequilibrium between MMP-3 promoter 5A/6A polymorphism and MMP-1 promoter 1G/2G polymorphism and that the 5A–1G haplotype was significantly associated with susceptibility to MI in Japanese.

The MMP-3 promoter with the 5A allele has higher transcriptional activity than that with the 6A allele [9]. Because the expression of MMP-3 is mainly regulated at the transcriptional level [14,15], the subjects with the 5A alleles will presumably have higher enzymatic activities than that with the 6A alleles. The positive disease association for MMP-3 5A allele in our study is relevant to a previous report in Japan that the 5A allele is a risk factor for acute myocardial

infarction [16]. On the other hand, the association between the 5A allele and the patients with coronary artery diseases without history of prior MI (non-MI CAD) was not observed in our study ($n = 156$; 6A/6A 110, 6A/5A 45, 5A/5A 1; allele frequency 0.15 in the non-MI CAD patients recruited from University of Tokyo Hospital). Why is the 5A allele of MMP-3 only associated with susceptibility to MI but not to non-MI CAD? Recent experimental study using MMP-3 deficient mice demonstrated that disruption of MMP-3 may promote plaque progression [17]. Consistently, clinical report in British patients showed that the weaker transcription of MMP-3 (6A allele) is associated the progression of atherosclerosis [18]. In contrast, increased expression of MMP-3 seems to induce plaque rupture [5]. The onset of MI is well known to be closely related to plaque rupture, not gradual plaque progression. Therefore, our observation that the 5A allele of MMP-3 is significantly associated with only MI, not non-MI CAD, is consistent with the above findings.

The MMP-1 promoter with the 2G allele shows higher transcriptional activity than that with the 1G allele [8]. Indeed, MMP-1 promoter 1G/2G polymorphism has been shown to be associated with the progression of ovarian cancer and melanoma through modulation of the expression of this extracellular matrix-degrading enzyme [19,20]. Judging from increased expression of MMP-1 in human atherosclerotic plaques [6], MMP-1 should also play an important role in the occurrence of MI. Thus, we examined the association of MMP-1 promoter 1G/2G polymorphism and susceptibility to MI. As a result, it was not associated with the susceptibility to MI in our study population. Why does only MMP-3, but not MMP-1, polymorphism significantly associate with the susceptibility to MI? One possible explanation is that MMP-3 has broad range of substrates including gelatins and other matrix proteins, however, MMP-1 can only degrade type 1 collagen. Further investigations are needed to clarify how each MMP is related to the different aspect of CAD.

The present study demonstrated that there is strong linkage disequilibrium between MMP-1 1G/2G and MMP-3 5A/6A polymorphism. MMP-1 and MMP-3 are closely located on chromosome 11q.22 and the distance between these polymorphisms is approximately 40–50 kb [21]. Of interest, linkage disequilibrium was found between the 1G allele of the MMP-1 promoter which is related to lower transcriptional activity of MMP-1 and the 5A allele of the MMP-3 promoter which is related to higher transcriptional activity of MMP-3.

Although many earlier studies have discussed these polymorphisms from the viewpoint of susceptibility to several diseases [18–20,22–24], none has mentioned the usefulness of haplotype of these polymorphisms for predicting the susceptibility to MI. In this study, to clarify whether each haplotype was associated with the disease susceptibility, we performed haplotype analysis of these polymorphisms. As a result, we found that the 5A–1G haplotype could be a significant genetic risk for MI. It is possible that this 5A–1G haplotype forms linkage disequilibrium with another functional polymorphisms existing around this genetic locus where lots of genes belonging to MMP family exist (MMP7–MMP20–MMP8–MMP10–MMP1–MMP3–MMP12–MMP13, in this order [21]). In any event, further investigations will be needed to search another SNPs around two genes and clarify the biological meaning of this 5A–1G haplotype.

In conclusion, we found in the present study that the 5A allele of the MMP-3 5A/6A polymorphism was an independent genetic risk for the occurrence of MI in Japanese. Moreover, we also found strong linkage disequilibrium between the MMP-1 1G/2G and the MMP-3 5A/6A genetic polymorphisms, and we demonstrated that the 5A–1G haplotype was associated with the susceptibility to MI in Japanese.

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信頼性の高い治験を迅速に実施するための東大病院の取り組み

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1 はじめに

ゲノム創薬による新薬の開発と個人の特性にあったテーラーメイド医療の確立が進むことが期待されている。一方で，グローバルな競争の激化，不十分な創薬環境，医療保険財政悪化等の影響から，日本の医薬品産業の国際競争力が低下することが懸念されている。これに対し，厚生労働省は，質の高い医薬品を提供するための医薬品開発の国際競争力の強化を目指して医薬品産業ビジョンを策定し，治験推進のための施策を打ち出している。具体的には，全国治験活性化3カ年計画治験推進事業として，大規模治験ネットワーク事業，治験コーディネーター養成事業，EBMのための臨床研究やトランスレーショナルリサーチの推進，治験施設整備，医師主導の治験を含む薬事法の改正等を進めている。

企業主導の治験については，ブリッジング試験から多国間治験への変革といったグローバル化が進む一方で，地域ネットワークやSMOを活用した治験など新たな展開が見られている。「遅い，高い，質が悪い」といわれた日本の治験は，新GCPの導入，医療機関における治験支援組織の整備，および関係者の意識改革等により，少なくとも「質」についてはかなり改善されたといわれている。しかし，「遅い」「高い」という問題については日本の医療環境を鑑みると解決は容易ではなく，国際治験を実施するうえで致命的問題となっている。また，「医師主導の治験」でも企業主導の治験と同レベルの「質」が要求され，医療機関の治験審査委員会の機能の充実や，実施状況管理体制の整備が必要となっている。このような治験を取り巻く状況変化に対応できるかで，治験を着実に実施する医師・医療機関が選別される時代がやってきた。本稿では，質の高い治験を迅速に推進するための東大病院の最近の取り組みを紹介したい。

2 東大病院の治験支援体制

2.1 臨床試験部の沿革と組織

東大病院においては治験の円滑な実施を支援するために，臨床試験部が治験審査委員会事務局として審査支援を行うと共に，治験事務局としての実施支援を行っている。臨床試験部は，平成13年4月従来からの治験に加えて，研究者主導臨床試験（当院では自主臨床試験と称している）をも支援する組織として，群馬大学と共に全国に先駆けて文部科学省から正式に許可された。平成14年4月には自主臨床試験実施支援を本格的に行うためのコンサルテーション部門を新設し，同年5月には後ほど述べるピアレビューシステムを導入し，現在に至っている（沿革は図1参照）。臨床試験部は病院長の直轄組織で，部長，副部長のもとに事務部門，治験薬・情報管理部門，コーディネーター部門，コンサルテーション部門の4部門からなっている（図2）。臨床試験部は上部の会議として「臨床試験部運営委員会」と「治験審査委員会」（Institutional Review Board：IRB）を有している。

昨今の治験をめぐる状況の変化に対応すべく，臨床試験部は病院長，臨床試験部運営委員会，治験審査委員