Human SLCO4C1 promoter activity was increased 1.49-fold (−2064) and 1.68-fold (−129) by 3-MC compared with controls (Figure 5B). The −129 construct exhibited the highest activity, and this segment contained XRE core motifs. Because AhR can also bind to a structurally divergent range of chemicals,[21] we next screened various compounds. The hepatic hydroxymethyl glutaryl–CoA reductase inhibitor (statin) fluvastatin (2.3-fold at 10 μM) and pravastatin (1.3-fold at 30 μM) and atypical AhR ligand flutamide (1.4-fold at 10 μM) upregulated the SLCO4C1 promoter activity (Figure 5C). Because of the comparable magnitude to 3-MC and its clinical availability, we further focused on statins. Deletion experiments showed that all constructs exerted potent promoter activation, but removal of the XRE core segment or mutation in the XRE core motifs abolished the response to fluvastatin (Figure 5D). Because there are various clinical reports on renoprotective effects of statins,[22] we further examined various statins on human SLCO4C1 transcription. Simvastatin, lovastatin, cerivastatin, itavastatin, mevastatin, atorvastatin, rosuvastatin, and pitavastatin upregulated SLCO4C1 transcription (Figure 5F).

Next, we determined the ligand-dependent recruitment of the AhR-XRE system by chromatin immunoprecipitation (ChIP) assay. Application of the antibody against AhR resulted in a positive band for both 3-MC and fluvastatin (Figure 5E, top). In addition, the nuclear recruitment of AhR protein was further confirmed by Western blotting with a strong band in the nuclear extract by 3-MC and fluvastatin (Figure 5E, bottom). These data suggested that statins regulate SLCO4C1 transcription through the AhR-XRE system.

Statins Increase Tubular Uremic Toxin Excretion

On the basis of our results, we next examined the effect of statins in renal failure. In human kidney proximal cells, application of fluvastatin and pravastatin significantly potentiated the SLCO4C1 mRNA by 1.72- and 1.73-fold, respectively (Figure 6A). The uptake of thyroid hormone T3, a representative ligand of SLCO4C1, was also significantly potentiated by fluvastatin and pravastatin by 1.3- and 1.4-fold, respectively (Figure 6B), suggesting the potentiation of SLCO4C1 function in the proximal tubules.

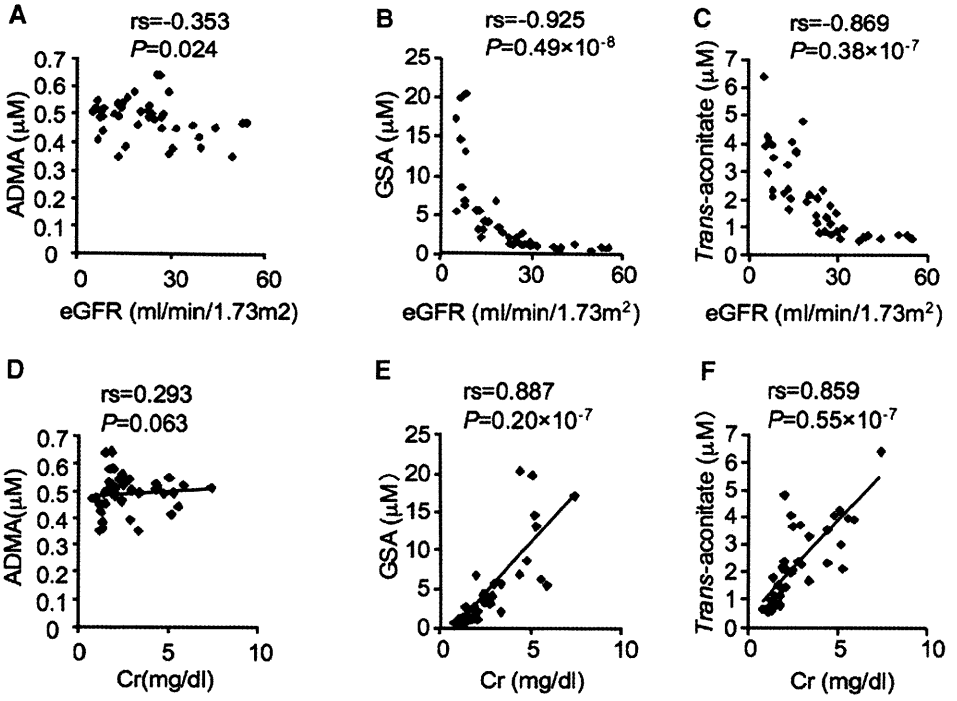

Figure 4. Relation between uremic toxins and eGFR as well as plasma creatinine in 41 patients with CKD is shown. (A through C) Correlations between eGFR and the plasma ADMA (A), GSA (B), and *trans*-aconitate (C) in patients with CKD. (D through F) Concentrations between plasma creatinine (Cr) and plasma ADMA (D), GSA (E), and *trans*-aconitate (F).

We next examined the effects of pravastatin *in vivo*. We and other groups reported that pravastatin reduced BP.[23,24] In addition, pravastatin has been reported to modulate DDAH activity and modulate ADMA concentration.[25] To avoid the effect on BP and to eliminate other pleiotropic effects of pravastatin, we administered low-dosage pravastatin to Nx Wistar rats and examined renal tubular function. After administration of pravastatin, BP was not changed but the mRNA level of rat slco4c1 was significantly increased in the kidney (Figure 7, A and B). Under this condition, the ADMA and *trans*-aconitate clearance were significantly increased in pravastatin-treated Nx rats without changing creatinine clearance, although the GSA clearance was not statistically significant (Figure 7, C through F). Furthermore, the mRNA level of DDAHs, protein arginine N-methyltransferases, or other transporters was not changed (data not shown). These data strongly suggested that pravastatin increased ADMA and *trans*-aconitate excretion in the proximal tubules. In addition, cardiac hypertrophy was decreased in the pravastatin-treated group (Figure 7G).

DISCUSSION

Here, we found that the plasma concentration of uremic toxins ADMA, GSA, and *trans*-aconitate were significantly reduced in

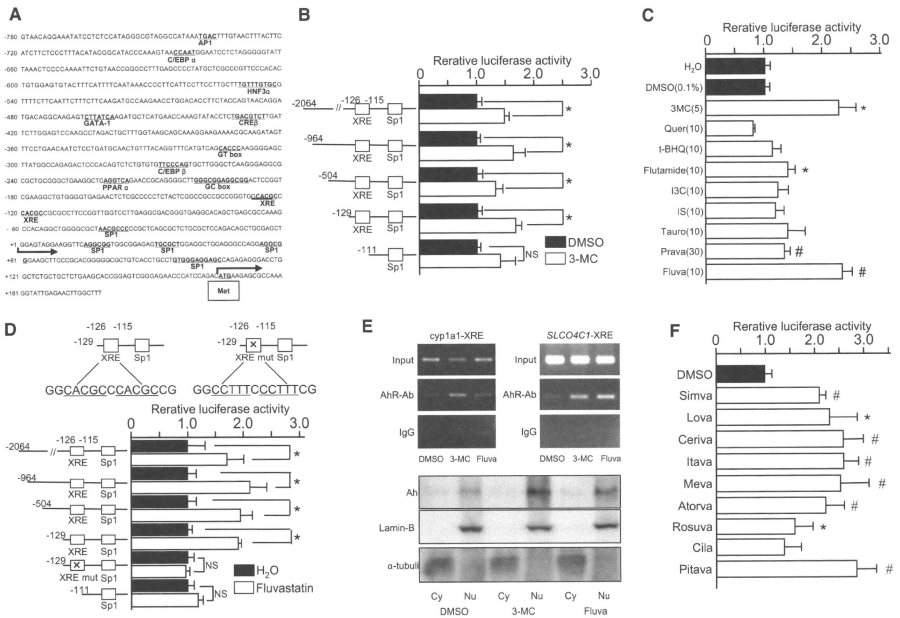

Figure 5. Transcriptional analysis and ligand screening are shown. (A) The 5′ region of human SLCO4C1. Potential *cis*-acting sequences are indicated. Met, first methionine. (B) Promoter activity of human SLCO4C1. Deletion constructs of the human SLCO4C1 promoter region were analyzed with 3-MC (5 μM). $^{*}P < 0.05$ (n = 3 to 4 per group). (C) Enhancement of promoter activity of human SLCO4C1 with various compounds (concentration as indicated, μM). Quer, quercetin; t-BHQ, *tert*-butylhydroquinone; I3C, indole-3-carbinole; IS, indoxyl sulfate; Tauro, taurocholic acid; Prava, pravastatin; Fluva, fluvastatin. $^{*}P < 0.05$ *versus* DMSO; $^{\#}P < 0.05$ *versus* H_2O (n = 3 to 4 per group). (D) Effect of fluvastatin (10 μM) on human SLCO4C1 transcription. Deletion constructs and loss-of-function mutation construct in XRE motifs of human SLCO4C1 were examined. $^{*}P < 0.05$ (n = 3 to 4 per group). (E) ChIP assay and Western blotting of 3-MC or fluvastatin-treated cells. (Top) After application of 3-MC (1 μM) or fluvastatin (10 μM), fixed cell extract was analyzed by mouse cyp1a1 XRE or human SLCO4C1 XRE PCR. (Bottom) Western blotting of nuclear and cytoplasmic fractions from HEK293T cells were stained with antibodies against AhR, Lamin B, or α-tubulin antibodies. Cy, cytosolic fraction; Nu, nuclear fraction. (F) Enhancement of human SLCO4C1 promoter activity with various statins (10 μM) using the minimal promoter region (−129). $^{*}P < 0.05$; $^{\#}P < 0.01$ (n = 3 to 4 per group).

TG(+)Nx rats. The guanidino compounds are a large group of structural metabolites of arginine, and the concentrations of GSA and ADMA are markedly increased in renal failure.[2,3] GSA accumulation causes various harmful effects, such as inhibition of platelet aggregation hemolysis and convulsions.[26] Likewise, ADMA is the most specific endogenous compound with inhibitory effects on NO synthesis, and it has also been implicated in the development of hypertension and adverse cardiovascular events.[6,7] *Trans*-aconitate, known as anti-feedant in brown plant hoppers,[27] is an inhibitor of aconitase and inhibits the TCA cycle[16]; however, its existence in mammals, especially in renal failure, was not previously known. Compounds that inhibit the TCA cycle are "poison." It is also widely known that fluoroacetate is a "suicide" substrate for aconitase. Acute fluoroacetate poisoning in humans mainly affects the central nervous system, cardiovascular system, and kidney, and the biochemical effects include TCA cycle blockade, respiratory failure, and metabolic acidosis and lactate accumulation.[28] *Trans*-aconitate administration also increased BP and generated oxidative stresses in rats. These data suggest that the overexpression of SLCO4C1 in the renal proximal tubules in TG(+) rats causes the beneficial effect of excretion of harmful uremic toxins such as ADMA, GSA, and *trans*-aconitate and proposes a new approach to decrease uremic toxins and to reduce the exacerbation of renal function in patients with CKD (Figure 8).

Here we show that statins function as a nuclear receptor ligand recruiting the AhR-XRE system and upregulating SLCO4C1 tran-

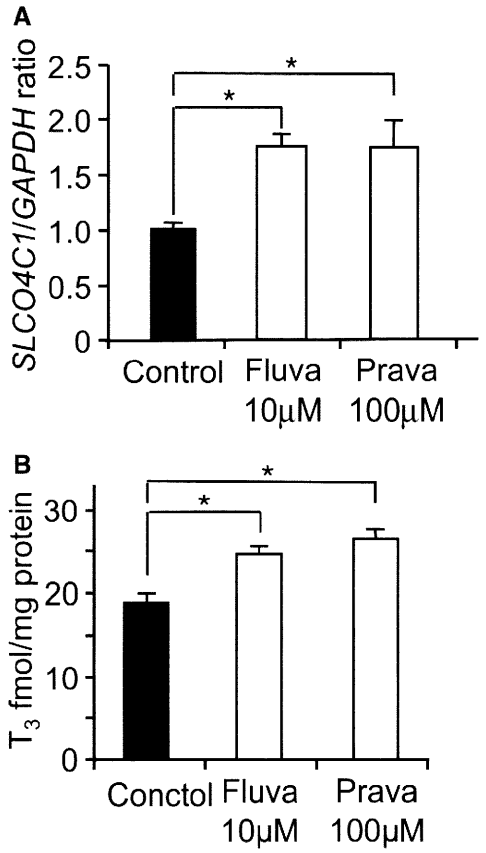

Figure 6. Effects of statins on SLCO4C1 expression and function *in vitro*. (A) Real-time PCR of SLCO4C1 in ACHN cells with fluvastatin (10 μM) or pravastatin (100 μM; $n = 3$ per group). (B) The uptake of T_3 by ACHN cells treated with fluvastatin (10 μM) and pravastatin (100 μM). *$P < 0.05$ ($n = 3$).

scription to facilitate the excretion of uremic toxins like a transgene phenotype. In patients with CKD, therapy with statins has the potential not only to lower cardiovascular morbidity and mortality but also to slow the progression of renal disease.[22] The effects are thought to be dependent on such mechanisms as a reduction of endothelial dysfunction, inhibition of inflammatory responses, and reduction of oxidative stress.[22,29] Recently, the relationship between statin administration and ADMA was examined in humans. The serum level of ADMA in metabolic syndrome was reduced by fluvastatin.[30] Thus, our data provide new scientific bases for renal protection to facilitate the excretion of uremic toxins in patients with CKD by drugs including statins as "transporter potentiators" (Figure 8). Because the significantly increased levels of GSA and ADMA were reported in patients with autosomal dominant polycystic kidney disease (ADPKD),[5] our data also support the clinical study and will be a new clue for further protection of renal damage in patients with ADPKD.

Cytochrome P-450 (CYP) comprises a superfamily of enzymes that catalyze oxidation of numerous xenobiotic chemicals, including drugs, toxic chemicals, and carcinogens, as well as endobiotic chemicals.[31] Among these CYP enzymes, cyp1a1 is important in the metabolism of carcinogens such as dioxin and halogenated aromatic hydrocarbons.[31] Because of the prominently catalyzing role, it has been believed that compounds that induce cyp1a1 activation are detrimental to humans and animals; however, it is also reported that induction of cyp1a1 is a sensitive but nonspecific indicator of AhR binding and activity, and the induction of cyp1a1 and activation of AhR are not synonymous with dioxin-like toxicity, including carcinogenesis.[32] Clinically, various weak AhR ligands, such as flutamide, omeprazole, and atorvastatin, were identified[32] but the Food and Drug Administration approves usage of these compounds, and in fact, they do not produce dioxin-like toxicities, including carcinogenesis in humans. Because statins have been used for a long time with a high safety and tolerability profile, induction of SLCO4C1 by statins in the kidney in patients with CKD and ADPKD may be a safe and new therapeutic tool to excrete uremic toxins and for reduction of renal inflammation.

We also found that the activation potency of the AhR-XRE system differs between cyp1a1 and slco4c1 in the kidney. In the rat liver, cyp1a1 was significantly induced by flutamide (329-fold) and omeprazole (79-fold), although renal cyp1a1 was weakly upregulated by flutamide (three- fold) and omeprazole (15-fold; Supplemental Figure 3, A and B). It is also reported that some statins significantly induced cyp1a1 in kidney but rather weakly in the liver, suggesting that statins act as AhR ligands mainly in the kidney.[32] Conversely, the renal activation of slco4c1 by flutamide and omeprazole was quite weak (Supplemental Figure 3C). Thus, further exploring for drugs that upregulate human SLCO4C1 only in the kidney much more potently than statins should be a new clinical tool for patients with CKD and ADPKD to decelerate renal damage and to delay initiating hemodialysis.

Metabolomics is an emerging tool that can be used to gain insights into cellular and physiologic responses. By CE-MS, we identified various renal failure–related compounds (Supplemental Figure 2, Supplemental Tables 1 through 4). In renal failure, indoxyl sulfate, creatinine, GSA, and guanidinoacetate were reported as uremic toxins.[4] Increase of citrulline and trimethyl N-oxide,[33] 3-methylhistidine,[34] N,N-dimethylglycine.[35] and allantoin[36] and decrease of carnitine,[37] Trp, and Tyr[38] were also reported in renal failure.

On the other hand, increase of *trans*-aconitate, 4-acetylbutyrate, hexanoate, argininosuccinate, α-aminoadipate, and pipecolate and decrease of desethylatrazine and methionine sulfoxide so far have not been reported in renal failure (Supplemental Figure 2). Thus, our data will be useful for clarifying the metabolic pathway of renal failure.

CONCISE METHODS

Materials

Pravastatin was provided by Daiichi-Sankyo (Tokyo, Japan). Other statins were purchased from Sequoia Sciences (St. Louis, MO).

Construction of Kidney-Specific TG Rats

The mutated coding region of human SLCO4C1[10] was inserted into the pGEM-sglt2–5pr-mut plasmid containing kidney-specific sglt2 pro-

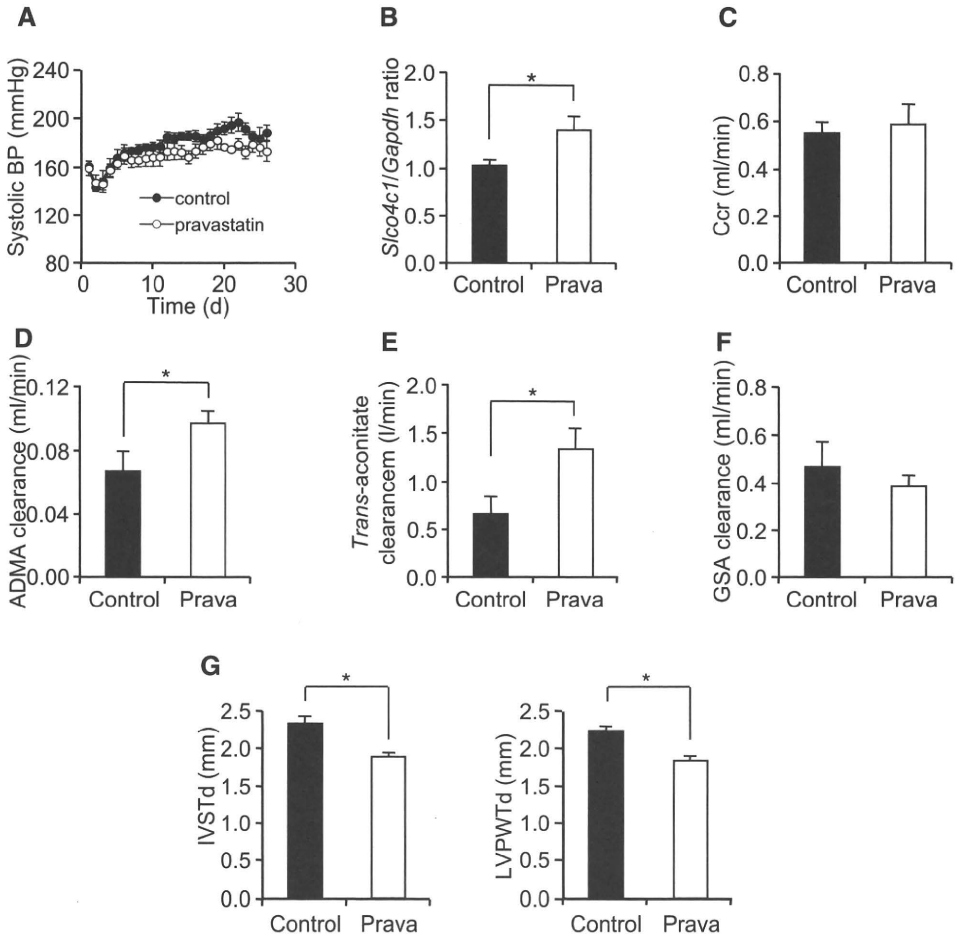

Figure 7. Effects of pravastatin *in vivo*. (A) BP in control and pravastatin-treated (0.1 mg/ml drinking water) rats after five-sixths Nx ($n = 6$ to 7 per group). (B) The mRNA expression of rat slco4c1 in the kidney after pravastatin administration ($n = 11$ per group). (C through F) Renal clearance of creatinine (C), ADMA (D), *trans*-aconitate (E), and GSA (F) 3 wk after five-sixths Nx ($n = 5$ to 7 per group). (G) Thickness of the interventricular septum (IVSTd) and left ventricular posterior wall at end-diastole (LVPWTd) before and after five-sixths Nx ($n = 6$ to 7 per group). *$P < 0.05$.

moter.[11] The linear purified plasmid was injected into the pronuclei of fertilized oocytes of Wistar rats. Pups were analyzed for the genomic integration by Southern blotting and by PCR amplification of tail DNA using the following primers: Forward (mouse sglt2) 5′-tccccccacttctgtttcccagtctatgt-3′ and reverse (human SLCO4C1) 5′-acgcgatctgcagaattagcttgggctc-3′. Reverse transcriptase–PCR was carried out using the same primers that can amplify the full length of human SLCO4C1 cDNA. Resultant TG(+) rats showed normal breeding and development with no obvious phenotypic abnormalities in body weight, water and food intake, and renal functions compared with TG(−) littermates, whose genetic background is the same as that of TG(+) rats except for expression of human SLCO4C1 (Supplemental Figure 1A). All animal experiments were approved by the Tohoku University Animal Care Committee.

Immunohistochemistry

The rabbit antiserum against 107 peptides of the *N*-terminus of human SLCO4C1 was raised and immunopurified. Western blotting and immunohistochemistry were performed as described previously,[39] and the quality was confirmed by peptide absorption (Supplemental Figure 1, B and D). The mouse mAb against CD68 was purchased from Serotec (Martinstried, Germany).

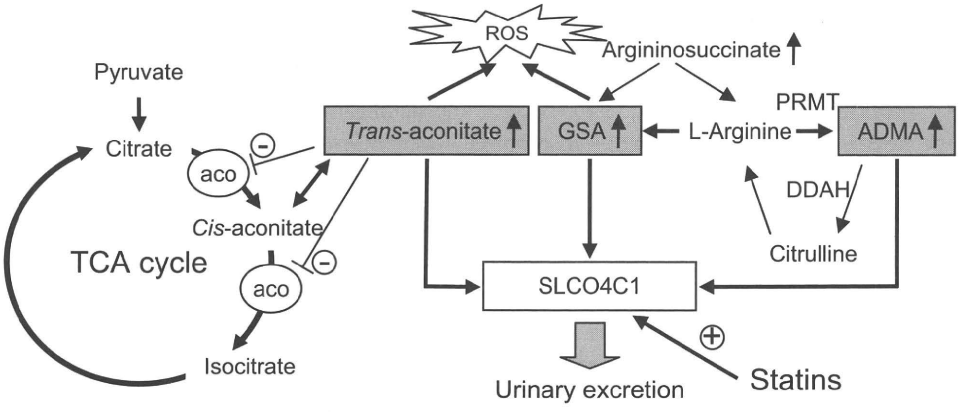

Figure 8. Uremic toxins and SLCO4C1 transporter in renal failure. ADMA is formed by protein arginine N-methyltransferase (PRMT) from arginine and degrades to citrulline by DDAH. Note that SLCO4C1 facilitates the excretion of GSA, ADMA, and *trans*-aconitate and that statins increase the expression and the function of SLCO4C1, resulting in reductions of the uremic toxins and BP. *Trans*-aconitase inhibits aconitase activity and induces reactive oxygen species (ROS). Aco, aconitase.

Nephrectomized Rat Model and BP Measurement

Five-sixths nephrectomized rats were generated as previously reported.[10] Briefly, male TG rats were intraperitoneally anesthetized with ketamine (30 mg/kg) and xylazine (2 mg/kg) and subjected to five-sixths renal ablation. At the time of surgery, rats were prepared for telemetric monitoring of BP (Data Sciences Int., St. Paul, MN).[40]

Echocardiogram

Rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) and studied with Doppler imaging by echocardiogram. The thickness of the interventricular septum and the left ventricular posterior wall at end-diastole were measured as described previously.[41]

CE-MS Method for Metabolome Analysis

A comprehensive and quantitative analysis of charged metabolites by CE-MS was performed.[13] Metabolites were first separated by CE on the basis of charge and size and then selectively detected using MS by monitoring over a large range of m/z values. Plasma and urine ADMA were measured by HPLC. Anionic and cationic compounds that were increased or decreased after Nx in both of the generated rat lines were nominated as statistically significant and are summarized in Supplemental Figure 2 (all analyzed CE-MS data are in Supplemental Tables 1 through 4). In the human plasma analysis, the protocols conformed to the ethical guidelines and approvals of both Tohoku University and Nagasaki University. Informed consent was obtained from each participant. The eGFR was calculated with the formula[42] eGFR (ml/min per 1.73 m^2) = $175 \times \text{creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female) $\times$ 0.741.

Measurement of Reactive Oxygen Species

The free radical formation within the human kidney proximal cell line HK-2 evoked by *trans*-aconitate (100 μM) was monitored by measurement of the changes in fluorescence resulting from the oxidation of dihydroethidium to ethidium as the increase of ethidium production (U/s)[43] using a 505-nm dichroic mirror with the 605/55-nm band-pass filter of an IX71 microscope (Olympus, Tokyo, Japan).

Transcriptional Assay

The human SLCO4C1 promoter DNA fragments were amplified by PCR, and the amplified fragments were inserted into the pGL3 basic luciferase expression vector (Promega, Madison, WI). The point mutation of two XREs was generated by PCR. Two micrograms of plasmid construct was transfected with 0.1 μg of *Renilla* Luciferase Reporter Vector PhRL-TK (Promega) as well as co-transfection with AhR and AhR nuclear translocator expression vector.[18] Forty-eight hours after ligand treatment, reporter assay was performed using Dual Luciferase Reporter Assay System (Promega). Incubation with activators of constitutive androstane receptor (clotrimazole and TCPOBOP), pregnane X receptor (rifampicin), and peroxisome proliferator–activated receptor α (bezafibrate, fenofibrate, clofibrate, and LTB_4) did not affect the SLCO4C1 transcription (data not shown).

ChIP Assay

ChIP assays were performed as described previously.[44] Briefly, cells either untreated or exposed to 3-MC (mouse HepaC1C7 cells) or fluvastatin (HEK293T cells) were crossed-linked with 1% formaldehyde, and protein-DNA complexes were immunoprecipitated using rabbit polyclonal antibody against AhR (BIOMOL, Plymouth, PA) or nonspecific anti-rabbit IgG. The recovered DNA was then subjected to PCR using primers that amplify regions containing the XRE elements of the human SLCO4C1 gene (forward primer 5′-AAGGGGAGCTTATGGCCAGAGACTC-3′ and reverse primer 5′-TCGCCTCAAGGACCAACCGGAAG-3′) or mouse cyp1a1 gene (forward primer 5′-CTATCTCTTAAACCCCACCCCAA-3′ and reverse primer 5′-CTAAGTATGGTGGAGGAAAGGGTG-3′). Nuclear and cytoplasmic fraction extracts were prepared and Western blotting was performed as described previously[39] using antibodies against AhR, Lamin B (Santa Cruz Biotechnology, Santa Cruz, CA), and α-tubulin (Sigma-Aldrich, St. Louis, MO).

Real-Time PCR Analysis

We performed real-time PCR analysis with probe sets from Applied Biosystems (Foster City, CA).

Statistical Analysis

The data are means ± SEM. We used an unpaired *t* test for comparisons between two groups. For multiple comparisons, we used two-way ANOVA with repeated measures in Figures 2A, 3H, and 7A and Supplemental Figure 1D and ANOVA on rank in Supplemental Figure 3, A through C. We derived *P* values for Supplemental Figure 1C using log-rank test. In Figure 4, Spearman rank correlation was calculated. $P < 0.05$ was considered to be significant.

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DISCLOSURES

None.

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Relationship between Estimated Glomerular Filtration Rate (eGFR) and Metabolic Syndrome in Japanese

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Abstract

We investigated the link between renal function as evaluated by estimated glomerular filtration rate (eGFR) and metabolic syndrome in Japanese. A total of 11,711 Japanese subjects, aged 20-79 years, were recruited in a cross-sectional clinical investigation. From this group, we further investigated the data on 1,576 subjects. eGFR was calculated using serum creatinine (Cr), age and sex. The diagnosis of metabolic syndrome was based on the Japanese criteria. In the first analysis, 288 men (7.8%) and 498 women (6.2%) were diagnosed with reduced eGFR (<60ml/min). eGFR was not correlated with anthropometric, body composition parameters in either sex. In the second analysis, in subjects without medications, 132 men (20.8%) and 15 women (1.6%) were diagnosed with metabolic syndrome. eGFR was lower in men with abdominal obesity and in women with hypertension was than in those without. Among Japanese not taking medications, lower eGFR may be a characteristic of men with abdominal obesity and of women with hypertension.

KEYWORDS: metabolic syndrome, estimated glomerular filtration rate (eGFR), abdominal circumference

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Original Article

Relationship between Estimated Glomerular Filtration Rate (eGFR) and Metabolic Syndrome in Japanese

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We investigated the link between renal function as evaluated by estimated glomerular filtration rate (eGFR) and metabolic syndrome in Japanese. A total of 11,711 Japanese subjects, aged 20-79 years, were recruited in a cross-sectional clinical investigation. From this group, we further investigated the data on 1,576 subjects. eGFR was calculated using serum creatinine (Cr), age and sex. The diagnosis of metabolic syndrome was based on the Japanese criteria. In the first analysis, 288 men (7.8%) and 498 women (6.2%) were diagnosed with reduced eGFR (< 60 ml/min). eGFR was not correlated with anthropometric, body composition parameters in either sex. In the second analysis, in subjects without medications, 132 men (20.8%) and 15 women (1.6%) were diagnosed with metabolic syndrome. eGFR was lower in men with abdominal obesity and in women with hypertension was than in those without. Among Japanese not taking medications, lower eGFR may be a characteristic of men with abdominal obesity and of women with hypertension.

Key words: metabolic syndrome, estimated glomerular filtration rate (eGFR), abdominal circumference

Chronic kidney disease (CKD) has become an important public health challenge in Japan and is a major risk factor for end-stage renal disease, cardiovascular disease and premature death [1, 2]. Identifying and treating risk factors for early chronic kidney disease may be the best approach to preventing and delaying adverse outcomes [1]. In Japan, clinical practice guidelines established by the Japanese Society of Nephrology estimate that 18.7% of adults have CKD, which is defined as kidney damage or a glomerular filtration rate (GFR) <60 ml/min/1.73m^2 for at least 3 months regardless of cause [3], and that 4.1% have moderate or severe CKD [4].

Metabolic syndrome is characterized by abdominal obesity, high blood pressure, dyslipidemia and impaired glucose tolerance [5]. In Japan, according to the criteria for this syndrome as defined in April 2005, 30.7% of men and 3.6% of women have metabolic syndrome [6, 7]. In some studies, CKD is closely related to body composition parameters and metabolic syndrome [8-18]. However, the link between renal function evaluated by estimated GFR (eGFR) and metabolic syndrome components using the Japanese criteria remains to be investigated.

In this study, we investigated renal function evaluated by eGFR in Japanese and evaluated the clinical impact of metabolic syndrome on eGFR in subjects not taking medications.

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Subjects and Methods

Subjects. In the first analysis, we used all data on 11,711 Japanese (3,674 men and 8,037 women) aged 20–79 years in a cross-sectional study. All subjects met the following criteria: (1) they had been wanting to change their lifestyle *i.e.*, diet and exercise habits, and had received an annual health checkup from June 1997 to May 2007 at Okayama Southern Institute of Health; (2) their creatinine (Cr) and anthropometric measurements had been taken as part of their annual health checkups; and (3) they provided informed consent (Table 1).

In the second analysis, among the 11,711 subjects, we further examined the data on 1,576 subjects (636 men and 940 women) who undertook fasting blood examination and blood pressure measurements and who were currently taking no medications; we also examined the Cr and anthropometric measurements of these second-analysis subjects (Table 2). In addition, medical staff subjectively evaluated these subjects' lifestyles, and encouraged subjects with fasting plasma glucose $\geqq 126$ mg/dl to begin taking medication.

The study was approved by the Ethics Committee

Table 1 Clinical profiles of subjects in the first analysis

	Men (n = 3,674)			Women (n = 8,037)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age	43.8 ± 14.2	20	79	42.9 ± 14.1	20	79
Height (cm)	168.9 ± 6.2	143.7	187.6	156.2 ± 5.7	134.9	179.3
Body weight (kg)	70.3 ± 11.7	39.1	175.7	55.1 ± 9.0	32.1	116.9
BMI (kg/m^2)	24.6 ± 3.7	13.6	61.5	22.6 ± 3.6	12.9	48.7
Body fat percentage (%)	24.3 ± 6.7	1.2	47.9	30.7 ± 7.0	3.9	56.2
Abdominal circumference (cm)	84.3 ± 10.2	58.0	157.0	72.3 ± 9.7	43.3	123.6
Hip circumference (cm)	94.2 ± 6.3	71.0	145.5	91.0 ± 6.0	58.5	132.0
Cr (mg/dl)	0.83 ± 0.15	0.39	2.57	0.61 ± 0.21	0.20	8.63
eGFR (ml/min/1.73m^2)	84.8 ± 18.7	20.2	191.3	90.6 ± 22.7	4.3	260.0

BMI: body mass index
Cr: creatinine
eGFR: estimated glomerular filtration rate

Table 2 Clinical profiles of subjects in the second analysis

	Men (n = 636)			Women (n = 940)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age	43.8 ± 11.2	20	78	45.7 ± 11.6	20	76
Height (cm)	169.1 ± 6.0	146.9	187.6	156.7 ± 5.5	139.3	176.3
Body weight (kg)	71.5 ± 11.2	40.1	121.7	56.0 ± 8.9	37.1	105.3
BMI (kg/m^2)	25 ± 3.5	16.4	43.3	22.8 ± 3.5	15.7	41.3
Body fat percentage (%)	24.4 ± 6.3	2.2	41.3	31.1 ± 6.6	10.6	50.1
Abdominal circumference (cm)	84.7 ± 9.5	58.8	123.0	72.5 ± 9.0	55.5	115.6
Hip circumference (cm)	94.9 ± 5.8	79.1	121.0	91.3 ± 6.1	60.0	122.0
Cr (mg/dl)	0.83 ± 0.14	0.50	1.85	0.62 ± 0.12	0.36	1.10
eGFR (ml/min/1.73m^2)	84.0 ± 16.8	36.0	146.5	84.5 ± 18.7	38.3	166.8
Systolic blood pressure (mmHg)	129.6 ± 15.7	90.0	205.0	121.3 ± 16.4	88.0	193.0
Diastolic blood pressure (mmHg)	81.2 ± 11.1	33.0	131.0	75.2 ± 10.2	44.0	120.0
Triglyceride (mg/dl)	142.5 ± 116.8	29.0	1,683.0	93.6 ± 14.7	70.0	331.0
HDL cholesterol (mg/dl)	55.4 ± 14.6	18.0	120.0	67.2 ± 16.4	28.0	151.0
Blood sugar (mg/dl)	100.6 ± 16.8	63.0	218.0	93.6 ± 14.7	70.0	331.0

BMI: body mass index
Cr: creatinine
eGFR: estimated glomerular filtration rate

of Okayama Health Foundation.

Anthropometric and body composition measurements. The anthropometric parameters were evaluated by using the following respective parameters such as height, body weight, body mass index (BMI), abdominal circumference, and hip circumference. BMI was calculated by weight/[height]^2 (kg/m^2). The abdominal circumference was measured at the umbilical level and the hip was measured at the widest circumference over the trochanter in standing subjects after normal expiration [19]. Body fat percentage was measured by an air displacement plethysmograph called the BOD POD Body Composition System (Life Measurement Instruments, Concord, CA, USA) [20, 21].

Blood pressure measurements. Each participant's blood pressure was measured after resting at least 15 min in the sitting position.

Blood sampling and assays. The level of Cr was measured with an automated biochemical analyzer (model 7700; HITACHI, Tokyo, Japan) and Accuras Auto CRE (Shino-Test Corporation, Tokyo, Japan). High-density lipoprotein (HDL) cholesterol [22], triglycerides (L Type Wako Triglyceride·H, Wako Chemical, Osaka, Japan) and plasma glucose (hexokinase method) were also measured at the Okayama Southern Institute of Health, Okayama Health Foundation. The accuracy of the measurements was maintained during the study period. eGFR was calculated using the following equation: eGFR (ml/min/1.73 m^2) $= 194 \times Cr^{-1.094} \times Age^{-0.287}$ (for men) and eGFR (ml/min/1.73 m^2) $= 194 \times Cr^{-1.094} \times Age^{-0.287} \times 0.739$ (for women) [23]. Reduced eGFR was defined as an eGFR < 60 ml/min/1.73 m^2.

Definition of metabolic syndrome. The syndrome was defined [6], among men with an abdominal circumference in excess of 85 cm and women with an abdominal circumference in excess of 90 cm [24], as having 2 or more of the following components: 1) dyslipidemia: triglyceride $\geq$ 150 mg/dl and/or HDL cholesterol $<$ 40 mg/dl; 2) hypertension: blood pressure $\geq$ 130/85 mmHg; 3) Impaired glucose tolerance: fasting plasma glucose $\geq$ 110 mg/dl.

Statistical analysis. Data are expressed as means $\pm$ standard deviation (SD) values. A comparison of parameters between the 2 groups was made using the unpaired *t*-test and covariance analysis. Simple correlation analysis was performed as well to test for the significance of the linear relationship among continuous variables: $p < 0.05$ was considered statistically significant. Statistical analysis was performed with StatView 5.0 (SAS Institute Inc., Cary, NC, USA).

Results

In the first analysis, the mean eGFR was 84.8 $\pm$ 18.7 ml/min/1.73 m^2 in men and 90.6 $\pm$ 22.7 ml/min/1.73 m^2 in women (Table 1). A diagnosis of reduced eGFR was made for 288 men (7.8%) and 498 women (6.2%). eGFR was not clearly correlated with anthropometric, body composition parameters in either sex (Table 3). eGFR in men with abdominal obesity (81.8 $\pm$ 17.8 ml/min/1.73 m^2) was lower than that in men without abdominal obesity (87.4 $\pm$ 19.1 ml/min/1.73 m^2), but the difference was not significant after adjusting for age ($p = 0.0675$). eGFR in women with abdominal obesity (83.8 $\pm$ 22.2 ml/min/1.73 m^2) was similar to that in women without abdominal obesity after adjusting for age (91.0 $\pm$ 22.6 ml/min/1.73 m^2) ($p = 0.8039$).

In the second analysis, we clarified the prevalence of metabolic syndrome among subjects who were not taking without medications (Table 4). Among the 1,576 Japanese subjects, 306 men (48.1%) had an abdominal circumference in excess of 85 cm and 48 women (5.1%) had an abdominal circumference

Table 3 Relationship between eGFR and anthropometric, body composition parameters

	Men		Women	
	r	p	r	p
Body weight (kg)	−0.017	0.2929	−0.110	<0.0001
BMI (kg/m^2)	−0.086	<0.0001	−0.174	<0.0001
Body fat percentage (%)	−0.146	<0.0001	−0.205	<0.0001
Abdominal circumference	−0.142	<0.0001	−0.233	<0.0001
Hip circumference (cm)	−0.006	0.7210	−0.060	<0.0001

Table 4 Comparison of eGFR between subjects with and without metabolic syndrome

Men	Abdominal obesity (+)	Abdominal obesity (−)	p	p (Afrer adjusting for age)
Number of subjects	306	330		
eGFR (ml/min/1.73m²)	83.8 ± 14.9	84.1 ± 18.4	0.7865	0.0055
	Impaired glucose torelance (+)	Impaired glucose torelance (−)		
Number of subjects	104	532		
eGFR (ml/min/1.73m²)	86.9 ± 16.4	83.4 ± 16.8	0.0479	0.0880
	Hypertension (+)	Hypertension (−)		
Number of subjects	347	289		
eGFR (ml/min/1.73m²)	82.7 ± 16.0	85.5 ± 17.6	0.0338	0.1106
	Dyslipidemia (+)	Dyslipidemia (−)		
Number of subjects	223	413		
eGFR (ml/min/1.73m²)	83.1 ± 16.6	84.4 ± 16.9	0.3501	0.6986
	Metabolic syndrome (+)	**Metabolic syndrome (−)**		
Number of subjects	132	504		
eGFR (ml/min/1.73m²)	83.6 ± 15.7	84.1 ± 17.1	0.7632	0.0830
Women	Abdominal obesity (+)	Abdominal obesity (−)		
Number of subjects	48	892		
eGFR (ml/min/1.73m²)	84.8 ± 16.7	84.5 ± 18.8	0.9179	0.2654
	Impaired glucose torelance (+)	Impaired glucose torelance (−)		
Number of subjects	50	890		
eGFR (ml/min/1.73m²)	86.0 ± 18.1	84.4 ± 18.8	0.5651	0.8745
	Hypertension (+)	Hypertension (−)		
Number of subjects	300	640		
eGFR (ml/min/1.73m²)	80.6 ± 17.0	86.3 ± 19.3	<0.0001	0.0222
	Dyslipidemia (+)	Dyslipidemia (−)		
Number of subjects	108	832		
eGFR (ml/min/1.73m²)	80.6 ± 20.0	85.0 ± 18.5	0.0223	0.2757
	Metabolic syndrome (+)	**Metabolic syndrome (−)**		
Number of subjects	15	925		
eGFR (ml/min/1.73m²)	81.5 ± 17.0	84.6 ± 18.8	0.5297	0.1077

Mean ± SD

exceeding 90cm. In addition, 132 men (20.8%) and only 15 women (1.6%) were diagnosed with the syndrome.

In subjects not taking medications, we also compared eGFR levels between the groups with and without each component of the Japanese definition of metabolic syndrome (Table 4). To avoid the influence of age, we used age as a covariate and compared eGFR between Japanese with and those without metabolic syndrome components using covariance analysis. eGFR in men with abdominal obesity and in women with hypertension was significantly lower than in subjects without these components of metabolic syndrome, even after adjusting for age. However, there were no significant differences in eGFR between the groups with or without other components of metabolic syndrome. In addition, eGFR in subjects with metabolic syndrome was similar to that in subjects without it, even after adjusting for age.

Discussion

Obesity is a significant risk factor for developing CKD and proteinuria [8–11]. Fox *et al.* reported that the odds ratio (OR) for developing new-onset kidney disease, defined as a GFR < 59.3ml/min/1.73m² in

women and 64.3 ml/min/m^2 in men, was 1.23, representing a 23% increase in BMI within 10 -years [8]. In Japan, it was also reported that BMI above 25 kg/m^2 was linked to proteinuria [9]. Bonnet *et al.* reported that abdominal obesity was related to the development of elevated albuminuria in both sexes, suggesting that the measurement of abdominal circumference might improve the identification of non-diabetic individuals at risk of developing microalbuminuria [10]. In addition, a greater waist-to-hip ratio was associated with a greater risk for diminished filtration, even when corrected for BMI [11]. In this study, the relationships between eGFR and anthropometric, body composition parameters were not clearly revealed in the first analysis. However, after adjusting for age by using covariance analysis, eGFR in men with abdominal obesity tended to be lower than that in men without abdominal obesity in the first and second analyses. Therefore, we could not accurately prove a link between eGFR and anthropometric, body composition parameters, unlike the case in previous studies.

This study is the first to reveal a relationship between eGFR and metabolic syndrome, defined by the new Japanese criteria of metabolic sundrome. Metabolic syndrome has important clinical and public health implications in Japan because it is a common disorder in that country [7]. Previous studies have documented that metabolic syndrome is an important risk factor for diabetes, coronary heart disease and stroke [25-27]. The present study shows new and important information about the relationship between eGFR and metabolic syndrome in a large sample of Japanese.

Subjects with metabolic syndrome, using the modified Adult Treatment Panel (ATP) III definition [28], showed higher urinary albumin excretion and left ventricular mass index, increased intima-media thickness, and a higher prevalence of microalbuminuria [12]. Compared with subjects with 0 or 1 component of the metabolic syndrome, subjects with 2, 3, 4, or 5 components of the syndrome had multivariate-adjusted odds ratios of 2.21, 3.38, 4.23, and 5.85 for CKD [13]. Using the Japanese criteria, we previously reported that the prevalence of proteinuria in subjects with metabolic syndrome was significantly higher than that in subjects without the syndrome [14]. Tanaka *et al.* [15], Ninomiya T *et al.* [16] and Iseki *et al.* [17] reported that metabolic syndrome, using the modified ATP III definition, was associated with CKD in Japanese. Although Tsuda *et al.* [18] revealed that the level of microalbuminuria in subjects with metabolic syndrome according to the Japanese criteria was significantly higher than that in subjects without the syndrome, the link between eGFR and metabolic syndrome using the Japanese criteria has not been investigated until now. In this study, although we evaluated eGFR in subjects without medications, the clinical impact of abdominal obesity in men and hypertension in women was noted in the second analysis. However, eGFR in subjects with metabolic syndrome was similar to that in subjects without the syndrome in either sex. eGFR was higher in subjects with impaired glucose tolerance than in those without, but not significantly. Glomerular hyperfiltration exists among Japanese type 2 diabetic patients with no evidence of overt proteinuria or hypertension [29]. In addition, according to the analysis of subjects without medications, the link between eGFR and metabolic syndrome and its components may be attenuated. Therefore, a significant difference in eGFR between subjects with and without metabolic syndrome might not be noted.

Potential limitations remain in this study. First, our study was a cross sectional and not a longitudinal study. Second, the 11,711 subjects, all of whom wanted to change their lifestyle, underwent measurements for this study: they were therefore more health-conscious than the average person. The selected 1,576 subjects underwent fasting blood examination and blood pressure measurements and were taking no medications; they were therefore more health-conscious than most of the subjects in the first analysis. Although some subjects were within the range of fasting plasma glucose levels at wthich medications are recommended, the prevalence of metabolic syndrome in this study was lower than in our previous report [7]. This was especially true in women, only 15 of whom were diagnosed as having metabolic syndrome. The small sample size in women with metabolic syndrome might make it difficult to compare eGFR between women with the syndrome and those without. Third, we could not accurately prove the mechanism between lower eGFR and metabolic syndrome components. Further prospective studies are needed in Japanese subjects using the new Japanese criteria.

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Short Communication

Decreasing Systolic Blood Pressure Is Associated with Improving Estimated Glomerular Filtration Rate (eGFR) with Lifestyle Modification in Japanese Healthy Women

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The link between changes in a subject's metabolic syndrome components and her estimated glomerular filtration rate (eGFR) was evaluated in healthy Japanese women. We used data for 53 Japanese women (46.0 ± 10.9 years) with a 1-year follow up. eGFR was defined by a new equation developed for Japan. There were no significant relationships between eGFR and clinical parameters at baseline. Subjects were given advice for dietary and lifestyle improvement. At the 1-year follow up, eGFR was significantly increased. In addition, changes in eGFR were weakly correlated with systolic blood pressure ($r = -0.306$, $p = 0.0260$). A decrease in systolic blood pressure may be associated with improving eGFR in Japanese women.

Key words: systolic blood pressure, estimated glomerular filtration rate (eGFR), metabolic syndrome, lifestyle modification

Chronic kidney disease (CKD) is a common disorder and has become a public health challenge [1]. For example, about 20% of adults have CKD, which is defined as kidney damage or a glomerular filtration rate (GFR) $< 60\,ml/min/1.73\,m^2$ for at least 3 months regardless of cause, and 4.1% have moderate or severe CKD [2]. We have also previously reported in a cross-sectional study that the estimated glomerular filtration rate (eGFR) [3] in men with abdominal obesity and in women with hypertension was significantly lower than that in subjects without these components of metabolic syndrome [4]. However, whether decreases in metabolic syndrome components are beneficial for improving eGFR, and what effects this has on eGFR remain to be investigated in a longitudinal study.

In this study, we evaluate the link between changes in eGFR and changes in metabolic syndrome components in Japanese women with a 1-year follow up.

Subjects and Methods

Subjects. We used data for 53 Japanese women, aged 46.0 ± 10.9 years, who met the following criteria: (1) received a health check-up including special health guidance and a follow-up check-up 1-year later, (2) received anthropometric measurements, fasting blood examination and blood pressure measurements as part of the annual health check-up, (3) received no medications for diabetes, hypertension, and/or dyslipidemia, and (4) provided written informed consent (Table 1).

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Table 1 Clinical characteristics and changes in parameters with 1-year follow up

	Baseline	Follow up	p
Number of Subjects	53		
Age	46.0 ± 10.9		
Height (cm)	156.1 ± 4.3		
Body weight (kg)	62.4 ± 8.8	60.9 ± 8.5	0.0002
Body mass index (kg/m^2)	25.6 ± 3.3	25.0 ± 3.3	0.0002
Abdominal circumference (cm)	78.7 ± 8.1	76.6 ± 8.3	0.0005
Systolic blood pressre (mmHg)	121.5 ± 14.1	119.8 ± 15.4	0.2772
Diastolic blood pressure (mmHg)	76.2 ± 9.4	74.6 ± 10.8	0.2245
Triglyceride (mg/dl)	98.2 ± 65.8	95.8 ± 62.1	0.7065
HDL cholesterol (mg/dl)	64.5 ± 14.5	64.6 ± 14.9	0.9362
Blood sugar (mg/dl)	95.1 ± 9.4	93.8 ± 9.3	0.2018
Cr (mg/dl)	0.58 ± 0.09	0.56 ± 0.10	0.0148
eGFR (ml/min/1.73m^2)	90.0 ± 17.9	94.2 ± 19.9	0.0215

Mean ± SD

At the first health check-up, all subjects were given instructions by well-trained medical staff on how to change their lifestyle as special health guidance. Nutritional instruction was provided with a well-trained nutritionist, who planned a diet for each subject based on their data and provided simple instructions (*i. e.* not to eat too much and to consider balance when they eat). Exercise instruction was also provided by a well-trained physical therapist, who encouraged each subject to increase their daily amount of steps walked.

Ethical approval for the study was obtained from the Ethical Committee of Okayama Health Foundation.

Anthropometric and body composition measurements. Anthropometric and body compositions were evaluated based on the following parameters: height, body weight and abdominal circumference. Body mass index (BMI) was calculated by weight / [height]^2, in kg/m^2. Abdominal circumference was measured at the umbilical level in standing subjects after normal expiration [5].

Blood pressure measurements at rest. Resting systolic and diastolic blood pressures were measured indirectly using a mercury sphygmomanometer placed on the right arm of the seated participant after at least 15 min of rest.

Urine examination. Urine samples were collected from the second- morning urine (before 10 a. m.) and subjected to examination within 1 h. The urine examination was performed using urine test strips (BAYER, Tokyo, Japan). The reagent strip was dipped directly into the urine sample. Just after dipping, the sample was graded as −: negative, ±: trace positive, +: positive (30mg/dl), 2+: positive (100mg/dl), 3+: positive (300mg/dl) or 4 +: positive (1,000mg/dl) by comparison with a standard color chart found on the container's label.

Blood sampling and assays. We measured overnight fasting serum levels of creatinine (Cr) (enzymatic method), high-density lipoprotein (HDL) cholesterol, triglycerides (L Type Wako Triglyceride · H, Wako Chemical, Osaka, Japan) and plasma glucose. eGFR was calculated using the following equation: eGFR (ml/min/1.73m^2) = $194 \times Cr^{-1.094} \times Age^{-0.287} \times 0.739$ (a constant derived specifically for women) [3]. Reduced eGFR was defined as an eGFR < 60ml/min/1.73m^2.

Definition of metabolic syndrome. Women with an abdominal circumference in excess of 90cm were defined as having metabolic syndrome if they also had 2 or more of the following components: 1) Dyslipidemia: triglycerides ≥ 150mg/dl and/or HDL cholesterol < 40mg/dl, 2) High blood pressure: blood pressure ≥ 130/85mmHg, 3) Impaired glucose tolerance: fasting plasma glucose ≥ 110mg/dl [5].

Statistical analysis. Data are expressed as means ± standard deviation (SD). A statistical analysis was performed using a paired t test: $p < 0.05$ was considered to be statistically significant. Pearson's correlation coefficients were calculated and used to test the significance of the linear relationship among con-

tinuous variables; stepwise multiple regression analysis was also used.

Results

The clinical parameters at the baseline and the 1-year follow up are summarized in Table 1. Anthropometric and body composition parameters such as body weight, BMI and abdominal circumference were significantly reduced with lifestyle modification after 1 year. Cr was significantly decreased and eGFR was significantly increased. No subject was diagnosed as having metabolic syndrome and only one subject was diagnosed with reduced eGFR from baseline to the 1-year follow up. In addition, 2 subjects were identified as positive (+) for proteinuria at baseline and 4 subjects were identified as trace positive at the 1-year follow up.

The relationship between eGFR and clinical parameters at baseline was evaluated. There were no significant relationships between eGFR and other clinical parameters at baseline (Table 2).

Table 2 Simple correlation analysis between eGFR and clinical parameters at baseline

	r	p
Body weight (kg)	0.082	0.5594
Body mass index (kg/m^2)	0.033	0.8165
Abdominal circumference (cm)	−0.154	0.2708
Systolic blood pressre (mmHg)	−0.167	0.2333
Diastolic blood pressure (mmHg)	−0.119	0.3958
Triglyceride (mg/dl)	0.123	0.3785
HDL cholesterol (mg/dl)	−0.063	0.6566
Blood sugar (mg/dl)	−0.193	0.1662

Table 3 Simple correlation analysis between changes in eGFR and changes in clinical parameters with 1-year follow up

	r	p
Body weight (kg)	0.188	0.1775
Body mass index (kg/m^2)	0.181	0.1945
Abdominal circumference (cm)	0.253	0.0672
Systolic blood pressre (mmHg)	−0.306	0.0260
Diastolic blood pressure (mmHg)	−0.112	0.4325
Triglyceride (mg/dl)	0.095	0.5006
HDL cholesterol (mg/dl)	0.227	0.1015
Blood sugar (mg/dl)	−0.214	0.1232

We further evaluated the relationship between changes in eGFR and changes in clinical parameters. Changes in eGFR were weakly correlated with changes in systolic blood pressure ($r = -0.306$, $p = 0.0260$) (Table 3, Fig. 1). However, changes in eGFR were not significantly correlated with changes in other metabolic components. We also used stepwise multiple regression analysis to evaluate the effect of changes in clinical parameters, *i.e.* body weight, BMI, abdominal circumference, systolic blood pressure, diastolic blood pressure, triglyceride, HDL cholesterol and blood sugar, on the change in eGFR, and found that only change in systolic blood pressure was significant [Change in eGFR = 3.632 − 0.349 (change in systolic blood pressure), $r^2 = 0.093$, $p = 0.0260$].

Finally, we further investigated the difference of change in eGFR between subjects who had different levels of systolic blood pressure at baseline [Group L, systolic blood pressure < 140 mmHg; Group H, systolic blood pressure ≥ 140 mmHg]. The changes in systolic blood pressure in Group H subjects (−1.20 ml/min/1.73 m^2) was lower than that in Group L subjects (4.9 ml/min/1.73 m^2) after 1 year, but not at a significant level ($p = 0.2822$).

Discussion

The main objective of this study was to explore the link between changes in eGFR and changes in metabolic syndrome components in Japanese women with a

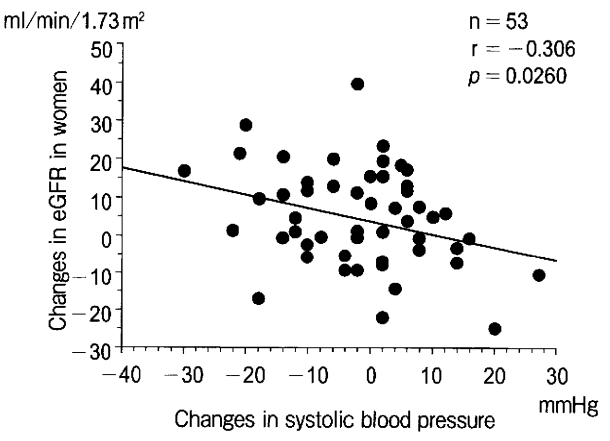

Fig. 1 Simple correlation analysis between changes in eGFR and changes in systolic blood pressure at 1-year follow up.

1-year follow up.

Tanaka *et al.* [6], Ninomiya T *et al.* [7] and Iseki *et al.* [8] reported that metabolic syndrome, using the modified ATP III definition [9], was associated with CKD in the Japanese population. Compared with subjects with 0 or 1 component of metabolic syndrome, subjects with 2, 3 and 4 or more components had odds ratios of 1.13, 1.90 and 2.79 for CKD [7]. In this study, no subject was diagnosed as having metabolic syndrome, using the Japanese criteria, either at baseline or at the 1-year follow up. We have previously reported that the prevalence of metabolic syndrome was 3.6% in Japanese women [10]. However, with lifestyle modification after the initial health check-up, eGFR was significantly increased even in women without metabolic syndrome at the 1-year follow-up.

Hypertension contributes to the development of renal injury and end-stage renal disease [11–15]. Even high-normal blood pressure has been shown to be significantly associated with development of CKD in both sexes. Yamagata *et al.* reported that the baseline-adjusted predictor of developing CKD included age, GFR, hematuria, hypertension, diabetes, serum lipids, obesity, smoking status and consumption of alcohol with 10-year follow up [11]. Tozawa *et al.* also reported a relative risk of 1.34 for end-stage renal failure for every increase of 10 mmHg in systolic blood pressure in 51,878 women investigated [12]. In the present study, there was no significant relationship between eGFR and systolic blood pressure at baseline. However, we revealed that, with lifestyle modification, changes in systolic blood pressure were correlated with changes in eGFR in women without metabolic syndrome. Therefore, the clinical impact of hypertension was noted.

Potential limitations remain in our study. First, the small sample size in our study makes it difficult to infer causality between eGFR and hypertension. Second, we also could not reveal the mechanism of the linkage between eGFR and hypertension. Further prospective studies are needed in Japanese subjects. Third, most of the enrolled subjects were not diagnosed as CKD at baseline. Therefore, the results in this study may not apply for patients with CKD.

In conclusion, a decrease in systolic blood pressure with lifestyle modification was associated with an increase in eGFR. Therefore, lifestyle modification may be a necessary and useful measure for the prevention of CKD.

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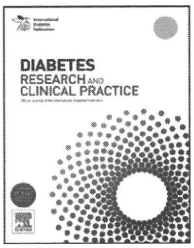

Microinflammation is a common risk factor for progression of nephropathy and atherosclerosis in Japanese patients with type 2 diabetes

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ABSTRACT

Aim: This study aimed to evaluate the change of serum levels of proinflammatory molecules in patients with type 2 diabetes and clarify the involvement of these molecules in diabetic nephropathy and atherosclerosis.

Methods: Sixty-six Japanese type 2 diabetic patients (T2DM) and 39 healthy control subjects were enrolled. We assessed clinical parameters, urinary albumin excretion rate (AER), brachial-ankle pulse wave velocity (baPWV), intima media thickness (IMT) and serum levels of proinflammatory molecules.

Results: Serum levels of IL-6, IP-10 and MCP-1 were significantly higher in T2DM than in control subjects. In T2DM, serum levels of high-sensitivity (hs) CRP, IP-10, hsTNF-α, VCAM-1 and E-selectin were positively correlated with AER. Serum levels of IP-10, hsTNF-α and VCAM-1 were positively correlated with baPWV. Serum levels of hsCRP, IL-6, IP-10 and hsTNF-α were positively correlated with IMT. Multiple linear regression analysis revealed that serum levels of hsTNF-α were independently associated with AER ($\beta = 0.235$, $P = 0.038$) and serum levels of IP-10 were independently associated with baPWV ($\beta = 0.209$, $P = 0.047$) and IMT ($\beta = 0.303$, $P = 0.032$).

Conclusion: Our results suggest that low-grade inflammation, microinflammation, may be a common risk factor for diabetic nephropathy and atherosclerosis in Japanese type 2 diabetic patients.

1. Introduction

Microinflammation, low-grade inflammation occurred on the vascular wall, is involved in the mechanism of atherosclerosis [1]. The elevation of serum C-reactive protein (CRP) level is known to be a risk factor for ischemic heart disease [2,3]. Proinflammatory molecules, such as interferon-γ inducible protein (IP)-10 are reported to be involved in the formation of atherosclerotic lesion [4]. Microinflammation is also occurred in patients with diabetes. Several reports indicated that CRP [5] and proinflammatory cytokines including interleukin (IL)-6 and tumor necrosis factor (TNF)-α [6], are elevated in patients with type 2 diabetes. Elevated levels of CRP and IL-6 predict insulin resistance and development of type 2 diabetes [7,8].

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