Fig. 2. Multivariate-adjusted odds ratios with 95% confidence intervals for prevalent CKD in the quartile groups after stratification for the presence or absence of hypertension, diabetes, and obesity. Logistic regression analysis was adjusted for age, gender, waist circumference, hypertension, obesity, diabetes, a current smoking habit, daily alcohol consumption, regular exercise habits, history of stroke and heart disease, as well as medication for dyslipidemia in participants with and without hypertension, diabetes, and obesity. The variable relevant to the subgroup was excluded from each model. Odds ratios and 95% confidence intervals for CKD are shown. Abbreviations: CKD, chronic kidney disease; TG/HDL; triglycerides/high-density lipoprotein cholesterol.

adjustment for age. Thus, we confirmed that no significant trends between history of cardiovascular disease and TG/HDL-C in men are influenced by the inverse trend for age in TG/HDL-C.

It is well-known that moderate CKD increases TG and decreases HDL-C levels [2–7]. A relationship between dyslipidemia and the incidence of CKD remains controversial [17–19]; however, there is a growing body of evidence to show that abnormalities in lipid metabolism contribute to the progression of renal disease [20–24]. Others have reported that the vicious cycle between renal dysfunction and dyslipidemia is activated in CKD, and this seems to contribute to cardiovascular and all-cause mortality [25,26].

In Asian population, Kang et al. showed that there were significant associations between TG/HDL-C and reduced eGFR or albuminuria among Korean adults [27,28]. Likewise, the present study clearly showed that TG/HDL-C was associated with the risk of both decreased eGFR and proteinuria on the basis of individual data from 216,007 Japanese participants. Therefore, these findings highlight the potential clinical value of the measurement of both TG and HDL-C for risk assessment of CKD in the Japanese population.

The mechanism by which the relationship between TG/HDL-C and the risk of CKD might be mediated is an area of great interest. Previous investigations reported a relationship between a high TG/HDL-C and elevated levels of small, dense LDL-C particles [12–14]. These LDL-C particles are highly atherogenic [29], and their level is considered a useful marker of insulin resistance [8–11], which, in turn, mediates risk factors for cardiovascular disease and CKD such as diabetes, hypertension, obesity, lipid abnormalities, and atherosclerosis [30]. These findings suggest us that the association between TG/HDL-C and CKD might be influenced by the relevant confounding factors such as diabetes, hypertension, and obesity. Because of the large number of the participants, we could perform stratified analyses to assess whether diabetes, hypertension, or obesity influenced the association between TG/HDL-C and CKD, and we found that the association between TG/HDL-C and CKD was robust regardless of the presence of these diseases, suggesting that TG/HDL-C is independently associated with the risk of CKD, regardless of the presence of known atherogenic variables. This finding is in accordance with previous studies suggesting insulin resistance as an independent risk factor for the progression of renal dysfunction in nondiabetic subjects [31].

On the other hand, the association between TG/HDL-C and CKD was stronger in participants with diabetes and hypertension than in participants without these diseases. It is thus possible that lipid disorders and diabetes or hypertension are linked in a vicious cycle

and that CKD is a consequence of diabetes or hypertension in individuals with dyslipidemia. Taken together, these results highlight the importance of TG/HDL-C as a relevant factor for CKD, especially in diabetic/hypertensive individuals, and we suggest aggressive management of dyslipidemia to prevent the incidence and progression of CKD. However, the pathophysiology of influence of TG/HDL-C requires further exploration.

Our study had some limitations. First, the cross sectional study design limits the interpretation of causality between TG/HDL-C and CKD. Second, single measurements of TG, HDL-C, serum creatinine, and dipstick measurements of urinary protein could have resulted in the misclassification of some comorbidities. Third, GFR was not directly measured, but was estimated with a serum creatinine-based equation, which could have over- or underestimated the actual GFR in the Japanese general population. Fourth, our study participants were generally healthy and were interested in their own health; therefore, the prevalence of dyslipidemia or CKD may have been underestimated. Fifth, although we argued the possible pathogenetic role of small, dense LDL, but we have not shown any data directly as to the small, dense LDL. One may argue that the availability of apolipoprotein B levels, which is not an unreasonable parameter to demand nowadays, might have helped our argument. However, we cannot provide the data because we had not examined the levels of apolipoprotein B and also had not stored any blood samples.

In conclusion, the present findings, representative of a general population of Japanese adults, indicated that high TG/HDL-C level significantly associated with prevalent CKD. Further prospective studies are needed to clarify the causative relationship between serum TG/HDL-C and CKD.

Contributors

Kazuhiko Tsuruya and Hisako Yoshida contributed to the study design, acquisition of data, statistical analysis, interpretation of data, and drafting of the manuscript. Masaharu Nagata contributed to the study design, acquisition of data, statistical analysis, and interpretation of data. Takanari Kitazono contributed to the critical revision of the manuscript and study supervision. Hideki Hirakata contributed to acquisition of data and critical revision of the manuscript. Kunitoshi Iseki, Toshiki Moriyama, Kunihiro Yamagata, Hideaki Yoshida, Shouichi Fujimoto, and Koichi Asahi contributed to acquisition of data and critical revision of the manuscript. Issei Kurahashi and Yasuo Ohashi contributed to acquisition of data, statistical analysis, and interpretation of data. Tsuyoshi Watanabe contributed to the funding, acquisition of data, critical revision of the manuscript and study supervision. All authors provided critical reviews of the draft and approved the final version.

Support and financial disclosure declaration

Acknowledgments

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Nephrology Dialysis Transplantation

Original Articles

Cerebral oxidative stress induces spatial working memory dysfunction in uremic mice: neuroprotective effect of tempol

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ABSTRACT

Background. Chronic kidney disease (CKD) is frequently associated with uremic encephalopathy and cognitive impairment. Recent studies have demonstrated that cerebral oxidative stress contributes to cognitive dysfunction. Although oxidative stress has been reported to increase in the uremic rat brain, the relationship between increased oxidative stress and cognitive impairment in uremia is unclear. In the present study, the effects of tempol (TMP), an antioxidant drug, were investigated in uremic mice.

Methods. CKD was induced in male C57BL/6 mice ($n = 8$) by left nephrectomy and 2/3 electrocoagulation of the right renal cortex. Working memory performance was tested by the radial arm water maze test. We have prepared two protocols ('time course study' and 'treatment study'). First, we examined the working memory test and histological examination of mouse brains after 4 and 8 weeks. Next, we investigated the effect of TMP (3 mM) against uremia-induced neurodegeneration and oxidative stress in the mouse brain.

Results. Eight weeks after CKD induction, vehicle-treated mice made significantly more errors than sham-operated control mice, while TMP improved working memory performance in CKD mice. CKD was associated with accumulation of 8-hydroxy-2'-deoxyguanosine in the hippocampal neuronal cells, but not in TMP-treated CKD mice. Increased numbers of pyknotic neuronal cells were observed in the hippocampus of CKD mice at 8 weeks, but pyknotic neuronal cell numbers were decreased under the influence of TMP in uremic mice.

Conclusions. The present study provided evidence that uremia is associated with spatial working memory dysfunction in mice and that treatment with TMP protects against cerebral oxidative stress and improves cognitive dysfunction in uremic mice, suggesting their potential usefulness for the treatment of cognitive dysfunction in uremia.

Keywords: chronic kidney disease, cognitive dysfunction, oxidative stress, uremia

INTRODUCTION

Uremic encephalopathy presents with diverse symptoms including headache, visual abnormalities, tremor, asterixis, multifocal myoclonus, chorea, seizure, clouding of consciousness, delirium and coma [1]. Several studies have suggested that the frequency of cognitive disturbance is higher than that previously suspected and can be detected even in moderate chronic renal insufficiency [2–4]. In addition, clinical studies have demonstrated a high risk for dementia and cognitive impairment in patients with chronic kidney disease (CKD) and those undergoing hemodialysis [3, 5–7].

The association between oxidative stress and cognitive dysfunction has previously been shown. Row *et al.* [8] reported

that intermittent hypoxia caused increased oxidative stress and deficits in spatial learning in rats. Oxidative stress is also implicated in neurodegenerative diseases with memory impairment such as Alzheimer's disease [9]. The hippocampus is considered important for learning and memory function. Moreover, hippocampal volume can be used to detect cognitive dysfunction and identify elderly people at risk for Alzheimer's disease [10]. However, the presence of hippocampal injury is unclear in human CKD. Deng et al. [11] reported that chronic renal failure (CRF) resulted in oxidative stress and increased tyrosine nitration in the cerebral cortex. Furthermore, antioxidant therapy alleviated CRF-induced oxidative stress and mitigated tyrosine nitration in rats with CRF. We have also focused on oxidative stress in the development of cognitive dysfunction in uremia and the effect of antioxidant drugs against uremia-induced cognitive dysfunction. Uremia is associated with depressed superoxide dismutase (SOD) levels and elevated NAD(P)H oxidase expression. This was shown by favorable responses to the administration of tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl; TMP), a nitroxide compound with SOD-mimicking activity [12]. Thus, in the present study, we hypothesized that TMP provides protection against chronic uremia-induced cognitive dysfunction through its antioxidative and neuroprotective effects on hippocampal neurons.

To verify this hypothesis, we developed a stable mouse model of uremic CKD and measured cognitive dysfunction using the radial arm water maze (RAWM) test (Figure 1). We undertook protocols to (i) determine the association of uremia-induced cognitive dysfunction with oxidative stress in uremic mice (Protocol 1) and (ii) examine the effect of antioxidants against uremia-induced cognitive dysfunction in uremic mice (Protocol 2).

MATERIAL AND METHODS

Preparation of mice

The present study was conducted in C57BL/6J male mice (8 weeks old; CLEA, Tokyo, Japan). All animal study protocols were reviewed and approved by the Kyushu University Animal Care Committee at the Center for Animal Care Facility. All experiments were conducted according to the National Institute of Health Guideline for the Care and Use of Laboratory Animals. Mice were kept at the Experimental Animal Center of Kyushu University Medical Institution and housed in groups of four or five per cage in a temperature-controlled room (23–25°C). All mice had free access to food and water during the experiments. Mice were sacrificed under anesthesia.

Induction of CKD in mice

CKD was induced in 8-week-old C57BL/6J male mice using a one-step procedure involving electrocoagulation of the surface of a surgically exposed right kidney and left nephrectomy. Details of this method have been reported previously [13, 14]. Briefly, electrocoagulation of the entire surface of the right kidney except for a 2-mm rim of renal tissue around the hilum was followed by left nephrectomy. All mice were
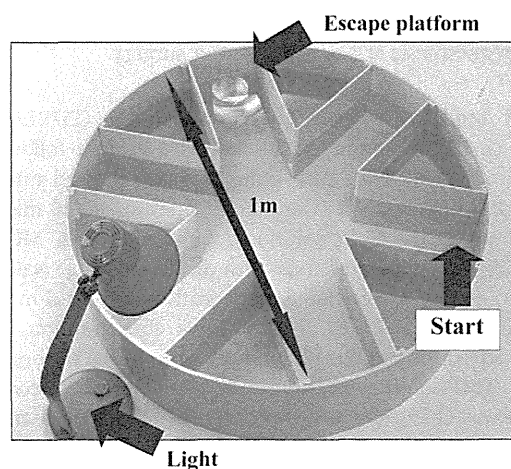
FIGURE 1: Working memory test–RAWM test. An image of the RAWM is shown. The RAWM consists of a circular pool measuring 1 m in diameter with six 19-cm-wide arms, radiating from an open central area, and a submerged escape platform, located at the end of one arm. The edges of the arms reached above the surface of the water, forming swim alleys surrounding a central open region. The platform was placed within one of the alleys (goal arm), and the mouse was started in one of the other swim arms. The light, a visual spatial cue, was placed on the floor of the testing room.

subjected to surgery under controlled ether anesthesia, with a surgical approach through small bilateral flank incisions, leaving the intestines and upper abdominal content undisturbed. Renal electrocoagulation was performed using a foot-operated single point cauterizer angled at 30° (PROG DS3-M; Muromachi Kikai Co., Tokyo, Japan). The right kidney was freed from the perirenal fat and adrenal gland prior to electrocoagulation and special care was taken not to injure the right ureter. After electrocoagulation, the kidney was placed into the renal fossa and completely covered with tissue from the abdominal wall. A left nephrectomy was performed under identical operating conditions. After each surgical procedure, the incisions were closed in layers with clips applied to the skin. The skin-to-skin operative time did not exceed 5 min. The control mice received a sham operation that included decapsulation of both kidneys. Special care was taken to avoid damage to the adrenals. All surgical procedures were performed by a single investigator, and the choice of treatment (TMP or vehicle water) was determined randomly.

Experimental protocols

Time course study—Protocol 1. Eight-week-old C57BL/6J male mice ($n = 32$) were randomly divided into two groups. All mice were allowed to drink water freely. One group was a sham-operated control group ($n = 16$), and the other group was CKD-operated group. After surgery on both kidneys, the 32 mice were divided into four groups: sham-operated mice observed for 4 weeks (Cont-4W: $n = 8$); sham-operated mice observed for 8 weeks (Cont-8W: $n = 8$); remnant kidney mice observed for 4 weeks (CKD-4W: $n = 8$) and remnant kidney

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mice for 8 weeks (CKD-8W: $n = 8$). All mice were sacrificed under ether anesthesia at 4 or 8 weeks after surgery.

Treatment study—Protocol 2. Eight-week-old C57BL/6J male mice ($n = 32$) were randomly subdivided into the following four groups ($n = 8$ for each group): sham-operated mice with drinking vehicle water (Cont-Veh); sham-operated mice with drinking 3 mM TMP solution (Sigma, St Louis, MO) (Cont-TMP); CKD-operated mice with drinking vehicle water (CKD-Veh) and CKD-operated mice with drinking 3 mM TMP solution (CKD-TMP). The effective concentration of TMP was determined by our preliminary study and a previous study [15]. TMP solution was administered in an opaque container and was changed every two days. All mice were sacrificed on day 56 (week 8).

Biochemical parameters

Blood samples were drawn from mice before sacrifice. Mice were fully anesthetized and held by the back. Then, blood samples were collected via tail vein, and samples for measurement of serum creatinine (SCr) and blood urea nitrogen (BUN) were immediately frozen at $-80°C$ until measurement. Measurements of SCr and BUN were performed at SRL Inc. (Tokyo, Japan). Hematocrit (Ht) was determined by centrifugation of microcapillary tubes at 10 000 rpm for 5 min.

RAWM test for spatial working memory assessment

For the RAWM test, an apparatus was constructed as previously described [16, 17]. The RAWM is a radial arm maze placed in a pool of water (Figure 1). In this experiment, the RAWM consisted of a circular pool measuring 1 m in diameter with 6 19-cm-wide arms radiating out from an open central area, with a submerged escape platform located at the end of one arm. Visual spatial cues including a light were placed on the wall of the testing room. The escape platform was placed on a different arm each day (the platform location does not change over one day), forcing the mice to use their memory to solve the task. A semi-random sequence from four of the remaining four arms was then selected as starting points for each day's four 'acquisition' trials (trial 1–4: T1–4). For any given acquisition trial, the animal was placed into the designated start arm facing the common circular swim area. For the ensuing 1-min trial, the animal was allowed to swim into arms, with each non-goal arm selection (i.e. error; swimming into an arm that did not contain the submerged platform) resulting in the mouse being returned (across the surface of the water) back to that trial's start arm to continue the trial. If a mouse entered the goal arm, but could not locate the submerged platform in that arm, an error was scored and the animal was returned to that trial's start arm to continue the trial (i.e. a 'win-stay strategy'). After the fourth trial, the mouse was placed in a cage for 30 min and then returned to the maze and administered the fifth 'memory retention' trial (T5) to assess short-term memory retention. T5 trial was then performed, wherein the start arm was the same as for the T4 trial. The number of errors in the fifth trial on the fifth day was used as the result of the RAWM test for each mouse.

Open field activity testing

To test activity and exploratory behavior, each mouse was placed in an open gray cylindrical box, consisting of a circular pool measuring 50 cm in diameter, which has lines (four horizontal and four vertical) to demarcate 16 squares. The total number of line crossings in a single 5-min trial was recorded [16].

Histological examination of mouse brains

Perfusion of the hippocampus was performed using a previously reported method with some modification [18]. Briefly, mice were perfused intracardially with a solution containing 4% paraformaldehyde (w/v) in 0.1 mol/L phosphate buffer, pH 7.3–7.5. The brain was dissected and then placed in the same fixative solution for 6 h at 4–5°C. Brains were then dehydrated in ethanol, transferred to 1:1 (v/v) ethanol–xylene, cleared in toluene and embedded in Paraplast (Fischer Scientific, Park Lane, PA). Serial sections (4 μm thick) were mounted on gelatin-coated glass slides. Paraplast was removed using toluene (2 × 10 min), and sections were passed through graded ethanol, washed with distilled water and colored with hematoxylin and eosin (H&E). To determine neuronal density, neuronal nuclei were counted in the 4-μm-thick sections to avoid double counting of neurons.

Enumeration of degenerated neuronal cells

H&E-stained sections of the mouse brain were examined by light microscopy (Eclipse E800M model; Nikon, Tokyo, Japan). The investigator was blinded to the groups and counted pyknotic cells in eight ×400 fields in the hippocampus region from each section (four sections per mouse) [19]. The number of pyknotic cells was presented per section (total counts in eight fields per section).

Immunohistochemical evaluation of hippocampus lesions for oxidative stress

All specimens were fixed in 10% formalin and routinely processed for paraffin. Formalin-fixed, paraffin-embedded tissue sections were serially cut at 4-μm and mounted on aminopropyltriethoxysilane-coated glass slides. Sections were deparaffinized in xylene and dehydrated through an ethanol series. Oxidative stress was examined by immunohistochemical staining for anti-8-hydroxy-2'-deoxyguanosine (8-OHdG). Brain sections from experimental mice were immunostained with anti-8-OHdG mouse monoclonal antibody (1:100, Japan Institute for the Control of Aging, Shizuoka, Japan) as described previously [19, 20]. For 8-OHdG staining, the glass slides were treated with 10 μg/mL proteinase K and 4N HCl. Sections were then pre-treated in 0.3% H_2O_2 in methanol for 30 min to inactivate endogenous peroxidase, preincubated with 5% skim milk to reduce nonspecific binding and incubated overnight at 4°C with anti-8-OHdG antibodies. After washing, the sections were incubated with biotinylated secondary antibodies for 1 h at room temperature followed by horseradish peroxidase (HRP)-conjugated streptavidin (100 μg/mL; Nichirei, Tokyo, Japan) for 30 min. HRP was visualized by reaction with 3, 3-diaminobenzidine tetrahydrochloride (Nichirei). Staining for 8-OHdG was analyzed from digitalized images using Image

J 1.43 imaging software (National Institute of Mental Health, Bethesda, MD) [21]. We selected four representative hippocampal regions from each mouse and produced image files. The image files were opened in gray scale mode. The plot area, mean density and plot number corresponding to the number of positively stained cells were then determined in the hippocampus using the 'Analyze Particles' command after setting an appropriate threshold.

Statistical analysis

All data are expressed as mean ± SEM in the text and figures. Data were analyzed and compared among groups by one-way ANOVA. When a statistically significant effect was found, post hoc analysis (Dunnett method) was performed to detect the difference between the groups. A value of $P < 0.05$ was considered statistically significant. Statistical comparisons were conducted using the StatView v5.0 program (Abacus Concept, Berkeley, CA).

RESULTS

Protocol 1

Characteristics of control and CKD mice. Table 1 shows time course of body weight (BW), BUN, SCr and Ht in normal control mice and uremic mice. SCr and BUN concentrations in the CKD-4W and CKD-8W mice were significantly higher than in the control mice ($P < 0.05$, respectively). There was no significant difference in all parameters between CKD-4W and CKD-8W mice. In addition, Ht and BW were significantly lower in CKD mice compared with control mice. These results indicate that experimental uremic mice are an appropriate model for the study of CKD.

Impaired spatial working memory in the RAWM test in uremic mice 8 weeks after induction of CKD. To determine the association of uremia with the spatial working memory dysfunction, we examined mice using the RAWM test (Figure 1). We trained mice for four days (training days) and then conducted the spatial working memory test on the fifth day. On memory retention test (T5), the numbers of errors of CKD-4W mice were comparable to those of Cont-4W mice, while CKD-8W mice showed significantly more errors than the Cont-8W mice, suggesting that CKD-8W mice showed impaired spatial working memory, probably due to

uremia (Figure 2A). Activity and exploratory behavioral studies were performed using an open field activity test to determine whether uremia-induced cognitive dysfunction was associated with a decline in activity or physical abnormalities. We measured the total number of lines crossed in a 5-min period after RAWM test. There was no significant difference in the number of crossed blocks among all groups. The CKD-8W mice did not exhibit a decrease in open field activity or physical performance compared with the control mice (Figure 2B).

Increased number of pyknotic neuronal cells in the hippocampus of uremic mice. To determine whether CKD was associated with neuronal degeneration, we examined the histological changes of neuronal cells using H&E-stained brain sections from all mice (Figure 3). At ×400 magnification, neuronal cells with a condensed and darkly stained cell body and nuclei were considered pyknotic neuronal cells (Figure 3B). The number of pyknotic neuronal cells in the cornu ammonis 3 (CA3) region of the hippocampus showed no difference between the control mice and CKD-4W mice but was significantly higher in the CKD-8W mice compared with the control mice (Figure 4).

Generation of oxidative DNA damage in the hippocampus of uremic mice. To determine the involvement of oxidative DNA damage in the pyknotic changes of neuronal cell nuclei in the hippocampus, we examined the accumulation of 8-OHdG in the hippocampus by immunohistochemistry. Strong 8-OHdG immunoreactivity was detected in the nuclei of hippocampal CA3 neurons in the CKD-8W mice (Figure 5A). In comparison, nuclei of hippocampal neurons of the control mice and CKD-4W mice exhibited weak immunoreactivity for 8-OHdG, and the numbers of positive cells were significantly lower in the control and CKD-4W mice than CKD-8W mice. (Figure 5B, $P < 0.05$).

Protocol 2

Characteristics of CKD-Veh and TMP-treated mice. SCr and BUN concentrations in the CKD-Veh and CKD-TMP mice were significantly higher than in the control mice at 8 weeks after operation ($P < 0.05$, respectively, Table 2). In addition, Ht and BW were significantly lower in all CKD mice compared with the control mice. All parameters were comparable between the CKD-Veh and CKD-TMP mice.

Table 1. Characteristics of control and CKD mice for Protocol 1

	Cont-4W (n = 8)	Cont-8W (n = 8)	CKD-4W (n = 8)	CKD-8W (n = 8)
	Sham operation		Nx+2/3EC	
BW (g)	26.8 (26.2–29.2)	28.4 (26.4–29.8)	25.1* (23.5–28.5)	25.8** (24.4–26.1)
BUN (mg/dL)	33.2 (30.2–39.0)	29.0 (22.4–37.8)	72.9* (58.0–102.0)	78.4** (60.8–80.8)
SCr (mg/dL)	0.09 (0.08–0.12)	0.09 (0.06–0.15)	0.26* (0.12–0.48)	0.28** (0.16–0.45)
Ht (%)	50.8 (48.5–52.0)	51.0 (50.0–53.0)	42.0* (40.0–46.5)	42.3** (38.5–44.1)

Data are medians with ranges.

Cont-4W, sham-operated control mice observed for 4 weeks; Cont-8W, sham-operated control mice observed for 8 weeks; CKD-4W, remnant kidney mice observed for 4 weeks; CKD-8W, remnant kidney mice observed for 8 weeks; CKD, chronic kidney disease; Nx+2/3EC, left nephrectomy and 2/3 electrocoagulation of the right renal cortex; BW, body weight; BUN, blood urea nitrogen; SCr, serum creatinine; Ht, hematocrit.

*$P < 0.05$ versus Cont-4W mice; **$P < 0.05$ versus Cont-8W mice.
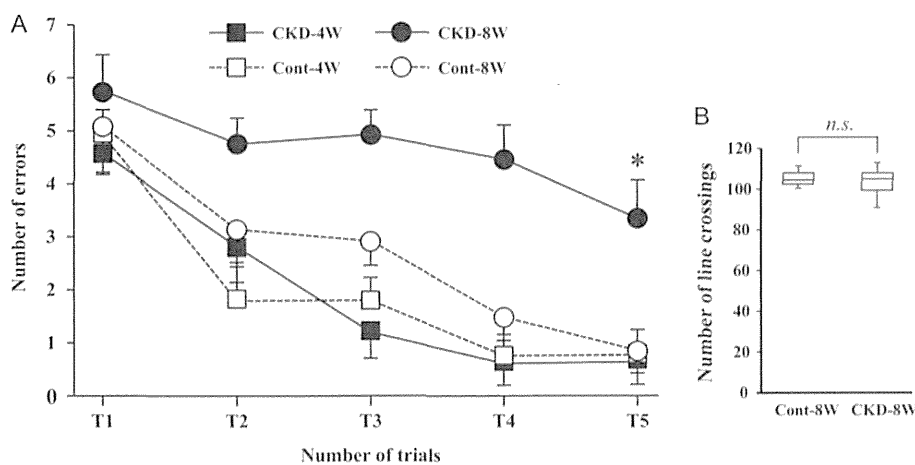
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FIGURE 2: Impaired spatial working memory in the RAWM test in uremic mice 8 weeks after induction of CKD. (A) The numbers of errors during RAWM test on the fifth day in Cont-4w (white square), CKD-4W (black square), Cont-8W (white circle) and CKD-8W (black circle) mice are shown. CKD-8W mice made significantly more errors than mice in the other three groups on the last trial (T5). Data are mean ± SEM of eight mice in each group. *P < 0.05 versus the other three groups. (B) Results of the open field activity test are shown. Activity and motor function in the CKD-8W mice are comparable with those in Cont-8W mice. Data are mean ± SEM. n.s.: not significant.
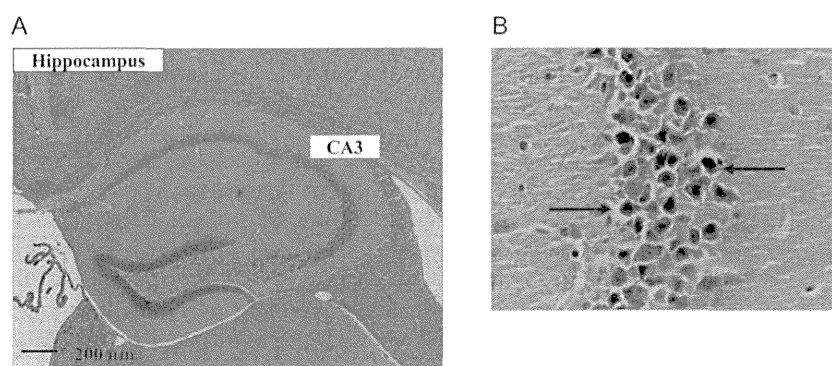

FIGURE 3: Light microscopic images of the hippocampal CA3 region. (A) Representative image of the entire hippocampus from a CKD-8W mouse. (B) Pyknotic neuronal cells in the CA3 region of the hippocampus from a CKD-8W mouse are shown. Arrows: pyknotic neuronal cell bodies. Magnification: ×40 (A) and ×400 (B).

Prevention of uremia-induced spatial working memory dysfunction by treatment with TMP. To determine the effect of TMP against the spatial working memory dysfunction induced by uremia, we administered the RAWM test to mice (Figure 1). During the memory retention test (T5), the CKD-Veh mice exhibited more errors compared with the control mice, and the treatment with TMP significantly decreased the number of errors to a level similar in control mice (Figure 6).

Prevention of uremia-induced pyknotic changes in neuronal cells by TMP treatment. To determine whether TMP ameliorated neuronal degeneration induced by CKD, we examined the histological changes of neuronal cells using H&E-stained brain sections from all mice (Figure 7A). The number of pyknotic neuronal cells in the CA3 region of the hippocampus was significantly higher in the CKD-Veh mice compared with the control mice. Treatment with TMP protected against the effects of CKD, as there was no significant

difference in the number of pyknotic neuronal cells among the control and CKD-TMP mice (Figure 7B).

TMP treatment prevented generation of oxidative DNA damage. To examine the effect of TMP against oxidative DNA damage, we performed immunohistochemistry for 8-OHdG (Figure 8A). Strong 8-OHdG immunoreactivity was detected in the nuclei of hippocampal neurons in the CKD-Veh mice. In comparison, nuclei of hippocampal neurons from the CKD-TMP mice exhibited weak immunoreactivity for 8-OHdG (Figure 8B, P < 0.05).

DISCUSSION

The present study provided evidence that uremia induces spatial working memory dysfunction in mice and that oxidative stress is associated with cognitive impairment in the
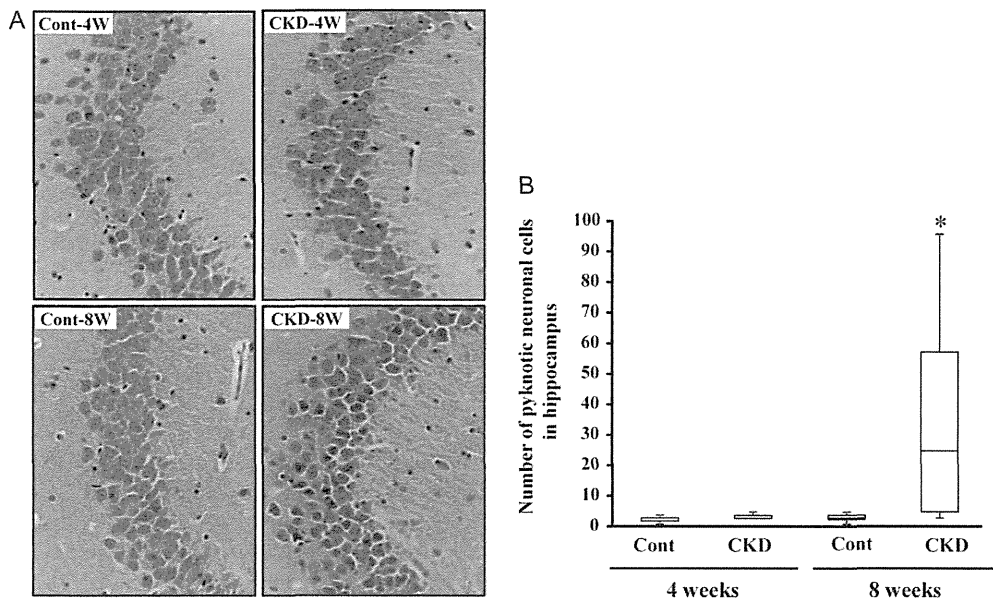
FIGURE 4: Increased number of pyknotic neuronal cells in the hippocampus of uremic mice. (A) Representative microphotographs of the hippocampus from each group are shown. Magnification: ×200. (B) Quantitative an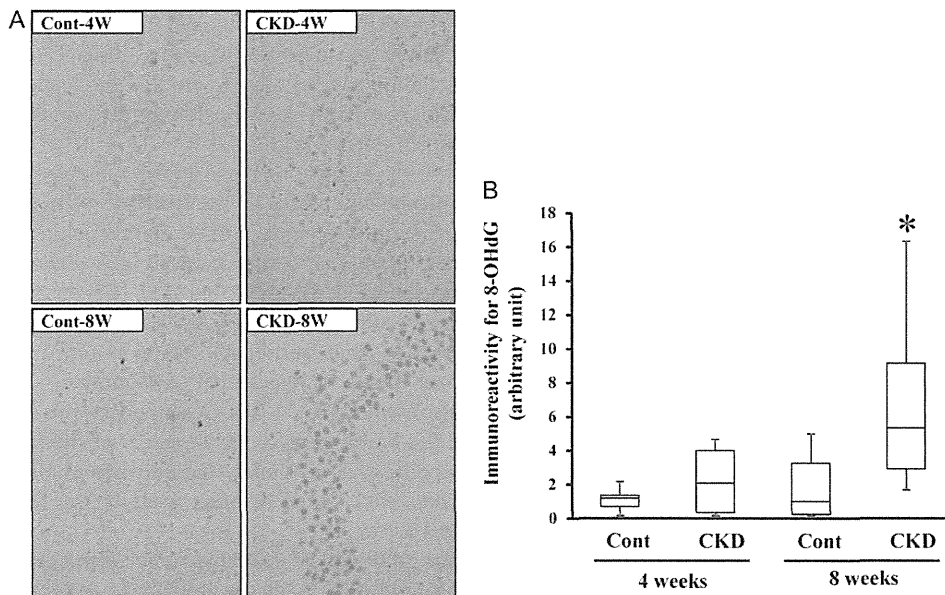alysis of pyknotic neurons in the hippocampus is shown. The number of pyknotic neuronal cells in the hippocampal CA3 is significantly higher in CKD-8W mice than control mice. The ends of the box represent the upper and lower quartiles; thus the box spans the interquartile range. The median is marked by a vertical line inside the box. The two lines outside the box that extend to the highest and lowest observations represent the whiskers. *P < 0.05 versus the other three groups.

FIGURE 5: Increased oxidative DNA damage in the hippocampus of uremic mice. (A) Representative microphotographs of 8-OHdG immunostaining in the hippocampal CA3 region of each group are shown. Magnification: ×200. (B) Quantitative analysis of 8-OHdG-positive neurons in the hippocampus is shown. Immunoreactivity for 8-OHdG in hippocampal CA3 is significantly higher in CKD-8W mice than control mice. The ends of the box represent the upper and lower quartiles; thus the box spans the interquartile range. The median is marked by a vertical line inside the box. The two lines outside the box that extend to the highest and lowest observations represent the whiskers. *P < 0.05 versus the other 3 groups.

uremic mouse model. To our knowledge, this is the first report to show that an antioxidant prevents spatial working memory dysfunction through inhibition of cerebral oxidative stress in uremic mice.

During uremia, the accumulation of neurotoxic metabolites, hormonal disturbances and an imbalance in excitatory and inhibitory neurotransmitters may be important in the pathogenesis of uremic encephalopathy [1, 22]. In addition

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Table 2. Characteristics of control and CKD mice for Protocol 2

	Cont-Veh (n = 8)	Cont-TMP (n = 8)	CKD-Veh (n = 8)	CKD-TMP (n = 8)
	Sham operation		Nx+2/3EC	
	Vehicle	TMP	Vehicle	TMP
BW (g)	28.2 (26.2–29.6)	28.0 (26.0–31.0)	25.7* (24.4–26.1)	26.0 (24.0–28.0)
BUN (mg/dL)	27.2 (22.8–34.5)	27.9 (20.5–35.9)	75.0* (59.8–93.6)	78.1* (63.1–101.2)
SCr (mg/dL)	0.09 (0.04–0.10)	0.09 (0.08–0.13)	0.30* (0.25–0.38)	0.29* (0.22–0.39)
Ht (%)	49.8 (48.5–52.0)	45.8 (43.0–48.5)	40.5* (35.0–43.0)	39.0* (27.0–42.5)

Data are medians with ranges.
Cont-Veh, sham-operated control mice with drinking water; Cont-TMP, sham-operated control mice with drinking tempol in water; CKD-Veh, CKD-operated mice with drinking vehicle water; CKD-TMP, CKD-operated mice with drinking tempol solution; CKD, chronic kidney disease; Nx+2/3EC, left nephrectomy and 2/3 electrocoagulation of the right renal cortex; BW, body weight; BUN, blood urea nitrogen; SCr, serum creatinine; Ht, hematocrit.
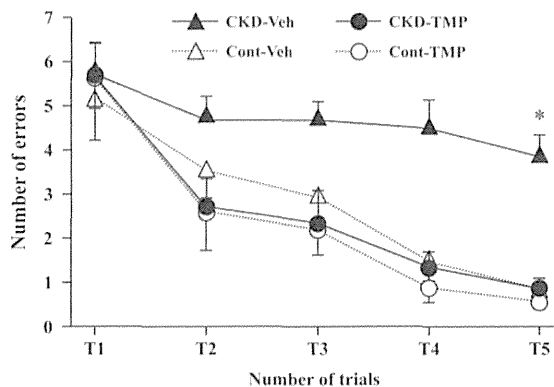*$P < 0.05$ versus Cont-Veh mice.

FIGURE 6: Effect of TMP on prevention of uremia-induced spatial working memory dysfunction. The numbers of errors during RAWM test on the fifth day in Cont-Veh (white triangle), CKD-Veh (black triangle), Cont-TMP (white circle) and CKD-TMP (black circle) mice are shown. The number of errors in CKD-TMP mice is significantly decreased to levels similar to those observed in control mice, and significantly lower than CKD-Veh mice. The ends of the box represent the upper and lower quartiles; thus the box spans the interquartile range. The median is marked by a vertical line inside the box. The two lines outside the box that extend to the highest and lowest observations represent the whiskers. *$P < 0.05$ versus the other three groups.

to these factors, the generation of reactive oxygen species (ROS) was recently implicated as a primary etiological factor in uremic encephalopathy [11, 23]. Furthermore, increased nitric oxide (NO) production in the uremic brain could be related to increased levels of guanidinosuccinic acid, a uremic toxin [24].

Protocol 1 was designed to establish the long-term effects of CKD on brain and cognitive dysfunction. A spatial working memory test was used to measure cognitive function. The RAWM test examines short-term working memory retention for the location of a submerged escape platform, the arm location of which is changed daily. Short-term working memory is generally the first cognitive function deficit in disorders of cognitive function. The water maze test is considered particularly appropriate for examining this important initial cognitive impairment in models of Alzheimer's disease [25].

The present study demonstrated that in the RAWM test, uremic mice in the CKD-8W mice could not reach the escape platform without more errors than the control mice, indicating uremia-induced spatial working memory dysfunction (Figure 2A). In this regard, one may argue that impairment in the RAWM test in uremic mice was attributable to generalized neuronal depression typically seen in uremia. However, the open field test did not demonstrate decreased physical performance or activity in these mice, indicating that these results are valid (Figure 2B). Overall, this study provided clear evidence that chronic uremia caused cognitive dysfunction in the CKD mouse model. There are limitations to the open field test. It does not completely exclude physical impairment and uremia-induced autonomic neuropathy and is influenced by several factors including general activity, anxiety and exploration [26]. In addition, anemia can impair cognitive function and motor/movement functions [27].

In the present study, histopathological analysis revealed the presence of significant differences in the structure of hippocampal CA3 neurons in mice with chronic uremia compared with control mice (Figure 4). Pyknotic neurons were observed in the hippocampus of CKD-8W mice. Liu et al. [19] reported that severe ischemic acute kidney injury induced similar neuropathological changes. Thus, neuropathological lesions of the hippocampus may cause hippocampal dysfunction. The present results also demonstrated that the hippocampus was the only area of the brain where large numbers of pyknotic neurons were found in CKD-8W mice, and only low numbers of pyknotic neurons were noted in other areas of the brain such as the cerebral cortex.

Among various products of oxidative DNA damage, 8-OHdG is the most important due to its abundance and mutagenicity. Immunostaining for 8-OHdG showed significantly higher numbers of 8-OHdG-positive cells in the hippocampus of CKD-8W mice than control mice and TMP-treated CKD mice (Figure 8B). Thus, it is suggested that oxidative DNA damage in neurons might affect cognitive function via mutation of neural genes in uremic mice.

Palumbo et al. [28] reported that chronic psychosocial stress-induced ROS in the hippocampus caused morphological changes in the hippocampal CA3 region and a decline in cognitive function. Thus, chronic stress such as ROS and the consequent oxidative damage may contribute to impaired
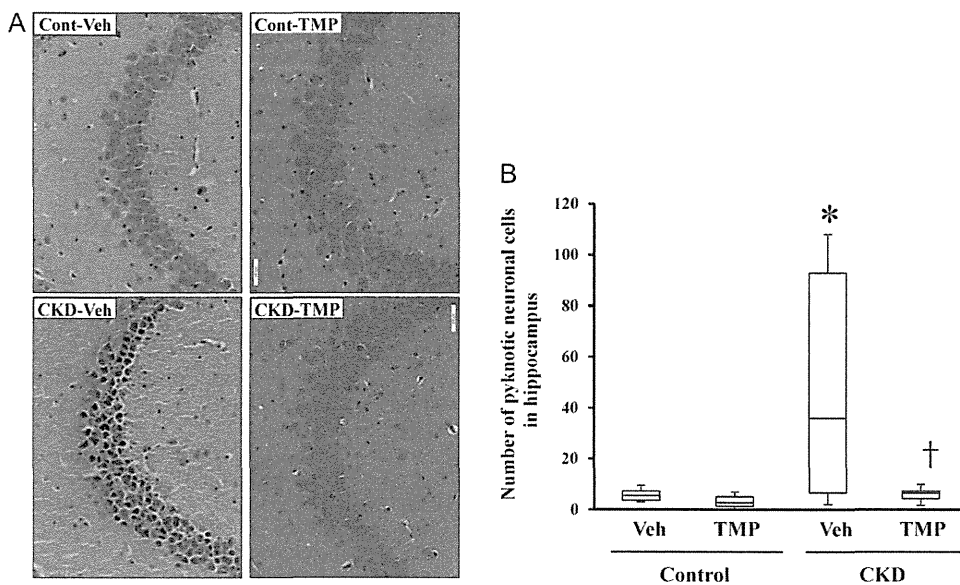
FIGURE 7: Effect of TMP on prevention of uremia-induced pyknotic changes in neuronal cells. (A) Representative microphotographs of the hippocampal CA3 region from each group are shown. Magnification: ×200. (B) Quantitative analysis of pyknotic neurons in the hippocampus is shown. The number of pyknotic neuronal cells in the hippocampal CA3 is significantly higher in CKD-Veh mice than TMP-treated and control mice. The ends of the box represent the upper and lower quartiles; thus the box spans the interquartile range. The median is marked by a vertical line inside the box. The two lines outside the box that extend to the highest and lowest observations represent the whiskers. *P < 0.05 versus Cont-Veh mice. †P < 0.05 versus CKD-Veh mice.
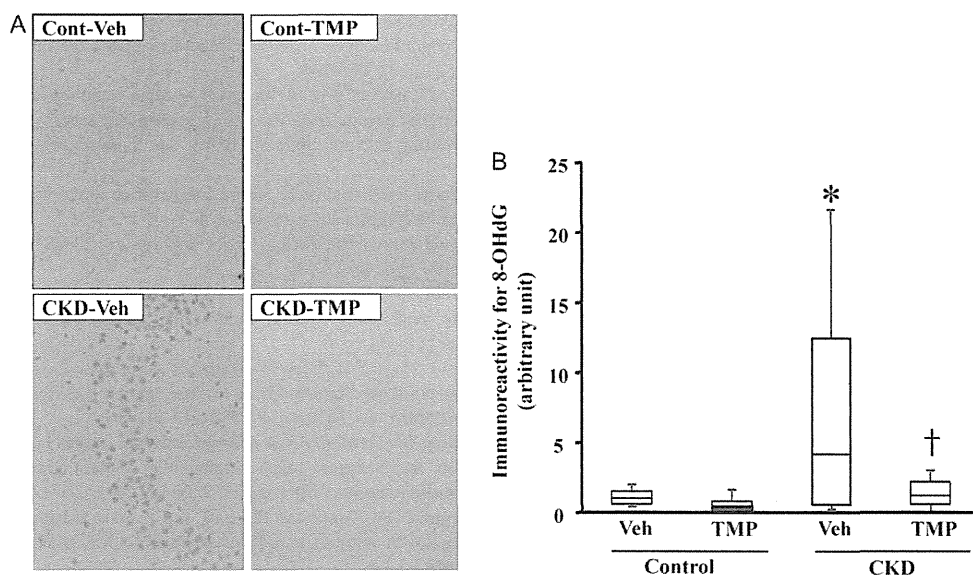
FIGURE 8: Effect of TMP on prevention of oxidative DNA damage generation. (A) Representative microphotographs of 8-OHdG immunostaining in the hippocampal CA3 region from each group are shown. Magnification: ×200. (B) Quantitative analysis of 8-OHdG-positive neurons in the hippocampal CA3 region is shown. 8-OHdG immunoreactivity in the hippocampal CA3 region is significantly higher in CKD-Veh mice than TMP-treated CKD mice. The ends of the box represent the upper and lower quartiles; thus the box spans the interquartile range. The median is marked by a vertical line inside the box. The two lines outside the box that extend to the highest and lowest observations represent the whiskers. *P < 0.05 versus Cont-Veh mice. †P < 0.05 versus CKD-Veh mice.

cognitive function. A previous study reported that oxidative stress in the diabetic rat brain caused cognitive dysfunction and that treatment with an antioxidant agent improved cognitive dysfunction [29].

TMP is a membrane-permeable radical scavenger and readily crosses the blood–brain barrier [30, 31]. In the present study, TMP was used to confirm that cognitive dysfunction in uremia is induced through oxidative stress, as TMP prevented cognitive

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dysfunction through inhibition of oxidative DNA damage in the hippocampus without improvement of renal function. Previous studies demonstrated that TMP decreased oxidative damage in cerebral mitochondria by targeting upstream peroxynitrite-derived free radicals, which would be expected to ameliorate Ca^{2+} overload, reduce calpain-mediated proteolysis and decrease neurodegeneration in traumatic brain damage [32]. Thus, our results may be explained by these mechanisms.

Asymmetric dimethylarginine (ADMA) derives from methylation of arginine residues in proteins. Hydrolysis of methylated proteins releases ADMA, which competitively inhibits NOS. Patients with end-stage renal disease have high levels of the endogenous NOS inhibitor ADMA. Dimethylamine dimethylaminohydrolase , the main enzyme responsible for degrading ADMA can be functionally impaired by oxidative stress [33]. Torre et al. [34] reported that NOS inhibition with L-NAME decreased spatial memory after common carotid artery occlusion. ROS cause excitotoxicity by facilitating glutamine release, which activates NMDA and non-NMDA receptors. This results in highly increased intracellular Ca^{2+} levels, activation of neuronal NO synthases, formation of peroxynitrite, protein nitration and mitochondrial damage, culminating in neuronal injury [12]. Taken together, our results suggest that TMP, an antioxidative agent, ameliorated cognitive dysfunction in uremic mice through the inhibition of oxidative stress. The neural and cognitive mechanisms of the symptom-reducing and/or recovery-promoting effects of an antioxidative agent in the uremic brain are currently unclear. The striking improvements observed in both behavioral and pathological outcomes following long-term antioxidative agent treatment warrant further analysis.

In conclusion, the present study demonstrated that inhibition of oxidative stress by TMP treatment provided significant protection against uremia-induced cognitive dysfunction. Although the precise mechanisms of the symptom-reducing and/or recovery-promoting effects remain unclear, the striking improvement of behavioral and pathological outcomes following TMP treatment has increased our understanding of the pathogenesis of uremic encephalopathy. Further investigation is required to elucidate the precise mechanisms and develop effective therapies for cognitive dysfunction in patients with CKD.

CONFLICT OF INTEREST

Part of this study was presented at the 41st and 42nd Annual Meetings of the American Society of Nephrology 30 (Philadelphia, USA, 2008; San Diego, USA, 2009).

(See related article by Kielstein and Hans-Gert Bernstein. The reversible part of cognitive impairment in chronic kidney disease: can mice help men break the TEMPOLimit? *Nephrol Dial Transplant* 2014; 29: 476–478.)

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Role of PCSK9 and IDOL in the pathogenesis of acquired LDL receptor deficiency and hypercholesterolemia in nephrotic syndrome

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ABSTRACT

Background. Nephrotic syndrome (NS) leads to elevation of serum total and LDL cholesterol. This is largely due to impaired LDL clearance, which is caused by hepatic LDL receptor (LDLR) deficiency despite normal LDLR mRNA expression, pointing to a post-transcriptional process. The mechanism(s) by which NS causes LDLR deficiency is not known. By promoting degradation of LDLR, Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) and inducible degrader of the LDL receptor (IDOL) play a major role in post-translational regulation of LDLR. We, therefore, tested the hypothesis that LDLR deficiency despite its normal gene expression in NS may be due to upregulation of hepatic PCSK9 and IDOL.

Methods. LDLR, IDOL and PCSK9 expressions and nuclear translocation of liver X receptor (LXR) that regulates IDOL expression were determined in the liver of rats with puromycin-induced NS and control (CTL) rats.

Results. Compared with the CTLs, the NS rats showed marked elevation of serum total and LDL cholesterol and a significant reduction in hepatic LDLR protein expression. This was accompanied by marked upregulation of hepatic PCSK9 and IDOL expressions and heightened LXR activation.

Conclusions. LDLR deficiency, hypercholesterolemia and elevated plasma LDL in NS are associated with upregulation of PCSK9 and IDOL. Interventions targeting these pathways may be effective in the management of hypercholesterolemia and the associated cardiovascular and other complications of NS.

Keywords: atherosclerosis, lipid disorders, liver X receptor, lipid disorders, proteinuria

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ORIGINAL ARTICLE

Sex differences in the association between serum uric acid levels and cardiac hypertrophy in patients with chronic kidney disease

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Several studies have documented an association between serum uric acid (SUA) concentration and cardiac hypertrophy in hypertensive patients; however, the association remains unclear in chronic kidney disease (CKD) patients. If there is an association between SUA and hypertrophy in these patients, it is unknown whether the association is different between men and women. Our aim in this study is to determine whether SUA is associated with cardiac hypertrophy in CKD patients, focusing on any sex differences. Two hundred sixteen CKD patients (117 men and 99 women) were enrolled in this cross-sectional study. Patients prescribed uric acid-lowering agents and those with congestive heart failure, valvular heart disease, or ischemic heart disease were excluded from this study. Left ventricular mass index (LVMI) and left ventricular hypertrophy (LVH) were assessed using echocardiography. The prevalence of LVH was 58% in men and 47% in women. In multivariate linear regression analysis, SUA levels did not correlate with LVMI in men, whereas SUA was independently associated with LVMI in women ($\beta = 0.27$, $P = 0.02$). Multivariate logistic regression analysis also revealed that diabetes mellitus (odds ratio (OR), 4.41; $P = 0.01$) was associated with LVH in men, whereas age (OR, 1.13; $P < 0.01$), hypertension (OR, 7.38; $P = 0.03$) and SUA (OR, 1.91; $P = 0.03$) were associated with LVH in women. In female CKD patients, SUA levels were associated with LVMI and LVH, whereas there was no association in male patients. These observations suggest that an association between SUA levels and the development of cardiac hypertrophy is more likely in women than in men.
Hypertension Research (2014) **37**, 246–252; doi:10.1038/hr.2013.134; published online 3 October 2013

Keywords: cardiac hypertrophy; chronic kidney disease; hyperuricemia; sex difference

INTRODUCTION

Patients with chronic kidney disease (CKD) have high cardiovascular (CV) morbidity and mortality, and the presence of CKD worsens CV disease outcomes.[1] Cardiac hypertrophy, that is, left ventricular hypertrophy (LVH) or increased left ventricular mass index (LVMI), is known to be a powerful, independent risk factor for future CV morbidity and mortality in patients with essential hypertension, as well as in the general population.[2–5] The prevalence of cardiac hypertrophy increases with declining renal function in CKD patients not undergoing dialysis and cardiac hypertrophy is independently associated with adverse CV events in these patients.[6–13] Thus, cardiac hypertrophy may be a major adverse CV consequence of CKD.

Several epidemiologic studies have suggested a positive association between serum uric acid (SUA) levels and CV disease in the general population,[14–17] in hypertensive patients[18,19] and in CKD

patients.[20,21] It has also been reported that higher SUA levels are associated with cardiac hypertrophy in hypertensive patients.[22–26] As approximately two-thirds of SUA is excreted through the kidneys, CKD patients develop hyperuricemia as the glomerular filtration rate declines.[27] It has been reported that high SUA is associated with cardiac hypertrophy in renal transplant recipients,[28] but very few studies have examined the relationship between SUA levels and cardiac hypertrophy in CKD patients.[29] Moreover, studies of sex differences in the relationship between SUA levels and CV disease are conflicting. Some studies have shown that SUA levels correlate significantly with cardiac hypertrophy only in female hypertensive patients,[22,23] whereas another report found a significant association between SUA and cardiac hypertrophy only in male hypertensive patients.[26] To date, there have been no studies of sex differences in the relationship between SUA and cardiac hypertrophy in CKD patients. Thus, we aimed to elucidate the association between SUA levels and

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cardiac hypertrophy in patients with CKD, focusing especially on the sex differences in this association.

METHODS

In this cross-sectional study, we enrolled 216 patients with CKD not on dialysis, who were admitted to our hospital for evaluation and education of CKD. Patients prescribed uric acid-lowering agents and those with congestive heart failure, valvular heart disease, ischemic heart disease or local asynergy on echocardiogram were excluded from this study.

All patients provided written informed consent to the protocol, which was approved by the Ethics Committee of the National Kyushu Medical Center Hospital. Blood samples were obtained from admitted patients in the early morning after an overnight fast for measurement of serum creatinine (SCr), C-reactive protein, hemoglobin (Hb), SUA and serum phosphorus levels. Daily proteinuria was also measured. The estimated glomerular filtration rate (eGFR; ml min^{-1} per 1.73 m^2) was calculated using the Modification of the Diet in Renal Disease equation for Japanese patients: $194 \times SCr^{-1.094} \times age^{-0.287} \times 0.739$ (if female).[30]

All enrolled patients were interviewed and clinically examined at presentation. Their medical histories and outpatient records were also evaluated in detail. Demographic information (age and sex), medication history, and atherosclerotic risk factors (hypertension, history of smoking, dyslipidemia and diabetes mellitus) at presentation were recorded for each patient. Hypertension was defined as systolic blood pressure $\geqslant 140$ mm Hg or diastolic blood pressure $\geqslant 90$ mm Hg, or use of antihypertensive drugs. Dyslipidemia was defined as plasma triglycerides $\geqslant 150$ mg dl^{-1}, plasma low-density lipoprotein cholesterol $\geqslant 140$ mg dl^{-1}, plasma high-density lipoprotein cholesterol < 40 mg dl^{-1} or the use of lipid-lowering drugs based on a history of dyslipidemia. Diabetes mellitus was defined as previous or current plasma fasting glucose $\geqslant 126$ mg dl^{-1} or the use of hypoglycemic agents. Cigarette smoking was evaluated as current or past. Body mass index was calculated as weight in kg divided by height in m^2. Blood pressure was measured at three separate times on the second day of hospitalization, with patients in a sitting position. The average of the three readings was recorded.

Left ventricular mass (LVM) was calculated from M-mode records taken on parasternal long-axis images, according to the following formula (derived by Reichek and Devereux[31]): LVM = 1.04([IVSd + LVPWd + LVDd]^3 −LVDd^3) −13.6, where IVSd and LVPWd are the thickness of the interventricular septum and of the posterior wall of the left ventricle (LV) in diastole, respectively, and LVDd is the diameter of the LV in diastole. LVMI was expressed as LVM per square meter of body surface area, calculated by the Du Bois formula:[32] body surface area = weight$^{0.425}$ × height$^{0.725}$ × 0.007184.

We defined LVH as LVMI of 134 g m^{-2} or greater for males and 110 g m^{-2} or greater for females.[33] Percent fractional shortening was calculated as (LVDd–LVDs)/LVDd × 100.

Statistical analysis

Continuous data are expressed as either the mean ± s.d. or the median (interquartile range), depending on their distribution. Categorical data are expressed as numbers (with %). The significance of differences between female < 45 years old and $\geqslant 45$ years old was examined using the χ^2-test for categorical data, the Wilcoxon rank sum test for nonparametric data and the unpaired Student's t-test for parametric data. Correlation coefficients were calculated according to Pearson's method.

Demographic, traditional, and non-traditional risk factors, serological data, LVMI, and the prevalence of LVH were compared across quartiles of SUA levels using the χ^2-test for categorical data, analysis of variance for continuous variables that are approximately normally distributed, and the Kruskal–Wallis test for skewed continuous variables analysis, followed by the Dunnett's test. A linear regression analysis was performed to elucidate the associations between LVMI and traditional and non-traditional risk factors. Covariates associated with LVMI in univariate analysis were analyzed by multivariate analysis to determine the independent risk factors for LVMI. A logistic regression model was also applied to elucidate the traditional and nontraditional risk factors associated with LVH. Covariates associated with LVH that were significant in

univariate analysis were selected as risk factors in multivariate analysis. The odds ratio and the 95% confidence interval were calculated for each variable. Data were analyzed using the JMP9 statistical package (SAS Institute, Cary, NC, USA). A P-value below 0.05 indicated a significant difference.

RESULTS

The median age of the 216 patients (117 men and 99 women) in this study was 61 years (range, 30–75 years). The primary causes of renal disease were chronic glomerulonephritis (44%, 95 patients), diabetic nephropathy (30%, 65 patients), hypertensive nephrosclerosis (15%, 32 patients), other defined causes (8%, 17 patients) and unknown (3%, 7 patients).

The clinical characteristics of the patients according to CKD stage are summarized in Table 1. The prevalence of hypertension, diabetes mellitus, smoking and dyslipidemia was significantly higher with advancing CKD stages. In addition, 121 patients (56%) had been treated with angiotensin-converting enzyme inhibitors (ACEIs) and/ or angiotensin II receptor blockers (ARBs), and this percentage was higher in advanced CKD stages. The percentage of patients receiving diuretics was also higher in more advanced CKD stages. Daily proteinuria in patients with CKD stages 4 and 5 was significantly higher than in patients with CKD stages 1 and 2. As kidney function declined, Hb levels decreased significantly, whereas SUA levels significantly increased in both sexes. LVMI was higher in more advanced CKD stages in both sexes. LVH was found in 53% of all subjects, with a significantly higher prevalence as kidney function decreased.

The study population was divided into quartiles by SUA level for each sex, as shown in Table 2a (men) and Table 2b (women). Among the male patients, the prevalence of hypertension was significantly higher with ascending SUA quartiles. No difference in systolic or diastolic blood pressure was found across quartiles. The eGFR values of patients in the second to fourth quartiles were significantly lower than those of patients in the first quartile. Figure 1 shows LVMI and prevalence of LVH according to quartiles of SUA in men. LVMI values were higher in fourth quartile patients than in first quartile patients. The prevalence of LVH did not increase significantly with ascending quartiles.

Among the female patients, patients in the fourth quartile had a higher median age than did those in the first quartile. The prevalence of hypertension and diabetes mellitus increased significantly with ascending quartiles. Patients in the third and fourth quartiles had significantly higher systolic blood pressure than those in the first quartile. The prevalence of ACEIs and/or ARBs and diuretic use was significantly higher with ascending quartiles. Patients in the second and fourth quartiles had significantly lower Hb compared with those in the first quartile. Patients in the third and fourth quartiles had significantly lower eGFR compared with those in the first quartile. As shown in Figure 2, LVMI was significantly higher in patients in the third and fourth quartiles than in those in the first quartile and the prevalence of LVH increased significantly with ascending quartiles. Table 3 shows the demographics, comorbidities, laboratory data and cardiac hypertrophy indices for females < 45 years of age and for those $\geqslant 45$ years of age. Female subjects $\geqslant 45$ years of age had significantly higher SUA and LVMI values, and a higher prevalence of LVH than did younger women. In addition, eGFR values in female subjects $\geqslant 45$ years of age were significantly lower than in those < 45 years of age.

Figure 3 shows the relationship between SUA and LVMI in men and women. In both sexes, LVMI was positively correlated with SUA levels ($R^2 = 0.06$, $P < 0.01$ in men; $R^2 = 0.28$, $P < 0.01$ in women).

Table 1 Clinical characteristics of patients according to chronic kidney disease stage

	All (n = 216)	CKD stages 1–2 (n = 58)	CKD stage 3 (n = 71)	CKD stage 4 (n = 46)	CKD stage 5 (n = 41)	P-values
Age	61 (49–70)	48 (40–59)	64 (51–72)*	64 (60–70)*	64 (57–73)*	<0.01
Male	117 (54)	23 (40)	44 (62)	24 (52)	26 (63)	0.04
Hypertension	157 (73)	20 (34)	54 (76)	44 (96)	39 (95)	<0.01
SBP (mm Hg)	134 ± 20	119 ± 14	134 ± 21*	136 ± 16*	151 ± 16*	<0.01
DBP (mm Hg)	76 ± 11	72 ± 10	76 ± 11	76 ± 12	80 ± 10*	<0.01
Diabetes mellitus	86 (40)	4 (7)	32 (45)	25 (54)	25 (61)	<0.01
Smoking	102 (47)	19 (33)	35 (49)	23 (50)	25 (61)	0.04
Dyslipidemia	144 (67)	30 (52)	51 (72)	32 (70)	31 (76)	0.04
ACEIs and/or ARBs	121 (56)	12 (21)	45 (63)	35 (76)	29 (71)	<0.01
Diuretics	51 (24)	2 (3)	17 (24)	13 (28)	19 (24)	<0.01
Body mass index (kg m^{-2})	22.1 (20.1–25.1)	22.2 (19.9–24.4)	22.0 (20.5–24.7)	22.8 (20.2–26.1)	22.0 (20.7–24.1)	0.88
Proteinuria (g day^{-1})	1.3 (0.5–3.7)	0.7 (0.3–1.6)	1.5 (0.2–4.1)	1.2 (0.4–4.7)**	2.6 (1.1–5.3)*	<0.01
Serum albumin (g dl^{-1})	3.5 (2.9–3.9)	3.7 (3.3–3.9)	3.5 (2.8–4.0)	3.5 (3.0–3.9)	3.1 (2.5–3.7)**	0.03
C-reactive protein (mg dl^{-1})	0.07 (0.03–0.15)	0.06 (0.03–0.13)	0.06 (0.02–0.12)	0.09 (0.04–0.31)	0.06 (0.03–0.15)	0.07
Hb (g dl^{-1})	11.4 ± 2.3	13.1 ± 1.7	11.8 ± 1.9*	10.7 ± 2.1*	9.1 ± 1.6*	<0.01
Serum phosphorus (mg dl^{-1})	3.6 (3.3–4.1)	3.4 (3.2–3.7)	3.5 (3.1–3.9)	3.8 (3.5–4.2)*	4.7 (4.0–5.3)*	<0.01
Serum uric acid (mg dl^{-1}) (males)	7.1 ± 1.5	6.0 ± 1.0	7.0 ± 1.5**	7.6 ± 1.5*	7.9 ± 1.4*	<0.01
Serum uric acid (mg dl^{-1}) (females)	6.3 ± 1.8	5.0 ± 1.1	6.0 ± 1.3**	7.5 ± 1.8*	8.0 ± 1.7*	<0.01
Fractional shortening (%)	40 ± 6	41 ± 5	39 ± 5	39 ± 5	39 ± 6	0.11
LVEF (%)	70 ± 7	72 ± 6	70 ± 7	69 ± 7	68 ± 7**	0.03
LVMI (g m^{-2}) (males)	143 (118–168)	112 (82–125)	140 (121–167)*	150 (108–178)*	159 (144–197)*	<0.01
LVMI (g m^{-2}) (females)	104 (86–137)	87 (78–102)	99 (84–125)	126 (104–149)*	146 (135–165)*	<0.01
LVH	115 (53)	11 (19)	38 (54)	30 (65)	36 (88)	<0.01

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; CKD, chronic kidney disease; DBP, diastolic blood pressure; Hb, hemoglobin; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; SBP, systolic blood pressure.
Values are expressed as the mean ± s.d., number (percent) or median (interquartile range).
*P<0.01, **P<0.05 *vs.* CKD stages 1–2.

Table 2a Clinical characteristics of male patients according to quartile of SUA

	All (n = 117)	1st Quartile (n = 29) (SUA, 3.7–6.1 mg dl^{-1})	2nd Quartile (n = 29) (SUA, 6.2–7.1 mg dl^{-1})	3rd Quartile (n = 30) (SUA, 7.2–8.0 mg dl^{-1})	4th Quartile (n = 29) (SUA, 8.1–11.9 mg dl^{-1})	P-values
Age	61 (51–70)	57 (44–66)	62 (52–71)	63 (53–73)	61 (55–70)	0.10
Hypertension	96 (82)	19 (66)	23 (79)	25 (83)	29 (100)	<0.01
SBP (mm Hg)	138 ± 21	134 ± 20	138 ± 21	135 ± 23	146 ± 16	0.06
DBP (mm Hg)	77 ± 11	75 ± 9	77 ± 11	77 ± 12	80 ± 10	0.31
Diabetes mellitus	58 (50)	14 (48)	16 (55)	15 (50)	13 (45)	0.89
Smoking	84 (72)	19 (66)	23 (79)	20 (67)	22 (76)	0.57
Dyslipidemia	83 (71)	21 (72)	22 (76)	18 (60)	22 (76)	0.50
ACEIs and/or ARBs	77 (66)	16 (55)	21 (72)	19 (63)	21 (72)	0.45
Diuretics	29 (25)	5 (17)	7 (24)	8 (27)	9 (31)	0.66
Body mass index (kg m^{-2})	22.3 (20.8–25.7)	21.4 (19.7–23.1)	23.1 (21.1–27.4)	22.5 (20.5–26.4)	22.9 (21.4–25.8)	0.14
Proteinuria (g day^{-1})	1.9 (0.7–4.9)	2.2 (0.7–5.7)	1.6 (0.3–5.0)	1.6 (1.0–4.3)	2.2 (1.0–5.1)	0.56
Serum albumin (g dl^{-1})	3.5 (2.6–3.9)	3.6 (2.6–4.1)	3.7 (2.9–4.0)	3.3 (2.8–3.8)	3.4 (2.5–3.9)	0.77
C-reactive protein (mg dl^{-1})	0.08 (0.04–0.17)	0.05 (0.03–0.13)	0.08 (0.05–0.17)	0.09 (0.03–0.23)	0.09 (0.05–0.27)	0.34
Hb (g dl^{-1})	11.8 ± 2.5	12.8 ± 2.6	11.9 ± 2.4	11.3 ± 2.3	11.1 ± 2.5**	0.03
eGFR (ml min^{-1} per 1.73 m^2)	34.7 (17.0–57.2)	59.0 (33.7–80.8)	38.9 (24.5–58.7)*	25.0 (13.7–43.1)*	17.5 (11.9–34.7)*	<0.01
Serum phosphorus (mg dl^{-1})	3.5 (3.2–4.1)	3.3 (3.1–3.7)	3.4 (3.1–3.8)	3.5 (3.2–4.1)	4.0 (3.4–4.5)*	0.01
Fractional shortening (%)	39 ± 6	39 ± 6	39 ± 6	39 ± 5	39 ± 6	0.96
LVEF (%)	69 ± 7	69 ± 6	68 ± 7	69 ± 6	68 ± 7	0.97

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; SUA, serum uric acid.
Values are expressed as the means ± s.d., number (percent) or median (interquartile range).
*P<0.01, **P<0.05 *vs.* first quartile of SUA.

Table 4 shows the relationship between clinical parameters and LVMI in each sex. In men, univariate linear regression analysis demonstrated that age, hypertension, diabetes mellitus, dyslipidemia, use of ACEIs and/or ARBs, use of diuretics, proteinuria, serum albumin, Hb, serum phosphorus, eGFR and SUA were associated with LVMI. Multivariate analysis showed that use of diuretics was an independent variable

Table 2b Clinical characteristics of female patients according to quartile of SUA

	All (n = 99)	1st Quartile (n = 23) (SUA, 2.7–4.9 mg dl⁻¹)	2nd Quartile (n = 26) (SUA, 5.0–6.0 mg dl⁻¹)	3rd Quartile (n = 23) (SUA, 6.1–7.2 mg dl⁻¹)	4th Quartile (n = 27) (SUA, 7.3–11.7 mg dl⁻¹)	P-values
Age	60 (46–69)	53 (42–65)	54 (40–67)	64 (50–71)	67 (58–71)**	0.01
Hypertension	61 (62)	7 (30)	11 (42)	18 (78)	25 (93)	<0.01
SBP (mm Hg)	128 ± 19	122 ± 16	119 ± 16	136 ± 19**	136 ± 17**	<0.01
DBP (mm Hg)	74 ± 11	73 ± 9	71 ± 10	74 ± 13	77 ± 10	0.21
Diabetes mellitus	28 (28)	3 (13)	5 (19)	9 (39)	11 (40)	0.07
Smoking	18 (18)	3 (13)	5 (19)	8 (35)	2 (7)	0.08
Dyslipidemia	61 (62)	12 (52)	14 (54)	16 (70)	19 (70)	0.39
ACEIs and/or ARBs	44 (44)	4 (17)	9 (35)	13 (57)	18 (67)	<0.01
Diuretics	22 (22)	1 (4)	3 (12)	7 (30)	11 (40)	<0.01
Body mass index (kg m⁻²)	21.8 (19.9–24.2)	21.0 (19.4–23.1)	21.3 (19.5–22.9)	21.9 (20.0–25.4)	23.0 (20.9–26.7)	0.17
Proteinuria (g day⁻¹)	1.0 (0.4–1.9)	1.1 (0.2–1.6)	0.7 (0.3–2.2)	1.4 (0.6–3.6)	1.1 (0.4–1.8)	0.41
Serum albumin (g dl⁻¹)	3.5 (3.1–3.9)	3.5 (3.0–3.9)	3.4 (3.0–3.6)	3.7 (3.1–3.9)	3.7 (3.3–4.1)	0.07
C-reactive protein (mg dl⁻¹)	0.06 (0.03–0.13)	0.08 (0.04–0.20)	0.05 (0.02–0.09)	0.04 (0.03–0.07)	0.07 (0.03–0.19)	0.22
Hb (g dl⁻¹)	11.0 ± 1.9	12.2 ± 1.3	10.6 ± 1.8**	11.0 ± 2.2	10.3 ± 1.8*	<0.01
eGFR (ml min⁻¹ per 1.73 m²)	42.7 (21.1–74.4)	74.0 (52.6–91.2)	60.5 (33.9–80.2)	39.2 (18.1–61.0)*	17.0 (13.2–38.2)*	<0.01
Serum phosphorus (mg dl⁻¹)	3.8 (3.4–4.1)	3.4 (3.1–3.6)	3.8 (3.4–4.1)	3.8 (3.5–4.1)	4.2 (3.8–4.8)*	<0.01
Fractional shortening (%)	41 ± 5	40 ± 5	40 ± 5	42 ± 5	41 ± 6	0.68
LVEF (%)	71 ± 6	71 ± 6	71 ± 7	72 ± 6	71 ± 7	0.93

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; SUA, serum uric acid.
Values are expressed as the means ± s.d., number (percent), or median (interquartile range).
*$P<0.05$, **$P<0.01$ vs. first quartile of SUA.
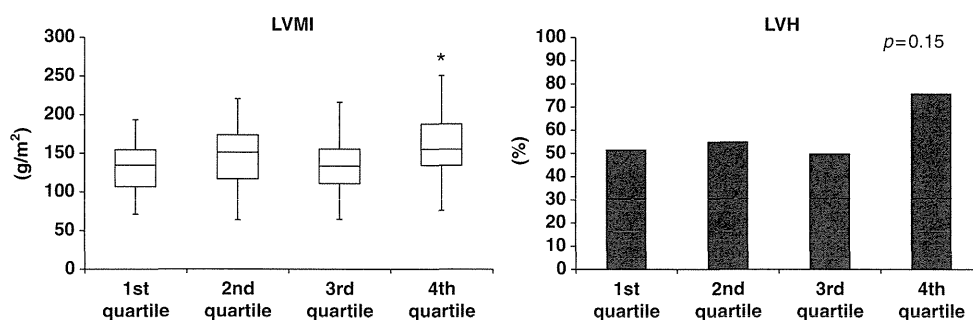
Figure 1 Box-and-whisker plot of left ventricular mass index (LVMI) and the prevalence of left ventricular hypertrophy (LVH) according to quartile of serum uric acid in men. The box represents the 25th, 50th and 75th percentiles; the line inside the box represents the median. *$P<0.01$ vs. quartile 1.
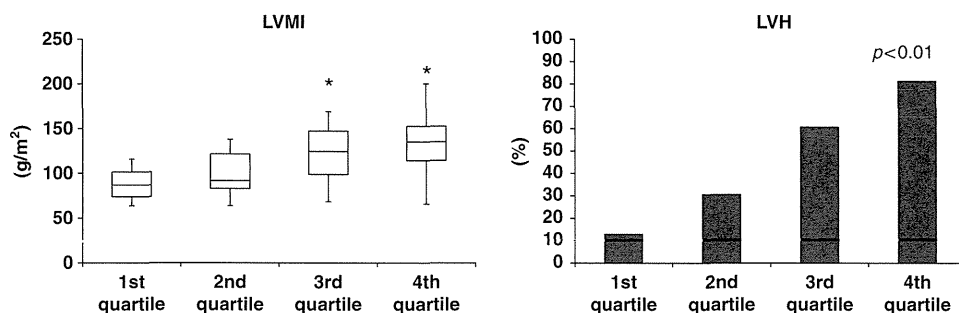
Figure 2 Box-and-whisker plot of left ventricular mass index (LVMI) and the prevalence of left ventricular hypertrophy (LVH) according to quartile of serum uric acid in women. The box represents the 25th, 50th and 75th percentiles; the line inside the box represents the median. *$P<0.01$ vs. quartile 1.

associated with LVMI. In women, multivariate linear regression analysis demonstrated that age, hypertension and SUA were independent variables associated with LVMI.

We analyzed the risk factors for LVH for each sex using a logistic regression model, as shown in Table 5. In men, univariate regression analysis showed that age, hypertension, diabetes mellitus, use of ACEIs and/or ARBs, use of diuretics, proteinuria, serum albumin, Hb, serum phosphorus, eGFR and SUA were associated with LVH. In multivariate analysis, only diabetes mellitus, but not SUA and the other variables, was independently associated with LVH. In women,

multivariate analysis showed that age, hypertension and SUA were independently associated with LVH.

DISCUSSION

We observed a high prevalence of cardiac hypertrophy among CKD patients in this study compared with a previous report showing LVH prevalence ranging from 36 to 41% among hypertensive patients.[34] The prevalence of cardiac hypertrophy was significantly higher with advancing CKD stage. In women, LVMI and the prevalence of LVH were significantly higher with ascending quartiles by SUA level. When male and female subjects were analyzed separately by multivariate analysis, elevated SUA levels were identified as an independent risk factor for both LVH and LVMI in females, whereas in males there was

Table 3 Clinical characteristics of female patients <45 years old and ⩾45 years old

	<45 (n = 21)	⩾45 (n = 78)	P-values
Age	39 (35–42)	65 (56–71)	<0.01
Hypertension	5 (24)	56 (72)	<0.01
SBP (mm Hg)	116 ± 14	132 ± 18	<0.01
DBP (mm Hg)	73 ± 11	74 ± 11	0.70
Diabetes mellitus	1 (5)	27 (35)	<0.01
Smoking	1 (5)	17 (22)	0.04
Dyslipidemia	6 (29)	55 (71)	<0.01
ACEIs and/or ARBs	6 (29)	38 (49)	0.09
Diuretics	0 (0)	22 (28)	<0.01
Body mass index (kg m^{-2})	20.7 (19.3–23.5)	21.9 (20.1–24.6)	0.20
Proteinuria (g day^{-1})	0.8 (0.3–1.7)	1.1 (0.4–2.2)	0.27
Serum albumin (g dl^{-1})	3.6 (3.3–3.8)	3.5 (3.1–3.9)	0.68
C-reactive protein (mg dl^{-1})	0.06 (0.03–0.09)	0.06 (0.03–0.14)	0.79
Hb (g dl^{-1})	11.3 ± 1.7	10.9 ± 1.9	0.45
eGFR (ml min^{-1} per 1.73 m^2)	74.0 (43.7–85.2)	36.7 (17.4–61.2)	<0.01
Serum phosphorus (mg dl^{-1})	3.5 (3.3–3.9)	3.9 (3.5–4.2)	0.07
SUA (mg dl^{-1})	5.1 (4.2–5.5)	6.4 (5.4–7.5)	<0.01
Fractional shortening (%)	39 ± 6	41 ± 5	0.24
LVEF (%)	70 ± 6	72 ± 6	0.33
LVMI (g m^{-2})	82 (71–92)	119 (93–144)	<0.01
LVH	2 (10)	45 (58)	<0.01

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; SBP, systolic blood pressure; SUA, serum uric acid. Values are expressed as the means ± s.d., number (percent), or median (interquartile range).

no significant relationship between SUA levels and cardiac hypertrophy.

This study did not clarify the mechanisms by which increased SUA levels caused cardiac hypertrophy. The role of SUA in the development of CV complications has not been fully investigated; however, some potential mechanisms have been proposed. It has been demonstrated that SUA impairs nitric oxide generation and induces endothelial dysfunction and smooth muscle cell proliferation.[35,36] It is also reported that SUA increases plasma levels of inflammatory mediators such as tumor necrosis factor alpha,[37] and potentially stimulates mitogen-activated protein kinases,[38] which are known to induce cardiac hypertrophy.[39,40] Moreover, uric acid appears to activate the renin–angiotensin system, thereby leading to the development of LVH.[41,42] Given these findings, cardiac hypertrophy may be directly attributable, at least in part, to increased SUA, via stimulation of endothelial dysfunction, smooth muscle cell proliferation, inflammation and activation of the renin–angiotensin system.

A recent study demonstrated that SUA levels were independently associated with CV morbidity, and that this association was stronger for patients in more advanced CKD stages.[20] In another recent study, an increased ratio of observed to predicted LVM was shown to be independently related to adverse CV events in patients with CKD stages 3–5.[11] Another report demonstrated that the combination of hyperuricemia with LVH is a powerful independent predictor for CV disease. The authors of that report speculated that the association between SUA and CV events in hypertensive patients might result from a direct association between SUA and LVMI.[25] Allopurinol has been found to decrease oxidative stress and to improve endothelial function in metabolic syndrome.[43] On the basis of these findings, SUA appears to contribute to the development of cardiac hypertrophy and CV events.

The sex-related effects of SUA on CV disease and cardiac hypertrophy are conflicting. This study demonstrated an association between SUA and cardiac hypertrophy only in female subjects. This finding agrees with previous observations in hypertensive patients.[22,23] Furthermore, the First National Health and Nutrition Examination Survey epidemiologic follow-up studies demonstrated a relationship between SUA and mortality (all-causes and due to CV events) only in women,[17] and found that the association between SUA and CV mortality was stronger in women than in men.[14] Given these findings, the influence of SUA on CV disease appears to be greater in women than in men. Although the reasons for these sex differences remain unclear, sex hormones may have a role.[44] This study found that female subjects ⩾45 years of age, who may be post-menopausal,
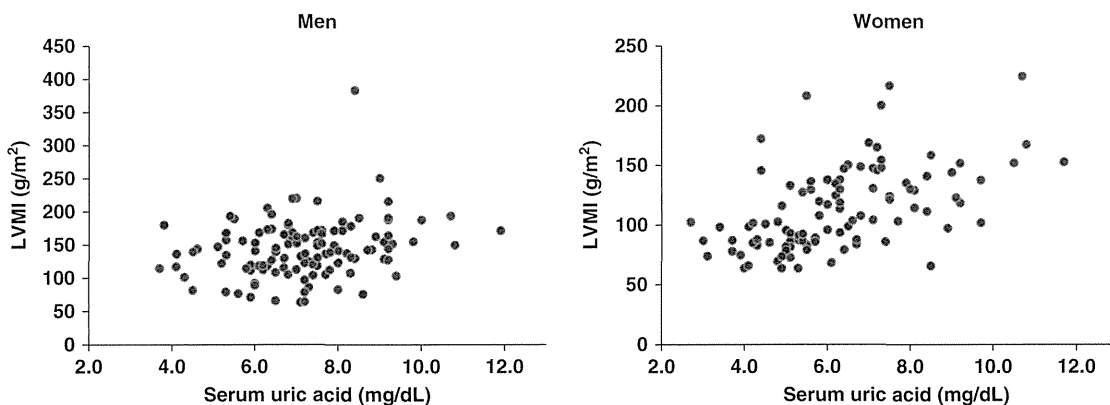
Figure 3 Relationship between serum uric acid and left ventricular mass index (LVMI) in men and women. A full color version of this figure is available at the *Hypertension Research* journal online.

Table 4 Relationship by sex between left ventricular mass index and clinical parameters

Variables	Men				Women			
	Univariate		Multivariate		Univariate		Multivariate	
	β	P-values	β	P-values	β	P-values	β	P-values
Age (years)	0.27	<0.01	0.20	0.06	0.42	<0.01	0.21	0.02
Hypertension	0.39	<0.01	0.13	0.25	0.61	<0.01	0.26	0.03
Diabetes mellitus	0.32	<0.01	0.07	0.52	0.44	<0.01	0.08	0.43
Smoking	0.15	0.10			0.17	0.09		
Dyslipidemia	0.20	0.03	−0.05	0.57	0.15	0.13		
ACEIs and/or ARBs	0.33	<0.01	−0.004	0.97	0.44	<0.01	0.08	0.42
Diuretics	0.37	<0.01	0.24	0.02	0.36	<0.01	0.01	0.92
Body mass index (kg m^{-2})	0.09	0.35			0.20	0.05		
Proteinuria (g day^{-1})	0.20	0.03	0.02	0.89	0.26	<0.01	0.15	0.08
Serum albumin (g dl^{-1})	−0.25	<0.01	0.05	0.72	−0.06	0.52		
C-reactive protein (mg dl^{-1})	0.04	0.66			0.07	0.52		
Hb (g dl^{-1})	−0.39	<0.01	0.03	0.84	−0.40	<0.01	−0.09	0.38
Serum phosphorus (mg dl^{-1})	0.36	<0.01	0.18	0.11	0.32	<0.01	0.03	0.77
eGFR (ml min^{-1} per 1.73 m^2)	−0.45	<0.01	−0.14	0.34	−0.47	<0.01	0.05	0.66
Serum uric acid (mg dl^{-1})	0.25	<0.01	0.03	0.78	0.53	<0.01	0.27	0.02

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate; Hb, hemoglobin.

Table 5 ORs by sex for left ventricular hypertrophy

Variables	Men						Women					
	Univariate			Multivariate			Univariate			Multivariate		
	OR	95% CI	P-values	OR	95% CI	P-values	OR	95% CI	P-values	OR	95% CI	P-values
Age (years)	1.04	1.01–1.07	0.01	1.03	0.98–1.10	0.22	1.11	1.06–1.16	<0.01	1.13	1.06–1.23	<0.01
Hypertension	20.9	5.59–136.5	<0.01	5.84	0.97–50.07	0.054	30.2	9.34–137.4	<0.01	7.38	1.20–57.37	0.03
Diabetes mellitus	9.36	4.06–23.3	<0.01	4.43	1.39–15.42	0.01	6.75	2.55–20.3	<0.01	1.42	0.26–8.39	0.68
Smoking	1.45	0.64–3.29	0.37				2.63	0.93–8.20	0.07			
Dyslipidemia	2.24	0.999–5.11	0.05				2.42	1.06–5.73	0.04	0.72	0.15–3.09	0.66
ACEIs and/or ARBs	6.22	2.74–14.89	<0.01	1.34	0.41–4.33	0.62	7.11	3.00–17.93	<0.01	3.92	0.87–20.25	0.07
Diuretics	9.49	3.06–41.85	<0.01	3.11	0.71–17.34	0.14	5.33	1.89–17.60	<0.01	0.81	0.13–4.89	0.82
Body mass index (kg m^{-2})	0.98	0.89–1.08	0.70				1.10	0.99–1.22	0.07			
Proteinuria (g day^{-1})	1.29	1.11–1.54	<0.01	0.97	0.75–1.28	0.83	1.21	0.99–1.53	0.06			
Serum albumin	0.33	0.18–0.57	<0.01	0.74	0.25–2.16	0.59	0.91	0.52–1.60	0.75			
C-reactive protein (mg dl^{-1})	0.70	0.23–1.93	0.48				2.06	0.91–12.4	0.09			
Hb (g dl^{-1})	0.67	0.55–0.80	<0.01	0.96	0.70–1.33	0.81	0.66	0.51–0.83	<0.01	0.77	0.48–1.22	0.12
Serum phosphorus (mg dl^{-1})	2.88	1.57–5.82	<0.01	1.46	0.59–4.00	0.43	3.05	1.56–6.83	<0.01	1.36	0.41–5.51	0.28
eGFR (ml min^{-1} per 1.73 m^2)	0.96	0.94–0.98	<0.01	1.00	0.96–1.04	0.99	0.96	0.95–0.98	<0.01	1.03	0.99–1.07	0.12
Serum uric acid (mg dl^{-1})	1.29	1.01–1.69	0.04	1.11	0.74–1.72	0.62	2.26	1.63–3.34	<0.01	1.91	1.14–3.61	0.03

Abbreviations: ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; CI, confidence interval; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; OR, odds ratio.

had higher SUA levels, and a higher prevalence of LVH, compared with those <45 years of age. Given that female subjects ≥45 years of age had lower kidney function compared with those <45 years of age, this observation may be partly attributable to the higher prevalence of advanced CKD stages in female subjects ≥45 years of age. In post-menopausal women, increased SUA levels may result from menopause-related metabolic changes, and might correlate with an increased risk of coronary artery disease.[45] It is possible that the role of SUA as a risk for CV disease becomes more obvious for patients with a generally lower risk profile, such as women.[22]

This study has some limitations. First, the sample size was relatively small. A larger cohort study will be needed to avoid study bias and to document more precisely the relationship between SUA and cardiac hypertrophy. Second, most of our subjects were treated with antihypertensive agents: 55% of subjects received ACEIs and/or ARBs, which are known to prevent cardiac hypertrophy.[46] However, univariate analysis showed that use of ACEIs and/or ARBs was associated with increased LVMI and higher prevalence of LVH in both sexes. It seems reasonable that ACEIs and/or ARBs were predominantly used for patients with cardiac hypertrophy.

Multivariate analysis showed that the use of diuretics was an independent variable associated with LVMI in men. This finding may reflect the tendency for patients with higher LVMI to receive diuretics more often.

In conclusion, our results show an independent association between SUA levels and LVH and LVMI in female subjects, but not in male subjects. This finding suggests that an association between SUA levels and the development of LVH and LVMI is more likely in women than in men.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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