Table 2. Primer sequences used for the gene expression analysis.

Gene	Forward primer sequence (5-3′)	Reverse primer sequence (5-3′)
IFN-γ	GAACTGGCAAAAGGACGGTA	CTGATGGCCTGGTTGTCTTT
TNF-α	AAATGGGCTCCCTCTCATCAGTTC	TCTGCTTGGTGGTTTGCTACGAC
IL-4	TCCTTACGGCAACAAGGAAC	GTGAGTTCAGACCGCTGACA
IL-17	ATCAGGACGCGCAAACATG	TGATCGCTGCTGCCTTCAC
β-actin	ACCACCATGTACCCAGGCATT	CCACACAGAGTACTTGCGCTCA

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Results

Characterization of Circulating Leukocytes in Peripheral Blood

After 10 Gy irradiation and allogeneic or syngeneic BMT, circulating leukocytes markedly decreased (by>95%) on day 7, but recovered by day 14 (Fig. 1A). In allogeneic BMT rats, circulating leukocyte levels approached control levels on day 21, however, then decreased by day 28. By contrast, circulating leukocytes in syngeneic BMT rats gradually recovered by day 28. Almost all circulating mononuclear cells in peripheral blood in allogeneic BMT rats originated from the donor transplant (Fig. 1B).

In syngeneic BMT rats, circulating CD6+ and CD8+ T-cell levels gradually recovered by day 28 (Fig. 1C and 1D). In allogeneic BMT rats, CD6+ and CD8+ T-cell levels recovered from day 14, and the number of CD6+ and CD8+ T-cells was significantly higher on day 21 than in syngeneic BMT rats. Thereafter, CD6+ and CD8+ T-cell levels decreased by day 28, which might be associated with recruitment to the GVHD organs. The number of CD4+ T-cells and CD68+ macrophages was similar in the peripheral blood of allogeneic and syngeneic BMT rats (Fig. 1E and 1F).

Development of Systemic Acute GVHD

After DA to Lewis allogeneic BMT, the body weight of Lewis recipient rats gradually decreased by day 28 by>20% compared with that of pretransplantation (Fig. 2A). By contrast, in non-BMT control rats and Lewis-to-Lewis syngeneic BMT control rats, the body weight gradually increased by day 28, although body weight decreased transiently in syngeneic BMT rats similar to that in allogeneic BMT rats on day 7. Based on macroscopic evaluations of allogeneic BMT rats, dermatitis occurred around day 21 and developed on day 28 with erythematous rush and alopecia (Fig. 3A and 3B). Diarrhea was also noted starting from day 21 to day 28. Liver function abnormalities were also detected with increased serum AST and ALT levels (Fig. 4), although T-Bil levels were within the normal range. The semiquantitative score of systemic acute GVHD gradually increased by day 28 (Fig. 2B). Light microscopic findings of the skin, liver, and intestine on day 28 demonstrated severe acute GVHD with infiltration of CD3+ T-cells (Fig. 3C-K). Only a minimal esterase+ neutrophils were present in inflammation (data not shown). Based on these clinical signs, laboratory data, and pathology, severe acute

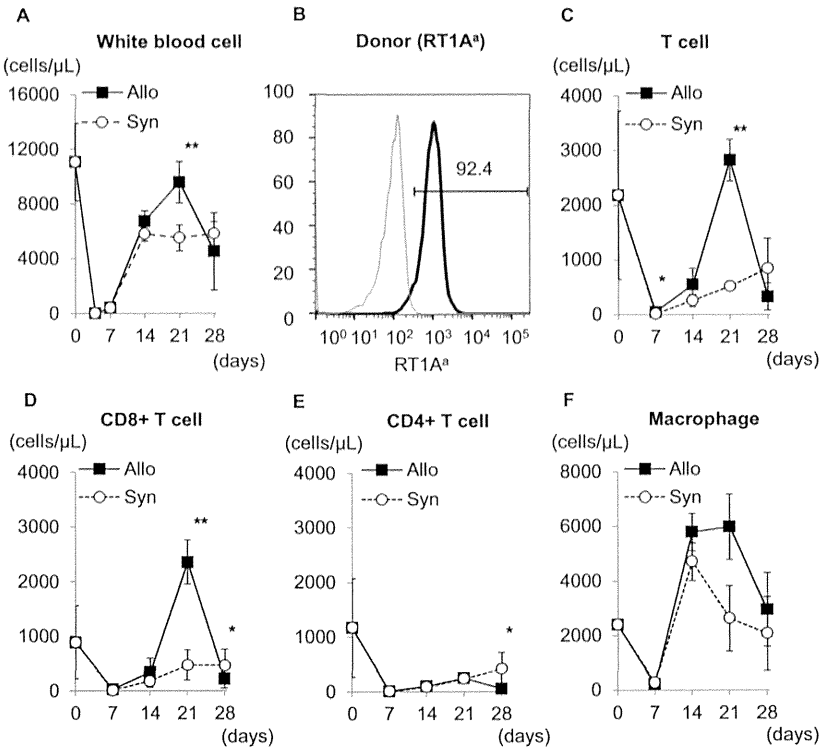

Fig. 1. Leukocytes in the peripheral blood after bone marrow transplantation (BMT). The total white blood cell (WBC) count in peripheral blood (A) decreased markedly on day 4, but recovered between day 7 and day 14 in both allogeneic and syngeneic BMT rats. The number of WBCs in the peripheral blood was higher on day 21 in allogeneic BMT rats than in syngeneic BMT rats. WBCs in the peripheral blood decreased again in allogeneic BMT rats on day 28, which may be because of recruitment of WBCs to GVHD organs. Almost all circulating leukocytes in allogeneic BMT rats on day 28 after BMT (B) expressed donor-type RT1A^a, indicating that circulating leukocytes in peripheral blood originated from the donor (Gray; no staining, Black; anti-RT1A^{a,b}). In peripheral blood, CD6+ T-cells (C), CD8+ T-cells (D), CD4+ T-cells (E), and ED1+ macrophages levels (F) recovered between day 7 and day 21 after BMT in both syngeneic and allogeneic BMT rats. The number of CD6+ T-cells and CD8+ T-cells was significantly higher on day 21 in allogeneic BMT rats than in syngeneic BMT rats. The number of CD4+ T-cells and CD68+ macrophages was similar in both syngeneic and allogeneic BMT rats. *$P<0.05$, **$P<0.01$.

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GVHD developed in the skin, liver, and digestive tract by day 28 after BMT in the DA-to-Lewis allogeneic BMT model. However, in the Lewis-to-Lewis syngeneic BMT rats and non-BMT control rats, only few CD3+ T-cells infiltrated the skin, liver, and digestive tract, and acute GVHD did not develop by day 28 (data not shown).

Development of Acute GVHD of the Kidney

In conjunction with the development of acute GVHD in the skin, liver, and digestive duct, renal function abnormalities developed by day 28. Serum BUN and urinary NAG levels increased on day 28 (Fig. 4), indicating renal dysfunction and proximal renal tubular injury. Urinary NAG levels were significantly increased in

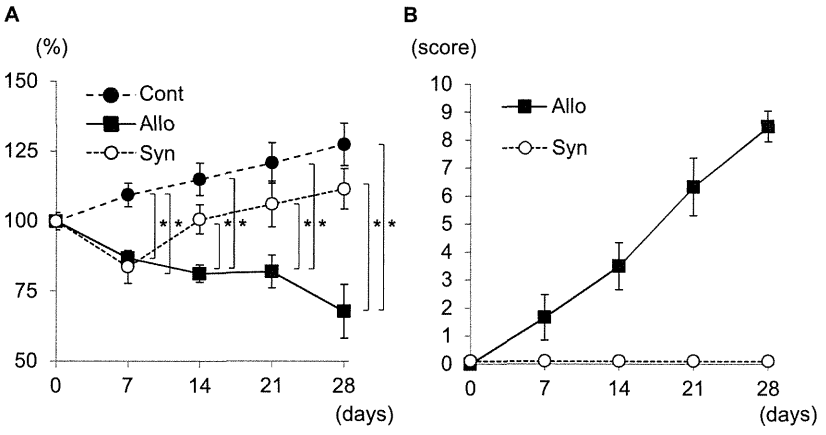

Fig. 2. Body weight and semiquantitative score of systemic acute GVHD after bone marrow transplantation (BMT). Comparison of body weight in percentage (A) on day 0, after radiotherapy and after BMT showed that this parameter decreased in syngeneic and allogeneic BMT rats on day 7 and continued gradually to decrease in allogeneic BMT rats by>20% on day 28. In addition, body weight was significantly lower in allogeneic BMT rats than in syngeneic BMT rats between day 14 and day 28 after BMT. The semiquantitative score of systemic acute GVHD (B) showed that symptoms associated with acute GVHD occurred from day 7 in allogeneic BMT rats, and developed by day 28 with score of 8.7 ± 0.5 (mean ± SD; score 0–10). In contrast, acute GVHD did not develop in syngeneic BMT rats by day 28. *$P<0.05$.

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allogeneic BMT rats on day 28 when serum creatinine (Cr) levels were within normal range. These findings indicated that the increase in urinary NAG levels was an early and sensitive marker of acute GVHD of the kidney that occurred before the increase in serum Cr levels. Urinary protein levels were not significantly different between non-BMT control rats (709.7 ± 72.3 mg/day), syngeneic BMT control rats (809.5 ± 174.8 mg/day), and allogeneic BMT rats (604.6 ± 141.4 mg/day).

Pathology of the kidney with acute GVHD indicated mononuclear cell infiltration to the interstitium (Fig. 5). Acute GVHD with mild renal inflammation was characterized by infiltration of mononuclear cells to the interstitium, mainly around small arteries and veins. Acute GVHD with moderate to severe renal inflammation was characterized by infiltration of inflammatory cells, which gradually expanded from the interstitium around small arteries to the peritubular interstitium. During mild to moderate renal inflammation, peritubular capillaritis and tubulitis was noted with infiltration of CD3+ T-cells and CD68+ macrophages (Fig. 5C). In addition, acute glomerulitis and acute endarteritis also developed in the kidney with marked renal inflammation (Fig. 6).

On day 28, infiltrating cells in the kidney were characterized by a large number of CD3+ T-cells and ED1+ macrophages in the interstitium (Fig. 7). CD3+ T-cells were mainly constituted with CD8+ T-cells (Figs. 7, 8A–C). CD4+ T-cells also infiltrated the kidney (Fig. 8D–F). Almost all infiltrating mononuclear cells involved in renal inflammation originated from the donor transplant (Fig. 8G–I). Only a minimal esterase+ neutrophils were present in inflammation (data not shown). In syngeneic BMT and non-BMT control rats, minimal numbers of

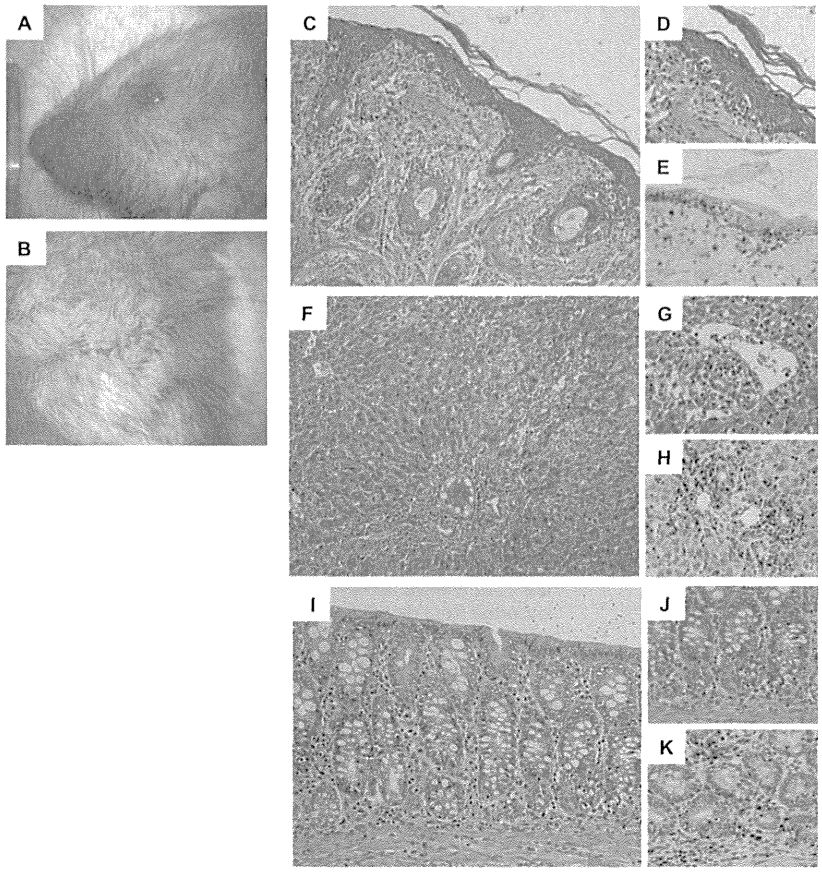

Fig. 3. Acute GVHD in the skin, liver, and intestine after allogeneic bone marrow transplantation (BMT). On day 28 after allogeneic BMT, macroscopic findings of the skin indicated erythematous rush and alopecia (A, B). Light microscopic findings showed inflammatory cells, mainly CD3+ T-cells, infiltrating the epidermis and the hair follicle in the dermis, indicating acute GVHD in the skin (C, D: HE stain, E: CD3 stain, C: ×400, D, E: ×800). Inflammatory cells, mainly CD3+ T-cells, infiltrated the portal areas and spread to the hepatic lobules with cholangiolitis and phlebitis in the portal and central veins, indicating acute GVHD in the liver (F, G: HE stain, H: CD3 stain, F: ×400, G, H: ×800). In the colon, erosion and inflammatory cell infiltration was noted with cryptitis and infiltration of CD3+ T-cells, indicating acute GVHD in the colon (I, J: HE stain, K: CD3 stain, I: ×600, J, K: ×800).

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CD3+ T-cells, CD8+ T-cells, and ED1+ macrophages infiltrated the kidney. IgM, IgG, and C3 depositions were not seen in the kidney (data not shown). The expression of rat MHC class II significantly increased in renal tubules in allogeneic BMT rats than those in syngeneic BMT and non-BMT control rats on day 28 (Fig. 7). In cytokine milieu in the kidney, the expressions of INF-γ and TNF-α were significantly increased on day 28 in allogeneic BMT rats compared with those in syngeneic BMT rats (Fig. 9). The expressions of IL-4 and IL-17 did not increase in the kidney in allogeneic and syngeneic BMT rats.

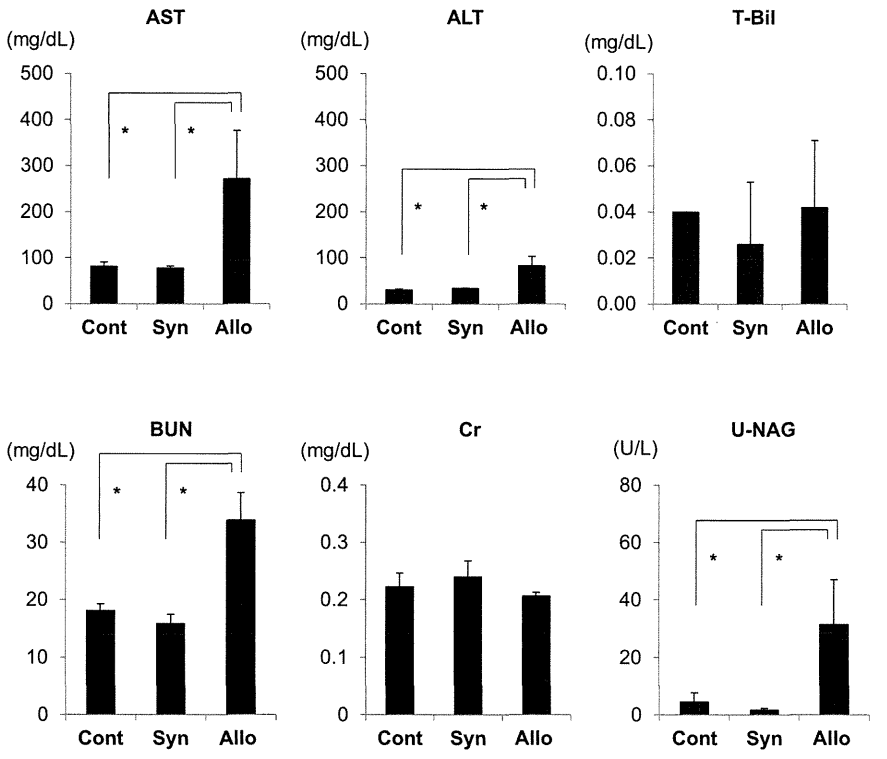

Fig. 4. Liver and renal dysfunction after allogeneic bone marrow transplantation (BMT). Liver function was determined using serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T-Bil) levels. Serum AST and ALT levels on day 28 were significantly increased in allogeneic BMT rats compared with those in syngeneic BMT rats, although serum T-Bil levels were similar in both groups. Renal function was assessed using serum blood urea nitrogen (BUN), serum creatinine (Cr), and urinary N-acetyl-β-D-glucosaminidase (NAG) levels. Serum BUN and urinary NAG levels on day 28 significantly increased in allogeneic BMT rats compared with those in syngeneic BMT and non-BMT control rats, although serum Cr levels were similar in these groups. Importantly, urinary NAG levels in allogeneic BMT rats were increased while the serum Cr levels were stable. *$P<0.05$.

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Discussion

The occurrence of GVHD is a major complication after HCT. In the present study, although the skin, liver, and digestive tract were found to be the main target organs of GVHD, we demonstrated that the kidney could also be a direct target organ of acute GVHD following BMT. Pathological examination revealed infiltration of mononuclear cells, CD3+ T-cells, mainly CD8+ T-cells, CD4+ T-cells, and macrophages, in the renal interstitium with peritubular capillaritis, tubulitis, acute glomerulitis, and endarteritis. These findings suggest the development of T cell-mediated injury in the renal microvasculature and renal tubules during acute GVHD of the kidney. Increased urinary NAG level was an early marker of acute GVHD in the kidney, whereas serum Cr and urinary protein levels were stable. Acute GVHD in rat BMT model in the present study was different condition from the clinical human GVHD after HCT, because human GVHD is induced in rich stem cell transplantation with a very close degree of

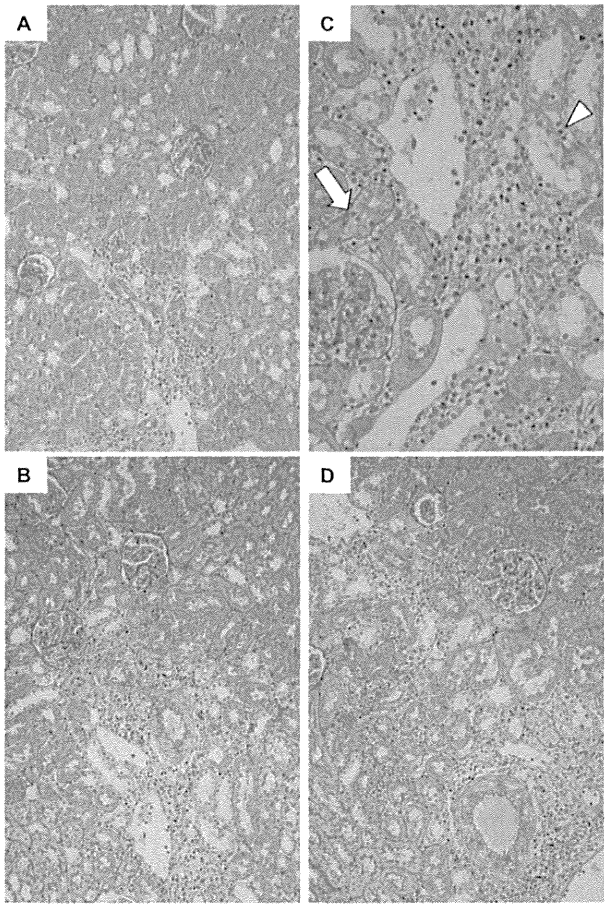

Fig. 5. Renal inflammation in acute GVHD after allogeneic bone marrow transplantation (BMT). In mild renal inflammation (A, PAS stain, ×200), mononuclear cells infiltrated the renal interstitium around the small vessels (arrow). In moderate renal inflammation (B, PAS stain, ×200), infiltration of mononuclear cells expanded into the peritubular interstitium. High magnification of renal inflammation (C, PAS stain, ×400), interstitial cell inflammation was often accompanied by tubulitis (arrow) and peritubular capillaritis (arrowhead). In severe renal inflammation (D, PAS stain, ×200), diffuse interstitial inflammation was noted in the renal cortex.

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HLA matching between donor and recipient, and use the immunosuppressant or conditioning regimens to prevent GVHD. Therefore, further studies are needed to evaluate the acute GVHD of the kidney in human GVHD after clinical HCT.

After allogeneic HCT, GVHD is a major cause of morbidity and mortality, responsible for 15–40% of mortality [15, 16]. In acute GVHD, the most commonly affected organs include the skin, liver, and gastrointestinal system [17, 18]. In the present study, recipient Lewis rats also showed typical acute GVHD in the skin, liver, and intestine after allogeneic DA rat BMT. In contrast, in the Lewis-to-Lewis syngeneic BMT control rats, acute GVHD did not develop, even in the skin, liver, or digestive tract. In the kidney, acute GVHD is a common etiology and independently increases the risk for acute kidney injury [19].

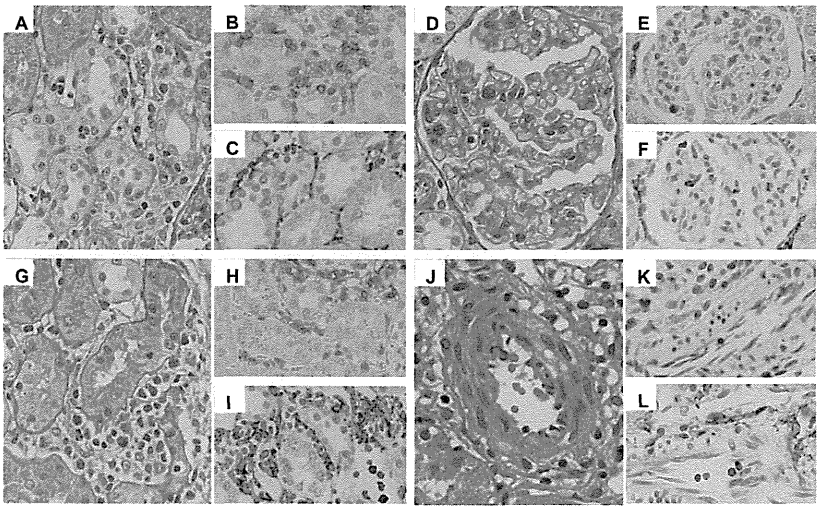

Fig. 6. Histopathological features in the kidney on day 28 after allogeneic bone marrow transplantation (BMT). In the kidney, 28 days after allogeneic BMT, tubulitis (A–C), peritubular capillaritis (D–F), acute glomerulitis (G–I), and endarteritis (J–L) were caused by the infiltration of mononuclear cells (A, D, G, J), CD3+ T-cells (B, E, H, K), and ED-1+ macrophages (C, F, I, L), indicating cell-mediated renal injury in acute GVHD. (A, D, G: PAS stain, ×600; J: HE stain, ×1000; B, E, H, K: CD3 stain, ×600; C, F, I, L: ED1 stain, ×600).

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However, GVHD can contribute to renal dysfunction indirectly through nephrotoxicity induced by a calcineurin inhibitor used in prophylaxis against GVHD, severe GVHD with diarrhea and dehydration, and cytomegalovirus reactivation [3, 5–7, 20]. However, it is generally accepted that acute GVHD of the kidney does not occur and acute GVHD itself cannot be involved directly in renal injury and dysfunction.

On the other hand, acute renal disorders after HCT are considered to be mediated by multiple factors [3, 5–7]. As patients with hematologic malignancy receive higher doses of chemotherapy and experience frequent infections during neutropenia, renal dysfunction may be attributable to GVHD as well as infiltration of underlying diseases [5]. High-dose chemotherapy and total body irradiation administered in the induction regimen may directly cause renal damage [21]. Rapid cytolysis of tumor and normal marrow can cause tumor lysis syndrome with renal injury due to hyperphosphatemia, as well as urate and xanthine nephropathy [22]. Infusion of cryopreserved marrow or blood progenitor cells may lead to renal insufficiency [23]. Post-transplantation infections often lead to acute renal insufficiency because they may be accompanied by hypotension and renal hypoperfusion. Antimicrobials used for prophylaxis and treatment of infections are also commonly nephrotoxic [3, 5–7, 22, 23]. In the present study, however, the relationship of all these factors with renal injury and inflammation could not be assessed, as our experiment did not use these nephrotoxic agents, except for lethal 10 Gy irradiation. In addition, the lethal 10 Gy irradiation could not have contributed to renal injury and

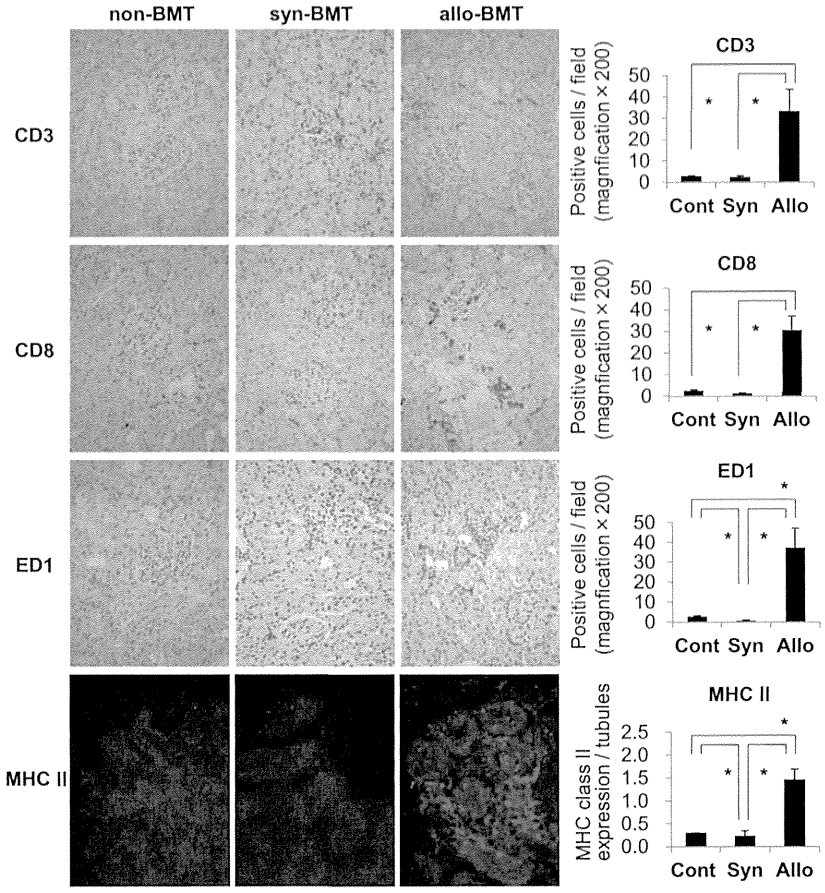

Fig. 7. The infiltrating cells in the kidney and the MHC class II expressions in renal tubules. In the kidney on day 28 in allogeneic (allo-BMT) bone marrow transplantation rats, CD3+ T-cells including CD8+ T-cells, and ED1+ macrophages infiltrated the interstitium. The number of CD3+ T-cells, CD8+ T-cells, and macrophages per ×200 magnification field on day 28 showed that infiltration of these cells in the kidney significantly increased in allogeneic BMT rats compared with that in the non-transplanted (non-BMT) control rats and syngeneic (syn-BMT) bone marrow transplantation control rats. In addition, the expression of MHC class II in renal tubules increased in the kidney on day 28 in allogeneic BMT rats. The expression of MHC class II in renal tubules was significantly increased in allogeneic BMT rats than those in non-BMT control and syngeneic BMT control rats. *$P<0.05$.

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inflammation in the present study, because syngeneic BMT rats that received lethal 10 Gy irradiation and syngeneic BMT showed minimal renal dysfunction and no obvious renal inflammation. Therefore, we considered that multiple factors excluding acute GVHD could not be associated with renal dysfunction and renal inflammation in our model.

Recently, several studies have reported that GVHD can involve renal insufficiency [8–11]. Membranous nephropathy after HCT may be associated with chronic GVHD [8, 9]. In a BMT mouse model of acute GVHD, in vivo imaging of the mice revealed that several non-classical organs are infiltrated by cytotoxic T-cells during GVHD, including the brain, kidney, and connective tissues [11]. In

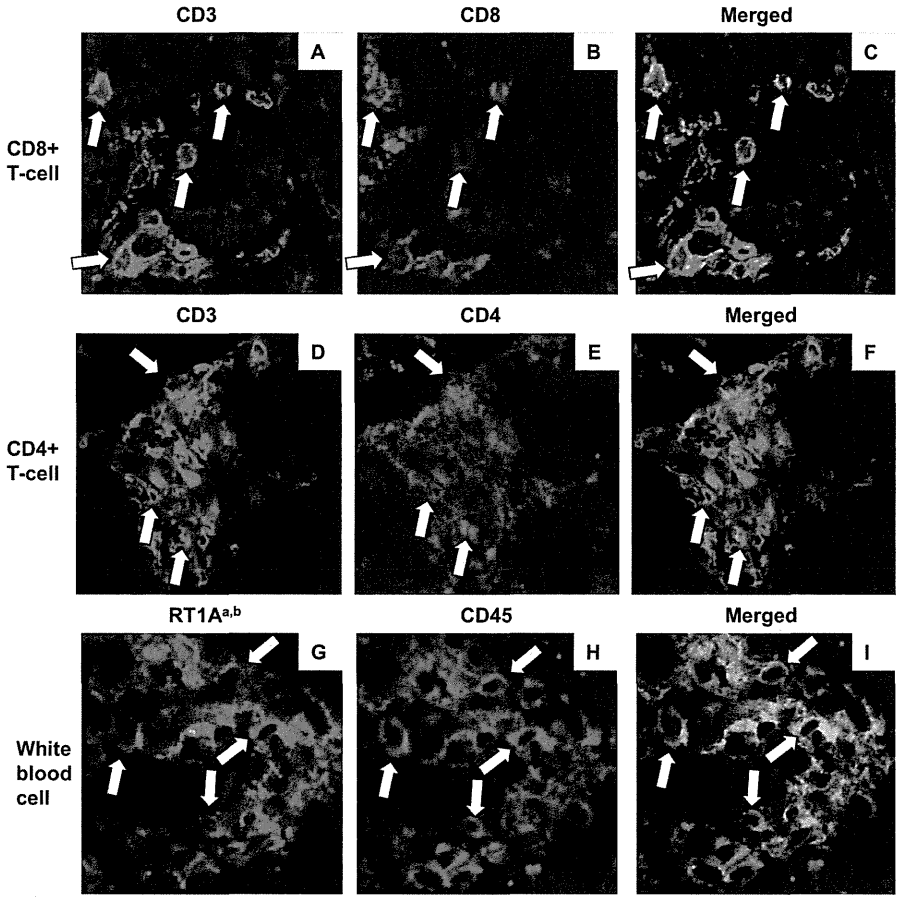

Fig. 8. Infiltrating cells in the kidney in acute GVHD after allogeneic bone marrow transplantation (BMT). Double immunofluorescence stain by fluorescence antibody technique against CD3+ (A) and CD8+ (B), and their merged image (C) indicated that, in the kidney with acute GVHD on day 28, CD8+ T-cells (arrow in A–C) infiltrated the kidney. In addition, CD4+ T-cells (arrow in D–F) were also noted in inflammation, indicating that not only class I-restricted T cell-mediated reactions but also class II-restricted T cell-mediated reactions developed in renal acute GVHD. Double immunofluorescence stain against $RT1A^{a,b}$ (G) and CD45 (H), and their merged image (I) indicated that, in the kidney with acute GVHD on day 28, almost all CD45+ leukocytes (arrow in G–I) were expressed rat $RT1A^{a,b}$, suggesting the infiltration of donor-type leukocytes in acute renal GVHD.

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autopsy cases after HCT, allogeneic HCT recipients with severe GVHD tended to have tubulitis and peritubular capillaritis [10]. These studies may suggest that some renal dysfunction is associated with GVHD.

In the present study, we found significant infiltration of donor leukocytes in the kidney, and that infiltration of CD3+ T-cells, CD8+ T-cells, CD4+ T-cells, and macrophages mediated renal inflammation with peritubular capillaritis, tubulitis, acute glomerulitis, and endarteritis in allogeneic BMT recipients with systemic acute GVHD. Our findings of acute GVHD in the kidney were quite similar to pathological findings, as acute T cell-mediated rejection of the kidney in allogeneic renal transplantation [24]. In allogeneic renal transplant rejection, the

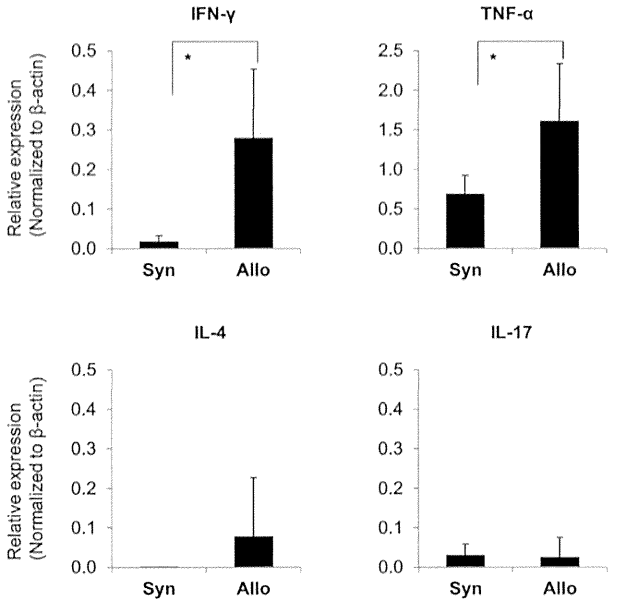

Fig. 9. Real-time reverse transcription-PCR analysis of cytokines in the kidney after bone marrow transplantation (BMT). *The expression of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)* was significantly up-regulated in the kidney on day 28 in allogeneic (Allo) BMT rats compared with that in the syngeneic (Syn) BMT rats. The expressions of interleukin 4 (IL-4) and IL-17 were not significantly different between these 2 groups. *$P<0.05$.

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pathology of tubulitis and peritubular capillaritis, acute glomerulitis, or endarteritis is considered the T cell-mediated immune injury for renal tubular epithelial cells and renal microvascular endothelial cells, respectively [25, 26]. The expression of MHC class II in renal tubules significantly increased in acute renal GVHD in the present study, and it showed similar findings to acute T- cell-mediated rejection in the kidney transplantation. Therefore, we considered that the pathology of the kidney in acute GVHD in the present study indicated T cell-mediated immunologic injury of renal tubules and renal microvasculature.

GVHD is caused by host-reactive T-cells derived from the donor bone marrow itself, or from the peripheral blood that contaminates the BM during its preparation [27, 28]. Donor-derived CD8+ cytotoxic T-cells have been identified as key players mediating GVHD pathogenesis [29–33]. CD8+ cytotoxic T-cell levels in peripheral blood predict the development of acute and severe GVHD [34]. In addition, CD4+ helper T-cells are also important effector cells of GVHD [35]. In the present study, renal inflammation in acute GVHD was accompanied by infiltration of CD8+ T-cells and CD4+ T-cells. CD8+ T-cells in the peripheral blood seemed to be increased during the development of acute GVHD, although they rapidly decreased after the full development of acute GVHD, in allogeneic BMT rats.

In the GVHD pathophysiology, both cellular factors (such as donor T-cells and macrophages) and soluble factors (cytokines) play a role in the development of

acute GVHD. Based on the cytokine profile, the Th1 cytokines (IFN-γ, IL-2, and TNF-α) have been implicated in the pathophysiology of acute GVHD [29–33]. The Th1 cytokines participate in the initiating events that culminate in GVHD, as well as amplify the disease process once established. The transcript levels of IFN-γ in CD8+ T-cells are a sensitive marker to detect active GVHD [36]. A series of clinical studies have demonstrated the correlation between circulating TNF-α levels or TNF receptor-1 levels following HCT and GVHD [37, 38]. In addition, several clinical studies have targeted TNF-α as part of a treatment strategy for acute GVHD [39]. In the present study, the expressions of IFN-γ and TNF-α mRNA increased in the kidney of allogeneic BMT rats compared with those in syngeneic BMT control rats. In our model, donor-derived CD8+ T-cells, CD4+ T-cells, and macrophages within Th1 cytokine milieu induced acute GVHD of the kidney that have classically been considered the main immune mechanism mediating GVHD pathogenesis. By contrast, in the present study, IL-4, one of the Th2 cytokines, was not significantly different between allogeneic and syngeneic BMT rats, which may be associated with the absence of antibody-mediated immune injury. Levels of IL-17 produced by Th17 cells, involved in many immunologic processes including several autoimmune diseases, were also not significantly different between allogeneic and syngeneic BMT rats.

Based on laboratory findings, serum BUN and urinary NAG levels increased in acute renal GVHD in the present model. Inflammatory damage to the renal tubules from GVHD may be associated with an increase in the urinary NAG levels. We speculate that urinary NAG levels may be an early marker of renal GVHD that can be detected when serum Cr and urinary protein levels are stable. Further studies are needed to clarify the occurrence of acute renal GVHD after clinical HCT, the correlation between acute renal GVHD and urinary NAG levels in human GVHD, and useful pre-emptive therapy to improve transplant outcome after clinical HCT.

In summary, the kidney may be a target organ of GVHD, and the increased urinary NAG levels after BMT may indicate the development of acute GVHD of the kidney. As the number of HCTs increases every year, hematologists, nephrologists, oncologists, and pathologists should work together to identify patients with acute GVHD of the kidney to both prevent its development and initiate therapy early to improve outcomes after HCT.

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Author Contributions

Conceived and designed the experiments: SH AS YM SN YK GK MF KN AM TK ST. Performed the experiments: SH AS YM SN YK GK MF KN AM TK ST. Analyzed the data: SH AS YM SN YK GK MF KN AM TK ST. Contributed reagents/materials/analysis tools: SH AS YM SN YK GK MF KN AM TK ST. Wrote the paper: SH AS YM SN YK GK MF KN AM TK ST. Pathological technical assistance: TA MK KW AI NK. Special adviser: YK YF.

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Therapeutic Apheresis and Dialysis

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Letters to the Editor

Birth Weight and End-Stage Diabetic Nephropathy in Later Life: A Japanese Multicenter Study

Dear Editor,

Low birth weight (LBW) of less than 2500 g is a surrogate marker for low nephron number, and emerging evidence suggests that it is an important risk factor for both the progression of renal diseases (1,2) and the incidence of end-stage renal disease (ESRD) (2,3). Individuals with an inborn nephron deficit are susceptible to progressive loss of kidney function due to aging-related glomerular alterations (1). However, most study participants have been younger than 40 (2,3), because little well-organized information on birth weight is available for the old general population as comparison subjects in most countries. Diabetes mellitus is currently the most frequent primary disease of patients receiving dialysis, and the mean age of initiation of RRT in DM nephropathy is over 60 years old in Japan. Furthermore, the effect of generational differences must be considered when analyzing the correlation between birth weight and ESRD (4). Therefore, birth weight for each individual should be compared to the mean birth weight from an age-matched population.

In January 2010 in nine centers in Ibaraki and Chiba prefectures in Japan, 1130 Japanese patients undergoing maintenance hemodialysis were identified. We surveyed the patients by using a self-completed questionnaire containing questions about birth weight. Data were obtained from 230 patients (20.3%). Mean birth weight in each year from 1903 to 1951 was estimated based on three nationwide surveys held in 1951, 1930 and 1903. Mean birth weight from 1951 to 2010 is available in the Annual Vital Statistics of National Population Dynamics Survey conducted by the Ministry of Health, Labor, and Welfare of Japan. We defined mean birth weight in each patient's birth year as age-matched mean birth weight. We divided our subjects into those with and without DM and used the age of 60 years old in each group as thresholds to further classify subjects as younger and older. We then compared the actual birth weight provided on the questionnaire to the age-matched mean birth weight in each group. Older patients with DM had significantly lower birth weight than age-matched mean birth weight, whereas no significant differences between them existed in younger patients in the DM group or in any patients in the non-DM group, which consists of 101 patients with chronic glomerulonephritis and 45 with miscellaneous diseases (Fig. 1).

Ichikawa et al. reported the dynamics of LBW and ESRD in Japan by using an ecological approach. However, they did not consider the effect of aging or the cause of ESRD in individuals (4). In contrast, we analyzed the effect of them by evaluating birth weight separately in younger and older individuals with or without DM. We considered that older diabetic patients with a small number of glomeruli due to significantly lower birth weight may have more severe long-term nephron damage than older non-diabetic patients. Therefore, we speculated that lower-than-mean birth weight would be a risk for ESRD in patients with DM in later life.

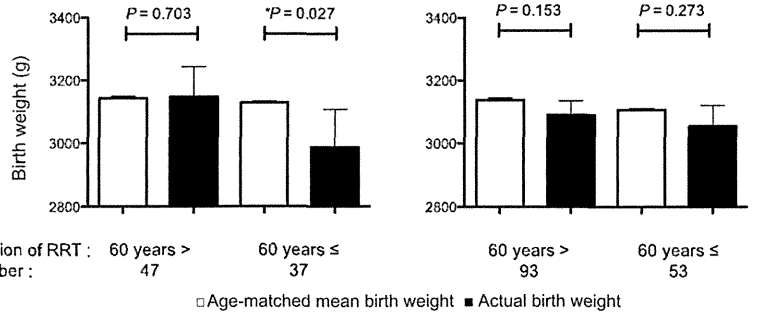

FIG. 1. Birth weight of elderly diabetic patients is lower than age-matched mean birth weight.
Patients receiving hemodialysis were categorized according to the presence of diabetes mellitus (DM) and the age of 60 years old at initiation of dialysis. Actual birth weight and age-matched mean birth weight was compared by using the Wilcoxon matched-pairs sign rank test. Values are expressed as mean ± standard error of the mean (SEM). $P < 0.05$ was considered to indicate a significant difference. RRT; renal replacement therapy.

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Conflict of interest: none declared.

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Atypical Vascular Access for Dialysis Patients via Persistent Left Superior Vena Cava

Dear Editor,

We report a case of a successful insertion and use of hemodialysis (HD) catheter into the persistent left superior vena cava (PLSVC). This anomaly results from failed obliteration of the left anterior cardinal vein during gestation as the most common congenital venous anomaly of the thoracic systemic venous return, occurring in 0.3% to 0.5% of individuals in the general population (1,2).

A 71-year-old male patient with elevated levels of total blood urea nitrogen (BUN) and potassium was diagnosed with end-stage renal disease requiring dialysis and a temporary vascular access. Several catheters inserted into the right and left jugular vein were pulled out by the patient who was extremely anxious/agitated in the first few days of treatment. At the third insertion of the catheter into the left jugular vein, the chest X-ray confirmed an unusual position of the catheter (Fig. 1A) as it glided along the left paramediastinal region, following the expected course of the descending aorta. We assumed the catheter was inserted into the left internal jugular vein (LIJV), without any complication except for mild initial resistance during guidewire insertion. Brisk dark/venous blood return from both catheter ports was noted, confirmed by the blood gas analysis. However, the computed tomography (CT) angiography through the catheter confirmed its position in PLSVC draining into the right atrium (Fig. 1B).

The catheter was successfully used for 2 months of HD treatment under continuous monitoring, with no evidence of arrhythmia or ischemia. He was discharged from hospital and was advised to undergo a regular ambulatory dialysis treatment. After 2 months he was re-admitted to our clinic as disoriented, with high potassium levels, and severely agitated during hemodialysis treatment. Five days later he died of cerebrovascular stroke, cardio-respiratory insufficiency and cardiac arrest. Unfortunately, an autopsy was not performed.

Insertion of jugular catheters may be difficult in the absence of ultrasound guidance and adequate patient cooperation, especially in cases of severe volume overload. From 2006 until 2012, approximately 1500 central venous catheters were inserted at our clinic with approximately 10% femoral and 90% jugular catheters (185 as tunneled). In cases of left IJV catheterization with difficulties during the procedure and unusual chest X-rays, catheter position into the PLSVC should be considered on CT scan. Although usually asymptomatic and hemodynamically insignificant (3), PLSVC can lead to serious complications (systemic embolization, arrhythmia, angina and

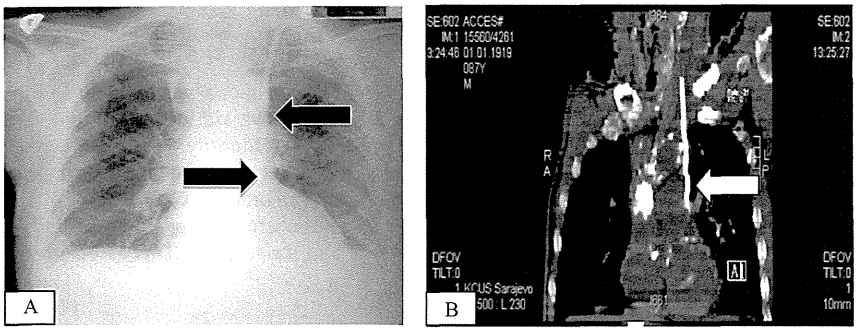

FIG. 1. (A) Position of the catheter tip after the third catheter placement through the left internal jugular vein (chest X-ray). (B) Chest computed tomography (CT) showing the hemodialysis catheter passing through the persistent left superior vena cava.

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Association of the triglycerides to high-density lipoprotein cholesterol ratio with the risk of chronic kidney disease: Analysis in a large Japanese population

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ABSTRACT

Objectives: To investigate the relationship between triglycerides to high-density lipoprotein cholesterol ratio (TG/HDL-C) and chronic kidney disease (CKD).
Methods: We used data from 216,007 Japanese adults who participated in a nationwide health checkup program. Men (n = 88,516) and women (n = 127,491) were grouped into quartiles based on their TG/HDL-C levels (<1.26, 1.26–1.98, 1.99–3.18, and >3.18 in men; <0.96, 0.96–1.44, 1.45–2.22, and >2.22 in women). We cross-sectionally assessed the association of TG/HDL-C levels with CKD [defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2 (low eGFR) and/or proteinuria (defined as urinary protein ≥1+ on dipstick testing)], low eGFR, and proteinuria.
Results: The prevalence of CKD, low eGFR, and proteinuria increased significantly with elevating quartiles of TG/HDL-C in both genders (all P for trend <0.001). Participants in the highest quartile of TG/HDL-C had a significantly greater risk of CKD than those in the lowest quartile after adjustment for the relevant confounding factors (odds ratio: 1.57, 95% confidence interval: 1.49–1.65 in men; 1.41, 1.34–1.48 in women, respectively). Furthermore, there were significant associations with low eGFR and proteinuria. In stratified analysis, the risk of CKD increased linearly with greater TG/HDL-C levels in participants with and without hypertension, diabetes, and obesity. Moreover, higher TG/HDL-C levels were relevant for CKD, especially in participants with hypertension and diabetes (P for interaction <0.001, respectively).
Conclusions: An elevated TG/HDL-C is associated with the risk of CKD in the Japanese population.

1. Introduction

Chronic kidney disease (CKD) is a global public health problem and a major risk factor for progressive kidney failure and cardiovascular morbidity and mortality [1]. Identifying and managing the risk factors associated with mild CKD may well be the best strategy to prevent and delay advanced outcomes of CKD [1].

Abnormal lipoprotein metabolism has been identified as a possible cause of CKD [2,3], and moderate CKD is associated with

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elevated levels of triglycerides (TG) and a decreased level of high-density lipoprotein cholesterol (HDL-C) [2–7].

Recent studies have shown that there is an association between TG/HDL-C and insulin resistance and that TG/HDL-C may be a better predictor of cardiovascular events than other lipid parameters, including TG, low-density lipoprotein-cholesterol (LDL-C), or the total cholesterol/HDL-C ratio [8–11]. In addition, TG/HDL-C has also been shown to predict the LDL particle size [12–14]. However, little is known about the association between TG/HDL-C and CKD. In the present study, we investigated the association between TG/HDL-C and CKD in a nationally representative group of Japanese adults.

2. Methods

2.1. Study population

This cross-sectional cohort study was conducted as a part of the prospective ongoing project entitled "Research on the Positioning of Chronic Kidney Disease in Specific Health Check and Guidance in Japan", and it was based on data obtained from the Japanese Specific Health Check and Guidance System. This annual health check program was initiated in 2008 by the Japanese government and it promotes the early diagnosis of metabolic syndrome and intervention strategies for the prevention of this disease. In 2008 and 2009, data were collected from 676,905 individuals participated in the health checkups. Men ($n = 278,017$) and women ($n = 383,586$) involved were between 20 and 101 years of age. For our study, data from 216,007 of the participants (88,516 men and 127,491 women) aged between 20 and 88 years were used for statistical analyses. (We excluded 460,898 participants because essential data, including information on proteinuria and serum creatinine levels, were unavailable.) This study was conducted in accordance with the Private Information Protection Law and ethical guidelines for epidemiology research published by the Ministry of Health, Labour and Welfare in 2005.

Table 1
Clinical features of all subjects.

Variables	Men ($n = 88,516$)	Women ($n = 127,491$)	P value
Age, years	63.8 ± 8.9	63.8 ± 8.5	0.77
Body mass index, kg/m^2	23.7 ± 3.0	22.8 ± 3.5	<0.001
Waist circumference, cm	85.3 ± 8.2	82.6 ± 9.8	<0.001
Systolic blood pressure, mmHg	131 ± 17	128 ± 18	<0.001
Diastolic blood pressure, mmHg	78 ± 11	75 ± 11	<0.001
Fasting blood glucose, g/dL	102 ± 25	95 ± 18	<0.001
Hemoglobin A1c, %	5.4 ± 0.8	5.3 ± 0.6	<0.001
LDL-C, mg/dL	121 ± 30	130 ± 30	<0.001
HDL-C, mg/dL	57 ± 15	66 ± 16	<0.001
TG, mg/dL	133 ± 96	107 ± 61	<0.001
TG/HDL-C	2.66 ± 2.59	1.83 ± 1.50	<0.001
Serum creatinine, mg/dL	0.84 ± 0.27	0.63 ± 0.19	<0.001
Estimated GFR, mL/min/1.73 m^2	74.7 ± 16.6	76.1 ± 16.3	<0.001
Low eGFR, %	17.7	11.4	<0.001
Proteinuria, %	8.2	4.0	<0.001
Chronic kidney disease, %	23.3	14.5	<0.001
Hypertension, %	51.4	42.7	<0.001
Diabetes mellitus, %	15.7	8.4	<0.001
Obesity, %	31.0	23.0	<0.001
Current smoker, %	25.3	5.9	<0.001
Daily drinker, %	44.9	8.3	<0.001
Regular exercise, %	47.6	39.8	<0.001
History of stroke, %	5.3	2.8	<0.001
History of heart disease, %	8.3	5.2	<0.001
Medication for hypertension, %	34.2	29.0	<0.001
Medication for diabetes mellitus, %	7.5	4.1	<0.001
Medication for dyslipidemia, %	12.9	22.9	<0.001

Low eGFR was defined as eGFR <60 mL/min/1.73 m^2. Proteinuria was defined as urinary protein of ≥1+ on dipstick testing. Chronic kidney disease was defined as low eGFR and/or proteinuria. Hypertension was defined as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or self-reported use of antihypertensive drugs. Diabetes was defined in accordance with American Diabetes Association guidelines as a fasting glucose concentration of ≥126 mg/dL, hemoglobin A1c concentration of ≥6.5%, or self-reported use of antihyperglycemic drugs. TG/HDL-C was calculated as TG (mg/dL) divided by HDL-C (mg/dL).
Abbreviations: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GFR, glomerular filtration rate; eGFR, estimated GFR.

2.2. Clinical evaluation and laboratory measurements

All participants completed a self-administered questionnaire that documented their medical history, current medications, smoking habits (current smoker or not), alcohol consumption (daily drinker or not), and regular exercise habits. A study physician physically examined every participant and checked the participants' reported medical history to ensure the accuracy of the information. The height and weight of participants were measured, and their body mass index (BMI) was calculated (kg/m^2). For these measurements, participants wore light clothing without shoes. Blood pressures were measured and blood as well as urine sampling was done at each participant's local medical institute, as stipulated by the health check program.

Blood samples were collected after participants fasted overnight and the blood was analyzed using an automated clinical chemical analyzer within 24 h of sampling. All blood analyses were conducted at a local, rather than a central, laboratory. Although the methods used for blood analyses were not calibrated between laboratories, the Japan Society of Clinical Chemistry-recommended methods for laboratory tests several years ago, and these recommendations have been widely adopted by laboratories across Japan. The enzymatic method was used to measure serum creatinine levels in fresh blood samples. Levels of LDL-C, HDL-C, and TG were determined enzymatically. Hemoglobin A1c (HbA1c) values were expressed as a National Glycohemoglobin Standardization Program equivalent value, which was calculated according to the following formula:

$$\text{HbA1c}(\%) = \text{HbA1c (Japan Diabetes Society)}\ (\%) + 0.4\%.$$

2.3. Definition of CKD, diabetes mellitus, obesity, hypertension, and TG/HDL-C

The estimated glomerular filtration rate (eGFR) was calculated using the following equation; eGFR (mL/min/1.73 m^2) = 194 × serum creatinine (mg/dL)$^{-1.094}$ × age (years)$^{-0287}$ × 0.739 (for women) [15]. Proteinuria was defined as urinary protein value of ≥1+ with dipstick testing. CKD was defined as an eGFR <60 mL/min/1.73 m^2 (low eGFR) and/or the presence of proteinuria. Hypertension was defined as a systolic blood pressure (SBP) of ≥140 mmHg, and/or a diastolic blood pressure (DBP) of ≥90 mmHg, or self-reported use of antihypertensive drugs. Diabetes mellitus was defined in accordance with the guidelines of the American Diabetes Association [16]; fasting glucose concentration ≥126 mg/dL, HbA1c concentration ≥6.5%, or self-reported use of antihyperglycemic drugs. TG/HDL-C was calculated as TG (mg/dL) divided by HDL-C (mg/dL). Male and female participants were separately grouped into quartiles based on their TG/HDL-C levels. TG/HDL-C levels for the quartile groups (Q) were as follows: $Q_1 < 1.26$, Q_2 1.26–1.98, Q_3 1.99–3.18, and $Q_4 > 3.18$ for men and $Q_1 < 0.96$, Q_2 0.96–1.44, Q_3 1.45–2.22, and $Q_4 > 2.22$ for women.

Table 2
Mean values or frequencies of relevant factors according to the quartiles of TG/HDL-C.

(A) Men

	TG/HDL-C				*P* for trend
	Q_1	Q_2	Q_3	Q_4	
	<1.26	1.26–1.98	1.99–3.18	>3.18	
	(n = 22,126)	(n = 22,126)	(n = 22,142)	(n = 22,122)	
Age, years	64.4 ± 8.7	64.4 ± 8.5	63.9 ± 8.8	62.3 ± 9.5	<0.001
Body mass index, kg/m^2	22.3 ± 2.8	23.4 ± 2.8	24.2 ± 2.9	24.9 ± 3	<0.001
Waist circumference, cm	81.2 ± 7.8	84.6 ± 7.8	86.7 ± 7.7	88.7 ± 7.7	<0.001
Systolic blood pressure, mmHg	129 ± 17	130 ± 17	131 ± 17	132 ± 17	<0.001
Diastolic blood pressure, mmHg	77 ± 11	78 ± 11	78 ± 11	79 ± 11	<0.001
Fasting blood glucose, g/dL	99 ± 21	101 ± 22	102 ± 24	106 ± 31	<0.001
Hemoglobin A1c,%	5.7 ± 0.7	5.7 ± 0.7	5.8 ± 0.8	5.9 ± 0.9	<0.001
LDL-C, mg/dL	110 ± 27	122 ± 28	128 ± 29	125 ± 33	<0.001
HDL-C, mg/dL	73 ± 15	60 ± 11	53 ± 10	45 ± 9	<0.001
TG, mg/dL	64 ± 16	95 ± 19	131 ± 27	242 ± 131	<0.001
TG/HDL-C	0.91 ± 0.23	1.60 ± 0.20	2.51 ± 0.34	5.61 ± 3.69	<0.001
Hypertension, %	45.3	50.9	53.7	55.5	<0.001
Diabetes mellitus, %	12.4	14.0	15.9	20.4	<0.001
Obesity, %	15.8	27.0	35.6	45.6	<0.001
Current smoker, %	19.1	23.2	25.9	32.8	<0.001
Daily drinker, %	52.1	45.5	41.4	40.5	<0.001
Regular exercise, %	53.3	49.7	46.4	40.8	<0.001
History of stroke, %	4.9	5.6	5.5	5.1	0.41
History of heart disease, %	7.9	8.5	9.0	7.8	0.95
Medication for hypertension, %	29.5	34.5	37	35.8	<0.001
Medication for diabetes mellitus, %	7.1	7.3	7.4	8.1	<0.001
Medication for dyslipidemia, %	9.2	12.8	14.6	15.1	<0.001

(B) Women

	TG/HDL-C				*P* for trend
	Q_1	Q_2	Q_3	Q_4	
	<0.96	0.96–1.44	1.45–2.22	>2.22	
	(n = 31,894)	(n = 31,817)	(n = 31,918)	(n = 31,862)	
Age, years	61.7 ± 9.7	63.7 ± 8.5	64.7 ± 7.7	64.9 ± 7.5	<0.001
Body mass index, kg/m^2	21.2 ± 2.9	22.3 ± 3.2	23.3 ± 3.4	24.3 ± 3.5	<0.001
Waist circumference, cm	77.8 ± 9.1	81.6 ± 9.4	84.3 ± 9.4	86.8 ± 9	<0.001
Systolic blood pressure, mmHg	124 ± 18	127 ± 17	129 ± 17	131 ± 17	<0.001
Diastolic blood pressure, mmHg	73 ± 11	74 ± 10	75 ± 10	76 ± 10	<0.001
Fasting blood glucose, g/dL	92 ± 14	94 ± 16	96 ± 17	99 ± 22	<0.001
Hemoglobin A1c, %	5.6 ± 0.5	5.7 ± 0.5	5.7 ± 0.6	5.9 ± 0.7	<0.001
LDL-C, mg/dL	118 ± 27	128 ± 28	135 ± 30	138 ± 32	<0.001
HDL-C, mg/dL	82 ± 15	70 ± 11	62 ± 10	51 ± 9	<0.001
TG, mg/dL	57 ± 13	82 ± 15	109 ± 20	180 ± 74	<0.001
TG/HDL-C	0.71 ± 0.16	1.18 ± 0.14	1.78 ± 0.22	3.66 ± 1.97	<0.001
Hypertension, %	31.5	40.1	46.3	53.0	<0.001
Diabetes mellitus, %	4.8	6.5	8.9	13.4	<0.001
Obesity, %	9.7	18.3	27.3	36.8	<0.001
Current smoker, %	5.1	5.2	5.7	7.5	<0.001
Daily drinker, %	12	8.6	6.6	5.9	<0.001
Regular exercise, %	40.5	40.3	40	38.5	<0.001
History of stroke, %	2.2	2.7	3.1	3.3	<0.001
History of heart disease, %	4.2	5.2	5.3	6.0	<0.001
Medication for hypertension, %	19.6	26.6	32.4	37.5	<0.001
Medication for diabetes mellitus, %	2.7	3.4	4.2	6.1	<0.001
Medication for dyslipidemia, %	17	21.9	25.8	27	<0.001

Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or self-reported use of antihypertensive drugs. Diabetes was defined in accordance with American Diabetes Association guidelines as a fasting glucose concentration of ≥126 mg/dL, hemoglobin A1c concentration of ≥6.5%, or self-reported use of antihyperglycemic drugs. TG/HDL-C was calculated as TG (mg/dL) divided by HDL-C (mg/dL).
Abbreviations: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

2.4. Statistical analyses

Independent two-tailed *t*-tests and chi-square tests were used for the analysis of continuous and categorical variables, respectively. We used a linear regression model to compare the mean values of possible risk factors between the quartile groups in each gender. The age- or multivariate-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for CKD, low eGFR, and proteinuria were determined by logistic regression model adjusted for potential confounding covariates. The confounding covariates used for adjustment included waist circumference, hypertension, obesity, diabetes, a current smoking habit, daily alcohol consumption, regular exercise habits, history of stroke and heart disease, and medication for dyslipidemia. We tested for heterogeneity in the relationship between subgroups by adding a multiplicative interaction term in our statistical model. All statistical analyses were

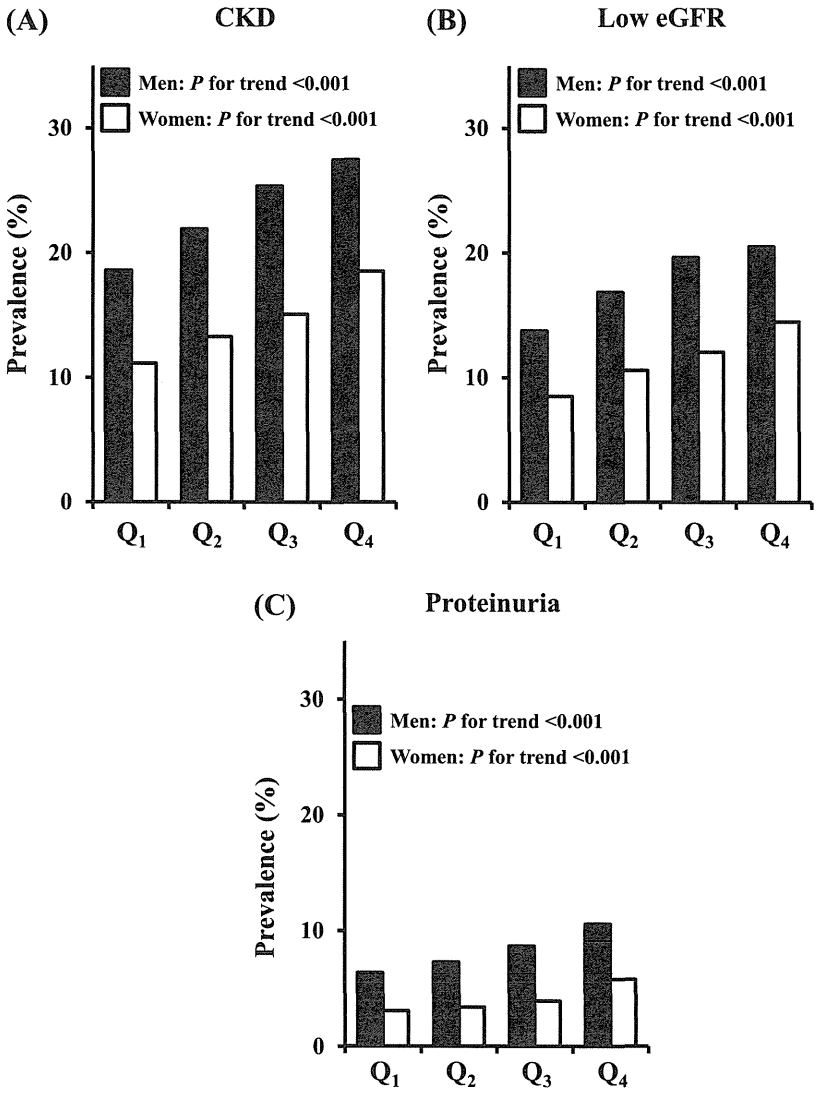

Fig. 1. The prevalence of CKD, low eGFR and proteinuria in participants with different TG/HDL-C. The prevalence of CKD (A), low eGFR (B) and proteinuria (C) increased as TG/HDL-C in men (closed bars) and women (open bars) increased. Low eGFR was defined as eGFR <60 mL/min/1.73 m^2. Proteinuria was defined as urinary protein value of ≥1+ as measured by dipstick testing. CKD was defined as a low eGFR and/or proteinuria. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; TG/HDL; triglycerides/high-density lipoprotein cholesterol.

performed with JMP version 9.0 software (SAS Institute, Inc., Cary, NC, USA).

3. Results

Table 1 shows the characteristics of the 216,007 participants (88,516 men and 127,491 women). The mean age of men and women was similar. Compared with women, a significantly higher percentage of men had proteinuria (as indicated by a value of ≥1+ on dipstick testing) and CKD. The mean serum levels of LDL-C and HDL-C, and the percentage of participants who took medication for dyslipidemia in women were significantly higher than those in men.

Table 2 shows the mean values or frequencies of potential risk factors in the quartile groups for men (Table 2A) and women (Table 2B). The frequency of hypertension, diabetes mellitus, obesity, a current smoking habit, and medication for hypertension, diabetes, and dyslipidemia also increased with higher TG/HDL-C levels in both genders. On the other hand, the mean values for HDL-C level as well as the frequencies for daily alcohol consumption and regular exercise habits decreased in both men and women. We observed opposite trends with regards to the association between age and TG/HDL-C; there was an inverse association between age and TG/HDL-C among men and a positive association between age and TG/HDL-C among women.

Fig. 1 presents the prevalence of CKD, low eGFR, and proteinuria among men and women according to TG/HDL-C levels. The prevalence of CKD increased 1.5–fold higher in the highest TG/HDL-C quartile group than in the lowest group in men (18.6% in Q_1, 21.9% in Q_2, 25.4% in Q_3, and 27.5% in Q_4, P for trend <0.001) and doubled in women (11.1%, 13.3%, 15.1%, and 18.5%, respectively, P for trend <0.001). Similar trends in the prevalence of low eGFR and proteinuria were also observed in both genders.

The age-adjusted or multivariate-adjusted ORs and 95% CIs for the presence of CKD, low eGFR, and proteinuria according to TG/HDL-C levels are shown in Table 3. The age-adjusted ORs for CKD,

Table 3
Age- and multivariate-adjusted ORs and 95% CIs for the presence of CKD, low eGFR, and proteinuria according to the quartiles of TG/HDL-C.

(A) Men					
	TG/HDL-C				*P* for trend
	Q_1	Q_2	Q_3	Q_4	
	<1.26	1.26–1.98	1.99–3.18	>3.18	
	(*n* = 22,126)	(*n* = 22,126)	(*n* = 22,142)	(*n* = 22,122)	
CKD					
Cases, *n* (%)	4115 (18.6)	4847 (21.9)	5618 (25.4)	6079 (27.5)	
Age-adjusted OR (95% CI)	Reference	1.23 (1.18–1.29)	1.55 (1.48–1.62)	1.90 (1.82–1.99)	<0.001
Multivariate-adjusted OR (95% CI)[a]	Reference	1.13 (1.08–1.18)	1.34 (1.28–1.40)	1.57 (1.49–1.65)	<0.001
Low eGFR					
Cases, *n* (%)	3045 (13.8)	3732 (16.9)	4349 (19.6)	4538 (20.5)	
Age-adjusted OR (95% CI)	Reference	1.28 (1.22–1.35)	1.62 (1.53–1.70)	1.92 (1.82–2.02)	<0.001
Multivariate-adjusted OR (95% CI)[b]	Reference	1.21 (1.15–1.28)	1.47 (1.39–1.55)	1.72 (1.63–1.82)	<0.001
Proteinuria					
Cases, *n* (%)	1404 (6.3)	1605 (7.3)	1915 (8.6)	2333 (10.5)	
Age-adjusted OR (95% CI)	Reference	1.15 (1.07–1.24)	1.41 (1.31–1.52)	1.81 (1.69–1.94)	<0.001
Multivariate-adjusted OR (95% CI)[c]	Reference	0.96 (0.89–1.03)	1.03 (0.95–1.11)	1.13 (1.04–1.21)	<0.001
(B) Women					
	TG/HDL-C				*P* for trend
	Q_1	Q_2	Q_3	Q_4	
	<0.96	0.96–1.44	1.45–2.22	>2.22	
	(*n* = 31,894)	(*n* = 31,817)	(*n* = 31,918)	(*n* = 31,862)	
CKD					
Cases, *n* (%)	3551 (11.1)	4226 (13.3)	4812 (15.1)	5901 (18.5)	
Age-adjusted OR (95% CI)	Reference	1.14 (1.09–1.20)	1.28 (1.22–1.34)	1.63 (1.56–1.71)	<0.001
Multivariate-adjusted OR (95% CI)[a]	Reference	1.08 (1.03–1.14)	1.16 (1.10–1.22)	1.41 (1.34–1.48)	<0.001
Low eGFR					
Cases, *n* (%)	2720 (8.5)	3370 (10.6)	3848 (12.1)	4614 (14.5)	
Age-adjusted OR (95% CI)	Reference	1.17 (1.11–1.23)	1.30 (1.23–1.37)	1.60 (1.52–1.68)	<0.001
Multivariate-adjusted OR (95% CI)[b]	Reference	1.13 (1.07–1.20)	1.23 (1.17–1.30)	1.47 (1.39–1.55)	<0.001
Proteinuria					
Cases, *n* (%)	974 (3.1)	1075 (3.4)	1250 (3.9)	1838 (5.8)	
Age-adjusted OR (95% CI)	Reference	1.09 (0.99–1.19)	1.25 (1.15–1.37)	1.88 (1.74–2.04)	<0.001
Multivariate-adjusted OR (95% CI)[c]	Reference	0.94 (0.86–1.03)	0.96 (0.88–1.05)	1.23 (1.13–1.34)	<0.001

Abbreviations: CKD; chronic kidney disease, TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate, OR; odds ratio, CI; confidence interval.

[a] Multivariate analyses were adjusted for age, waist circumstance, hypertension, obesity, diabetes, current smoking, daily alcohol consumption, regular exercise habits, history of stroke and heart disease, and medication for dyslipidemia (Model 1 covariates).

[b] Multivariate analyses were adjusted for the presence of proteinuria in addition to Model 1 covariates.

[c] Multivariate analyses were adjusted for eGFR in addition to Model 1 covariates.

low eGFR, and proteinuria significantly increased as the quartiles of TG/HDL-C increased (*P* for trend <0.001). In both men and women, ORs for Q_2, Q_3, and Q_4 were significantly higher than the ORs for Q_1. We also calculated the ORs for CKD, low eGFR, and proteinuria after adjustment for age, waist circumference, hypertension, obesity, diabetes, a current smoking habit, daily alcohol consumption, regular exercise habits, history of stroke and heart disease, and medication for dyslipidemia. Proteinuria was adjusted in the analysis of eGFR, and vice versa. The OR for the presence of CKD increased progressively with higher TG/HDL-C levels in men [Q_2: OR 1.13 (95% CI 1.08–1.18); Q_3: 1.34 (1.28–1.40); Q_4: 1.57 (1.49–1.65); *P* for trend <0.001] and in women [1.08 (1.03–1.14), 1.16 (1.10–1.22), 1.41 (1.34–1.48), respectively; *P* for trend <0.001]. Furthermore, there were significant associations with risk of low eGFR and proteinuria in both genders. We also performed stratified analyses to assess the association between TG/HDL-C and CKD according to the presence of hypertension, diabetes, or obesity, and found that the risk of CKD increased linearly with greater TG/HDL-C levels in participants with and without hypertension, diabetes, and obesity. Moreover, higher TG/HDL-C level was a relevant factor for CKD, especially in participants with hypertension and diabetes (*P* for interaction <0.001, respectively) (Fig. 2).

4. Discussion

CKD is a major public health problem, and the identification of risk factors for the development of CKD may well be useful for early intervention and prevention strategies. The results of our large cohort study, which represented the general population of Japan, show that higher TG/HDL-C level in Japanese adults is significantly associated with the risk of CKD. We found that an elevated TG/HDL-C level was an independent and relevant factor for CKD in both men and women, even after adjustment for the relevant potential confounding factors. In stratified analyses, elevated TG/HDL-C levels were significantly associated with the likelihood of having CKD, independently of hypertension, diabetes, and obesity. Higher TG/HDL-C levels were relevant for CKD, especially in participants with hypertension and diabetes.

It seems strange that the trends for history of stroke and heart disease are not statistically significant in men. We suspected that it might be attributed to the inverse trend between age and TG/HDL-C, thus examined the association of TG/HDL-C with history of stroke and heart disease with adjustment for age. As a result, significant positive trends for history of stroke (*P* for trend <0.001) and heart disease (*P* for trend <0.001) were observed in TG/HDL-C after