(Figure 4A–D). These results indicate that PDGF induced the expression of Col4 through the activation of Src/Smad1 signal transduction.

Silencing of c-Src in MCs inhibits PDGF-mediated phosphorylation of Smad1 and synthesis of Col4

To further confirm the role of c-Src in PDGF-induced upregulation of Smad1 and Col4 expression, c-Src gene silencing by siRNA was performed. c-Src silencing suppressed the PDGF-induced phosphorylation of Smad1 and the synthesis of Col4. In contrast, GAPDH protein levels, used as a loading control, were not affected across the samples (Figure 4E–H). We confirmed the result of knockdown experiments with PDGF stimulation by using three c-Src siRNAs (Src siRNA-1, -2, and -3) (Figure S2). We showed the representative data from using Src siRNA-3 in Figure 4E–H. From these results, c-Src may be significantly involved in PDGF-mediated Col4 expression.

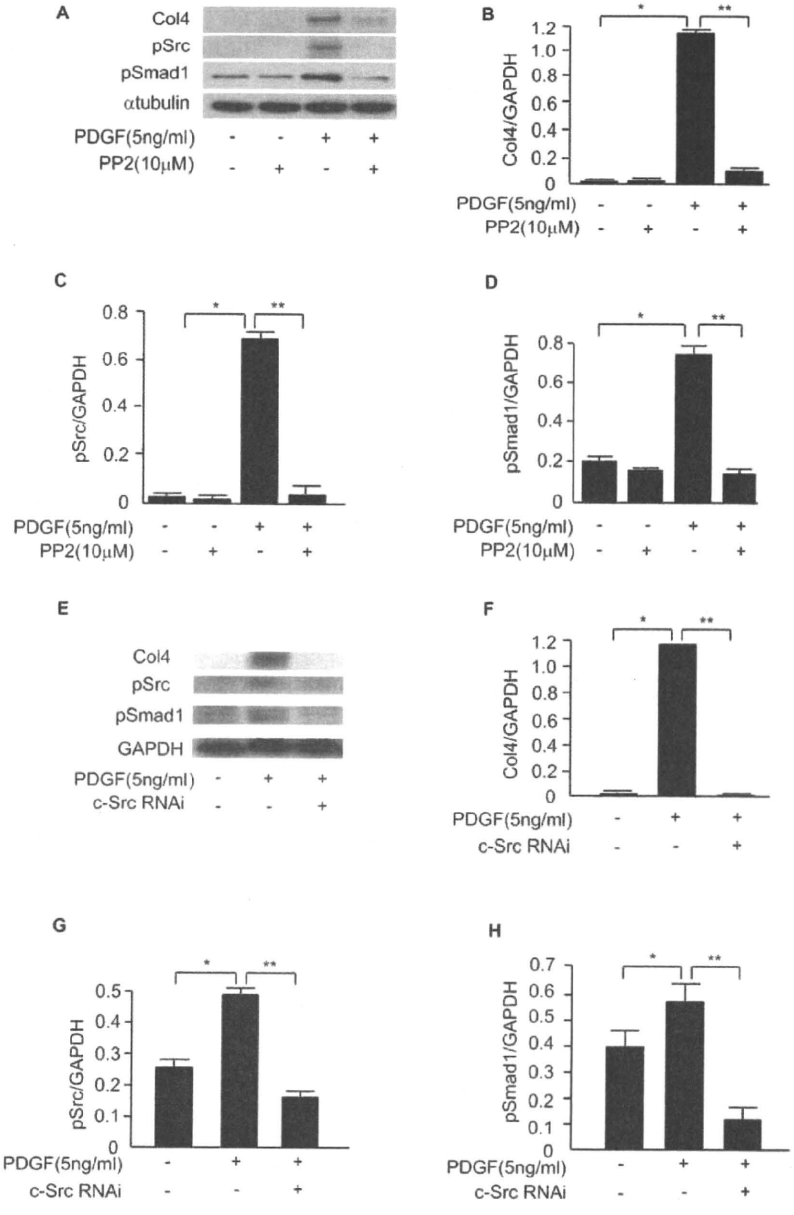

Figure 4. Activation of c-Src and Smad1 is regulated by PDGF in MCs. (A) Effect of PP2 on pSrc, pSmad1 and Col4. MCs were preincubated with PP2 (10 μM) or DMSO for 48 h before exposure to PDGF (5 ng/ml, 12 h). (B) Optical densitometry of Col4 in western blot. *$P<0.001$ and **$P<0.001$. (C, D) Optical densitometry of pSrc (*$P<0.001$ and **$P=0.003$) and pSmad1 (*$P=0.002$, **$P=0.002$) in western blot analyses. (E) Effects of RNAi-mediated silencing of c-Src on pSrc, pSmad1 and Col4 under stimulation of PDGF (5 ng/ml, 12 h). (F–H) Optical densitometry of Col4 (*$P<0.001$, **$P<0.001$), pSrc (*$P<0.001$, **$P<0.001$), and pSmad1 (*$P=0.02$, **$P=0.002$) in western blot. Data represent mean values ± S.D. of at least three independent experiments.
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Activated c-Src is associated with PDGFR in MCs

To clarify the intracellular interaction between PDGF signaling pathway and c-Src/Smad1 axis, the effects of constitutively active form of c-Src (caSrc) transfected in MCs was examined. Transient transfection of MCs with caSrc could induce phosphorylation of Smad1 wihtout stimulation of PDGF, and subsequently upregulated Col4 expression (Figure 5A). In contrast, transfection of the dominant negative Src (dnSrc) did not show these regulations. Moreover, we performed knockdown analysis using Smad1 siRNAs to confirm the role of Smad1 in the regulatory effect of PDGF-induced Col4 expression. Knockdown study revealed that Smad1 acts downstream of PDGF-c-Src signaling pathway in the induction of Col4 (Figure 5B). Furthermore we have explored the possibility that c-Src, while interacting directly with PDGF receptor, could transduce the PDGF signals in MCs. For this purpose, PDGF receptor was immunoprecipitated from whole cell lysates after PDGF stimulation. Anti-c-Src immunoblot revealed that c-Src really associates with PDGFR only when stimulated by PDGF (Figure 5C).

TGFβ signaling pathway partially mediated PDGF-induced Smad1/Col4 expression in MCs

Transforming growth factor beta (TGFβ) is an important growth factor in the modulation of cell proliferation as well as PDGF in a variety of cells. In addition, several studies reported that PDGF may increase the production of TGFβ and the expression of TGFβ type I receptor [25,26]. To elucidate the the molecular basis of the influence of PDGF on TGFβ signaling pathway, we performed TGFβ-neutralizing antibody assay for PDGF-stimulated MCs. PDGF increased the expressions of TGFβ and activin receptor-like kinase 5 (ALK5) and activated Smad1. However, these changes by PDGF could not be inhibited by neutralizing anti-TGFβ antibody (Figure 6A), indicating that PDGF, but not TGFβ, upregulates expression of ALK5, pSmad1, pSrc, and Col4. In particular, pSmad1 is phosphorylated by ALK1, but not by ALK5, therefore, we investigated the effects of high concentration of PDGF on MCs. At concentration of 50 ng/ml, PDGF increased the expressions of ALK1 as well as other proteins (Figure 6B). Interestingly, an addition of neutralizing anti-TGFβ antibody suppressed not only ALK1 expression, but also expressions of pSmad1 and Col4 (Figure 6B). These results suggest that PDGF has the potential to enhance TGFβ signal transduction through ALK1 as well as ALK5.

TGFβ signaling pathway partially mediated PDGF-induced Smad1/Col4 expression in MCs

To further elucidate the regulatory mechanisms controlling the cross-talk between PDGF and TGFβ in the activation of Smad1

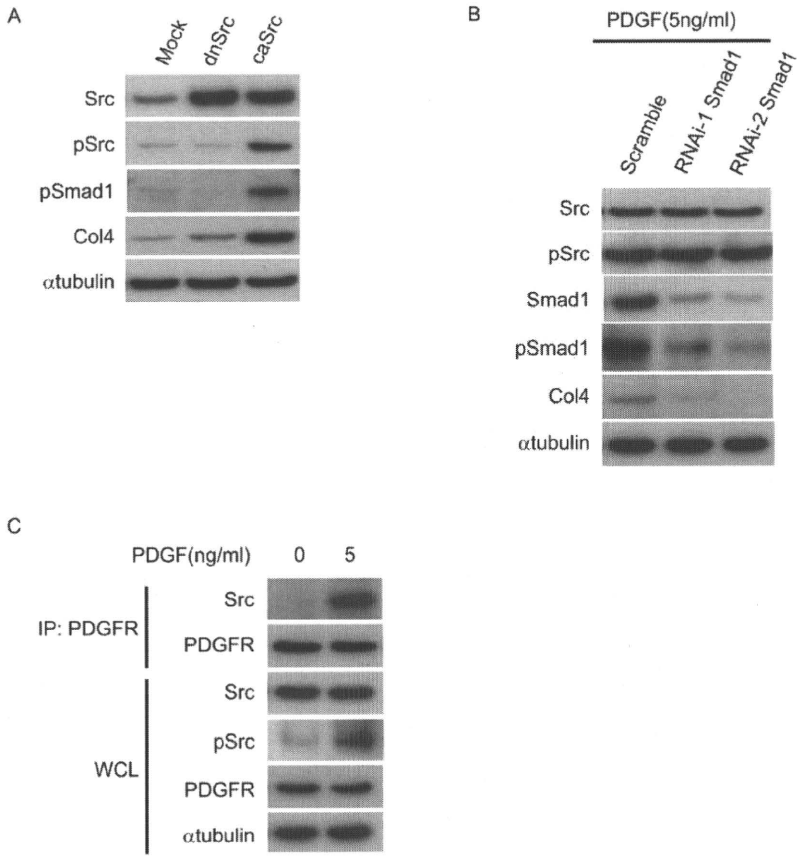

Figure 5. Activated c-Src is associated with PDGF Receptor (PDGFR) in MCs. (A) Western blot analyses of MCs transfected with constitutively active c-Src (caSrc), dominant negative c-Src (dnSrc), and empty vector (Mock). One of three independent experiments is shown. (B) Effects of RNAi-mediated silencing of Smad1 on pSmad1 and Col4 after 5 h stimulation of PDGF (5 ng/ml). Scrambled siRNA (Scramble) was used as a control. One of three independent experiments is shown. (C) MCs were serum-starved for 10 h and then incubated with 5 ng/ml of PDGF for 5 min. Whole cell lysates (WCL) were immunoprecipitated with polyclonal anti-PDGFR antibody and subjected to anti-Src immunoblot.
doi:10.1371/journal.pone.0017929.g005

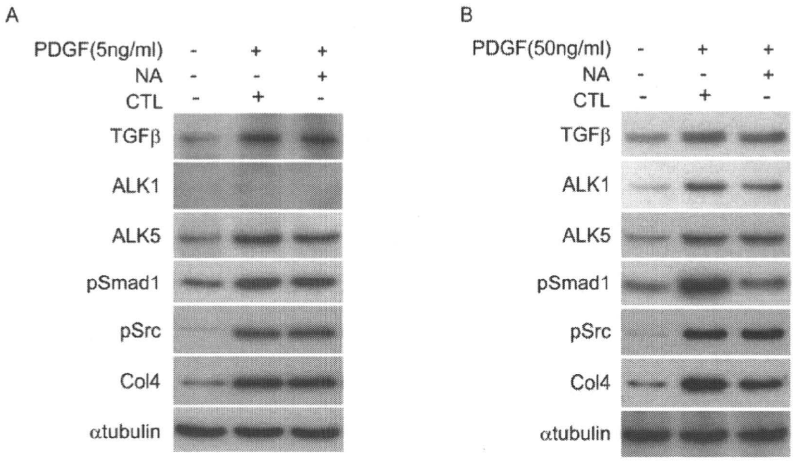

Figure 6. PDGF modulated TGFβ-Activin Receptor-like Kinases (ALKs) signaling pathways in MCs. (A, B) MCs were treated with neutralizing antibody for TGFβ (10 μg/ml) (NA) or control normal IgY (CTL) for 24 h prior to treatment with PDGF at indicated concentrations for 24 h. Equal amounts of cell lysates were subjected to Western blot. One of three independent experiments is shown.
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and induction of Col4 in MCs, we examined whether LDL receptor related protein-1 (LRP1) is involved in the signal pathways. Because Boucher et al. reported that LRP1 is tightly involved in the pathogenesis of atherosclerosis by regulating signaling of TGFβ and PDGF, and their receptors [27,28], knockdown analysis using LRP1 siRNAs was perfomed to examine the role of LRP1 in the regulatory effect of PDGF-induced Col4 expression and PDGF-activated TGFβ signaling pathway in MCs. Knockdown of LRP1 enhanced the downstream pathway of PDGF (Figure 7A) with the exception of ALK1 (Figure 7B). These results suggest that LRP1 has a significant inhibitory effect on PDGF signaling pathway leading to production of Col4 in MCs.

PDGF signaling pathway is partially involved in the AngII-induced c-Src/Smad1 signal activation in MCs

We previously reported that AngII activates the c-Src/Smad1 signaling pathway in the development of diabetic nephropathy and cultured MCs [23]. To investigate whether AngII signals influence the regulatory mechanisms of PDGF-induced c-Src/Smad1 signal transduction, we examined the inhibitory effects of APB5 and AngII receptor blocker (ARB) on the activation of c-Src, Smad1, and Col4 by AngII and PDGF, respectively. APB5 clearly attenuated the AngII-induced c-Src/Smad1/Col4 signal (Figure 8A). In contrast, ARB treatment slightly reduced PDGF-induced activation of the signal (Figure 8B). These data suggest that PDGF signaling pathway is activated by AngII in MCs.

Discussion

Cellular proliferation and extracellular matrix accumulation are characteristic features of progressive glomerular diseases, a major cause of end-stage renal failure in humans throughout much of the world. Glomerulosclerosis followed by mesangial proliferative glomerulonephritis is characterized by mesangial matrix expansion

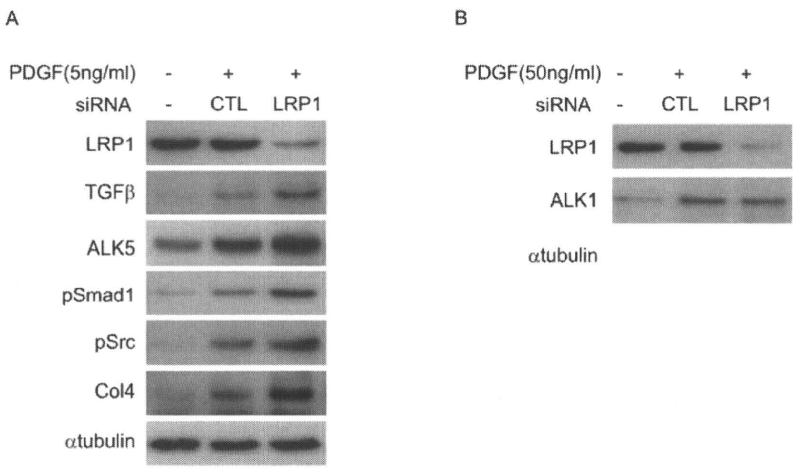

Figure 7. LRP1 modulated both PDGF and TGFβ signaling pathways in MCs. (A, B) Effects of PDGF stimulation and RNAi-mediated silencing of LRP1 after 5 h stimulation of PDGF at indicated concentrations on MCs. Scrambled siRNA (Scramble) was used as a control (CTL). Equal amounts of cell lysates were subjected to Western blot. One of three independent experiments is shown.
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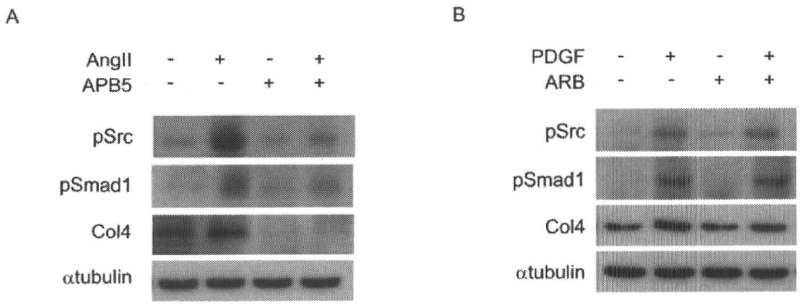

Figure 8. Molecular cross-talk between PDGF and AngII signaling pathways in MCs. (A) Effects of APB5 on pSrc, pSmad1 and Col4. MCs were preincubated with APB5 (100 ng/ml) or control rat IgG for 24 h before exposure to AngII (0.1 μM, 30 min). (B) Effects of olmesartan (ARB) on pSrc, pSmad1 and Col4. MCs were preincubated with olmesartan (10 μM) or methanol for 48 h before exposure to PDGF (5 ng/ml, 12 h). Equal amounts of cell lysates were subjected to Western blot. One of three independent experiments is shown.
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and phenotypic change of MCs [3]. In the expanded mesangial matrix, Col4 is a major component of ECM and is overproduced in glomerulosclerosis [6]. In addition, phenotypic modulation is a commonly observed feature in the progression of many renal diseases leading to CKD and ESRD. Expression of SMA is a well-known marker for the activation of MCs in most glomerular diseases. We previously reported that Smad1 upregulated the expression of Col4 and SMA [5,6] and thereby participates in the development of glomerulosclerosis in experimental glomerulonephritis [4]. However, the molecule that activates Smad1 in glomerulonephritis has not been fully elucidated. Since PDGF has been consistently implicated in cell proliferation and extracellular matrix accumulation, which characterize progressive glomerular disease [29], and since c-Src is an important component of the PDGF signaling pathway [30], we first investigated whether c-Src is induced in glomeruli of proliferative glomerulonephritis. In Thy1 GN, Col4 is strongly expressed in the sclerotic lesions of glomeruli, as previously described [4,21]. We show here that c-Src and Smad1 are heavily phosphorylated in the nuclei of glomerular cells in Thy1 GN. This phosphorylation parallels the progress of glomerulosclerosis and peaks on day 6, when Col4 and SMA expression levels have peaked. These results suggest that c-Src has a potential to be involved in the development of glomerulosclerosis in mesangial proliferative glomerulonephritis.

c-Src was identified as the first proto-oncogene, and a great deal of work has been carried out to elucidate its role in biological systems [31–33]. The two main areas in which Src inhibitors have been applied are regulating bone resorption [34,35] and both tumor growth and metastasis [36,37]. Most previous studies have shown that the role of Src family members is related to inflammatory responses. Additionally, the small chemical inhibitors that effectively and specifically block Src kinases could have great clinical implications for diseases with acute inflammatory responses [38,39]. In a rat renal ischemia-reperfusion injury model, increased active Src expression was found in the injured rat kidney after reperfusion [40]. To our knowledge, however, no report has demonstrated that c-Src is involved in the development of glomerulosclerosis in glomerular diseases. In the rat proliferative glomerulonephritis model, administration of PP2 completely abolished the phosphorylation of c-Src and Smad1 and resulted in the amelioration of glomerulosclerosis. Therefore, the activation of c-Src signal transduction plays a pivotal role in glomerulosclerosis, implicating it as a novel target of the therapeutic strategies for glomerulonephritis. Moreover, our findings show a new side of PP2 as an anti-glomerular disease agent.

In addition, PDGF is known to contribute to the development of both experimental and human glomerulonephritis [12,13]. Src kinase activation has been reported to contribute to PDGF-dependent cell-cycle proliferation, mitogenesis, and chemotaxis [24,29,30]. Thus, to investigate the molecular mechanisms underlying the progression of proliferative glomerulonephritis, we used cultured MCs under PDGF stimulation. PDGF induced phosphorylation of c-Src and Smad1 as well as Col4 expression, and these changes were blocked by PP2. The interaction between PDGFR and c-Src may be important for the phosphorylation of c-Src. In addition, the siRNA silencing experiments confirmed that c-Src regulated Smad1 activation. These findings suggest that c-Src activation is a key event in the PDGF-induced phosphorylation of Smad1, followed by the subsequent overproduction of Col4 in proliferative glomerulonephritis. In addition, PDGF activated TGFβ signaling pathways by induction of TGFβ and its type I receptors, ALK1 and ALK5. In particular, the induction of ALK1 may be an important event, because ALK1 transduce TGFβ signals to Smad1. Furthermore, several recent reports demonstrated that LRP1 has an inhibitory effect on TGFβ signaling pathway as well as PDGF signaling pathway [27,28]. As expected, LRP1 silencing exhibited additional effect on the activation of TGFβ signals by PDGF. Hence, LRP1 represents a promising new therapeutic target for the control of proliferative glomerular diseases. Moreover, our previous study demonstrated that AngII stimulated this Src-Smad1 axis independent of p44/42 MAP kinase activation and that the AngII receptor blocker ARB blocked this pathway. Because it is generally accepted that the AngII blockade significantly delays the progression of proliferative glomerulonephritis [41,42], our previous findings implied that the inhibition of the Src-Smad1 axis may partially explain the AngII-induced progression of proliferative glomerunonephritis. PDGF-induced activation of c-Src/Smad1 signaling pathway leading to Col4 production also plays an important role downstream of AngII stimulation, whereas ARB treatment did not fully suppressed the effect of PDGF. Chemical inhibitors directly or indirectly targeting Src kinases have been developed as potential drugs for the treatment of cancer [43]. It was recently reported that the inhibition of c-Src by these chemical inhibitors helps to prevent ischemia-reperfusion-induced injury in organs [38,39]. The present study raises the possibility that using these chemical inhibitors to block Src signal transduction could be a promising option for ameliorating proliferative glomerulonephritis as well as for the already reported effects of these inhibitors on excessive inflammatory cells, monocytes and macrophages [44,45]. Another report by Severgnini et al. demonstrated that c-Src controls

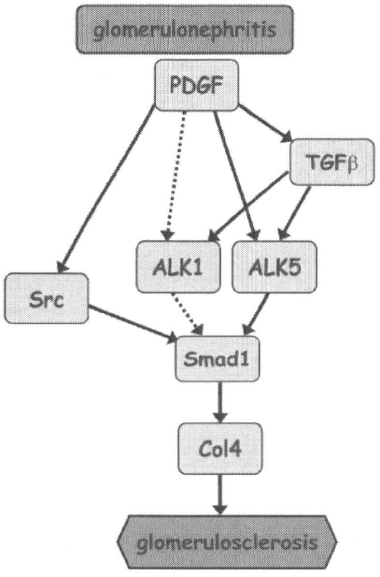

Figure 9. Proposed model for PDGF effects on Smad1 activation and Col4 expression in glomerulonephritis. Activation of Smad1 by PDGF mediates at least two different signal transduction pathways, TGFβ-ALK5-Smad1 and Src-Smad1. ALK1 may potentially activate Smad1 when exposed to high concentration of PDGF (broken arrows). The expression of ALK5 is induced by PDGF and is largely independent of TGFβ. Excessive activation of these signaling pathways may result in Col4 overproduction leading to the development of glomerulosclerosis in glomerulonephritis.
doi:10.1371/journal.pone.0017929.g009

STAT3 activation in acute lung injury [46]. In addition, we previously reported that STAT3 is involved in the development of glomerulosclerosis in experimental proliferative glomerulonephritis [4]. In light of these previous findings, our results highlight the importance of c-Src in the development of glomerulosclerosis in glomerulonephritis. Combining with our overall findings summarized in Figure 9, we can speculate that Smad1-mediated production of Col4 leading to mesangial expansion is a critical event in the development of glomerulosclerosis.

In conclusion, our present study indicates that c-Src activates Smad1-induced ECM production and phenotypic alteration, and is involving in the progression of proliferative glomerulonephritis leading to glomerulosclerosis. Further understanding of the Src/Smad1 pathway and the molecules involve in this pathway is critical for the clarification of glomerulosclerosis and to pave the way for a strategy to treat progressive glomerulonephritis.

Supporting Information

Figure S1 Time course of renal function in Thy1 GN. Urine volume (*P = 0.042) (A), serum BUN (*P = 0.014) (B), and UAE (*P = 0.017) (C) in Thy1 GN. Data represent mean values ± S.D. of at least three independent experiments; n = 6 for each experimental group.
(TIF)

Figure S2 Knockdown of c-Src expression. MCs were transfected with three different siRNAs specific for c-Src and with scrambled siRNA with or without PDGF stimulation. Effects of RNAi-mediated silencing of c-Src on pSrc, pSmad1 and Col4 under stimulation of PDGF (5 ng/ml, 12 h) were analyzed by Western blot. GAPDH served as a loading control.
(TIF)

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Author Contributions

Conceived and designed the experiments: TD H. Abe. Performed the experiments: AM H. Abe KN TM MA KT TT. Analyzed the data: H. Abe H. Arai NI AF TK TD. Wrote the paper: AM H. Abe.

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ORIGINAL ARTICLE

Japan Renal Biopsy Registry: the first nationwide, web-based, and prospective registry system of renal biopsies in Japan

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Abstract

Background The Committee for the Standardization of Renal Pathological Diagnosis and the Working Group for Renal Biopsy Database of the Japanese Society of Nephrology started the first nationwide, web-based, and prospective registry system, the Japan Renal Biopsy Registry (J-RBR), to record the pathological, clinical, and laboratory data of renal biopsies in 2007.

Methods The patient data including age, gender, laboratory data, and clinical and pathological diagnoses were recorded on the web page of the J-RBR, which utilizes the system of the Internet Data and Information Center for Medical Research in the University Hospital Medical Information Network. We analyzed the clinical and pathological diagnoses registered on the J-RBR in 2007 and 2008.

Results Data were collected from 818 patients from 18 centers in 2007 and 1582 patients from 23 centers in 2008, including the affiliated hospitals. Renal biopsies were obtained from 726 native kidneys (88.8%) and 92 renal grafts (11.2%) in 2007, and 1400 native kidneys (88.5%) and 182 renal grafts (11.5%) in 2008. The most common clinical diagnosis was chronic nephritic syndrome (47.4%), followed by nephrotic syndrome (16.8%) and renal

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transplantation (11.2%) in 2007. A similar frequency of the clinical diagnoses was recognized in 2008. Of the native kidneys, the most frequent pathological diagnosis as classified by pathogenesis was immunoglobulin (Ig) A nephropathy (IgAN) both in 2007 (32.9%) and 2008 (30.2%). Among the primary glomerular diseases (except IgAN), membranous nephropathy (MN) was the most common disease both in 2007 (31.4%) and 2008 (25.7%). *Conclusions* In a cross-sectional study, the J-RBR has shown IgAN to be the most common disease in renal biopsies in 2007 and 2008, consistent with previous Japanese studies. MN predominated in the primary glomerular diseases (except for IgAN). The frequency of the disease and the clinical and demographic correlations should be investigated in further analyses by the J-RBR.

Keywords Glomerulonephritis · Tubulointerstitial disorder · Renal vascular disease · Renal grafts · National registry

Introduction

There has been no national registry of renal biopsies in Japan. The Committee for the Standardization of Renal Pathological Diagnosis and the Working Group for Renal Biopsy Database in the Japanese Society of Nephrology established the first nationwide, web-based, and prospective registry system, the Japan Renal Biopsy Registry (J-RBR), to record pathological, clinical, and laboratory data regarding all renal biopsies performed in 2007.

To date, the epidemiological and clinical data of renal diseases are available from nationwide registries of renal biopsies from the United Kingdom [1], Italy [2], Denmark [3], Spain [4], the Czech Republic [5], and Australia [6]. The role of a renal biopsy registry has been recently encouraged [7]. In Japan, several surveys were temporarily conducted for patients with restricted renal diseases, including primary glomerulonephritis [8], idiopathic membranous nephropathy (MN) [9], and immunoglobulin (Ig) A nephropathy (IgAN) [10]. However, there has been no web-based, nationwide, or prospective registry system of overall renal biopsies in Japan. The aim of the current study was to provide data to investigate the epidemiology and frequency of renal diseases with a histological diagnosis for patients registered in 2007 and 2008 on the J-RBR.

Subjects and methods

Registry system and patients

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for Renal Biopsy Database in the Japanese Society of Nephrology participated in this study. The report includes the data from patients on the J-RBR, registered prospectively from January to December of 2007 and 2008. Patient data including age, gender, laboratory data, and the clinical and pathological diagnoses were electronically recorded at each institution and registered on the web page of the J-RBR utilizing the system of Internet Data and Information Center for Medical Research (INDICE) in the University Hospital Medical Information Network (UMIN). The ethical committee of the Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences comprehensively approved the study, and a local committee of participating centers and their affiliated hospitals individually approved the study. Written informed consent was obtained from the patients at the time of biopsy or before participation in the study. The J-RBR is registered to the Clinical Trial Registry of UMIN (registered number UMIN000000618) and is available in Japanese and English.

Clinical or renal histopathological diagnosis and laboratory data

Three classifications, clinical diagnosis, histological diagnosis by pathogenesis, and histological diagnosis by histopathology, were selected for each case (Supplementary Table) from the J-RBR. The classification of clinical diagnoses was determined as follows: acute nephritic syndrome, rapidly progressive nephritic syndrome, recurrent or persistent hematuria, chronic nephritic syndrome, nephrotic syndrome, renal disorder with metabolic disease, renal disorder with collagen disease or vasculitis, hypertensive nephropathy, inherited renal disease, acute renal failure, drug-induced nephropathy, renal transplantation, and others. The definitions of the former five clinical diagnoses were based on the clinical syndromes and glomerular histopathology in the classification of glomerular diseases [11]. Acute nephritic syndrome was defined as a syndrome characterized by the abrupt onset of hematuria, proteinuria, hypertension, decreased glomerular filtration, and edema. Rapidly progressive nephritic syndrome was defined as an abrupt or insidious onset of hematuria, proteinuria, anemia, and rapidly progressing renal failure. Recurrent or persistent hematuria included the insidious or abrupt onset of gross or microscopic hematuria with little or no proteinuria and no evidence of other features of nephritic syndrome. Chronic nephritic syndrome was defined as slowly developing renal failure accompanied by proteinuria, hematuria, with or without hypertension. Nephrotic syndrome was defined as massive proteinuria >3.5 g/day and hypoalbuminemia of <3 g/dL of serum albumin with or without edema or hypercholesteremia.

The renal histological diagnosis is classified either according to pathogenesis (A) or by histopathology (B) as follows: (A) primary glomerular disease (except IgAN), IgAN, purpura nephritis, lupus nephritis, myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA)-positive nephritis, protein 3 (PR3)-ANCA-positive nephritis, anti-glomerular basement membrane antibody nephritis, hypertensive nephrosclerosis, thrombotic microangiopathy, diabetic nephropathy, amyloid nephropathy, Alport syndrome, thin basement membrane disease, infection-related nephropathy, transplanted kidney, and others; (B) minor glomerular abnormalities, focal and segmental glomerulosclerosis (FSGS), MN, mesangial proliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, membranoproliferative glomerulonephritis (MPGN) (type I, III), dense deposit disease, crescentic and necrotizing glomerulonephritis, sclerosing glomerulonephritis, nephrosclerosis, acute interstitial nephritis, chronic interstitial nephritis, acute tubular necrosis, transplanted kidney, and others. IgAN (Berger disease) was separated from primary glomerular diseases on the basis of basic glomerular alterations in the classification of glomerular diseases [11]. Clinical data, including urinalysis, daily proteinuria, serum creatinine concentrations, total protein, albumin, and total cholesterol values were also recorded, but only the frequency of the disease is described here.

Statistics

Data were expressed as mean ± SD as appropriate. Statistical analyses were performed using the JMP software program, version 8 (SAS Institute Inc., Cary, NC, USA).

Results

Baseline characteristics of registered biopsies

Data were collected from 818 patients from 18 centers in 2007 and 1582 patients from 23 centers in 2008, including the affiliated hospitals. Renal biopsies were obtained from

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Table 1 Number of participating renal centers and registered renal biopsies on the Japan Renal Biopsy Registry (J-RBR) in 2007 and 2008

Year	2007	2008	Total
Renal centers	18	23	23
Total biopsies	818	1582	2400
Average age (y)	44.6 ± 20.7	44.2 ± 21.1	44.4 ± 21.0
Male	430	851	1281
Female	388	731	1119
Native kidneys	726	1400	2126
Average age (y)	45.2 ± 21.4	44.8 ± 22.0	44.9 ± 21.5
Male	378	751	1129
Female	348	649	997
Renal grafts	92	182	274
Average age (y)	40.5 ± 13.5	39.4 ± 16.3	39.8 ± 15.4
Male	52	100	152
Female	40	82	122

726 native kidneys (88.8%) and 92 renal grafts (11.2%) in 2007 and 1400 native kidneys (88.5%) and 182 renal grafts (11.5%) in 2008 (Table 1). The average age of the patients was 44.6 ± 20.7 years of age in 2007 and 44.2 ± 21.1 years of age in 2008. A higher number of male patients than female patients were registered in both years (male patients 52.6% in 2007 and 53.8% in 2008). The distribution of the total number of renal biopsies according to age and gender are presented in Fig. 1, and reveals a different age and gender distribution in native kidneys and renal grafts.

The frequency of clinical diagnoses

The clinical diagnosis and renal histological diagnosis as classified by pathogenesis and by histopathology were determined for each biopsy. A clinical diagnosis of chronic nephritic syndrome was the most frequent, followed by nephrotic syndrome and renal transplantation in 2007, which was similar in 2008 (Table 2). In native kidneys, the majority of the cases corresponded to chronic nephritic syndrome, followed by nephrotic syndrome and recurrent or persistent hematuria or renal disorder with collagen disease or vasculitis in 2007 (Table 2). Similar frequencies of chronic nephritic syndrome, nephrotic syndrome and renal disorder with collagen disease or vasculitis were observed in 2008 (Table 2).

The frequency of pathological diagnoses

Pathological diagnoses were classified by pathogenesis (Table 3) and histopathology (Table 4). In the classification of pathogenesis, IgAN was diagnosed most frequently, followed by primary glomerular disease (except IgAN) and renal grafts both in 2007 and 2008 (Table 3). In the present cohort, except for renal grafts, the frequency of IgAN was 32.9%, followed by primary glomerular disease (except IgAN) (26.3%) and diabetic nephropathy (5.9%) in 2007 (Table 3). A slightly lower frequency of IgAN was present (30.2%), but similar frequencies of primary glomerular disease (except IgAN) (26.3%) and diabetic nephropathy (5.1%) were observed in 2008 (Table 3).

In the pathological diagnoses as classified by histopathology, mesangial proliferative glomerulonephritis was primarily observed in 2007 and 2008 (Table 4). In the present cohort, except for renal grafts, the frequency of mesangial proliferative glomerulonephritis was the highest followed by MN, minor glomerular abnormalities, nephrosclerosis, and crescentic and necrotizing glomerulonephritis in 2007 (Table 4). In 2008, mesangial proliferative glomerulonephritis was the most frequently diagnosed,

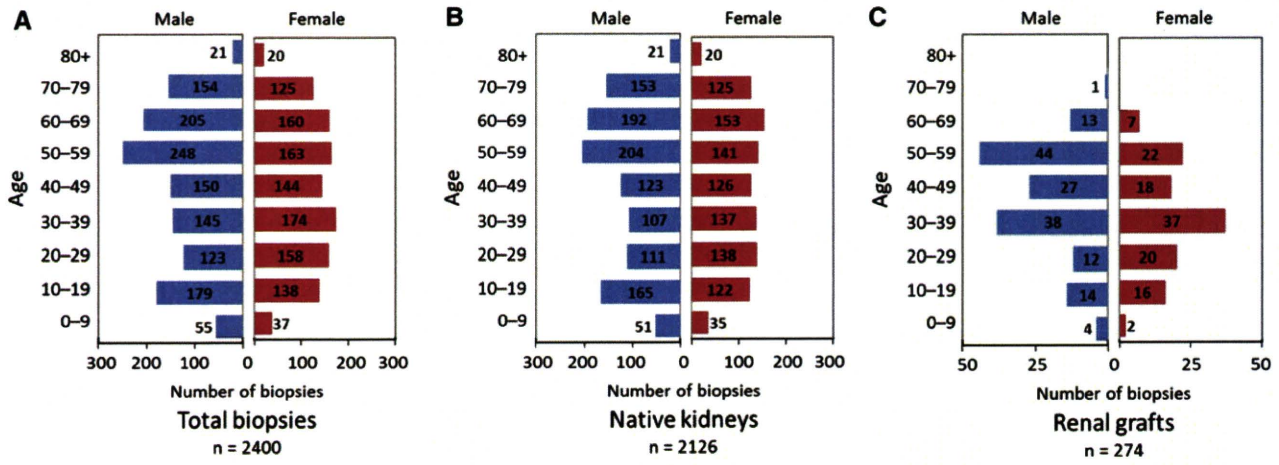

Fig. 1 Distribution of age ranges and gender in total renal biopsies (**a**), native kidneys (**b**), and renal grafts (**c**) in the combined data of 2007 and 2008

Table 2 Frequency of classification of clinical diagnoses

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Chronic nephritic syndrome	388	47.4	768	48.5	1156	48.2
Nephrotic syndrome	138	16.9	259	16.4	397	16.5
Renal transplantation	92	11.2	182	11.5	274	11.4
Renal disorder with collagen disease or vasculitis	41	5.0	87	5.5	128	5.3
Rapidly progressive nephritic syndrome	33	4.0	80	5.1	113	4.7
Recurrent or persistent hematuria	41	5.0	33	2.1	74	3.1
Renal disorder with metabolic syndrome	29	3.5	46	2.9	75	3.1
Hypertensive nephropathy	14	1.7	30	1.9	44	1.8
Acute nephritic syndrome	15	1.8	20	1.3	35	1.5
Acute renal failure	7	0.9	13	0.8	20	0.8
Drug-induced nephropathy	3	0.4	11	0.7	14	0.6
Inherited renal disease	5	0.6	8	0.5	13	0.5
Others	12	1.6	45	2.8	57	2.4
Total	818	100.0	1582	100.0	2400	100.0

Table 3 Frequency of pathological diagnoses as classified by pathogenesis

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
IgA nephropathy	239	29.2	424	26.8	663	27.6
Primary glomerular disease (except IgA nephropathy)	191	23.3	369	23.3	560	23.3
Renal graft	93	11.3	179	11.3	272	11.3
Diabetic nephropathy	43	5.2	71	4.5	114	4.8
Hypertensive nephrosclerosis	31	3.7	61	3.9	92	3.8
Lupus nephritis	29	3.5	59	3.7	88	3.7
MPO-ANCA-positive nephritis	25	3.0	58	3.7	83	3.5
Purpura nephritis	18	2.2	39	2.5	57	2.4
Amyloid nephropathy	12	1.4	22	1.4	34	1.4
Infection-related nephropathy	16	1.9	16	1.0	32	1.3
Thin basement membrane disease	11	1.3	5	0.3	16	0.7
Alport syndrome	1	0.1	9	0.6	10	0.4
PR3-ANCA-positive nephritis	1	0.1	7	0.4	8	0.3
Thrombotic microangiopathy	3	0.3	2	0.1	5	0.2
Anti-glomerular basement membrane antibody-type nephritis	0	0.0	4	0.3	4	0.2
Others	105	12.8	257	16.2	362	15.1
Total	818	100.0	1582	100.0	2400	100.0

with minor glomerular abnormalities being the second, and MN being the third (Table 4).

Primary glomerular disease (except IgAN) and nephrotic syndrome

In the cohort of primary glomerular disease as classified by pathogenesis, MN was predominant, followed by mesangial proliferative glomerulonephritis, minor glomerular abnormalities, and FSGS in 2007 (Table 5). In 2008, MN was still the most frequently diagnosed, present at the same frequency as minor glomerular abnormalities (Table 5).

In nephrotic syndrome as classified by clinical diagnosis, primary glomerular disease (except IgAN) was predominant, followed by diabetic nephropathy, amyloid nephropathy, IgAN, and lupus nephritis in 2007 (Table 6). A similar ordering of the disease frequencies was noted in 2008 (Table 6). Among the primary glomerular diseases

Table 4 Frequency of pathological diagnoses as classified by histopathology

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Mesangial proliferative glomerulonephritis	326	39.8	607	38.4	933	38.9
Renal graft	90	11.0	171	10.8	261	10.9
Membranous nephropathy	74	9.0	128	8.1	202	8.4
Minor glomerular abnormalities	52	6.3	143	9.0	195	8.1
Crescentic and necrotizing glomerulonephritis	32	3.9	87	5.5	119	5.0
Nephrosclerosis	38	4.6	77	4.9	115	4.8
Focal segmental glomerulosclerosis	32	3.9	65	4.1	97	4.0
Membranoproliferative glomerulonephritis (type I and III)	20	2.4	32	2.0	52	2.2
Chronic interstitial nephritis	24	2.9	21	1.3	45	1.9
Endocapillary proliferative glomerulonephritis	18	2.2	27	1.7	45	1.9
Sclerosing glomerulonephritis	10	1.2	33	2.1	43	1.8
Acute interstitial nephritis	7	0.9	18	1.1	25	1.0
Acute tubular necrosis	5	0.6	6	0.4	11	0.5
Dense deposit disease	1	0.1	5	0.3	6	0.3
Others	89	10.8	162	10.2	251	10.5
Total	818	100.0	1582	100.0	2400	100.0

Table 5 Frequency of pathological diagnoses as classified by histopathology in primary glomerular disease (except IgA nephropathy)

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Membranous nephropathy	60	31.4	95	25.7	155	27.7
Minor glomerular abnormalities	33	17.3	95	25.7	128	22.9
Mesangial proliferative glomerulonephritis	45	23.6	82	22.2	127	22.7
Focal segmental glomerulosclerosis	24	12.6	53	14.4	77	13.8
Membranoproliferative glomerulonephritis (type I and III)	13	6.8	19	5.1	32	5.7
Crescentic and necrotizing glomerulonephritis	5	2.6	6	1.6	11	2.0
Endocapillary proliferative glomerulonephritis	1	0.5	6	1.6	7	1.3
Nephrosclerosis	2	1.0	4	1.1	6	1.1
Dense deposit disease	1	0.5	3	0.8	4	0.7
Sclerosing glomerulonephritis	2	1.0	1	0.3	3	0.5
Others	5	2.6	5	1.4	10	1.8
Total	191	100.0	369	100.0	560	100.0

(except IgAN) in nephrotic syndrome, MN was dominant followed by minor glomerular abnormalities, such as minimal change nephrotic syndrome (MCNS), FSGS, and MPGN (type I and III) in 2007 (Table 7). In 2008, the frequency of minor glomerular abnormalities was predominant, followed by MN (Table 7).

Clinical diagnosis of MN, minor glomerular abnormalities, and FSGS

Subanalyses of subjects with a clinical diagnosis of MN, minor glomerular abnormalities, and FSGS were performed since these were the most common forms of primary glomerular diseases (except IgAN) (Tables 8, 9, 10). Nephrotic syndrome was the most common clinical diagnosis in MN and minor glomerular abnormalities (Tables 8, 9), whereas chronic nephritic syndrome was the most common in FSGS (Table 10). In the pathogenesis of minor glomerular abnormalities (total 195 cases), primary glomerular diseases (except IgAN) comprised 65.6% (128 cases), followed by others 13.8% (27 cases), IgAN 8.2% (16 cases) and thin basement membrane disease 5.1% (10 cases). In the pathogenesis of FSGS (total 97 cases), primary glomerular diseases (except IgAN) comprised 79.4%

Table 6 Frequency of pathological diagnoses as classified by pathogenesis in nephrotic syndrome

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Primary glomerular disease (except IgA nephropathy)	91	65.9	179	69.1	270	68.0
Diabetic nephropathy	15	10.9	15	5.8	30	7.6
Amyloid nephropathy	9	6.5	13	5.0	22	5.5
IgA nephropathy	8	5.8	9	3.5	17	4.3
Lupus nephritis	4	2.9	8	3.1	12	3.0
Purpura nephritis	1	0.7	4	1.5	5	1.3
Infection-related nephropathy	3	2.2	1	0.4	4	1.0
Thrombotic microangiopathy	1	0.7	0	0.0	1	0.3
MPO-ANCA-positive nephritis	0	0.0	1	0.4	1	0.3
Hypertensive nephrosclerosis	0	0.0	1	0.4	1	0.3
Others	6	4.3	28	10.8	34	8.6
Total	138	100.0	259	100.0	397	100.0

Table 7 Frequency of pathological diagnoses as classified by histopathology in primary glomerular disease (except IgA nephropathy) in nephrotic syndrome

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Minor glomerular abnormalities	29	31.9	79	44.1	108	40.0
Membranous nephropathy	40	44.0	56	31.3	96	35.6
Focal segmental glomerulosclerosis	10	11.0	25	14.0	35	13.0
Membranoproliferative glomerulonephritis (type I and III)	7	7.7	13	7.3	20	7.4
Mesangial proliferative glomerulonephritis	1	1.1	4	2.2	5	1.9
Crescentic and necrotizing glomerulonephritis	2	2.2	1	0.6	3	1.1
Endocapillary proliferative glomerulonephritis	1	1.1	0	0.0	1	0.4
Others	1	1.1	1	0.6	2	0.7
Total	91	100.0	179	100.0	270	100.0

Table 8 Frequency of clinical diagnoses in membranous nephropathy

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Nephrotic syndrome	44	59.5	66	51.6	110	54.5
Chronic nephritic syndrome	20	27.0	47	36.7	67	33.2
Renal disorder with collagen disease or vasculitis	7	9.5	9	7.0	16	7.9
Renal disorder with metabolic syndrome	1	1.4	1	0.8	2	1.0
Recurrent or persistent hematuria	1	1.4	0	0.0	1	0.5
Renal transplantation	0	0.0	1	0.8	1	0.5
Rapidly progressive nephritic syndrome	0	0.0	1	0.8	1	0.5
Acute nephritic syndrome	0	0.0	1	0.8	1	0.5
Drug-induced nephropathy	0	0.0	1	0.8	1	0.5
Others	1	1.4	1	0.8	2	1.0
Total	74	100.0	128	100.0	202	100.0

Table 9 Frequency of clinical diagnoses in minor glomerular abnormalities

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Nephrotic syndrome	29	55.8	82	57.3	111	56.9
Chronic nephritic syndrome	9	17.3	43	30.0	52	26.7
Recurrent or persistent hematuria	6	11.5	10	7.0	16	8.2
Renal disorder with collagen disease or vasculitis	1	1.9	5	3.5	6	3.1
Rapidly progressive nephritic syndrome	1	1.9	0	0.0	1	0.5
Renal disorder with metabolic syndrome	1	1.9	0	0.0	1	0.5
Acute nephritic syndrome	1	1.9	0	0.0	1	0.5
Drug-induced nephropathy	1	1.9	0	0.0	1	0.5
Inherited renal disease	0	0.0	1	0.7	1	0.5
Others	3	5.8	2	1.4	5	2.6
Total	52	100.0	143	100.0	195	100.0

Table 10 Frequency of clinical diagnoses in focal segmental glomerulosclerosis

Classification	2007		2008		Total	
	n	%	*n*	%	*n*	%
Chronic nephritic syndrome	18	56.3	32	49.2	50	51.5
Nephrotic syndrome	10	31.3	26	40.0	36	37.1
Inherited renal disease	2	6.3	0	0.0	2	2.1
Renal disorder with collagen disease or vasculitis	1	3.1	1	1.5	2	2.1
Rapidly progressive nephritic syndrome	1	3.1	1	1.5	2	2.1
Renal transplantation	0	0.0	1	1.5	1	1.0
Recurrent or persistent hematuria	0	0.0	1	1.5	1	1.0
Renal disorder with metabolic syndrome	0	0.0	1	1.5	1	1.0
Others	0	0.0	2	3.1	2	2.1
Total	32	100.0	65	100.0	97	100.0

Table 11 Profile of IgA nephropathy

IgA nephropathy	2007	2008	Total
Total native kidney biopsies (*n*)	239	421	660
Average age (y)	36.5 ± 19.0	36.4 ± 18.2	36.4 ± 18.5
Male (*n*)	112 (46.9%)[a]	219 (52.0%)[a]	331 (50.2%)[a]
Average age (y)	37.1 ± 18.9[b]	37.2 ± 19.3[b]	37.2 ± 19.1[b]
Female (*n*)	127 (53.1%)	202 (48.0%)	329 (49.8%)
Average age (y)	36.1 ± 19.2	35.4 ± 17.0	35.7 ± 17.8

[a] Ratio indicates percentage of each gender in each biopsy category

[b] Not significant as compared to another gender

(77 cases), followed by others 11.3% (11 cases) and hypertensive nephrosclerosis 4.1% (4 cases).

Subanalysis of IgAN

The profile, classification of clinical diagnosis, and the pathological diagnosis of IgAN, the most frequent glomerulonephritis on the J-RBR, were further analyzed (Tables 11, 12, 13). The percentage of IgAN detected in total biopsies and native kidneys was 27.5 and 31.0% in 2007 and 2008, respectively. The average age was the fourth decade in both genders. There was no difference in the proportion based on gender (Table 11). The majority of the clinical and pathological diagnoses were chronic

Table 12 Frequency of classification of clinical diagnoses in IgA nephropathy

Clinical diagnosis	2007		2008		Total	
	n	%	*n*	%	*n*	%
Chronic nephritic syndrome	197	82.4	387	91.9	584	88.5
Recurrent or persistent hematuria	23	9.6	17	4.0	40	6.1
Nephrotic syndrome	8	3.3	9	2.1	17	2.6
Rapidly progressive nephritic syndrome	8	3.3	1	0.2	9	1.4
Acute nephritic syndrome	2	0.8	4	0.9	6	0.9
Hypertensive nephropathy	0	0.0	2	0.5	2	0.3
Renal disorder with metabolic disease	1	0.4	0	0.0	1	0.2
Acute renal failure	0	0.0	1	0.2	1	0.2
Total	239	100.0	421	100.0	660	100.0

nephritic syndrome (Table 12) and mesangial proliferative glomerulonephritis (Table 13), respectively.

Other diseases

Rare diseases such as Alport syndrome, Fabry disease, lipoprotein glomerulopathy, and dense deposit disease (one case each) were registered in 2007, and one subject was diagnosed with POEMS syndrome in 2008.

Discussion

The J-RBR obtained data from 818 and 1582 patients with kidney disease and renal transplantation who submitted renal biopsies in 2007 and 2008, respectively. The main objectives of the registry were, based on the histopathological findings, to establish the frequency of glomerulopathies, tubulointerstitial diseases, renal vascular disorders, and renal grafts in renal biopsies in Japan. Data for all patients with histopathological evidence of renal disease at the participating centers were collected on standard forms and registered on the J-RBR program in the UMIN-INDICE. Chronic nephritic syndrome was the most frequent clinical diagnosis in both years of the registry. IgAN was the most frequently diagnosed disease in renal biopsies in 2007 and 2008, consistent with previous reports [8]. In patients with nephrotic syndrome, primary glomerular diseases (except IgAN) were predominant in both years.

Regarding the classification of clinical diagnosis in native kidney biopsies, more than half were diagnosed with chronic nephritic syndrome, which was usually accompanied by urinary abnormalities, as shown in Table 2. The frequency of clinical diagnosis may reflect the prevalence of renal biopsy in Japan. Indications of renal biopsy in Japan included urinary abnormalities such as mild-to-moderate proteinuria with or without hematuria, massive proteinuria such as nephrotic syndrome, rapidly progressive glomerulonephritis, and renal allografts (a protocol or episode biopsy). Solitary hematuria may be indicated after urological examinations. In Japan, all students in primary and junior high schools routinely undergo an annual urinalysis by the dip-stick test as one of the national health programs. Therafter students in high schools and universities and employees of companies submit to a urinalysis as part of a nationwide screening program. This social system promotes the early referral to nephrologists and may thus influence the frequency of chronic nephritic syndrome according to the clinical diagnoses of the J-RBR.

In the present study, IgAN was the most frequently diagnosed by pathological findings, which is consistent with a previous report [8]. The frequency of IgAN was 32.9% in 2007 and 30.2% in 2008 in native kidneys of patients registered on the J-RBR, which was less than that in the previous nationwide survey [8]. IgAN is the most common biopsy-proven renal disease among primary glomerulopathies in Asia as described in reports from

Table 13 Frequency of pathological diagnoses as classified by histopathology

Pathological diagnosis by histopathology	2007		2008		Total	
	n	%	*n*	%	*n*	%
Mesangial proliferative glomerulonephritis	228	95.4	398	94.5	626	94.8
Minor glomerular abnormalities	0	0.0	16	3.8	16	2.4
Crescentic and necrotizing glomerulonephritis	2	0.8	3	0.7	5	0.8
Sclerosing glomerulonephritis	3	1.3	0	0.0	3	0.5
Nephrosclerosis	1	0.4	1	0.2	2	0.3
Membranous nephropathy	1	0.4	1	0.2	2	0.3
Membranoproliferative glomerulonephritis (type I and III)	1	0.4	0	0.0	1	0.2
Others	3	1.3	2	0.5	5	0.8
Total	239	100.0	421	100.0	660	100.0

Korea [12] and China [13]. In the United States, IgAN is the most common primary glomerulopathy in young adult Caucasians and the most common cause of end-stage renal disease, while it was found to be rare in African Americans in whom FSGS remained more common [14]. In Australia, IgAN, FSGS, lupus nephritis, and vasculitis are the most common renal diseases in adults with a male predominance, excepting lupus nephritis [6]. In Europe, IgAN is the most frequent primary glomerulonephritis in several countries [2, 4, 5, 15], while MN is the most frequent in Macedonia [16], MPGN in Romania [17], and non-IgA mesangial proliferative glomerulonephritis in Serbia [18]. FSGS is the most frequent renal disease in a recent report from Brazil [19]. Because there is a different policy of renal biopsy practice in each country, it may not be easy to compare the different databases across countries. Instead, the changing frequency patterns of renal disease in the same country over a certain time period are useful to treat disease and reduce chronic kidney disease burden [20].

The frequency of nephrotic syndrome was 19.0% in 2007 and 18.5% in 2008 for patients registered on the J-RBR. Primary renal diseases were present in approximately two-thirds of all patients with nephrotic syndrome. MN was the most common primary nephrotic syndrome in 2007 (44.0%) and MCNS was the most common in 2008 (44.1%). The reason for this difference may depend on the cohort of registered biopsies in both years, since the number of patients registered was not as large as other registries [2, 4, 13, 19].

For the registry of patients with end-stage renal disease in Japan, there has been a nationwide and yearly statistical survey of chronic dialysis patients since 1968, conducted by the Japanese Society for Dialysis Therapy in Japan [21]. The combined data of the J-RBR with this dialysis registry will allow us to evaluate the long-term outcome of patients with various renal diseases in the near future. Similarly, the combined renal transplant registry data allows the evaluation of patient outcome. A sizeable frequency of renal grafts was registered on the J-RBR. Consequently, the future analysis of renal grafts, including the frequency of the protocol and episode biopsies and the precise histological diagnosis, will be necessary.

There is no overall registry of renal biopsies in Japan at the moment. It is noteworthy that the J-RBR is web-based, and a prospective registry system that can easily increase the number of participating centers and enlarge the number of patients enroled in the future. We cannot conclude that the present sample of patients on the J-RBR in 2007 and in 2008 is actually representative of the nationwide frequency of glomerular, tubulointerstitial, or renal vascular diseases or renal grafts in Japan. However, in the near future, investigation of a larger cohort or a population-based analysis of the rate of each renal disease may reveal the actual frequency of the disease and the distribution of age ranges by utilizing the J-RBR system.

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Appendix

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Tacrolimus Therapy as an Alternative to Thiopurines for Maintaining Remission in Patients With Refractory Ulcerative Colitis

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Background: Although the efficacy of tacrolimus for inducing remission of refractory ulcerative colitis (UC) is established, its efficacy for maintaining remission of UC has not been evaluated.

Aim: The aim of this study was to evaluate the efficacy of tacrolimus compared with thiopurines for maintaining remission in patients with refractory UC.

Methods: Twenty-four UC patients treated with tacrolimus and 34 treated with thiopurines to maintain remission were enrolled as the tacrolimus group and the thiopurine group, respectively. In the tacrolimus group, 82.8% of the patients were treated with tacrolimus for induction of the remission, whereas 70% of the patients in the thiopurine group were induced remission with either corticosteroid or cytapheresis. Proportions of patients who kept steroid-free remission between the tacrolimus and the thiopurine groups were compared. Maintenance of remission using tacrolimus or thiopurines was defined as no need for other therapies other than aminosalicylates without relapse for at least 3 months. Secondarily, to determine whether the response to thiopurines affects the long-term efficacy of tacrolimus maintenance therapy, the overall cumulative relapse-free survival based on the Kaplan-Meier method was estimated in thiopurine-naive or thiopurine-intolerant patients and thiopurine-refractory ones in the tacrolimus group.

Results: Remission was successfully maintained in 17 patients (70.8%) of the tacrolimus group, and 28 patients (82.4%) of the thiopurine group. The overall cumulative relapse-free survival of thiopurine-naive or thiopurine-intolerant patients in the tacrolimus group was similar to that in the thiopurine group, and significantly higher than that of thiopurine-refractory patients in the tacrolimus group.

Conclusion: Maintenance therapy with tacrolimus for patients with UC could be considered an alternative to thiopurine therapy.

Key Words: ulcerative colitis, tacrolimus, thiopurines, remission maintenance therapy

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Ulcerative colitis (UC) is an idiopathic, chronic, and inflammatory disorder characterized by diarrhea, rectal bleeding, abdominal pain, fever, anemia, and body weight loss.[1] Corticosteroid (CS) therapy is used for patients with UC who do not respond to aminosalicylates or those with a severe attack.[1,2] Although most patients with UC initially respond to CS, approximately 20% of patients with UC become steroid-dependent within 1 year after initiating CS therapy.[3] CS is not used as maintenance therapy for patients with UC because of undesirable side-effects such as opportunistic infections, diabetes mellitus, osteoporosis, etc.[4] Therefore, steroid-free remission is an important issue for patients with UC.

Tacrolimus is effective for patients with UC refractory to or dependent on CS, and is usually used as a rescue and bridging therapy before initiating azathioprine (AZA) or 6-mercaptopurine (6-MP) therapy.[5–11] Several studies have reported the long-term outcomes after tacrolimus therapy for the induction of remission[6–9]; in all of these case series, however, patients received maintenance therapy with other agents, including thiopurines, and infliximab after the induction of clinical remission. To our knowledge, except for a couple of case reports,[6,12] there are no reports on the effect of tacrolimus as maintenance therapy for patients with refractory UC. Here, we evaluated the efficacy of tacrolimus therapy for maintaining remission in patients with refractory UC in comparison with thiopurines, currently the most widely used treatment to maintain steroid-free remission in patients with refractory UC.[1,2]

PATIENTS AND METHODS

Patients

Between April 2001 and October 2009, 42 patients with UC who were resistant to or could not be treated with conventional therapy were treated with tacrolimus, and 29 (69.0%) achieved clinical remission. Of those, 23 patients received tacrolimus subsequently for the maintenance of remission. In addition, 1 patient in whom tacrolimus therapy failed for inducing remission was maintained remission with tacrolimus after the induction of remission

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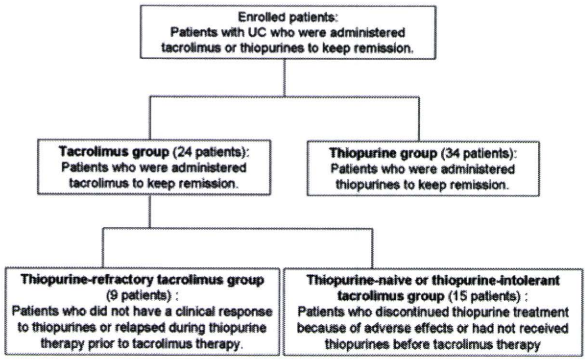

FIGURE 1. A flow chart of patients in different groups and subgroups. UC indicates ulcerative colitis.

by inflixmab. These 24 patients with UC who were treated with tacrolimus to maintain remission after achieving remission were enrolled in this retrospective, observational, single-center study (tacrolimus group). To compare the effect of tacrolimus for maintaining remission of UC, 34 patients who were administered thiopurines to maintain remission during the same period were enrolled as the thiopurine group.

In addition, tacrolimus group was divided into 2 subgroups according to the response to thiopurines; that is, thiopurine-refractory tacrolimus group (9 patients did not have a clinical response to thiopurines or relapsed during thiopurine therapy before tacrolimus administration) and thiopurine-naive or thiopurine-intolerant tacrolimus group (3 patients discontinued thiopurine treatment because of adverse effects and 12 patients had not received thiopurines before tacrolimus therapy). Flow chart of the patients in different groups and subgroups is shown in Figure 1.

This study was reviewed and approved by the Institutional Review Board of Kyoto University. Patients were informed about the potential risks and benefits of tacrolimus therapy and provided written consent to its use. In all cases, the diagnosis was established according to standardized criteria by prior clinical assessment, radiology, endoscopy, and histology.

Definition of Response

Disease activity was measured using a modified Truelove Witts severity index (MTWSI)[13]; details are shown in Table 1. Clinical remission was retrospectively defined as an estimated MTWSI score of 4 or less. Relapse was defined as an increase in the MTWSI score to 5 or higher with additional therapies required. To remove the influence of other drugs as much as possible when comparing the efficacy of tacrolimus and thiopurines for steroid-free remission, maintenance of remission was defined as no need for concomitant treatment other than aminosalicylates and topical steroid therapy in addition to tacrolimus or thiopurines without relapse for at least 3 months.

Patients were classified as steroid-resistant or steroid-dependent in accordance with the earlier published definition of Ogata et al.[5]

Treatment

Tacrolimus was administered in its oral formulation. Dosage was adjusted to produce trough tacrolimus whole-blood levels of 10 to 15 ng/mL to induce remission. After inducing clinical remission, tacrolimus whole-blood trough concentrations were maintained at a lower level, between 5 and 10 ng/mL.[9] The initial dose of tacrolimus was 0.1 mg/kg body weight per day. The mean doses of tacrolimus for inducing and maintaining remission were 7.7 mg/d (range: 2.0 to 12.0 mg/d) and 5.7 mg/d (range: 2.0 to 10.0 mg/d), respectively.

For thiopurine maintenance therapy, the dose of AZA or 6-MP was adapted to achieve white blood cell counts between 3000 and 5000/μL, or 6-thioguanine nucleotide concentrations between 250 and 500 pmol/8×10^8 red blood cells. Twenty-four and 10 patients were treated with AZA and 6-MP, respectively. The mean initial doses of AZA and 6-MP were 30.5 mg/d (range: 25 to 100 mg/d) and 13.3 mg/d (range: 5 to 30 mg/d), respectively. The mean doses of AZA and 6-MP for the remission phase were 53.0 mg/d (range: 25 to 100 mg/d) and 25.0 mg/d (range: 5 to 40 mg/d), respectively.

Assessment and Statistics

The primary endpoint of this study was the proportion of patients in whom remission was successfully maintained. Secondary endpoints included relapse-free survival and treatment safety.

Proportions between groups were compared by Fisher exact test and continuous variables were compared by Mann-Whitney *U* test. Relapse-free survival was assessed using the Kaplan-Meier method. Relapse-free survival of patients who could not maintain remission without concomitant therapies other than aminosalicylates and topical steroid therapy were set as 0 month. A *P* value of less than 0.05 was considered to be statistically significant.

TABLE 1. Modified Truelove Witts Severity Index[13]

Score	0	1	2	3	4	5
Bowel movement	0-2	3-4	5-6	7-9	≥10	
Nocturnal diarrhea	No	Yes				
Visible blood in stool (%)	0	< 50	≥50	100		
Abdominal tenderness	None	Mild	Moderate	Severe		
Abdominal pain/cramping	None	Mild	Moderate	Severe		
Need for antidiarrheals	No	Yes				
General status	Perfect	Very good	Good	Average	Poor	Terrible
Fecal incontinence	No	Yes				

Remission category: 4 or less.

RESULTS

Patient Characteristics

Baseline characteristics of the patients in each group and subgroup of the tacrolimus group are shown in Tables 2 and 3, respectively. Except for treatment duration, these baseline characteristics of the tacrolimus group were similar to those of thiopurine group (Table 2).

All patients in the tacrolimus group were treated with aminosalicylates. Four patients (16.7%) were steroid resistant, 17 patients (70.8%) were steroid dependent, and the remaining 3 patients (12.5%) had not been treated with CS. Two patients had received infliximab for the induction of remission. No other patients had been treated with biologics before tacrolimus maintenance therapy.

Therapies for the induction of remission that preceded remission maintenance therapy are summarized in Table 4. In tacrolimus group, 3 patients received 2 courses of tacrolimus maintenance therapy and 1 patient received 3 treatment courses, and in thiopurine group, 6 patients received 2 courses of thiopurine maintenance therapy. Therefore, a total 29 and 40 remission induction therapies were performed before remission maintenance therapy in tacrolimus and thiopurine group, respectively.

Maintenance of Remission

In tacrolimus group, remission ratio at 3 and 6 months was 70.8% (17 of 24 patients) and 54.2% (13 of 24 patients), respectively. In thiopurine group, remission ratio at 3 and 6 months was 82.4% (28 of 34 patients) and 73.5% (25 of 34 patients), respectively. Proportions of patients who were stayed in remission at 3 and 6 months were not statistically different between the 2 groups ($P = 0.3494$ and 0.1647, respectively; Fisher exact test). The mean durations of steroid-free remission in the tacrolimus and the thiopurine groups were 9.2 months (range: 0 to 50.7 mo) and 14.0 months (range: 0 to 51.6 mo), respectively. There was no statistical difference between the 2 groups again ($P = 0.1114$; Mann-Whitney U test).

Although there was no significant difference between the 2 groups, the duration of remission in the tacrolimus group tended to be shorter than that in thiopurine group. Therefore, to determine whether the response to thiopurine therapy affects the long-term efficacy of tacrolimus maintenance therapy, the overall cumulative relapse-free survival based on the Kaplan-Meier method was estimated in the thiopurine-naive or thiopurine-intolerant tacrolimus group and the thiopurine-refractory tacrolimus group. Relapse-free survival in patients in the thiopurine-naive or thiopurine-intolerant tacrolimus group was comparable with that in the thiopurine group ($P = 0.5594$; log-rank test) and was significantly higher than that in the thiopurine-refractory tacrolimus group ($P = 0.0104$; log-rank test; Fig. 2).

Adverse Effects

The frequency of adverse events during tacrolimus therapy is shown in Table 5. Tacrolimus withdrawal was

TABLE 2. Patients' Baseline Characteristics in the Tacrolimus and the Thiopurine Groups

	Tacrolimus Group	Thiopurine Group	P
Age at diagnosis [median (range)] (y)	23.4 (11.9-74.7)	29.3 (15.3-60.8)	0.4334
Age at start of the therapy [median (range)] (y)	27.5 (15.9-78.1)	36.2 (16.8-65.0)	0.3619
Disease duration prior the therapy [median (range)] (y)	3.6 (0.4-22.8)	3.6 (0.8-31.6)	0.7116
Follow-up duration [median (range)] (mo)	23.0 (5.1-87.1)	34.4 (3.4-86.0)	0.4207
Treatment duration [median (range)] (mo)	13.2 (1.8-53.7)	25.7 (3.4-61.5)	0.0187
Sex			0.2913
Men (%)	12 (50)	22 (64.7)	
Women (%)	12 (50)	12 (35.3)	
Disease extent			1.0000
Extensive (%)	17 (70.8)	24 (70.6)	
Left-sided (%)	7 (29.2)	10 (29.4)	
Response to corticosteroids			0.3428
Steroid resistance (%)	4 (16.7)	5 (14.7)	
Steroid dependence (%)	17 (70.8)	28 (82.4)	
Steroid naive (%)	3 (12.5)	1 (2.9)	

Numbers of patients are shown unless specified.

TABLE 3. Baseline Characteristics of Patients in Each Tacrolimus Subgroup

	Thiopurine-refractory Group	Thiopurine-naive Intolerant Group	P
Age at diagnosis [median (range)] (y)	21.7 (15.3-56.0)	23.8 (11.9-74.7)	0.4207
Age at start of the therapy [median (range)] (y)	30.4 (16.5-67.7)	27.2 (15.9-78.1)	0.7565
Disease duration prior the therapy [median (range)] (y)	4.8 (1-22.8)	3.4 (0.4-4.8)	0.0131
Follow-up duration [median (range)] (y)	12.67 (5.1-61.2)	44.6 (6.5-87.1)	0.1440
Treatment duration [median (range)] (mo)	12.67 (5.1-44.4)	15.6 (1.8-53. 7)	0.5711
Sex			0.4003
Men (%)	3 (33.3)	9 (60.0)	
Women (%)	6 (66.7)	6 (40.0)	
Disease extent			1.0000
Extensive (%)	6 (66.7)	11 (73.3)	
Left-sided (%)	3 (33.3)	4 (26.7)	
Response to corticosteroids			0.8241
Steroid resistance (%)	1 (11.1)	3 (20.0)	
Steroid dependence (%)	7 (77.8)	10 (66.7)	
Steroid naive (%)	1 (11.1)	2 (13.3)	

Numbers of patients are shown unless specified.

TABLE 4. Therapies for the Induction of Remission Before Maintenance Therapies

	Tacrolimus Group	Thiopurine Group
Prednisolone (%)	1 (3.4)	20 (50.0)
Tacrolimus (%)	24 (82.8)	11 (27.5)
Infliximab (%)	2 (6.9)	1 (2.5)
Cytoapheresis (%)	2 (6.9)	8 (20.0)

Numbers of patients are shown.