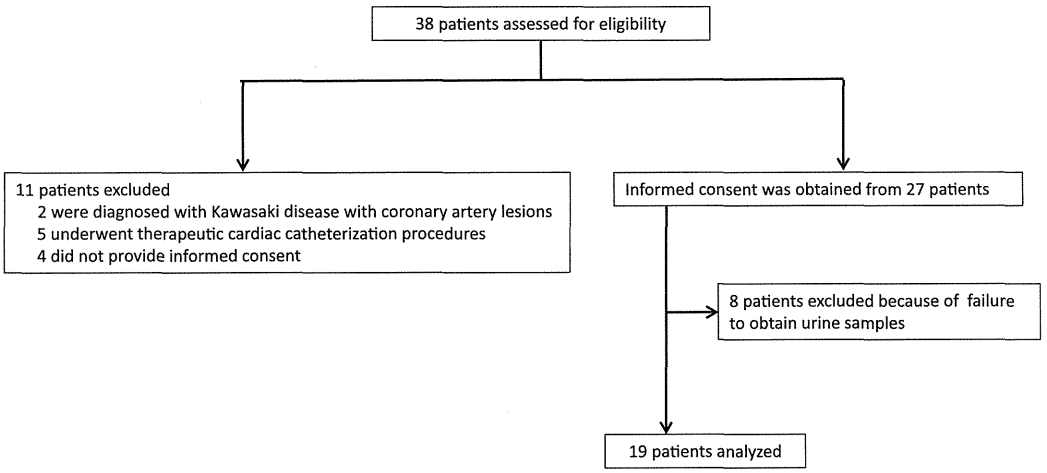

Figure 1. The study profile.

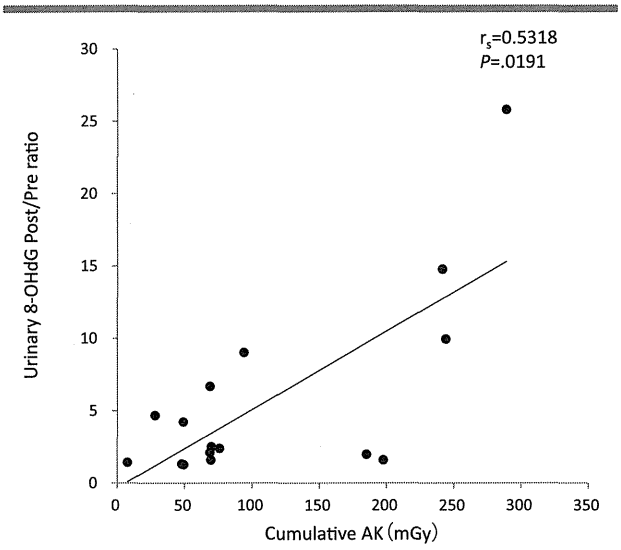

Figure 4. The correlation between cumulative AK and ratio of post- to precardiac catheterization urinary 8-OHdG levels.

Table II. Results of single regression analysis of associations between post:pre 8-OHdG and the variables

	r_s	*P* value
Age (y)	0.36	.22
Body weight (kg)	0.26	.29
Body height (cm)	0.25	.30
Body mass index (kg/m^2)	−0.13	.61
Body surface area (m^2)	0.23	.35
Qp:Qs	0.053	.85
Pp:Ps	−0.030	.92
Dose of contrast medium (mL/kg)	−0.12	.62
Duration of catheterization study (min)	0.40	.10
FT (min)	0.35	.14
Cumulative AK (mGy)	0.53	.020

Pathogenesis of childhood idiopathic nephrotic syndrome: a paradigm shift from T-cells to podocytes

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Background: **Nephrotic syndrome is the most common cause of kidney disease in children, but its pathogenesis remains unclear. This article reviews the novel aspects of the mechanisms underlying massive proteinuria in minimal-change disease, which is the most common form of childhood nephrotic syndrome.**

Data sources: **This article integrates the findings of a PubMed database search for English language articles published in the past 40 years (from September 1974 to February 2014) using the key words "pathogenesis", "minimal change nephrotic syndrome" or "idiopathic nephrotic syndrome".**

Results: **Unknown humoral factors associated with T-cell dysfunction have been thought to play an important role in the pathogenesis of minimal-change disease. However, recent findings are changing this paradigm, i.e., visceral glomerular epithelial cells (podocytes) may be involved via expression of molecules such as CD80 and angiopoietin-like 4.**

Conclusions: **Recent evidence suggests that minimal-change disease results from interactions between humoral factors and dysfunctional podocytes. In addition to immunosuppressant drugs that target lymphocytes, a biological agent such as an antibody against the abnormal molecule(s) expressed by podocytes may provide novel drug treatment for minimal-change disease.**

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Key words: **angiopoietin-like 4;**
CD80;
cytokine;
minimal change nephrotic syndrome;
podocyte

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Review Article

Introduction

Nephrotic syndrome (NS) is characterized by heavy proteinuria (urine protein:creatinine ratio ≥2000 mg/g or ≥300 mg/dL, or 3+ protein on urine dipstick), hypoalbuminemia (≤2.5 g/dL), and edema.[1] Leakage of massive amounts of serum proteins into the urine leads to a hypercoagulable state, a higher rate of infectious disease, and the dysregulation of fluid balance. However, 80%-90% of children with NS achieve complete remission after glucocorticoid (GC) therapy. The annual incidence of NS is estimated to be 2-7 per 100 000 children, with a cumulative prevalence of 16 per 100 000 children.[2,3]

Childhood NS is classified into three groups: idiopathic (INS, 90% of cases), secondary (10%), and congenital (<1%). INS is further classified into the two major histological variants: minimal-change disease (MCD, 85%) and focal segmental glomerulosclerosis (FSGS, 10%). It is still debatable whether MCD and FSGS represent different ends of the same disease spectrum with the same underlying pathophysiological processes, or whether they are two distinct disease entities.[4,5] Secondary NS is defined as NS associated with a systemic disease, such as lupus nephritis, Henoch-Schönlein purpura nephritis, or Alport syndrome. Congenital NS is defined as heavy proteinuria starting before the age of 3 months, and may be associated with congenital infections (such as syphilis, toxoplasmosis, or cytomegalovirus) or with mutations of the genes coding for podocyte proteins (such as *NPHS1*, *NPHS2*, and *WT1*).[6]

MCD, which is almost equivalent to steroid-sensitive INS, accounts for the vast majority of cases of childhood NS, but its pathogenesis remains unknown. In this review, we discuss recent research findings and the paradigm shift regarding the likely pathogenesis of MCD (including steroid-sensitive INS).

Old paradigm for the pathogenesis of MCD

In 1974, Shalhoub[7] proposed that MCD was a disorder of T-cell function resulting in increased plasma levels of lymphocyte-derived permeability factor. This hypothesis

was based on the absence of immune complexes in glomeruli, the rapid response to steroid therapy, the association of MCD with Hodgkin's disease, and the observation that measles infection often induced remission of MCD. The massive proteinuria and hypoalbuminemia that characterize MCD were thought to result from increased permeability of the glomerular capillary wall due to T-cell activation triggered by stimuli such as viral infection or allergens.[3] The most compelling evidence came from experience with renal allografts: NS disappeared when MCD kidneys were transplanted into patients without NS.[8] The following clinical findings further support the concept that vascular permeability factors produced from activated T-cells play an important role in MCD: in patients with MCD, there is a risk of recurrence of the disease when transplanted;[9] placental transfer of proinflammatory cytokines from a mother to a newborn results in neonatal NS;[10] the potential of apheresis monotherapy to induce and maintain complete remission of MCD suggests that circulating factors play an important role in the pathogenesis of MCD.[11]

Role of cytokines in the pathogenesis of MCD

Since Koyama et al[12] found that injection of supernatants from T-cell hybridomas from patients with MCD relapse into rats caused immediate proteinuria and glomerular podocyte foot process fusion, researchers have tried to identify the circulating factors released from T-cells that increase the glomerular permeability to serum proteins. Cytokines were considered to be the most likely pathogenic factors. Cytokines are small proteins (molecular weight 8-80 kDa) that function as soluble mediators in an autocrine or paracrine manner, which are produced by both immune and non-immune cells. Patients with MCD relapse were found to have increased levels of various cytokines in the serum or urine including interleukin (IL)-2,[13] soluble IL-2 receptor,[13-16] interferon-gamma,[13,17] IL-4,[17,18] IL-12,[19] IL-18,[20] tumor necrosis factor (TNF)-α,[21] and vascular endothelial growth factor (VEGF).[22] Isolation of peripheral blood mononuclear cells (PBMCs) from patients with MCD relapse and measurement of the *in vitro* mitogen-stimulated production of cytokines in the cultured cell supernatants demonstrated increased production of various cytokines including IL-1,[23] IL-2,[17,24] IL-4,[17,18] IL-10,[24] IL-12,[25] IL-18,[20] and TNF-α.[26] Yap et al[27] also reported increased expression of IL-13 mRNA in patients with MCD relapse. However, few studies have investigated the direct effects of specific cytokines on the development of proteinuria. VEGF is known to increase capillary permeability by stimulating the release of nitric oxide and thereby inducing endothelial cell fenestration, but VEGF infusion does not induce proteinuria in rats.[28,29] Other cytokines that may be involved in the pathogenesis of MCD include IL-13 and TNF-α. IL-13-transfected rats develop nephrotic proteinuria and display an MCD-like phenotype.[30] However, proteinuria does not occur in many pathological conditions that are associated with increased levels of IL-13 such as asthma, psoriasis, and allergic dermatitis. Infusion of TNF-α has been reported to result in increased urinary albumin excretion in rats.[29] Furthermore, Raveh et al[31] reported remission of MCD after treatment with infliximab, a chimeric TNF-α monoclonal antibody, in a 13-year-old boy with MCD that was refractory to the standard treatment protocols. However, there is controversy over the relationship between increased production of TNF-α and capillary permeability,[32] and infliximab therapy carries a risk of inducing proteinuria secondary to membranous nephropathy.[33]

Role of T-cells in the pathogenesis of MCD

The aberrant T-cells in MCD are thought to be T helper (Th) type 2 cells,[34-37] because MCD is often associated with atopy and allergy,[38-40] which are caused by Th2 immunologic responses. The increased serum immunoglobulin (Ig) E level and preservation of IgG4 observed in MCD are also characteristic of a Th2 response.[41-43] However, some observations do not support this hypothesis.[24,44] Our study of Th cell subsets in children with MCD using 3-color flow cytometry found no significant differences in the proportions of Th cell subsets [such as Th0 (naive T-cells), Th1, or Th2 CD4+ cells] or the Th1/Th2 ratio among patients with relapse of NS, patients with remission of NS, and normal controls.[45] It has therefore not been definitively established that MCD is a Th2-dependent disorder.

There are clinical reports of MCD remission after depletion of B-cells using monoclonal antibodies or the anti-CD20 drug rituximab (RTX).[46-48] These reports suggest that there are not any underlying T-cell disorders in MCD. Furthermore, the increased nitric oxide production by B-cells exclusively found in the relapse phase of MCD further supports the possibility of B-cell involvement.[49] These findings indicate that T-cells may not play a central role in the pathogenesis of MCD.

Limitations to the hypotheses focusing on lymphocyte-derived permeability factors

As described above, studies of alterations in cytokine production in MCD reported variable results. The differences may have resulted from the different immunogenetic characteristics of the patients, or the heterogeneity of the stimulated cells in non-

physiological environments. Lack of documentation of biopsy findings, inclusion of steroid-treated patients, and differences in methodology among studies makes it difficult to determine the factors associated with glomerular permeability. Furthermore, the complex interactions among cytokines make it very difficult to determine which cytokine is increased first.

New paradigm for the pathogenesis of MCD

A new paradigm for the pathogenesis of MCD has emerged since the discovery by Kestila et al[50] in 1998 that mutations in the gene *NPHS1*, which encodes the podocyte-expressed immunoglobulin superfamily protein nephrin, cause congenital NS in humans. This landmark study led to a substantial increase in our understanding of glomerular biology and physiology. Additionally, the development of proteinuria in lipopolysaccharide (LPS)-injected severe combined immunodeficient mice, which are devoid of T- and B-cells, suggests that this mouse model of MCD may be independent of T- or B-cells.[51] Based on these findings, visceral glomerular epithelial cells (podocytes) have attracted particular attention as a key player in the pathogenesis of MCD.[52,53]

Podocyte ultrastructure is the final barrier to urinary protein loss

Podocytes are terminally differentiated cells that line the outer aspect of the glomerular basement membrane (GBM). Podocytes form the final barrier to urinary protein loss by the formation and maintenance of podocyte foot processes (FPs) and the interposed slit diaphragms (SDs) (Fig. 1).[54] The SDs are the main selectively permeable barrier in the kidney.[55] Podocyte FPs contain a contractile and dynamic apparatus consisting of actin, myosin II, α-actinin-4, talin, vinculin, and synaptopodin.[56,57] The FPs are anchored to the GBM via α3/β1-integrin[58] and dystroglycans.[59] Our knowledge of SD structure is based on genetic studies of familial NS, which led to identification of SD proteins such as podocin, nephrin, α-actinin-4, and TRPC6. The genes for these proteins may be mutated in inherited NS.[6] In contrast, no mutation has been found in MCD characterized by selective albuminuria associated with FP effacement, where the expression of these proteins is unchanged or downregulated.[60,61] It is easily understandable that various proteins can leak from the impaired SD between podocytes due to reduced nephrin expression in congenital NS of the Finnish type or podocyte detachment in FSGS.[62] However, it remains unclear how albumin can diffuse across the effaced podocyte FP in MCD. The effaced podocyte FPs extensively cover the glomerular capillary wall and podocyte SD density is decreased by 80% at most, with half of the slits displaying a tight-junction-like structure.[63] These structural observations raise questions regarding the route by which albumin passes through the glomerular wall. Tojo et al[62] proposed a receptor-mediated albumin transport mechanism that transports albumin through the podocyte cell body by endocytosis and exocytosis. This mechanism of receptor-mediated transport through podocytes may explain the selective albuminuria observed in MCD.

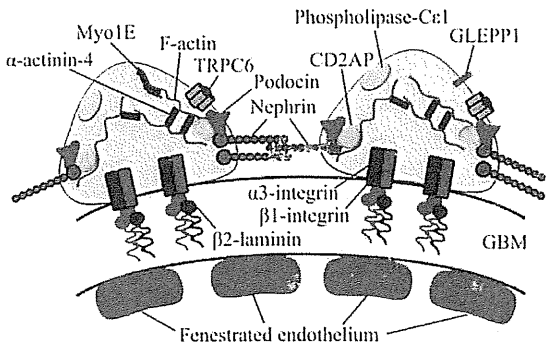

Fig. 1. Podocyte proteins that form the foot process ultrastructure and influence glomerular permselectivity.[54] Myo1E: myosin 1E; TRPC6: transient receptor potential cation channel 6; CD2AP: CD2-associated protein; GLEPP1: glomerular epithelial protein 1; GBM: glomerular basement membrane. This figure was reproduced with permission from Springer Science Business Media.

Hypotheses for the pathogenesis of MCD with a central focus on podocytes

In the last decade, several hypotheses have been proposed that focus on the role of the podocyte in the mechanism underlying the proteinuria in MCD.[53,64-69] Two representative hypotheses are discussed below.

CD80 expression on podocytes as a key player in the induction of proteinuria

CD80, also known as B7.1, is a T-cell costimulatory molecule that is involved in both activation and termination of the T-cell response. Activation of CD80 on antigen-presenting cells and binding to the CD28 receptor on T-cells has a key role in T-cell activation. In contrast, binding of CD80 to cytotoxic T-lymphocyte-associated (CTLA)-4 terminates the T-cell response.[70] CTLA-4 is expressed on the membrane of the Foxp3$^+$ T-regulatory cell (Treg), and Treg may further inhibit the immune response by release of soluble CTLA-4, IL-10, and transforming growth factor-β (TGF-β). CTLA-4 also suppresses CD80 expression on antigen-presenting cells. Experimental results suggest that direct activation

of podocytes, independent of T-cell involvement, can induce CD80 expression and proteinuria. Injection of LPS into mice increased CD80 expression on podocytes by binding to toll-like receptor (TLR)-4 in association with the development of proteinuria and FP effacement, and LPS also induced CD80 expression in cultured podocytes, with actin reorganization and shape change.[51] CD80 expression can also be induced by T-cell cytokines such as IL-13,[27] and by polyinosinic-polycytidylic acid.[71] Polyinosinic-polycytidylic acid stimulates TLR3 and is structurally similar to the double-stranded RNA found in some viruses, which may be related to the observation that MCD relapse is frequently preceded by an upper respiratory tract infection.[72] Furthermore, MCD is associated with pronounced expression of CD80 on podocytes, and increased urinary excretion of CD80.[66,67] Shimada et al[53] proposed the "two-hit" podocyte immune disorder underlying MCD. Briefly, the "first hit" is induction of podocyte expression of CD80 in response to a circulating factor (such as a cytokine, allergen, or microbial product). The increased CD80 expression on podocytes results in shape change and proteinuria, although the underlying mechanism is unclear.

The proteinuria induced by LPS injection in mice is only transient,[51] and it is hypothesized that CD80 expression in humans is also usually transient because of autoregulatory mechanisms mediated by the T cells and/or podocytes. The "second hit" is dysfunction of this autoregulatory mechanism, resulting in persistent CD80 expression and proteinuria. CD80 expression is inhibited by both CTLA-4 and IL-10[70,73] resulting in resolution of proteinuria. If dysfunctional Tregs in MCD patients cannot turn off podocyte CD80 expression by secretion of soluble CTLA-4, IL-10, and TGF-β, proteinuria may persist. However, this mechanism has not been confirmed in humans. Consistent with this hypothesis, the urinary soluble CD80/CTLA-4 ratio was reported to be >100-fold higher in patients with MCD relapse than in patients with remission,[66] and impaired function of Tregs was observed in MCD patients.[74,75] Evidence that abatacept (CTLA-4-immunoglobulin fusion protein) may inhibit the pathogenesis of rheumatoid arthritis at several levels via selective modulation of CD80/CD86 co-stimulatory molecules expressed by a variety of activated cell types also provides direct and indirect support for this hypothesis.[76]

It is currently unknown why children with MCD may have defective Treg or podocyte autoregulatory function, but this may be related to delayed or ineffective maturation of the T-cell response, possibly because of genetic or environmental factors. Further research is required to determine the mechanism by which CD80 signaling alters podocyte function and disrupts the glomerular barrier.

Podocyte expressed angiopoietin-like 4 (Angptl4) as a key player in the induction of proteinuria

Recent studies[52,65,77] found that qualitative and quantitative changes in the expression of Angptl4 in podocytes can induce most of the characteristic features of MCD, including dyslipidemia. Angptl4 is a glycoprotein that shares some structural and functional similarities with angiopoietins, and is expressed in many tissues. Angptl4 inhibits endothelium-bound lipoprotein lipase activity,[78] resulting in increased plasma triglyceride levels. Clement et al[65] reported that glomerular expression of Angptl4 is highly upregulated in the serum and podocytes in experimental models of MCD and in the human disease. Podocyte-specific transgenic overexpression of Angptl4 (NPHS2-Angptl4) in rats induced nephrotic-range proteinuria (over 500-fold increase in albuminuria), loss of GBM charge, and FP effacement. Angptl4 secreted from podocytes in some forms of NS was also shown to lack normal sialylation. Based on these findings, it has been proposed that podocytes secrete a hyposialylated form of Angptl4 in MCD,[52,65,77] whereas a sialylated form of Angptl4 is secreted by extrarenal organs (mostly skeletal muscle, the heart, and adipose tissue) in response to an elevated plasma ratio of free fatty acids to albumin when proteinuria reaches the nephrotic range.

High serum levels of sialylated Angptl4 were found in MCD as well as other glomerular diseases, and these circulating pools of Angptl4 may reduce proteinuria by interacting with glomerular endothelial β5-integrin.[77] Although the precise mechanism underlying the proteinuria caused by podocyte-specific overexpression of Angptl4 is still unclear, the high isoelectric point,[79] positively charged form of Angptl4 may play a key role.[79] Analysis of glomerular protein extracts from the rat model of MCD induced by puromycin aminonucleoside shows that Angptl4 is overproduced in two distinct forms: a positively charged form that migrates at a high pI (8-8.5) and a neutral form that migrates at or just less than a pI of 7. Both high-pI and neutral Angptl4 secreted by podocytes bind to the GBM to alter protein-protein interactions. Progressive accumulation and clustering of Angptl4 in the GBM likely activates signals at the podocyte-GBM interface and induces foot-process effacement, resulting in proteinuria.[52] The relationship between Angptl4 and CD80 has not yet been determined.[52] In a mouse model of MCD, injection with LPS (an activator of TLR4) increased both the expression of Angptl4 in adipose tissue[80] and CD80 on podocytes.[51] It is therefore possible that some pathogenic stimuli such as LPS activate common pathways to induce expression of Angptl4 and CD80 on podocytes.

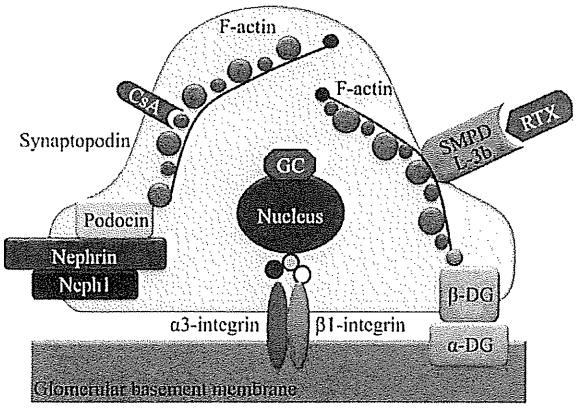

Fig. 2. Podocyte as a target for therapies in minimal-change disease. GC inhibit the nuclear factor-kappa B transcription factor signaling in the podocyte. The calcineurin-dependent dephosphorylation of synaptopodin, which in turn leads to destabilization of the podocyte actin cytoskeleton. CsA inhibits this reaction resulting in its stabilization. RTX binds to the CD20 molecule and the podocyte protein SMPDL-3b, which mediates stabilization of the actin cytoskeleton. GC: glucocorticosteroids; CsA: cyclosporine A; RTX: rituximab; SMPDL-3b: sphingomyelin phosphodiesterase acid-like 3b; DG: dystroglycans.

Podocytes as a novel therapeutic target

Because MCD was historically considered to be a disease of T-cell dysfunction, it was thought that the immunosuppressant drugs used for the treatment of MCD such as GCs, cyclophosphamide, azathioprine, chlorambucil, mycophenolate mofetil, levamisole, cyclosporine A (CsA), tacrolimus, and RTX acted by correcting lymphocyte dysfunction, especially of T-cells. However, it has become clear over the past decade that some of these drugs have direct effects on podocytes, and novel putative modes of action of GCs, CsA, and RTX have been proposed (Fig. 2).[81,82]

Even though 80%-90% of children with MCD achieve complete remission after treatment with GCs, the mechanism by which GCs induce remission is still unknown. As GC receptor expression is ubiquitous, any cell type can theoretically be affected by these drugs. Identification of the podocyte as the key player in MCD resulted in investigation of the effects of GCs on podocytes that may explain their efficacy in MCD. An initial study[83] found that dexamethasone had potent effects on human podocyte structure and function. GCs inhibit the intracellular signaling of nuclear factor κB (NF-κB) transcription factor, which is known to be important in the podocyte.[84] We also confirmed that podocyte NF-κB has a role in the development of proteinuria in a mouse model of MCD induced by puromycin aminonucleoside. Pretreatment with dehydroxymethylepoxyquinomicin (DHMEQ), which potently inhibits the DNA-binding activity of NF-κB, reduced the proteinuria and reversed the serum abnormalities. Electron microscopic analyses indicated that DHMEQ can inhibit podocyte FP effacement by blocking the translocation of podocyte NF-κB from the cytoplasm to the nucleus.[85]

The calcineurin inhibitor CsA is widely used in the treatment of MCD, especially when there is an insufficient response to GCs. Although it was thought that the efficacy of CsA in MCD was due to the inhibition of intracellular signaling in the activated T cells, Faul et al[86] challenged this by demonstrating its action on podocytes. They reported that CsA acts on podocytes by the calcineurin-dependent dephosphorylation of synaptopodin, which in turn leads to destabilization of the podocyte actin cytoskeleton. The net effect is that CsA can stabilize the actin cytoskeleton in podocytes and thereby reduce proteinuria directly. Using sera collected from patients with FSGS recurrence, Fornoni et al[87] recently demonstrated that RTX, a monoclonal antibody directed against CD20 expressed on B-cells, also recognizes CD20 and binds to sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b) on podocytes. They confirmed that RTX prevented proteinuria in nephrotic patients by preserving SMPDL-3b expressed on podocytes and by preventing disruption of the actin cytoskeleton of the podocytes.

The recent findings discussed above suggest that the molecules expressed by podocytes in MCD are therapeutic targets for GCs, CsA, and RTX. Novel therapeutic agents directed against these molecules may support the stabilization and reconstruction of podocytes in MCD. For example, inhibition of CD80 expression on podocytes could be of therapeutic interest in MCD, FSGS, and glomerular diseases. Yu et al[88] recently reported treatment of five patients with biopsy-proven CD80 positive podocytes (four with FSGS recurrence and one with primary FSGS) with abatacept, which is a CTLA-4 agonist and CD80 inhibitor, resulting in sustained remission of NS in all cases. Considering both *in vitro* and *in vivo* findings, they suggested that CD80 interferes with the binding of talin to β-integrin, thereby preventing β-integrin activation.[88] However, other findings may not support this suggestion.[89,90]

Limitations to the hypotheses focusing on podocyte-related molecules

There are several limitations to the hypotheses focusing on podocytes. First, a variety of pathogenic mechanisms contribute to the development of MCD, and there is a growing body of evidence suggesting that pathogenic mechanisms in other cells are also involved, such as alterations in the NF-κB/inhibitory κB regulatory feedback loop of PBMCs,[91] increased

vascular permeability resulting from increased hemopexin production by the liver,[92,93] and increased production of oxygen radicals or nitric oxides by leukocytes.[49,94] It has been suggested that c-maf inducing protein (c-mip) increases in the podocytes in MCD, and that c-mip interferes with podocyte signaling by preventing the interaction of nephrin with the tyrosine kinase Fyn.[69] Second, there is still debate over whether MCD and FSGS represent different ends of the same disease spectrum with the same underlying pathophysiological processes, or whether they are two distinct disease entities.[4,5] There is no clear distinction between steroid-sensitive INS[61] and steroid-resistant INS (FSGS), and findings from studies of each of these conditions are extrapolated to the other condition. Third, importance of the role of CD80 in MCD has been proposed mainly by one group,[53,66-68,71,95,96] and has not been confirmed by other researchers. Finally, the finding that most patients with genetic FSGS did not benefit from CsA[97] suggests that CsA may not have a stabilizing effect. The proposed mechanism of action of RTX on podocytes may also need to be reconsidered in light of the recent suggestion that phagocytes and other inflammatory cells not only remove anti-CD20-opsonized B cells, but also remove autoreactive T-cells that interact with the autoantigen-presenting B-cells.[98]

Conclusions

Historically, MCD was commonly thought to be caused by T-cell dysfunction. However, recent evidence suggests that lymphocytes and podocytes are both involved in the pathogenesis of this condition, and that MCD results from interactions between humoral factors and dysfunctional podocytes. A biological agent such as an antibody against one or more of the molecules expressed by podocytes could provide a novel drug for the treatment of MCD, in addition to immunosuppressant drugs that target lymphocytes.

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ORIGINAL ARTICLE

Insignificant impact of VUR on the progression of CKD in children with CAKUT

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Abstract
Background Vesicoureteral reflux (VUR) is associated with an increased risk of kidney disorders. It is unclear whether VUR is associated with progression from chronic kidney disease (CKD) to end-stage kidney disease (ESKD) in children with congenital anomalies of the kidney and urinary tract (CAKUT).
Methods We conducted a 3-year follow-up survey of a cohort of 447 children with CKD (stage 3–5). Rates of and risk factors for progression to ESKD were determined using the Kaplan–Meier method and Cox regression respectively.
Results Congenital anomaly of the kidney and urinary tract was the primary etiology in 278 out of 447 children; 118 (42.4 %) had a history of VUR at the start of the cohort study. There were significantly more boys than girls with VUR, whereas the proportions were similar in children without VUR. The types of urinary anomalies/complications of the two groups were significantly different. Three-year renal survival rates of the groups were not significantly different, irrespective of CKD stage. Age <2 years and age after puberty, stage 4 or 5 CKD, and heavy proteinuria, but not history of VUR, were significantly associated with progression to ESKD.
Conclusions History of VUR at the start of follow-up was not associated with the progression of stage 3–5 CKD in children with CAKUT.

Keywords Chronic kidney disease · Cohort study · Congenital anomalies of the kidney and urinary tract · End-stage kidney disease · Vesicoureteral reflux

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Introduction

Congenital anomalies of the kidney and urinary tract (CAKUT), particularly hypoplastic and dysplastic kidneys, are the most common causes of advanced chronic kidney disease (CKD) in children. Children with hypoplastic and dysplastic kidneys often display other structural and physiological abnormalities in the urinary tract system, such as vesicoureteral reflux (VUR). It is probable that VUR predisposes children to urinary tract infections (UTIs) [1–4], although in children with normal kidneys, it was noted that childhood UTIs were unlikely to be associated with the development of CKD [3]. In some studies, VUR was reported to be associated with an increased risk of pyelonephritis, renal scarring, and end-stage kidney disease (ESKD) [5–7], while other studies have reported conflicting results [8–10]. Currently, however, the impact of VUR on the progression of CKD to ESKD in children remains controversial, and it is debated whether VUR is a benign or nonbenign condition [8, 9]. Indeed, some researchers have proposed that VUR is not a risk factor for renal scarring after UTI [10], while another report showed that children with higher-grade VUR (grade III or higher) were more likely to develop renal scarring than children with lower-grade VUR [5]. This controversy extends to the management and treatment of VUR and associated conditions/UTIs, and whether to administer prophylactic antibiotics to prevent recurrent UTIs or consider surgical correction of the ureterovesical junction responsible for VUR [11–13].

In 2010, we started a prospective study of a cohort of 447 Japanese children (aged 3 months to 15 years) with stage 3–5 CKD and reported that 62 % (n=278) of the children had CAKUT [14]. In a subsequent follow-up study, the 1-year renal survival rates, defined as cases that did not progress to ESKD or CKD-related death, were 98.3, 80.0, and 40.9 % for stage 3, 4, and 5 CKD respectively, and risk factors for ESKD were advanced CKD stage, age (<2 years and after the start of puberty), and severe proteinuria [15].

As part of the cohort study, we sent questionnaires to participating institutions to document the clinical characteristics of CKD patients, including history of VUR and its management, history of other urinary tract anomalies and subsequent complications, and history of UTIs. Therefore, the data obtained in this questionnaire provided a valuable opportunity to examine the association, if any, between congenital anomalies of the kidney and VUR. Because we have now accumulated 3 years of follow-up data for our initial cohort of 447 children with stage 3–5 CKD, we also assessed the outcomes of children with a history of VUR at 1 April 2010, namely at the start of the cohort study, in terms of the 3-year renal survival rate. Additionally, we evaluated whether VUR is associated with progression to ESKD. Our objective in this study was to examine the association between a history of VUR at the start of the follow-up and the progression of CKD to ESKD in children with congenital anomalies of the kidney.

Materials and methods

Study design and subjects

The study design and patient population are described in more detail in our previous reports [14, 15]. In August 2010, we sent surveys to 1,190 Japanese institutions asking them to report on their cases of pediatric CKD managed as of 1 April 2010. The first survey documented the number of children with stage 3–5 CKD at each institution. The respondents were asked to review their medical records to determine the numbers of patients with a confirmed diagnosis of CKD, or patients with abnormal serum creatinine values. A total of 925 out of 1,190 institutions (77.7 %) responded to the first questionnaire. In the second survey, questionnaires were sent to 130 institutions treating children with stage 3–5 CKD, as the remaining 795 institutions reported that they did not treat children with stage 3–5 CKD. Respondents were asked to record the clinical characteristics of their patients. In the second survey, 119 out of 130 institutions provided data for 479 children treated within 6 months of 1 April 2010. Of these, 447 children at 113 institutions were eligible, and 278 had a primary etiology of CAKUT [14, 15]. In the other 169 children, CAKUT was not the primary etiology. These children are referred to as children without CAKUT in this study. To determine pubertal stage, the patients were divided into three age groups for boys (<2, ≥2 to <10.8, and ≥10.8 years) and girls (<2, ≥2 to <10.0, and ≥10.0 years), where 10.8 and 10.0 years correspond to the mean age of Japanese boys and girls, respectively, at the start of puberty [16].

Survey on the etiology of chronic kidney disease

The second survey inquired about the factors that led to the discovery of CKD, the presence and type of CAKUT, what led to the detection of CAKUT, and the history/severity of VUR. Among 73 institutions that provided data on VUR assessments, 55 adopted the conventional bottom–up method, and 11 adopted the so-called top–down method (in which 99mTc-dimercaptosuccinic acid renal scans are performed before voiding cystourethrography) [17]. At the other 7 institutions, the method was selected based on the patient's situation. VUR was graded according to the International Classification of Vesicoureteral Reflux [4, 18], into unilateral VUR, low-grade bilateral VUR (with at least one side classified as mild, i.e., grades I and II), and high-grade bilateral VUR (with both sides classified as severe). For the purposes of the present study, we identified all patients with CAKUT and divided them according to the history of VUR, as documented in the second survey.

Survey on patient outcomes at 3 years

To assess the outcomes of patients at 3 years, another survey was sent to the participating institutions (n=113) in July 2013, with a deadline of September 2013. The survey recorded similar information to that recorded in our 1-year follow-up survey [15], and included patient characteristics, cardiac function, blood/urine parameters, renal outcomes, and CKD complications. The present survey also recorded urological complications and bladder dysfunction. As before, all surveys were to be returned using the envelopes provided and data entry was conducted by an independent data center (Japan Clinical Research Support Unit, Tokyo). In the third survey, 91 out of 113 institutions provided data for 384 of the 447 children covered by the second survey.

As previously described, stages 3, 4, and 5 CKD were defined as serum creatinine levels (measured enzymatically) more than twice, four times, and eight times respectively the median normal levels in age- and sex-matched Japanese children [14, 15, 19]. Using the Schwartz equation [20], we verified the accuracy of the classification, yielding a weighted κ-value of 0.71 (95 % confidence interval [CI] 0.65–0.77).

Statistical analyses

The characteristics of children with or without VUR were compared using unpaired t tests for continuous variables and Chi-squared tests for categorical variables. The 3-year renal survival rates were assessed using the Kaplan–Meier method, where death was also considered as an event. The date of measurement of serum creatinine closest to 1 April 2010 represented the starting point (i.e., t=0 years). The log-rank test was used to compare survival rates in patients with and those without VUR at each stage. Cox's proportional hazard regression model was used to identify possible predictors of CKD progression by calculating hazard ratios with 95 % confidence intervals. Values of $P<0.05$ were considered statistically significant. All statistical analyses were carried out using SAS system version 9 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the children with CAKUT

Of 278 children with CAKUT and stage 3–5 CKD, 60 children (21.6 %) had obstructive urological malformations, which included the posterior urethral valve, stricture of the urethra, hydronephrosis, hydroureter, and cloacal anomalies [14]. The characteristics of 278 children with CAKUT according to history of VUR are presented in Table 1. A history of VUR at 1 April 2010 was present in 118 children (42.4 %), and was absent in 115 children (41.4 %), or unknown/not evaluated in 45 children (16.2 %); data were not provided for 9 children (3.2 %). Among 118 children with VUR, 39 (33.3 %) were previously diagnosed with unilateral VUR, 10 (8.8 %) with low-grade bilateral VUR, and 59 (50.4 %) with high-grade bilateral VUR. The other 10 children were reported by the physician to have been diagnosed with VUR, but the classification was not stated. Among 169 children without a primary etiology of CAKUT, 6 had a history of VUR, of whom 3 had neurogenic bladder, 2 had nephronophthisis, and 1 had polycystic kidney disease. The mean ages at April 2010 were comparable in children with/without VUR. Furthermore, the distributions of stage 3–5 CKD were comparable in the two groups, as were the distributions of proteinuria, the use of antihypertensive drugs, and hypertension. However, there were significantly more boys than girls with VUR, whereas the proportions of boys and girls were approximately equal in children without VUR. Sixty children underwent surgical treatment for the correction of VUR. A large proportion of children with VUR had a history of UTI (66.9 %), and about half of the children with VUR had a history of≥2 episodes of UTIs. These percentages were significantly greater than those in children without VUR or in whom voiding cystourethrography was not performed. In addition, there were significant differences in the distribution of the types of urinary anomalies/complications between the groups, with hydronephrosis, megaureter, bladder dysfunction, and posterior urethral valve anomalies being significantly more frequent in children with VUR. Surgical treatment was performed in 58 children with VUR; the characteristics of children who did or did not undergo surgical treatment are presented in Supplementary Table 1.

The factors that led to the detection of CKD are listed in Table 1. Fetal/neonatal ultrasonography was the most common reason that led to the detection of CKD in both groups. However, the history of a UTI led to the detection of CKD in a significantly greater proportion of children with VUR than children without VUR (23.7 % vs 7.0 %, $P<0.001$). The rates of other factors that led to the detection CKD (e.g., incidental finding, failure to thrive, and blood analysis in the neonatal period) were comparable in the two groups.

Renal survival rates

The 3-year renal survival rates in children with CAKUT according to CKD stage are shown in Fig. 1a. As expected, renal survival rates declined with increasing CKD stage at the start of the survey. Figure 1b shows the renal survival rates according to CKD stage and history of VUR at 1 April 2010. The renal survival rates were not significantly different in children with VUR and children without VUR at each CKD stage. The renal survival rate was unaffected by the laterality or severity of VUR (Fig. 1c).

Table 1 Characteristics of children with congenital anomalies of the kidney and urinary tract (CAKUT) according to history of vesicoureteral reflux (VUR)

Variable	History of VUR		*P* value	Unknown VUR status
	Yes	No		
n	118	115		45
Sex, *n* (%)				
Male	91 (77.1)	58 (50.4)	<0.001*	26 (57.8)
Female	27 (22.9)	57 (49.6)		19 (42.2)
Age in April 2010, years	8.04±4.63	8.73±4.60	0.251**	7.23±4.27
Age at diagnosis, years	1.48±2.76	3.01±3.99	0.001**	2.08±3.25
CKD stage 3/4/5, *n* (%)				
Stage 3	77 (65.3)	79 (68.7)	0.432*	33 (73.3)
Stage 4	36 (30.5)	28 (24.4)		10 (22.2)
Stage 5	5 (4.2)	8 (7.0)		2 (4.4)
SCr (mg/dL)	1.66±1.13	1.66±1.24	0.960**	1.40±0.92
eGFR abbreviated (mL/min/1.73 m^2)[a]	38.30±16.98	39.79±16.69	0.513**	38.30±16.98
eGFR complete (mL/min/1.73 m^2)[b]	41.81±12.69	38.98±13.32	0.238**	41.81±12.69
History of UTI, *n* (%)	79 (66.9)	15 (13.0)	<0.001*	5 (11.1)
History of ≥2 UTIs, *n* (%)	56 (47.5)	9 (7.8)	<0.001*	2 (4.4)
Proteinuria (g/g creatinine)	1.23±2.66	0.94±1.01	0.376**	1.23±2.66
Heavy proteinuria, *n* (%)[c]	9 (7.6)	12 (10.4)	0.662*	4 (8.9)
Hypertension, *n* (%)[d]	21 (17.8)	20 (17.4)	0.916*	7 (15.6)
Use of antihypertensive drugs, *n* (%)	24 (20.3)	16 (13.9)	0.171*	1 (2.2)
Urinary anomalies/complications, *n* (%)				
Single kidney	18 (15.3)	19 (16.5)	0.791*	2 (4.4)
MCDK	3 (2.5)	9 (7.8)	0.068	2 (4.4)
Hydronephrosis	40 (33.9)	12 (10.4)	<0.001*	5 (11.1)
Megaureter	19 (16.1)	0 (0.0)	<0.001*	0 (0.0)
Bladder dysfunction	19 (16.1)	2 (1.7)	<0.001*	1 (2.2)
Posterior urethral valve	17 (14.4)	3 (2.6)	<0.001*	0 (0.0)
Duplication of pelvis and ureter	4 (3.4)	2 (1.7)	<0.426*	0 (0.0)
Factors leading to the detection of CKD, *n* (%)				
Fetal/neonatal ultrasonography	31 (26.3)	37 (32.2)	0.678*	11 (24.4)
UTI	28 (23.7)	8 (7.0)	<0.001*	3 (6.7)
Incidental finding	13 (11.0)	18 (15.7)	0.298*	8 (17.8)
Failure to thrive, weight loss, or general fatigue	9 (7.6)	13 (11.3)	0.337*	3 (6.7)
Blood analysis in the neonatal period, asphyxia, neonatal shock, or another event	9 (7.6)	9 (7.8)	0.955*	7 (15.6)

Values are *n* (%) or means±standard deviation

VUR vesicoureteral reflux, *CKD* chronic kidney disease, *SCr* serum creatinine, *eGFR* estimated glomerular filtration rate, *UTI* urinary tract infection, *MCDK* multicystic dysplastic kidney, *BUN* blood urea nitrogen

*Chi-squared test

***t* test

[a] Abbreviated Schwartz equation [20], eGFR=41.3 [height (m)/SCr (mg/dL)]

[b] Complete Schwartz equation [20], eGFR=39.1 [height (m)/SCr (mg/dL)]$^{0.516}$ ×[1.8/cystatin C (mg/L)]$^{0.294}$ ×[30/BUN (mg/dL)]$^{0.169}$ ×[1.099 if male]×[height (m)/1.4]$^{0.188}$

[c] Urine protein/creatinine ratio >2.0 g/g urine creatinine

[d] Systolic blood pressure >95th percentile

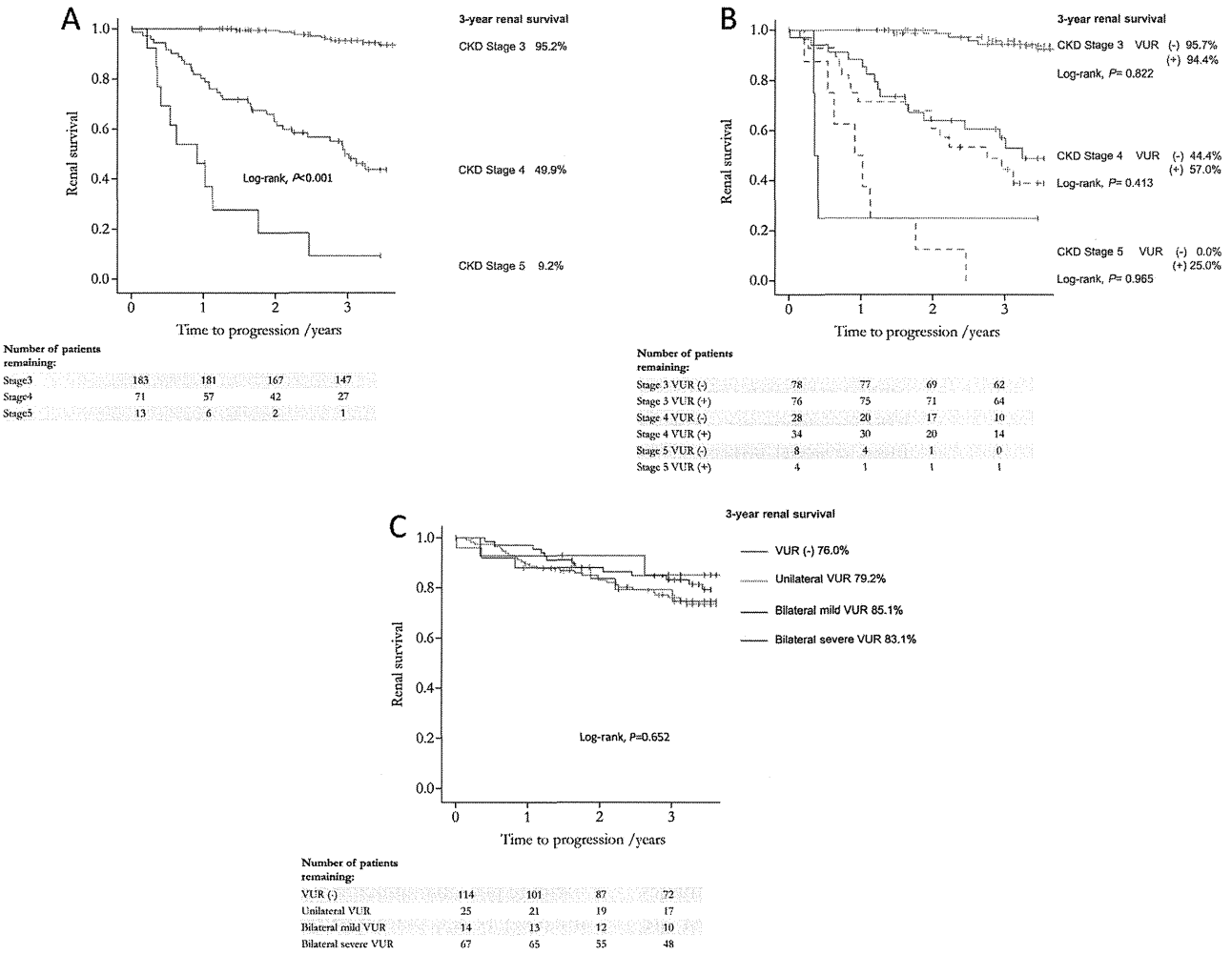

Fig. 1 Renal survival rates at 3 years according to the stage of chronic kidney disease (*CKD*) in patients with congenital anomalies of the kidney and urinary tract (CAKUT). **a** Renal survival rates according to CKD stage (n=267). **b** Renal survival rates according to the presence (n=114) or absence (n=114) of vesicoureteral reflux (*VUR*) for each CKD stage. *Solid lines* children with VUR [VUR (+)]; *dashed lines* children without VUR [VUR (−)]. **c** Renal survival rates according to the laterality and severity of VUR (n=220, those without information on VUR grade were excluded). Children were assessed for progression to end-stage kidney disease or death

Risk factors for progression to end-stage kidney disease

The risk factors for progression to ESKD at 3 years in 278 children with CAKUT, as determined by Cox regression, are shown in Table 2. Age <2 years and age after puberty (vs 2 years to the start of puberty), stage 4 or 5 CKD (vs stage 3 CKD), and heavy proteinuria (urine protein/creatinine ratio >2.0 g/g urine creatinine) were significant risk factors for progression to ESKD. Consistent with the Kaplan–Meier analysis of renal survival rates, the Cox regression model showed that the history of VUR was not associated with progression to ESKD (hazard ratio: 1.19; 95 % confidence interval: 0.53–2.64; P=0.675). Replacing history of VUR with the maximum severity of VUR (none, unilateral, bilateral mild, or bilateral severe) or latest VUR status at 1 April 2010 did not appreciably affect the associations observed (data not shown).

When we repeated this analysis only in children with a history of VUR, the risk factors for progression to ESKD included age and CKD stage, but not sex, heavy proteinuria, or history of surgical treatment of VUR (Supplementary Table 2).

Discussion

This 3-year prospective cohort study of children with stage 3–5 CKD caused by CAKUT revealed that the history of VUR at the start of follow-up did not influence the progression to ESKD in these children. However, there were significant differences in the sex distribution and the frequencies of urinary anomalies/complications between children with VUR and those without VUR. These features suggest that the main cause of kidney dysfunction and its progression in these

Table 2 Risk factors for end-stage kidney disease at 3 years (Cox regression model; n=278)

Variable	HR	95 % CI	*P* value
Female (vs male)	1.75	0.70–4.37	0.232
Age			
Age <2 years (vs 2 years to the start of puberty)	5.31	0.89–28.83	0.053
Age after puberty (vs 2 years to the start of puberty)	6.25	2.53–15.44	<0.001
CKD stage			
Stage 4 (vs stage 3)	37.45	11.56–121.31	<0.001
Stage 5 (vs stage 3)	249.38	43.20–1439.70	<0.001
History of VUR[a]	1.19	0.53–2.64	0.675
Heavy proteinuria[b]	5.08	1.98–13.05	<0.001

HR hazard ratio, *CI* confidence interval, *CKD* chronic kidney disease, *VUR* vesicoureteral reflux

[a] The associations did not change appreciably when history of VUR was replaced with the severity of VUR (none, unilateral, bilateral mild, or bilateral severe) or the maximum grade of VUR

[b] Urine protein/creatinine ratio >2.0 g/g urine creatinine

children might be the presence of a hypoplastic/dysplastic kidney, not VUR.

Notably, a history of VUR at the start of follow-up did not influence the progression of stage 3–5 CKD to ESKD in our cohort. However, it was of interest that there were several statistically significant and clinically relevant differences in the characteristics of children with VUR versus children without VUR. First, although the two groups of children were of similar age, there were significantly more boys than girls with VUR, whereas the proportions of boys and girls were approximately equal in children without VUR. Second, children with VUR were also more likely to have urinary tract anomalies/complications, including hydronephrosis and posterior urethral valve, compared with children without VUR. These differences, in addition to the nonsignificant effect of VUR on the progression of CKD, suggest that it might be important to consider the background etiology other than VUR, such as hypoplastic/dysplastic kidneys.

It is interesting that the majority of children with VUR had a history of UTIs, and about half of the children had a history of multiple UTIs, consistent with the notion that VUR might be a major risk factor for UTI [21–24]. However, despite the evidence supporting an association between UTIs and VUR, there is no conclusive evidence that either contributes to the progression of CKD to end-stage kidney disease in children. In addition, there is currently a limited consensus on how to treat UTIs, primary VUR, and associated anomalies in children [21, 23].

In the present study, risk factors for progression to ESKD included age <2 years and age after puberty (vs 2 years to the start of puberty), stage 4 or 5 CKD (vs stage 3 CKD), and heavy proteinuria (urine protein/creatinine ratio >2.0). These factors are identical to those identified in our previous study [15], which analyzed data on children with CKD with a 1.49-year follow-up period, and were confirmed to be risk factors in those with CAKUT with a longer follow-up period. Importantly, however, the 3-year renal survival rate was not markedly affected by a history of VUR at the start of follow-up in children with stage 3–5 CKD. These findings were also supported by the results of Cox regression, which further revealed that history of VUR was not a risk factor for progression to ESKD in this cohort of children. Our results suggest that VUR itself might not markedly affect the prognosis of children with CAKUT and CKD, addressing the controversy surrounding whether VUR is a benign or nonbenign condition, and helping to clarify the relationship between VUR and progression to ESKD in children with stage 3–5 CKD.

Some potential limitations warrant a mention. First, we did not observe the course of these children during stage 1–2 CKD. It is conceivable that VUR influences the emergence of CKD or progression through the early stages (i.e., stages 1–2). We must also consider that we used "history of VUR" (i.e., the diagnosis of VUR at any time) in the analyses examining the influence of VUR on the progression of CKD. Although similar results were obtained using the "severity of VUR" and the latest VUR status at the start of follow-up, many cases of VUR spontaneously resolve in clinical practice. Indeed, the severity of VUR is changeable over time; thus, the grade at any one time is difficult to know, especially given the invasiveness of the examination for VUR. Additionally, many children with a history of VUR underwent surgical treatment, which may have attenuated the pathological effects of VUR. However, the surgical treatment of VUR was not associated with progression to ESKD (Supplementary Table 2, Supplementary Figure). A similar limitation also applies to the analysis of UTIs, which was assessed as the history of UTIs before 2010. Another limitation is the duration of the survey period; 3 years may be too short to detect progression of CKD to ESKD in some children, depending on the cause of CKD.

In conclusion, about 40 % of children with CAKUT, who account for approximately 60 % of pediatric patients with stage 3–5 CKD, also had a history of VUR. However, the

history or severity of VUR was not associated with increased risk of progression of CKD to ESKD during a follow-up of 3 years. We found marked differences in the general and clinical characteristics of children with VUR vs children without VUR in terms of sex distribution and the proportions of children with urinary tract anomalies. Therefore, while children with CKD caused by CAKUT may have diverse backgrounds, the presence or absence of VUR may enable different backgrounds to be distinguished. Nevertheless, we observed no association between a history of VUR at the start of follow-up and the progression of CKD to ESKD in these children, which may implicate some background etiological factor other than VUR, such as the hypoplastic/dysplastic kidney itself, as the main cause of CKD and its progression to ESKD.

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Ethics The study was conducted in accordance with the principles of the Declaration of Helsinki and the ethical guidelines issued by the Ministry of Health, Labour, and Welfare, Japan. The study was approved by the ethics committee of the Tokyo Metropolitan Children's Medical Center (ID: 23–49). Because data were reported using patient medical records, informed consent was not obtained in accordance with the above guidelines.

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Conflict of interest Kenji Ishikura has received lecture fees from Novartis Pharma and Asahi Kasei Pharma. Osamu Uemura has received lecture fees from Asahi Kasei Pharma, Kyowa Hakko Kirin, Takeda Pharmaceutical, and Siemens Group in Japan. Yuko Hamasaki has received research grants from Novartis Pharma, and lecture fees from Novartis Pharma, Astellas Pharma, and Pfizer Japan. Hideo Nakai has received a research grant from Astellas Pharma. Ryojiro Yasuo Ohashi has received research grants from Kyowa Hakko Kirin and Chugai pharmaceutical. Tanaka has received lecture fees from Pfizer Japan and Asahi Kasei Pharma. Koichi Nakanishi has received lecture fees from Novartis Pharma, Asahi Kasei Pharma, and Astellas Pharma. Kazumoto Iijima has received research grants from Novartis and Pfizer Japan, and lecture fees from Novartis, Asahi Kasei Pharma, and Pfizer Japan. Masataka Honda has received lecture fees from Novartis Pharma, Asahi Kasei Pharma, Takeda Pharmaceutical, and Chugai Pharmaceutical. Drs Ito, Harada, Hattori, and Mr Kaneko have no conflicts of interest to declare.

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LETTER TO THE EDITOR

Mean and standard deviation of reference glomerular filtration rate values in Japanese children

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Keywords Children · Creatinine · Cystatin C · Glomerular filtration rate · Reference levels

To the Editor

We recently reported a study in *Clinical and Experimental Nephrology,* titled "Reference glomerular filtration rate levels in Japanese children: using the creatinine and cystatin C based estimated glomerular filtration rate" [1]. In this article, we reported the median, 2.5, and 97.5 percentile of glomerular filtration rate (GFR) reference values by age in 1137 Japanese children. Previously, we established serum creatinine (Cr)-based and cystatin C (cysC)-based estimated GFR (eGFR) equations for use in Japanese children and adolescents, aged 2–18 years [2], and 1 month–18 years including infants [3], respectively. In addition, we reported reference GFR levels in Japanese children using these eGFR equations in children aged 3 months–16 years, nonparametrically [1], using reference values of serum Cr [4] and cysC [5] in Japanese children. The medians of reference GFRs were 91.7, 98.5, 106.3, and 113.1 mL/min/1.73 m^2 in children aged 3–5 months, 6–11 months, 12–17 months, and 18 months–16 years, respectively (Table 1). The rise in GFR from birth to adulthood is well known, however, few reports give detailed GFR reference values in children by age. It is important for pediatricians who examine pediatric chronic kidney disease patients to know the values of normal renal function in children. Consequently, we provided this information.

Table 1 The median, 2.5, 97.5 percentile, average and standard deviation of GFR reference value in each age group between 3 months and 16 years

Age	*n*	2.5 percentile	50 percentile	97.5 percentile	Mean	SD
3–5 months	17	76.6	91.7	106.7	91.7	9.5
6–11 months	47	75.7	98.5	133.0	100.8	15.8
12–17 months	31	83.3	106.3	132.6	106.6	13.7
18 months–16 years	1042	83.5	113.1	156.7	115.2	18.3

However, we consider that we should formally announce the means and standard deviations of the GFR reference values, because we will use these data to know the *Z* scores of GFR parametrically in future studies. The means and standard deviations of reference GFRs were 91.7 and 9.5, 100.8 and 15.8, 106.6 and 13.7, and 115.2 and 18.3 mL/min/1.73 m^2 in children aged 3–5 months, 6–11 months, 12–17 months, and 18 months–16 years, respectively (Table 1). We hope these data will be used in the practice and research of pediatric CKD patients with the goal of high-quality diagnosis and treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that no conflict of interest exists.

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Review Article

Urinary screening and urinary abnormalities in 3-year-old children in Japan

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Abstract In Japan, urinary screening for preschool children has been obligatory since 1961. The system was reconsidered and has been under review since 2012, because many problems in the system had been identified, and its usefulness was uncertain. In the process, the following were analyzed: (i) frequency of urinary abnormalities identified on screening; (ii) diseases identified from urinary abnormalities; (iii) clinical course of children found to have urinary abnormalities; and (iv) screening for asymptomatic urinary tract infection (UTI) as a way of screening for congenital anomalies of the kidney and urinary tract. A computerized literature search was conducted, and study reports issued by the Ministry of Health, Labour and Welfare study group, and data of Akita City and Chiba City were reviewed. The prevalence of abnormal results at the first urinalysis was high, but at the second urinalysis the prevalence decreased in the range 1/6–1/20. The prevalence of tentative diagnosis at the third urinalysis was similar to the school urinary screening results. Serious illness was not found in children who had hematuria alone. In contrast, diseases requiring immediate attention were found in children with proteinuria, although the prevalence of proteinuria was not high. The dipstick method for leukocyturia was inefficient. The importance of two consecutive urinalyses before detailed examination, the lack of usefulness of screening for hematuria in 3-year-old children, and the importance of proteinuria were confirmed. Screening for asymptomatic UTI using urinary leukocytes was very inefficient.

Key words congenital anomalies of the kidney and urinary tract, end-stage renal disease, proteinuria, screening, three-year-old children.

In Japan, the Child Welfare Law, passed in 1961, mandated urinary screening for preschool children, usually 3-year-old children. The purpose of the urinary screening for preschool children was to prevent progression to end-stage renal disease (ESRD) or to ameliorate the quality of life of children who are expected to develop ESRD. To accomplish this, congenital anomalies of the kidney and urinary tract (CAKUT), other than glomerulonephritis, need to be detected in the early stage. This is because CAKUT comprised 40.7% of the primary diseases in patients aged <15 years who newly started dialysis in 2005 in Japan, and the percentage has been increasing.[1] Since 1961, the urinary screening has been conducted by each local government using their own methods, although the systems have been considered to have many problems. In 2008 and 2009, questionnaires about the urinary screening system were sent to persons in charge of child health in each local government in Japan and to councilors of The Japanese Society for Pediatric Nephrology.[2] The screening system protocol was found to differ from government to government, and, in many areas, children who screened positive for urinary abnormalities were advised only to go to an outpatient office. Thus, the final diagnoses of such children were not available, and it was not possible to evaluate the screening system of each area. Furthermore, many councilors answered that the current screening system was not very useful.

In this situation, it was necessary to reconsider the urinary screening system for preschool children to ensure that it can carry out its primary aim. In 2012, a study group under the Ministry of Health, Labour and Welfare was organized, and a review of the screening system was started, based on the data reported so far. In the process, the following were analyzed: (i) frequency of urinary abnormalities identified on screening; (ii) the diseases identified from urinary abnormalities; (iii) the clinical course of the children found to have urinary abnormalities; and (iv) screening for asymptomatic urinary tract infection (UTI) as a way of screening for CAKUT.

Urinary screening for preschool children is a system unique to Japan. The important points about the urinary screening system, which are also critical at clinical sites, and urinary abnormalities seen in the 3-year-old children in Japan are reported.

Methods

The first urinalysis was performed using dip-and-reagent strips. The criteria for +/− or + differed from area to area. Furthermore,

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