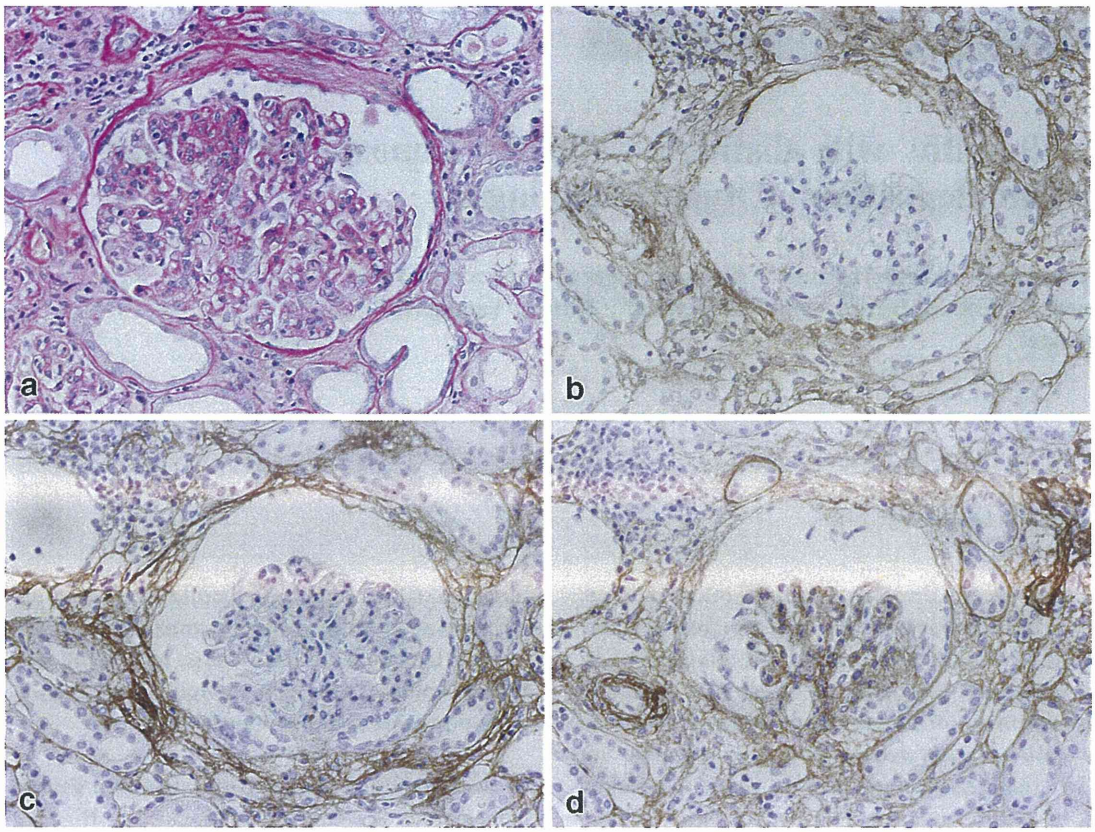

Fig. 1 Light microscopic findings and immunohistochemical demonstration of type I, III, and IV collagen. **a** Lobulation and enlargement of the glomerulus, occasional thickening of the glomerular capillary walls, and narrowing of the capillary lumen by swollen endothelial cells were observed. There was no glomerular deposition of type I (**b**) and III (**c**) collagen, whereas glomerular deposition of type IV (**d**) collagen was observed. The increased deposition of type I, III, and IV collagen was observed in the periglomerular and interstitial fibrotic lesions. **a** PAS staining, original magnification, ×200. **b**–**d** Serial sections, original magnification, ×200

abnormalities in the patellas or nails. Laboratory investigation revealed proteinuria of 6,170 mg/day and microhematuria. The concentration of hemoglobin was 13.0 g/dL; serum total protein, 5.6 g/dL; albumin, 2.9 g/dL; creatinine, 0.67 mg/dL; urea nitrogen, 13 mg/dL; uric acid, 6.7 mg/dL; and cholesterol, 315 mg/dL. Serum electrolytes, blood glucose, hemoglobin A1c (HbA1c), immunoglobulin A (IgA), IgM, C3, C4, and CH50 were within normal limits, but IgG was slightly decreased to 789 mg/dL. No evidence of auto-antibodies, HBV or HCV infection, cryoglobulinemia, Bence Jones proteinuria, or serum monoclonal peak was detected. Findings of chest and abdominal radiography were normal. Increases in plasma aldosterone concentration (PAC) to 17.6 ng/dL and PAC/plasma renin activity (PRA) ratio to 25.1, the lack of suppression of the PAC/PRA ratio of 25.5 at 2 h after administration of 50 mg of captopril, and the presence of a right adrenocortical adenoma 15 mm in diameter, as demonstrated by computed tomography, suggested the presence of primary aldosteronism, but the patient refused to undergo adrenal venous sampling. Nephrotic syndrome was diagnosed, and a percutaneous renal biopsy was performed.

Under light microscopy, the specimen showed 13 glomeruli. Of the 13, three glomeruli showed global obsolescence. The other glomeruli showed lobulation and enlargement, with occasional thickening and double contours of the glomerular capillary walls, and narrowing of the capillary lumen by swollen endothelial cells (Fig. 1a). Congo red staining was negative for amyloid. No significant intraglomerular fibrin deposition was noted by phosphotungstic acid hematoxylin (PTAH) staining. Immunofluorescence microscopy showed no depositions of IgG, IgA, IgM, C3, C1q, or κ and λ light chains. Electron microscopy showed fibrillar subendothelial and mesangial electron-dense deposits, mesangial cell interposition, and swelling and vacuolization of endothelial cells resulting in capillary lumen narrowing (Figs. 2, 3). Some deposits were also

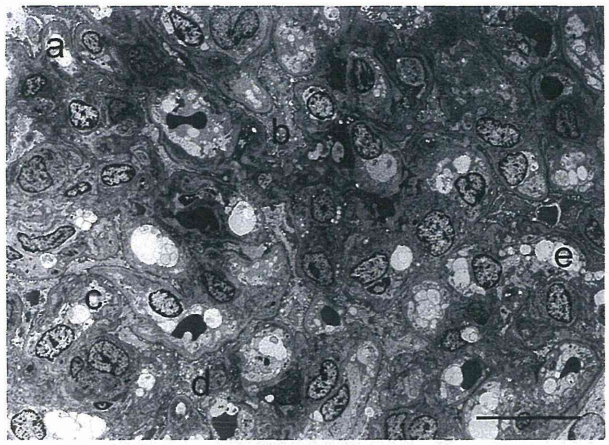

Fig. 2 Electron micrograph showing the distribution of subendothelial and mesangial electron-dense deposits at low magnification. Mesangial cell interposition, and swelling and vacuolization of endothelial cells resulting in capillary lumen narrowing were also noted. Some deposits were observed in the midportion of the glomerular basement membranes. The deposits found in the glomerular capillary area marked with *d* demonstrated the curvilinear fibrillar appearance. The fibrillar structures of the deposits in the five glomerular capillary areas, which were marked with *a–e*, are shown at high magnification in Fig. 3a–e, respectively. The length of the *bar* in the *lower right* represents 20 μm

observed in the midportion of the glomerular basement membranes. The banded structure was observed in some curvilinear fibrillar deposits at high magnification (Fig. 3f).

Immunohistochemical testing for type I, III, and IV collagen was conducted using the EnVision/HRP kit (Dako, Glostrup, Denmark) in consecutive kidney sections. Microwave irradiation was performed to enhance antigen retrieval. To stain type I and IV collagen, the samples were pretreated with proteinase K (Dako) at 37 °C for 45 min. The primary antibodies were mouse monoclonal anti-human type I collagen (Daiichi Fine Chemical Co., Takaoka, Japan), anti-human type III collagen (Daiichi Fine Chemical Co.), and anti-human type IV collagen (Dako) antibodies. There was no deposition of type I and III collagen in the glomeruli, although glomerular deposition of type IV collagen was observed in the sclerosing glomeruli (Fig. 1b–d). Increased deposition of type I, III, and IV collagen was also observed in the periglomerular and interstitial fibrotic lesions and in the glomerular tuft lesions adhesive to the Bowman's capsules.

The patient was treated with 500 mg/day of methylprednisolone pulse therapy for three consecutive days followed by 40 mg/day of prednisolone for 4 weeks. The patient's blood pressure was maintained at approximately 130/80 mmHg by combination therapy consisting of imidapril hydrochloride, nifedipine, furosemide, and spironolactone. However, the nephrotic syndrome persisted, and the serum level of creatinine increased progressively. The dose of prednisolone was decreased gradually and hemodialysis was initiated 23 months after the renal biopsy. The serum levels of procollagen III peptide were <0.5, 0.9, and 1.0 U/mL (normal range: <1.0 U/mL) at 12, 18, and 22 months after renal biopsy, respectively; we did not measure serum procollagen III peptide at the time of the renal biopsy.

Discussion

Renal diseases with organized deposits are divided into those with amyloid origins and those with non-amyloid origins. Among those with non-amyloid origins, the deposits which are composed of immunoglobulin components are seen in fibrillary glomerulonephritis, immunotactoid glomerulopathy, cryoglobulinemic glomerulonephritis, and light and/or heavy chain-deposition disease; those without immunoglobulin components are seen in fibronectin glomerulopathy, nail–patella syndrome, collagenofibrotic glomerulopathy, fibrin tactoids in inflammatory glomerular diseases, and diabetic nephropathy [1, 2]. The electron-dense deposits in fibronectin glomerulopathy, an autosomal dominant hereditary disorder characterized by large mesangial and subendothelial electron-dense deposits composed of fibronectin, are predominantly amorphous and granular, but they may contain electron-lucent areas and scattered, focal fine filaments 10–14 nm in diameter [3, 4].

The deposition of banded fibrils of type III collagen is noted in nail–patella syndrome and collagenofibrotic glomerulopathy [1]. Nail–patella syndrome is a hereditary disorder caused by a genetic mutation of LMX1B, an LIM-homeodomain transcription factor that plays a key role in limb development [5]; it is clinically characterized by the association of nail hypoplasia or dysplasia, absence or delayed development of the patella, dysplasia of the knees and elbows, and iliac horns. Bundles of fibrillar type III collagen are usually deposited in the midportion of the glomerular basement membrane, but in some patients, deposits are found in the subepithelial or subendothelial space. However, the possibility of nail–patella syndrome was excluded in the present case because there were no deformities of the nails and bones.

Collagenofibrotic glomerulopathy, also called collagen type III glomerulopathy, is a rare disease characterized by accumulation of curled subendothelial and mesangial deposits containing type III collagen fibrils. It is associated with a marked elevation of serum procollagen III peptide [6], an indicator of increased type III collagen synthesis [7, 8]. Most adult cases are sporadic, and many have been reported in Japan, suggesting a geographical or racial predilection [1, 6, 9]. Other cases have been reported, mainly from Europe, of children with an autosomal

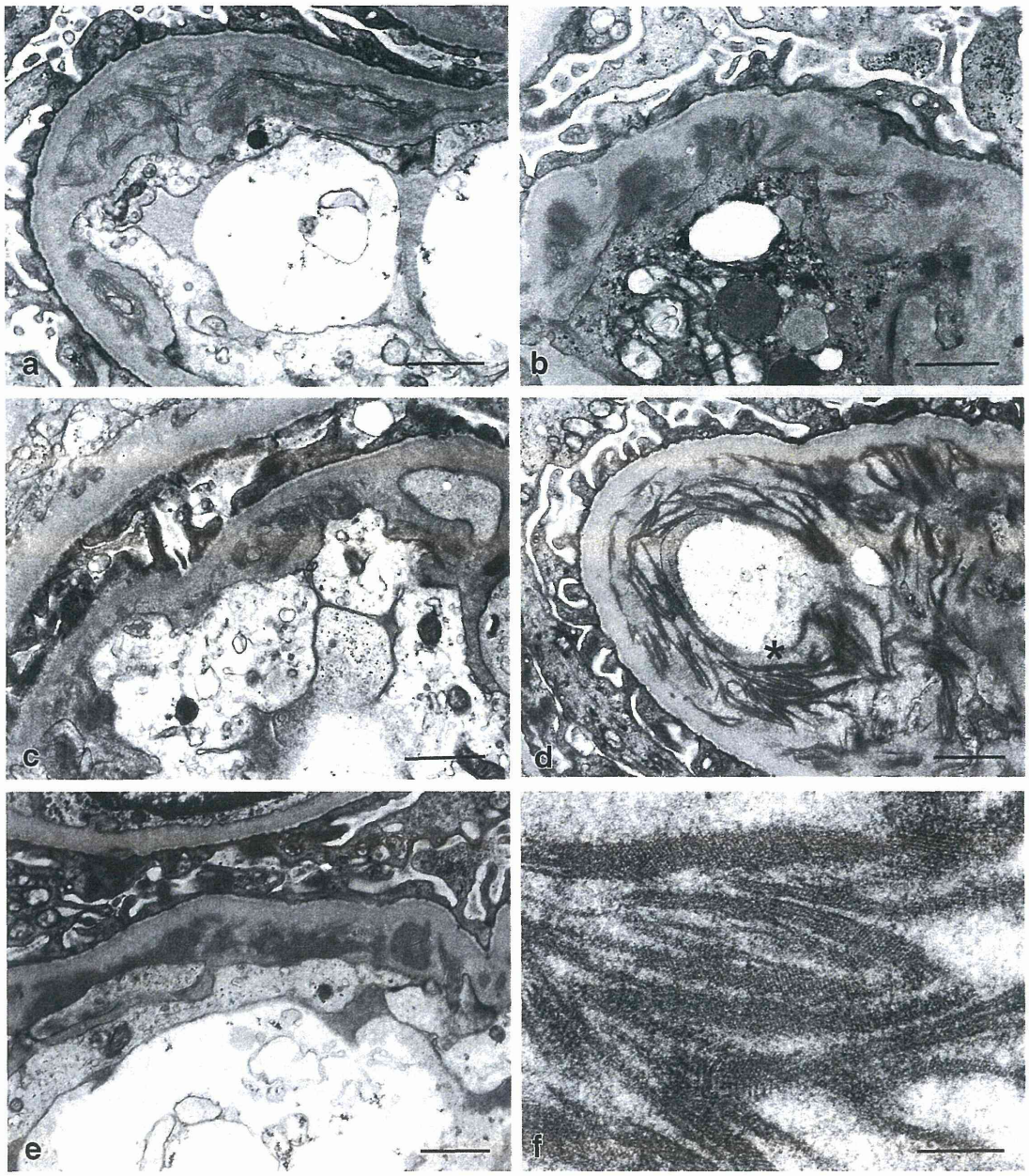

Fig. 3 Electron micrographs showing fibrillar structures of the deposits in the glomerular capillary areas marked with **a**–**e** in Fig. 2 are shown at high magnification in **a**–**e**, respectively. **f** Higher magnification of the curvilinear fibrillar deposits marked with an *asterisk* in **d** revealed the banded structure in the fibrils. The length of the *bars* in the *lower right* in **a**–**e** represents 1 μm and that in **f** represents 200 nm

recessive mode of transmission and early disease progression, which suggests a difference in gene penetrance in different populations [10–12]. One case with collagenofibrotic glomerulopathy has been associated with factor H deficiency [13]. There is no evidence of a sex predilection.

Although some banded fibrils in the present case mimicked the type III collagen fibers seen in collagenofibrotic glomerulopathy, no significant glomerular deposition of type I or III collagen was observed despite the increased deposition of type IV collagen in the sclerosing glomeruli and that of type I, III, and IV collagen in the periglomerular and interstitial fibrotic lesions and the glomerular tuft lesions adhesive to Bowman's capsule. Glomerular accumulation of type I collagen has also been demonstrated in collagenofibrotic glomerulopathy [14, 15]. One case of collagenofibrotic glomerulopathy with massive glomerular deposition of both

type III and V collagen has been reported [16]. However, the present case differs from collagenofibrotic glomerulopathy because of the lack of glomerular type III collagen deposition; we did not investigate type V collagen deposition. In addition, the serum procollagen III peptide level is increased to 10–100 times the normal level in patients with collagenofibrotic glomerulopathy, and it is nearly doubled in patients with chronic renal failure [6, 11]; however, no significant increases in procollagen III peptide levels were noted in the present case. Taken together, this glomerulopathy did not fulfill the diagnostic criteria for collagenofibrotic glomerulopathy.

There may be deposits of fibrin in inflammatory glomerular diseases, and the polymerized fibrin usually forms amorphous electron-dense masses. Rarely, there are fibrin tactoids with characteristic periodicity [2]. Fibrin tactoids were excluded in our case because no significant intraglomerular fibrin deposition was demonstrated by PTAH staining. Our patient had no clinical manifestation of diabetes mellitus. Recently, Ohtani et al. reported a case with progressive glomerulopathy with unusual organized deposits of non-amyloid non-immunoglobulin origins with striated structures [17]. However, most striated deposits were lumpy and only a few deposits showed fibrillar structure in their case.

This patient had a longstanding history of smoking and hypertension. In the smoking-related nodular or diffuse mesangial glomerulosclerosis, the endothelial cell injuries which resemble chronic thrombotic microangiopathy characterized by endothelial swelling, subendothelial widening, new basement membrane formation and cellular interposition have been demonstrated [18, 19]. Although no organized deposits are generally seen in smoking-related glomerulopathy, Salvatore et al. recently found a case with focal fibrillary degeneration of the collagen matrix along the subendothelial zone out of 10 cases with smoking-related glomerulopathy [19]. However, the possibility that the fibrillar deposits in the present case were relevant to the fibrillary degeneration of the collagen matrix in smoking-related diffuse mesangial glomerulosclerosis is unlikely because of the lack of intraglomerular deposition of types I and III interstitial collagen.

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Compliance with ethical standards

Conflict of interest All the authors have declared no competing interest.

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ORIGINAL ARTICLE

Transition of adolescent and young adult patients with childhood-onset chronic kidney disease from pediatric to adult renal services: a nationwide survey in Japan

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Abstract

Background Transition of adolescent and young adult (AYA) patients with childhood-onset chronic kidney diseases (C-CKD) from pediatric to adult renal services has received increasing attention. However, information on transition of Japanese patients with C-CKD is limited.

Methods The Transition Medicine Working Group, in collaboration with the Japanese Society for Nephrology, the Japanese Society for Pediatric Nephrology and the Japanese Society of Pediatric Urology, conducted a retrospective cross-sectional study in 2014 on issues concerning the transition of Japanese patients with C-CKD.

Results Few institutions in Japan had transition programs and/or transition coordinators for patients with C-CKD. Refusal to transfer by patients or their families, lack of concern about transition and inability to decide on transfer were common reasons for non-transfer of patients still followed by pediatric renal services. Around 25 % of patients who had ended or interrupted follow-up by pediatric renal services presented to adult renal services because of symptoms associated with C-CKD. Patients with various types of childhood-onset nephrourological diseases were transferred from pediatric to adult renal services. IgA nephropathy, minimal change nephrotic syndrome and congenital anomalies of the kidney and urinary tract were the most frequent primary kidney diseases in adult patients with C-CKD.

Conclusion These survey results indicate the need for introduction of transitional care for Japanese AYA patients with C-CKD. Consensus guidelines for the optimal clinical management of AYA patients with C-CKD are required to ensure the continuity of care from child to adult renal services.

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Keywords Transition · Childhood-onset chronic kidney disease · Adolescent and young adult patients · Pediatric renal services · Adult renal services

Introduction

Transition is a process involving purposeful, planned efforts to prepare pediatric patients to move from caregiver-directed care to disease self-management as adults [1]. This transition process has been shown important in the management of childhood-onset chronic diseases [2, 3]. Transition of adolescent and young adult (AYA) patients with childhood-onset chronic kidney diseases (C-CKD) from pediatric to adult renal services has also attracted a good deal of attention in the field of nephrology [4, 5]. Recently, the International Society of Nephrology (ISN) and the International Pediatric Nephrology Association (IPNA) developed a consensus statement on the transition of C-CKD patients from pediatric to adult renal services [6]. Since health-care systems vary across countries, the consensus statement by the ISN and IPNA may provide a basis for the development of locally appropriate recommendations for clinical practice [6].

To better apply this consensus statement to Japanese AYA patients with C-CKD, information is needed on the transition of Japanese patients with C-CKD from pediatric to adult renal services. To date, however, information on this transition in Japanese patients with C-CKD is limited [7]. To clarify the issues related to transition of Japanese AYA patients with C-CKD, the Transition Medicine Working Group, in collaboration with the Japanese Society for Nephrology (JSN), the Japanese Society for Pediatric Nephrology (JSPN) and the Japanese Society of Pediatric Urology (JSPU), conducted a nationwide survey of Japanese patients with C-CKD. This report describes the basic information on the transition of these patients.

Patients and methods

Data collection

The Transition Medicine Working Group, in collaboration with JSN, JSPN and JSPU, conducted a retrospective cross-sectional study of Japanese patients with C-CKD aged >20 years in October 2014.

Questionnaires were collected in two steps. The first questionnaires were sent to institutions at which council members of the JSN, JSPN, and JSPU practiced. As shown in Table 1, these questionnaires asked whether there were patients with C-CKD aged >20 years, as well as whether there were transition programs and/or transition coordinators, on September 30, 2014. The second questionnaires were sent to the institutions that reported having patients with C-CKD aged >20 years. These questionnaires asked for data on individual patients, included their primary kidney disease, age at transfer, reasons for transfer or non-transfer, reasons for presenting at adult renal services after ending or interrupting follow-up at pediatric renal services, and patient educational level and employment status.

Patients with C-CKD aged >20 years were divided into a transfer and a non-transfer group. Patients in the transfer group were those transferred from pediatric renal services to adult renal services with medical, social, and/or educational information prepared by pediatric renal services. Patients in the non-transfer group were those who were not transferred from pediatric to adult renal services. This group included patients still followed by pediatric renal services and those who had ended or interrupted follow-up by pediatric renal services, but later presented to adult renal services without medical, social, and/or educational information prepared by pediatric renal services.

This survey was in accordance with the ethical principles in the 1964 Declaration of Helsinki, and with the ethical guidelines for epidemiological studies issued by the Ministry of Health, Labour and Welfare of Japan. The survey was also approved by the central ethics board of Tokyo Women's Medical University (approval number; 3186) before study commencement.

Results

Patients collected in this survey

The first questionnaires were sent to 401 institutions, with 208 (51.9 %) responding; of these, 146 institutions had patients with C-CKD aged >20 years. The second questionnaires were therefore sent to these 146 institutions, with 117 (80.1 %) responding.

These institutions reported a total of 3138 patients, divided into a transfer group ($n = 1260$) and a non-transfer group ($n = 1878$), as shown in Fig. 1. Of the 1260 patients in the transfer group, 735 were cited by pediatric renal services and 525 by adult renal services. Of the 1878 patients in the non-transfer group, 1631 were still followed by pediatric renal services, whereas the other 247 had ended or interrupted follow-up by pediatric renal services, but later presented to adult renal services.

Transition program and transition coordinator

Of 101 responding pediatric institutions, four (4.0 %) had transition programs for patients with C-CKD and three

Table 1 First questionnaires

First questionnaires to pediatric renal services
1. Do you have patients with C-CKD aged >20 years who were transferred from pediatric renal services to adult renal services with medical, social, and/or educational information prepared by pediatric renal services during the periods of January 1, 2010 to September 30, 2014?
2. Do you have patients with C-CKD aged >20 years who were still followed by pediatric renal services at the time of September 30, 2014?
3. Do you have any transition programs and/or transition coordinators at the time of September 30, 2014?
First questionnaires to adult renal services
1. Do you have patients with C-CKD aged >20 years who were transferred from pediatric renal services to adult renal services with medical, social, and/or educational information prepared by pediatric renal services at the time of September 30, 2014?
2. Do you have patients with C-CKD aged >20 years who had ended or interrupted follow-up by pediatric renal services, but later presented to adult renal services without medical, social, and/or educational information prepared by pediatric renal services at the time of September 30, 2014?
3. Do you have any transition programs and/or transition coordinators at the time of September 30, 2014?

The first questionnaires were sent to institutions at which council members of the Japanese Society for Nephrology (JSN), the Japanese Society for Pediatric Nephrology (JSPN), and the Japanese Society of Pediatric Urology (JSPU) practiced

C-CKD childhood-onset chronic kidney diseases

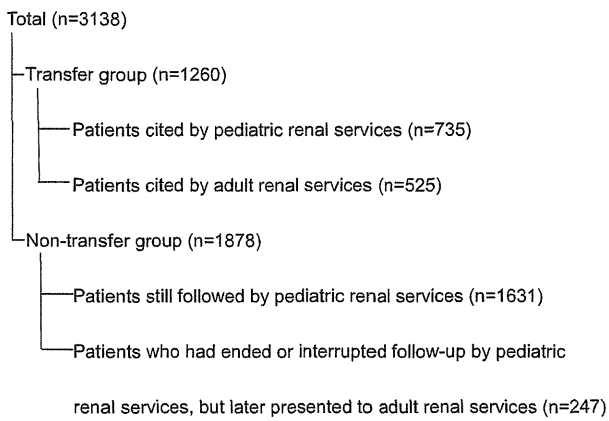

Fig. 1 Patients involved in this survey

(3.0 %) had transition coordinators within their pediatric renal services. Of 107 adult institutions, none had transition programs for patients with C-CKD and only one (0.9 %) had a transition coordinator within their adult renal services.

Primary kidney disease in the transfer and non-transfer groups

Primary kidney diseases were categorized as (1) primary glomerulonephritis (GN) or nephrotic syndrome (NS); (2) secondary GN, vasculitis and hemolytic uremic syndrome (HUS); (3) congenital anomalies of the kidney and urinary tract (CAKUT); (4) tubulointerstitial nephritis (TIN); (5) hereditary and metabolic diseases; (6) hypertension; (7) urologic diseases and tumors, and (8) other conditions. Primary kidney diseases of the transfer ($n = 1260$) and non-transfer ($n = 1878$) groups are shown in Table 2.

The most frequent primary kidney disease in both the transfer (23.5 %) and non-transfer (22.6 %) groups was IgA nephropathy (IgAN), followed by minimal change NS (16.7 and 15.0 %, respectively), CAKUT (12.9 and 10.7 %, respectively), focal segmental glomerulosclerosis (5.3 and 5.4 %, respectively), lupus nephritis (4.7 and 3.8 %, respectively), IgA vasculitis nephritis (3.8 and 3.5 %, respectively) and Alport syndrome (3.9 and 3.2 %, respectively).

Age at transfer in the transfer group patients

The distribution of age at transfer from pediatric to adult renal services in the transfer group patients enrolled in this study ($n = 1260$) is shown in Fig. 2. The peak age at transfer was 20–24 years, with 65.5 % of these patients transferred from pediatric to adult renal services before age 25 years. The remaining 34.5 % were transferred after age 25 years.

Current ages of non-transfer group patients still followed by pediatric renal services

Current ages of non-transfer group patients who were still followed by pediatric renal services ($n = 1631$) are shown in Fig. 3. The peak of age was 20–24 years old, with the number of patients decreasing with age. Of this group, 43.3 % were followed by pediatric renal services after age 25 years.

Reasons for transfer in the transfer group patients

The reasons for transfer from pediatric to adult renal services in the transfer group patients enrolled in this

Table 2 Primary kidney diseases in patients included in this survey

	Transfer, *n* (%)	Non-transfer, *n* (%)
Primary GN·NS	718 (57.1)	1115 (59.3)
IgA nephropathy	295 (23.5)	425 (22.6)
Mesangioproliferative GN	22 (1.7)	46 (2.4)
Membranoploliferative GN	25 (2.0)	42 (2.2)
Membranous GN	1 (0.1)	19 (1.0)
Crescentic GN	3 (0.2)	3 (0.2)
Dense deposit disease	3 (0.2)	2 (0.1)
Minimal change NS	211 (16.7)	281 (15.0)
Focal segmental glomerulosclerosis	67 (5.3)	102 (5.4)
Other	91 (7.2)	195 (10.4)
Secondary GN·vasculitis·HUS	129 (10.2)	172 (9.2)
Lupus nephritis	59 (4.7)	71 (3.8)
IgA vasculitis nephritis	48 (3.8)	65 (3.5)
ANCA- mediated GN	3 (0.2)	13 (0.7)
HUS	8 (0.6)	8 (0.4)
Atypical HUS	3 (0.2)	0 (0.0)
Other	8 (0.6)	15 (0.8)
CAKUT	162 (12.9)	201 (10.7)
Hypoplastic/dysplastic kidney	83 (6.6)	116 (6.2)
Reflux nephropathy	50 (4.0)	42 (2.2)
Obstructive nephropathy	9 (0.7)	12 (0.6)
Other	20 (1.6)	31 (1.7)
TIN	7 (0.6)	21 (1.1)
Drugs	3 (0.2)	7 (0.4)
Autoimmune	0 (0.0)	2 (0.1)
Infection	1 (0.1)	0 (0.0)
Other	4 (0.2)	12 (0.6)
Hereditary·metabolic	150 (11.9)	198 (10.5)
Alport syndrome	49 (3.9)	61 (3.2)
Autosomal dominant PKD	10 (0.8)	12 (0.6)
Autosomal recessive PKD	5 (0.4)	3 (0.2)
PKD	3 (0.2)	8 (0.4)
Nephronophthisis	5 (0.4)	17 (0.9)
Congenital nephrotic syndrome	4 (0.3)	7 (0.4)
Denys-Drash syndrome	1 (0.1)	4 (0.2)
Prune belly syndrome	2 (0.2)	1 (0.1)
Cystinosis	2 (0.2)	1 (0.1)
Fabry disease	1 (0.1)	2 (0.1)
Nail-Patella syndrome	2 (0.2)	0 (0.0)
Other	66 (5.2)	82 (4.4)
Hypertension	5 (0.4)	13 (0.7)
Renovascular	3 (0.2)	10 (0.5)
Other	2 (0.2)	3 (0.2)
Urologic·Tumor	23 (1.8)	61 (3.2)
Neurogenic bladder	19 (1.5)	39 (2.1)
Urolithiasis	2 (0.2)	11 (0.6)
Wilms tumor	2 (0.2)	5 (0.3)
Others	0 (0.0)	6 (0.3)
Miscellaneous	27 (2.1)	66 (3.5)

Table 2 continued

	Transfer, *n* (%)	Non-transfer, *n* (%)
Cortical necrosis	3 (0.2)	6 (0.3)
Other	24 (1.9)	60 (3.2)
Unknown	13 (1.0)	13 (0.7)
Missing	26 (2.1)	18 (1.0)
Total	1260 (100)	1878 (100)

GN glomerulonephritis, *NS* nephrotic syndrome, *HUS* hemolytic uremic syndrome, *ANCA* anti-neutrophil cytoplasmic autoantibody, *CAKUT* congenital anomalies of the kidney and urinary tract, *TIN* tubulointerstitial nephritis, *PKD* polycystic kidney disease

Fig. 2 Age at transfer from pediatric to adult renal services in transfer group patients ($n = 1260$)

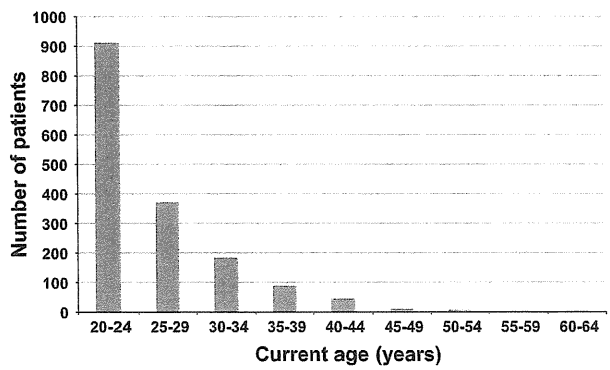

Fig. 3 Current age of non-transfer group patients who were still followed by pediatric renal services ($n = 1631$)

study ($n = 1260$) are shown in Fig. 4. Proposed transfer by pediatric nephrologists and pediatric urologists was the most common reason. Life events, including employment, furtherance of education at a higher level, moving to new addresses, marriage and pregnancy, were also common reasons for transfer. In contrast, only 11.2 % of these patients reported a desire of patients or their families to transfer from pediatric to adult renal services.

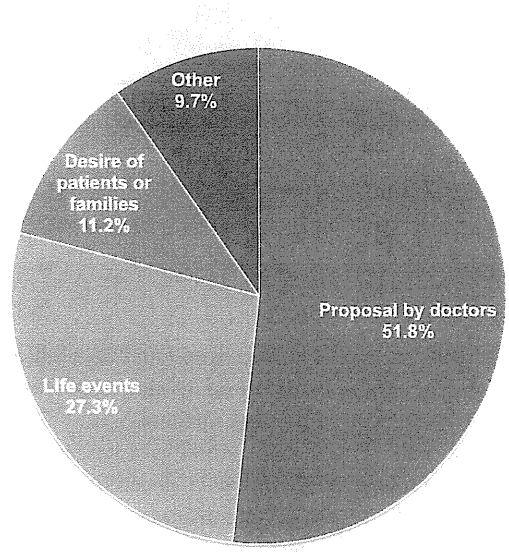

Fig. 4 Reasons for transfer from pediatric to adult renal services in transfer group patients ($n = 1260$)

Reasons for inability to transfer from pediatric to adult renal services

Figure 5 shows the reasons that non-transfer group patients still followed by pediatric renal services ($n = 1631$) were unable to transfer from pediatric to adult renal services. The most common reason was refusal of patients or their families to transfer, followed by lack of concern about transition, and inability of pediatric nephrologists and urologists to decide on transfer. For 14.1 % of these patients, pediatric nephrologists and urologists believed that there were no appropriate adult nephrologists to oversee the transfer. In addition, 3.6 % of patients were unable to transfer because of their extra-renal comorbidities, including mental retardation.

Reasons for presenting at adult renal services after ending or interrupting follow-up by pediatric renal services

The survey identified 247 patients in the non-transfer group who had ended or interrupted follow-up by

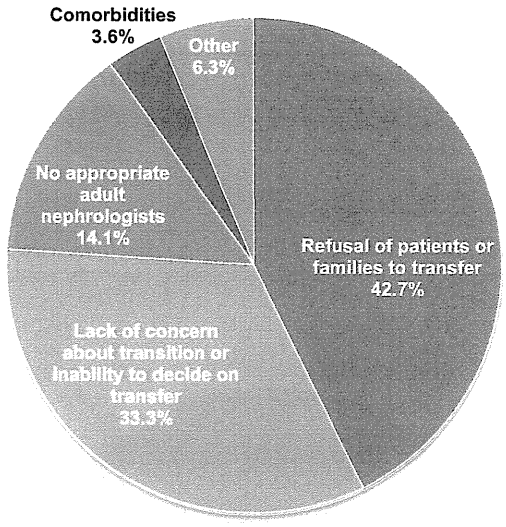

Fig. 5 Reasons for inability to transfer from pediatric to adult renal services in patients still followed by pediatric renal services ($n = 1631$)

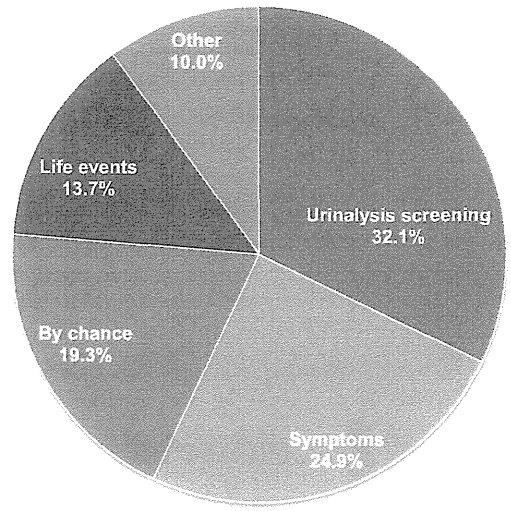

Fig. 6 Reasons for presenting at adult renal services in patients who had ended or interrupted follow-up by pediatric renal services, but later presented to adult renal services ($n = 247$)

pediatric renal services, but later presented to adult renal services. The reasons for presenting at adult renal services are shown in Fig. 6. Of these patients, 32.1 % were rediagnosed with C-CKD by urinalysis screening and 19.3 % were rediagnosed by chance. In addition, 13.7 % presented to adult renal services in association with life events, including furtherance of education at a higher level, moving to new addresses, marriage and pregnancy. Noteworthy, 24.9 % of these patients presented to adult renal services because of the symptoms associated with their C-CKD, including relapse of NS, edema, and worsened kidney function.

Educational levels

Since adult renal services had insufficient information about patients' educational levels, the latter was evaluated using data from pediatric renal services. The educational levels of patients in the transfer ($n = 735$) and non-transfer ($n = 1631$) groups are shown in Fig. 7. Of the patients in these two groups, 42.9 and 44.3 %, respectively, were in college or graduate school.

Employment status

Employment status of patients in the transfer ($n = 1260$) and non-transfer ($n = 1878$) groups was evaluated using data from both pediatric and adult renal services (Fig. 8). Of the patients in these two groups, 20.7 and 24.0 %, respectively, were unemployed at the time of this survey.

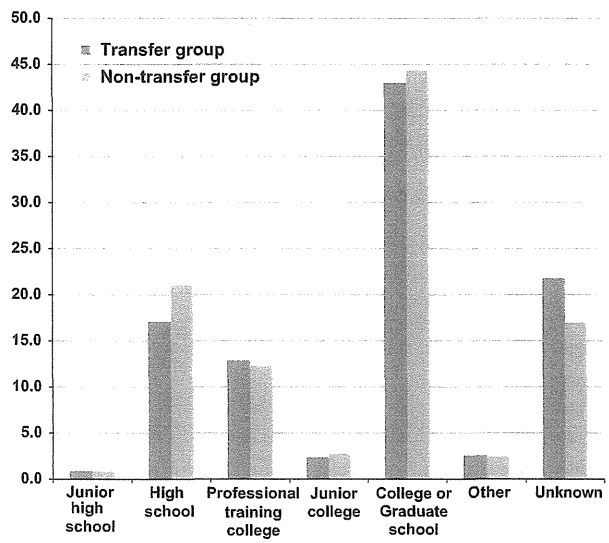

Fig. 7 Educational levels of patients in the transfer ($n = 735$) and non-transfer ($n = 1631$) groups. Since adult renal services had insufficient information about patients' educational levels, evaluation was performed using data from pediatric renal services

Discussion

This nationwide survey throughout Japan was conducted in collaboration with pediatric nephrologists, pediatric urologists and adult nephrologists. Because good communication between pediatric and adult services is vital to ensure a successful transition of AYA patients [6], the present nationwide survey may provide important information on

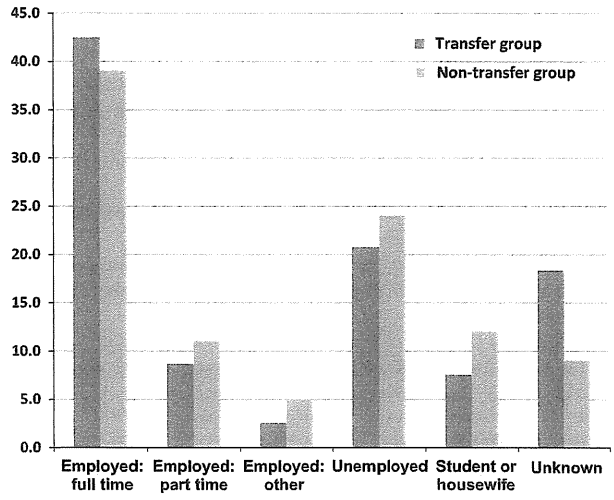

Fig. 8 Employment status of patients in the transfer ($n = 1260$) and non-transfer ($n = 1878$) groups

beginning and facilitating transitional care for AYA patients with C-CKD in Japan.

This survey showed that few institutions in Japan had transition programs and/or transition coordinators for patients with C-CKD. Although it is recognized the adolescent/young adult years embrace ages 14–24 years in terms of brain development and maturation [6], 34.5 % of the transfer group patients in this survey were transferred from pediatric to adult renal services after age 25 years, and 43.3 % of patients in the non-transfer group were still being followed by pediatric renal services after age 25 years. Only 11.2 % of patients in the transfer group reported transferring from pediatric to adult renal services because of the desire of patients or their families for transfer. In contrast, the most common reasons for non-transfer in non-transfer group patients still being followed by pediatric renal services included refusal of patients or their families for transfer, lack of concern about transition and inability to decide on transfer. Moreover, 24.9 % of patients who had ended or interrupted follow-up by pediatric renal services presented to adult renal services because of the symptoms associated with their C-CKD. Taken together, these results indicate the need for introduction of transitional care for Japanese AYA patients with C-CKD. Since successful transition requires communication and collaboration among pediatric and adult subspecialists, nurses, other health-care professionals, as well as patients and their families [2, 5], the consensus statement on health-care transition from pediatric to adult renal services, which was recently crafted by the Transition Medicine Working Group in collaboration with the JSN and JSPN [8], may provide a basis for the development and promotion of transitional care for AYA patients with C-CKD in Japan.

The present survey also showed that patients with various types of childhood-onset nephrourological diseases were transferred from pediatric to adult renal services, although adult nephrologists are likely less familiar with congenital anomalies and hereditary conditions [6]. IgAN, minimal change NS and CAKUT were the most frequent primary kidney diseases in adult patients with C-CKD, both among those who were and were not transferred from pediatric to adult renal services in Japan. The biggest problem in transitional care is the treatment gap between pediatric and adult services [9]. In fact, the difference in the steroid regimen between pediatric and adult patients with steroid-sensitive NS has been reported to be a barrier to transition [7]. Additionally, the biggest danger in transition is the avoidable loss to follow-up and care of AYA patients with potentially reversible diseases [10]. Therefore, consensus guidelines for the optimal clinical management of AYA patients with C-CKD are required to ensure the continuity of care from child to adult renal services.

Transition is an active, multifaceted process that attends to the medical, psychosocial, and educational needs of AYA patients as they move from child-focused to adult-focused healthcare [1]. Transition is also regarded as a pathway to employment for AYA patients with chronic health conditions and other disabilities [11]. The present survey did not include medical and psychosocial information, but asked about educational levels and employment status of Japanese patients with C-CKD. The survey results showed that around 43–44 % of patients with C-CKD had attended colleges and graduate schools, whereas around 21–24 % were unemployed at the time of this survey. The percentage of survey patients in colleges and graduate schools was slightly lower than in the Japanese general population (about 50 %) [12], whereas the rate of unemployment in survey patients was higher than in the Japanese general population (about 7–9 %) [13]. Further studies are needed to examine the effects of transitional care on long-term outcomes, including medical, psychosocial, educational, and employment outcomes, in AYA patients with C-CKD [14].

In conclusion, the present nationwide survey results indicate the need for introduction of transitional care for Japanese AYA patients with C-CKD. Consensus guidelines for the optimal clinical management of AYA patients with C-CKD are required to ensure the continuity of care from child to adult renal services.

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Compliance with ethical standards

Conflict of interest Motoshi Hattori has received a research grant from Astellas Pharma and Chugai Pharma. Masayuki Iwano has received lecture fees from Otsuka Pharma and has a research grant from Otsuka Pharma, Astellas Pharma and Eli Lilly Japan. Hirokazu Okada has received a research grant from Chugai Pharma, Torii Pharma and Takeda Pharma. Yoshio Terada has received a research grant from Chugai Pharma and Daiichi Sankyo Pharma. Shouichi Fujimoto has received a research grant from Chugai Pharma, Dainippon Sumitomo Pharma and Nikkiso. Takao Masaki has received lecture fees from Chugai Pharma and Mitsubishi Tanabe Pharma and has a research grant from Asahi Kasei Pharma, Astellas Pharma, Genzyme, Otsuka Pharma, Kyowa Hakko Kirin, JMS, Mochida Pharma, Mitsubishi Tanabe Pharma, Takeda Pharma, Terumo, Torii Pharma, Daiichi Sankyo Pharma, Bayer Yakuhin and Baxter. Shoichi Maruyama has a research grant from Astellas Pharma, Alexion Pharma, Genzyme, Otsuka Pharma, Kyowa Hakko Kirin, Daiichi Sankyo Pharma, Dainippon Sumitomo Pharma, Takeda Pharma and Torii Pharma. Seiichi Matsuo has a research grant from Astellas Pharma, Alexion Pharma, Daiichi Sankyo Pharma, Otsuka Pharma, Kyowa Hakko Kirin, MSD, Baxter, Dainippon Sumitomo Pharma, Boehringer Ingelheim Japan, Takeda Pharma, Mitsubishi Tanabe Pharma, Teijin Pharm, Mochida Pharma and Torii Pharma. Drs Sako, Honda, Akioka, Ashida, Kawasaki, Kiyomoto, Hirano, and Fujieda have no conflicts of interest to declare.

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ORIGINAL ARTICLE

Analysis of the genes responsible for steroid-resistant nephrotic syndrome and/or focal segmental glomerulosclerosis in Japanese patients by whole-exome sequencing analysis

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Steroid-resistant nephrotic syndrome (SRNS) represents glomerular disease resulting from a number of different etiologies leading to focal segmental glomerulosclerosis (FSGS). Recently, many genes causing SRNS/FSGS have been identified. These genes encode the proteins associated with the formation and/or maintenance of glomerular filtration barrier. Next-generation sequencing is used to analyze large numbers of genes at lower costs. To identify the genetic background of Japanese patients, we studied 26 disease-causing genes using whole-exome sequencing analysis in 24 patients with SRNS and/or FSGS from 22 different Japanese families. We finally found eight causative gene mutations, four recessive and four dominant gene mutations, including three novel mutations, in six patients from five different families, and one novel predisposing mutation in two patients from two different families. Causative gene mutations have only been identified in ~20% of families and further analysis is necessary to identify the unknown disease-causing gene. Identification of the disease-causing gene would support clinical practices, including the diagnosis, understanding of pathogenesis and treatment.
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INTRODUCTION

Idiopathic nephrotic syndrome (NS) is a kidney disorder characterized by high excretion of protein in the urine, hypoalbuminemia and edema. In Europe and the United States, 2 in 100 000 children will develop idiopathic NS in a single year.[1,2] Approximately 80% of patients are steroid responsive and have a favorable prognosis, and the other 20% of children with NS are resistant to steroid therapy and have an unfavorable prognosis.[2,3] Steroid-resistant NS (SRNS) represents glomerular disease resulting from a number of different etiologies leading to focal segmental glomerulosclerosis (FSGS).[4]

Recently, many genes causing SRNS/FSGS have been identified. These genes encode the proteins associated with formation and/or maintenance of the glomerular filtration barrier.[5–10] Genetic backgrounds of SRNS/FSGS may be different in each ethnic group. There are currently no detailed reports of genotype–phenotype correlations, including the responsibility to the ordinal treatment, complication, prognosis and post-transplant recurrence of SRNS/FSGS. These data will provide useful information for clinical practice and genetic counseling.[11] Next-generation sequencing is used to analyze a large number of genes associated with SRNS/FSGS and can be carried out at lower costs compared with the individual sequence analysis of many genes.[12] To identify the genetic background of Japanese patients, we studied 26 disease-causing genes using whole-exome sequencing analysis in 24 patients with SRNS and/or FSGS from 22 different families. We finally identified eight causative gene mutations in six patients from five different families and one predisposing gene mutation in two patients from two different families.

MATERIALS AND METHODS

Subjects

We studied 24 patients with SRNS and/or FSGS (male-to-female ratio, 12:12; ages from 2 to 42 years; median age, 12 years) from 22 different Japanese families (Supplementary Table 1). The institutional review board of the Yamagata University School of Medicine study approved this study, and written informed consent was obtained from the patients or parents of all children. The definitions and criteria for NS and steroid resistance were those used by the International Study of Kidney Disease in Children.[13,14] NS was defined as heavy proteinuria (urinary protein excretion $\geqslant 40\ mg\ m^{-2}\ h^{-1}$) with hypoalbuminemia $\leqslant 25\ g\ l^{-1}$. Steroid resistance was defined as a failure to achieve a response despite 4 weeks of prednisolone therapy ($2\ mg\ kg^{-1}$ per day prednisolone).

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Molecular analysis

We extracted genomic DNA from peripheral blood using a standard method.

We analyzed all the exons of 26 genes: *NPHS1*, *NPHS2*, *NPHS3 (PLCE1)*, *CD2AP*, *TRPC6*, *ACTN4*, *MYH9*, *ARHGAP24*, *MYO1E*, *IFN2*, *WT1*, *LMX1B*, *SMARCAL1*, *LAMB2*, *MT-TL1*, *COQ6*, *COQ2*, *PDSS2*, *SCARB2*, *ZMPSTE24*, *PMM2*, *ALG1*, *PTPRO*, *GPC5*, *APOL1* and *ITGB4*,[9–11,15] using a next-generation sequencer. Exon capture was performed with the SureSelect Human All Exon Kit v.5 (Agilent Technologies, Santa Clara, CA, USA). Exon libraries were sequenced with the HiSeq 2000 platform (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Paired 100-base pair reads were aligned to the reference human genome (UCSC hg19) using the Burrows–Wheeler Aligner (version 0.7.3a).[16] Probably, PCR duplicates were removed with the Picard 1.88 (http://picard.sourceforge.net/). We picked up the sequences of 26 genes previously identified as the responsible genes for SRSN. Single-nucleotide variants and indels were identified using the Unified Genotyper module of the Genome Analysis Tool Kit (GATK) 2.5. 2 (Broad Institute of MIT and Harvard, Cambridge, MA, USA),[17] with the Best Practice Variant Detection with the GATK v.3 (https://www.broadinstitute.org/gatk/index.php). Single-nucleotide variants and indels were annotated against the set of RefSeq database, NHLBI-ESP 6500 exomes and dbSNP135 with the ANNOVAR (8 May 2013).[18] We predicted the functional effects of mutations using two PolyPhen scores (HumDiv and HumVar models) provided by PolyPhen-2 version 2.2.2,[19] Grantham,[20] PhastCons[21] and GERP[22] scores provided by SeattleSeq SNP annotation 137 (http://snp.gs.washington.edu/SeattleSeqAnnotation137/). We performed direct sequencing to confirm the mutations identified by exome sequencing in the patients and some of family members and to find additional gene mutations detected in cases 10, 11, 12 and 15. PCR was performed with the primers designed based on the genome database. The sequence reactions were analyzed on an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems).

RESULTS

We studied 26 disease-causing genes using whole-exome sequencing analysis in 24 patients with SRNS and/or FSGS from 22 different families and found 10 nucleotide changes in eight genes (Supplementary Table 1 and Table 1). Based on the database and functional prediction analyses, we finally identified eight causative gene mutations in six patients from five different families and one predisposing gene mutation in two patients from two different families (Table 2).

For the genes of recessive types, we detected a heterozygous *NPHS2* p.R168H mutation[23] in Case 15 and a heterozygous *LAMB2* c.1405 +1g>a mutation (splicing mutation)[24] in Case 12 (Table 2). We also detected three polymorphic nucleotide changes as follows: *PTPRO* p. S338F (rs.200478856), *NPHS1* p.A219V (rs.757417823) and *NPHS2* p. Q287R (rs.200042397). Direct sequencing revealed that Cases 15 and 12 were a compound heterozygote of *NPHS2* p.[R138X];[R168H] mutation[25,26] and a compound heterozygote of *LAMB2* p.[abnormal splicing];[G699R] mutation, respectively. Case 15 presented as congenital NS, and steadily progressed to end-stage renal failure (ESRF) at age 11 years. Case 12 manifested the symptoms in infantile period and progressed rapidly to ESRF at age 2 years, but he did not have any ocular symptoms. Cases 15 and 12 had no relapse of FSGS after renal transplantation.

In the analysis of the genes of dominant types, we found five gene mutations in six cases from five families. Cases 10 and 11 carrying a novel *CD2AP* p.R74M mutation were unrelated and presented different clinical features. Case 10 partially responded to steroid and cyclosporin A therapy; however, he progressed to ESRF. He received cadaveric renal transplantation, but had a recurrence of FSGS. Case 11 frequently relapsed, but he was responsive to therapy and had a complete remission. We performed direct sequencing of all coding regions of *CD2AP* in Cases 10 and 11, but we did not find any additional mutations.

Case 16 carrying a novel heterozygous *TRPC6* p.E875V mutation presented FSGS at age 12 years, did not respond to steroid and cyclosporin A and progressed to ESRF. Her grandfather died of renal disease at age 30 years and her father presented proteinuria.

Case 19 was a daughter of Case 20, and Cases 19 and 20 presented the symptoms at school age and progressed to ESRF. They were complicated with bicornuate uterus and had a novel heterozygous *WT1* p.[K141Q; P249T] mutation.

Table 1 Nucleotide changes detected in 24 patients with FSGS and/or SRNS from 22 different families

No.	Gene	Mutation		Status	PolyPhen-2 (HumDiv)	PolyPhen-2 (HumVar)	Grantham score	PhastCons score	GERP score	References and information
1	*PTPRO*	c.1013c>t	p.S338F	Heterozygote	1	0.998	155	0.996	5.23	rs.200478856
6	*NPHS1*	c.656c>t	p.A219V	Heterozygote	0.692	0.148	64	0.334	−0.75	Sibling of case 7 rs.757417823
7	*NPHS1*	c.656c>t	p.A219V	Heterozygote	0.692	0.148	64	0.334	−0.75	Sibling of case 6 rs.757417823
15	*NPHS2*	c.412c>t	p.R138X	Heterozygote	NA	NA	NA	1	4.91	Grantham[20]
		c.503g>a	p.R168H	Heterozygote	1	0.999	29	1	4.52	
22	*NPHS2*	c.860a>g	p.Q287R	Heterozygote	0.989	0.979	43	1	5.34	rs.200042397
12	*LAMB2*	c.1405+1g>a	ab. spl.	Heterozygote	0.996	0.755	43	0.719	3.44	Siepel *et al.*[21]
		c.2095g>c	p.G699R	Heterozygote	0.986	0.593	125	0.992	5.49	rs.28364667
10	*CD2AP*	c.221g>t	p.R74M[a]	Heterozygote	0.966	0.641	91	1	4.65	
11	*CD2AP*	c.221g>t	p.R74M[a]	Heterozygote	0.966	0.641	91	1	4.65	
16	*TRPC6*	c.2624a>t	p.E875V[a]	Heterozygote	1	0.982	121	1	5.89	
19	*WT1*	c.421a>c	p.K141Q[a]	Heterozygote	0.960	0.545	53	1	2.58	Daughter of case 20
		c.745c>a	p.P249T[a]		0.035	0.027	38	1	5.62	
20	*WT1*	c.421a>c	p.K141Q[a]	Heterozygote	0.960	0.545	53	1	2.58	Mother of case 19
		c.745c>a	p.P249T[a]		0.035	0.027	38	1	5.62	
24	*IFN2*	c.550g>a	p.E184K	Heterozygote	1	0.999	56	0.709	4.48	Cooper *et al.*[22]

Abbreviations: Ab. spl., abnormal splicing; FSGS, focal glomerular sclerosis; GERP, Genomic Evolutionary Rate Profiling; NA, not analyzed; SRNS, steroid-resistant nephrotic syndrome.
[a]Novel mutations.

Table 2 Gene mutations and clinical features

No.	*Sex*	*Age at onset*	*Response for treatment*	*Pathology*	*Prognosis*	*Age at ESRF*	*Gene*	*Causative or predisposing mutation*	*Clinical features*
15	M	At birth	No trials	FSGS	ESRF	11y3m	*NPHS2*	c.[412c>t];[503g>a]	p.[R138X]; [R168H]	No relapse after renal transplantation at age 11y4m Sister with FSGS died of renal failure at age 5y
12	M	1y9m	Resistant	FSGS	ESRF	2y2m	*LAMB2*	c.[1405+1g>a]; [2095g>c]	p.[ab. spl.]; [G699R]	No relapse after renal transplantation at age 7y2m No ocular symptoms No affected family members
10	M	5y4m	Initially responsive	FSGS	ESRF	7y6m	*CD2AP*	c.221g>t	p.R74M[a]	FSGS relapsed after renal transplantation at age 8y1m No affected family members
11	M	5y2m	Responsive Frequently relapse	FSGS	Complete remission	—	*CD2AP*	c.221g>t	p.R74M[a]	No affected family members
16	F	12y5m	Resistant	FSGS	ESRF	23y11m	*TRPC6*	c.2624a>t	p.E875V[a]	Hemodialysis at age 23y Father had proteinuria; grandfather died of renal disease at age 30y
19	F	6y7m	No trials	FSGS	ESRF	16y6m	*WT1*	c. [421a>c;745c>a]	p.[K141Q[a]; P249T[a]]	Daughter of case 20; Bicornuate uterus Grandfather died of renal disease at age 42y
20	F	10y	Unknown	NA	ESRF	42y	*WT1*	c. [421a>c;745c>a]	p.[K141Q[a]; P249T[a]]	Mother of case 19; Bicornuate uterus Father died of renal disease at age 42y
24	M	10y	Resistant	FSGS	ESRF	16y11m	*IFN2*	c.550g>a	p.E184K	No relapse after renal transplantation at age 20y No affected family members

Abbreviations: ab. spl., abnormal splicing; ESRF, end-stage renal failure; FSGS, focal glomerular sclerosis; m, months; y, years.
[a]Novel mutations.

Case 24 carrying a heterozygous *IFN2* p.E184K mutation presented FSGS at age 10 years, did not respond to steroid, cyclosporin A and plasma exchange and progressed to ESRF at age 16 years.[27] He did not have any signs of Charcot-Marie-Tooth disease (CMT).

The patients carrying those dominant gene mutations manifested the symptoms in childhood period; they were older than the patients carrying the genes of recessive types. The patients carrying the mutations except *CD2AP* mutation did not respond to therapy and did not relapse FSGS after renal transplantation (Supplementary Table 1 and Table 2).

DISCUSSION

We studied 26 disease-causing genes using whole-exome sequencing analysis in 24 patients with SRNS and/or FSGS from 22 different families and finally found eight causative gene mutations, four recessive and four dominant gene mutations, in six patients from five different families, and one predisposing mutation in two patients from two different families (Table 2). Recently, Sadowski *et al.* studied 1783 families with SRNS that manifested before 25 years of age and detected a single-gene cause in 526 families (29.5%).[10] Our study was very small, but detected gene mutations in 22.7% of families.

Cases 15 and 12 carrying the recessive gene mutations manifested the symptoms during infantile period. Case 15 was a compound heterozygote of *NPHS2* p.[R138X];[R168H] mutation[25,26] (Table 2). Podocin encoded by *NPHS2* is expressed mainly in the podocytes and localized to the intercellular junction of the podocytes foot processes. Podocin is a lipid raft component of the slit diaphragm that is essential for the localization of nephrin and other components. Most missense mutations including the p.R168H mutation alter the trafficking to the plasma membrane[25] and the p.R138X mutation was demonstrated to be present at the plasma membrane but fail to recruit nephrin in lipid rafts.[26] Case 15 was resistant to therapy, progressed to ESRF and had no recurrence of FSGS after renal transplantation as reported previously.[28] Case 12 was a compound heterozygote of *LAMB2* c.[1405+1g>a];[2095g>c]; that is, p.[abnormal splicing];[G699R] mutation[24] (Table 2). Laminins are the major noncollagenous constituent of basement membranes and laminin β-chain encoded by *LAMB2*, a component of laminins, is highly expressed in the basement membrane of muscles at the neuromuscular junctions, kidney glomerulus and vascular smooth muscle. Mutations with complete loss of function are associated with the features of Pierson syndrome and missense mutations cause mild and variable phenotypes.[29] The homozygous c.[1405+1g>a] mutation was reported in the patient showing Pierson syndrome.[24] The p.G699R is registered as a rare variation (frequency is 0.00274), but is predicted to have a deleterious functional effect using several prediction programs, PolyPhen-2 (HumDiv), MutationTaster score (data not shown) and MutationAssessor score (data not shown). These suggest that a compound heterozygous *LAMB2* p.[abnormal splicing];[G699R] mutation is a causative mutation of FSGS.

For the dominant type, Cases 10 and 11 were heterozygous for *CD2AP* p.R74M mutation. CD2AP is an adapter protein with an SH3 domain, is expressed in lymphoid cells, and interacts with the T-cell adhesion protein CD2.[30] CD2AP is also expressed in the slit diaphragm of the podocytes and interacts with podocin and nephrin to form a signaling complex.[31,32] Mice with null mutation die of massive proteinuria shortly after birth and mice with heterozygous null mutation developed glomerular change similar to FSGS at 9 months of age.[30] For the human, only one case carrying homozygous p.R612X mutation was clearly demonstrated in association with renal disease.[33] Several patients with heterozygous *CD2AP* mutations have

been reported in association with adult onset FSGS.[30,34] However, segregation data was not available in all mutations and the heterozygous mutations were also detected even in unaffected individuals. Case 10 presented FSGS and relapsed FSGS after renal transplantation. In contrast, Case 11 frequently relapsed, but he was responsive to therapy and had a complete remission. In addition to their clinical difference, Cases 10 and 11 had no affected family members. These suggested that a heterozygous *CD2AP* p.R74M mutation is not a disease-causing mutation, but is a predisposing factor towards glomerular disease.

Case 16 had a novel heterozygous *TRPC6* p.E875V mutation. TRPC6 is a cation channel and is widely distributed in the body including the podocytes cell bodies and the slit diaphragms. The majority of mutations associated with FSGS are gain-of-function mutations, which can increase intracellular calcium influx and lead to the apoptosis of the podocyte.[35] Case 16 had similarly affected family members, but we could not confirm the linkage because their specimens were not available for analysis.

Cases 19 and 20 had heterozygous *WT1* p.[K141Q; P249T] mutation. These mutations were located to exons 1 and 2. Nearly all mutations of WT1 associated with FSGS are located in exons 8 and 9. However, SNPs located in *WT1* (promoter to exon 5) and *WITI* (gene immediately 5′ to *WT1*) were significantly associated with FSGS.[36] and Gebeshuber *et al.*[37] reported that transgenic expression of the microRNA miR-193a in mice rapidly induced FSGS via downregulation of WT1. Those reports suggest that a *WT1* mutation located in other than exons 8 and 9 will cause FSGS. Cases 19 and 20 had bicornuate uterus as genitourinary malformations, which may be associated with the sexuality and/or the type of the *WT1* mutation.[38]

Case 24 had a heterozygous *IFN2* p.E184K mutation and was not complicated with CMT as reported previously.[27] IFN2 is a member of the formin family and has a domain structure similar to the diaphanous formins: a diaphanous-inhibitory domain (DID), formin homology 1 and 2 domains and a diaphanous autoregulatory domain. IFN2 is strongly expressed in podocytes and Schwann cells. *IFN2* mutations were detected in the patients with isolated FSGS and the patients with FSGS associated with CMT. All mutations are distributed in different parts of the DID; however, the mutations detected in patients with FSGS and CMT are located in the inner face of the central core of the DID.[39,40] The p.E184K mutation is located in the DID, but not in the inner face of the central core of the DID. The clinical phenotype of the IFN2 mutation may depend on the site and/or type of the mutation.

Detection of an SRSN-causing mutation will support clinical practices, including the diagnosis, understanding of pathogenesis and treatment. It will also avoid an unnecessary steroid therapy, introduce supplement therapy for the patients with a defect in CoQ biosynthesis[41,42] and provide a prenatal diagnosis in severe cases. However, causative gene mutations have been identified only in ~ 20% of families and further analysis is necessary to identify the unknown disease-causing gene.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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●総　説●

特発性ネフローゼ症候群の発症機序

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（受付日：平成27年8月18日　採用日：平成27年9月10日）

要　旨

小児期発症特発性ネフローゼ症候群の発症機序についてはこれまで様々な検討がなされてきた。先天性ネフローゼ症候群や幼少期発症ステロイド抵抗性ネフローゼ症候群の原因の多くは糸球体上皮細胞の機能に関する遺伝子の異常が原因であることが判明しているが，微小変化型ネフローゼ症候群（MCNS）については未だ原因が不明な部分が多く，様々な仮説が提唱されるに至っている。古くからT細胞の異常が想定されるようになり，それ以来血中に存在するサイトカイン等の循環因子が糸球体に作用して濾過バリアーの透過性を亢進させると考えられてきた。しかし最近ではB細胞をターゲットとするリツキシマブの有効性が明らかとなり，T細胞以外のメカニズムも想定されるようになった。また遺伝的素因の検討により，MCNSの原因となるいくつか遺伝子変異が同定されている。MCNSの発症機序に関する様々な原因仮説を整理し，その解明に向けた今後の課題を議論する。

序　言

小児期発症特発性ネフローゼ症候群の中で微小変化型ネフローゼ症候群（minimal change nephrotic syndrome: MCNS）は最も頻度の高いものである。その発症機序についてこれまで様々な検討がなされており，特に免疫系の関与が注目されてきた。70年代からMCNSの原因としてT細胞の異常が想定されるようになり，その後血中に存在するT細胞由来のサイトカイン等の循環因子が糸球体に作用して濾過バリアーの透過性を亢進させる機序が想定されてきた。しかし最近ではB細胞をターゲットとするリツキシマブの有効性が明らかとなり，T細胞以外の関与も想定されている。またMCNSと関連する遺伝的素因の検討により，原因となるいくつかの遺伝子変異が同定されている。本項では特発性ネフローゼ症候群のうち，特にMCNSの発症機序に関する様々な仮説（**表1**）を中心に整理し，その解明に向けた今後の課題を議論する。

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特発性ネフローゼ症候群とT細胞機能の関わり

1970年代から微小変化型ネフローゼ症候群へのT細胞の機能異常の関与が考えられてきた[1]。T細胞仮説の根拠は，糸球体に液性因子（免疫グロブリンや補体）の沈着がないこと，T細胞機能を抑制する免疫抑制薬（糖質コルチコイド，シクロスポリン，シクロフォスファミドなど）が効果があること，T細胞機能を低下させることで知られる麻疹感染に引き続いて寛解する症例があること，またT細胞腫瘍にMCNSが合併することがあることなどであり[2]，現在も多くの支持を得ている。ネフローゼ症候群患者のT細胞を不死化し，その上清をラットに投与すると蛋白尿と足突起の消失が見られるが，コントロールのT細胞上清ではそのような変化が認められないことも，ネフローゼのT細胞由来液性因子の存在を強く示唆する根拠と考えられてきた[3]。

T細胞には単球・マクロファージから抗原を提示され，免疫反応を調節するヘルパーT細胞（CD4抗原陽性）と，ウイルス感染細胞などを傷害するキラーT細胞（CD8抗原陽性）がある。さらにヘルパーT細胞には，Th1細胞とTh2細胞とがあり，この両者ではどのようなサイトカインを分泌しているか，あるいはどのようなエフェクター機能を有しているかが異なる。Th1細胞はIL-2，IFN-γとTNF-βを産生し，Th2細胞はIL-4，IL-5，IL-6，IL-9，IL-10，IL-13を産生する。これまで様々なグルー

Key words: 特発性ネフローゼ症候群 / 微小変化型ネフローゼ症候群 / 糸球体上皮細胞

表1　ネフローゼ症候群の原因仮説

仮説	時期	根拠	エビデンス	文献
T細胞	1970年代～	ステロイドやCyA等の薬剤の有効性	確定的なデータが不足	1-3
Th1<Th2	1980年代～	サイトカインプロファイル	報告によりまちまち	3-6
サイトカイン	1990年代～	T細胞由来循環因子	報告によりまちまち	4-6
IL-13	2000年代～	MCNS患者で血中濃度上昇	データの蓄積が必要	7-11
c-mip	2010年代～	MCNSでの発現量上昇	十分なデータがない	12, 13
TNFα	2000年代～	阻害薬の有効性	十分なデータがない	14
NF-κB	2000年～	ステロイドの有効性	十分なデータがない	15
Treg	2000年後半～	抑制性T細胞の機能の変化	データの蓄積が必要	16, 17
DNAメチル化	2010年代～	MCNSでの変化	データの蓄積が必要	18, 19
B細胞	2010年代～	リツキシマブの有効性	データの蓄積が必要	20, 21
Hemopexin	2000年代～	MCNS患者で活性上昇	十分なデータがない	22-24
Angptl4	2010年代～	MCNS患者で血中濃度上昇	高脂血症と関与する可能性	25, 26
suPAR	2010年代～	FSGS患者で血中濃度上昇	否定的	27-33
CD80	2000年代～	アバタセプトの有効性？	データの蓄積が必要	34-39
ポドサイト因子	2000年代～	家族性の症例の存在，GWASデータ	いくつかの因子は原因と確定	40-49

プによりMCNS患者における血中サイトカイン濃度の動態が検討されてきた[4]。MCNS患者の寛解期では正常コントロールとサイトカイン濃度は大きな違いがないがネフローゼ発症時にはIL-4やIL-13濃度が上昇している，すなわちTh2優位の変動が見られるとする報告がある一方，これらの変動については否定的な報告もあり，一定した見解には至っていない[5][6]。結果が一致しない理由としては患者の背景が同一でない可能性や検体採取の方法，タイミングの問題などの解析の標準化がされていないことに加え，適切な培養細胞やモデル動物が存在しないというin vitro系の限界も大きく関与している。現状ではTh1あるいはTh2の優位性によりネフローゼ症候群が発症するという確立した根拠はない。

一方で，T細胞におけるmRNA発現パターンの検討によりYapらはネフローゼ症候群患者のT細胞でIL-13のmRNA発現レベルが上昇していることを見出した[7]。その後血中IL-13濃度上昇やT細胞での発現上昇は他のグループによっても確認された[8][9]。糸球体上皮細胞にはIL-13の受容体が発現しており，培養糸球体上皮細胞にIL-13を添加することによりバリアー機能が低下する[10]。さらにIL-13をラットで強発現させるとMCNS様の腎症を生じることから[11]，MCNS患者におけるIL-13の上昇が病態に何らかの影響を及ぼしている可能性がある。しかしIL-13についても必ずしもMCNS患者で血中濃度が高くないとする報告もあり，腎局所でのサイトカイン濃度の検討なども含めた今後の検証が必要である。

またT細胞のcDNAの解析によりMCNSでc-mip(c-maf inducing protein)と呼ばれる分子の発現が上昇していることが報告されている[12]。その後の解析でこの分子の発現はT細胞だけでなく糸球体上皮細胞でもネフローゼの再発時に発現が上昇することが明らかになった[13]。c-mipを糸球体上皮細胞で強制発現させたマウスは蛋白尿を生じること，またc-mipがスリット膜によるチロシンキナーゼシグナルを修飾することからc-mipがMCNSでの糸球体上皮細胞障害のメディエーターである可能性が示唆されている[13]。

また，TNFα阻害薬がネフローゼ患者に効果があったとする報告[14]や，NF-kB経路がMCNS患者の血球中で活性化されているなどの報告[15]もあるが，症例数が少なく，その後の追試の報告がなされていない現状である。

T細胞には制御性T細胞と呼ばれる免疫応答に対して抑制的な作用を持つ集団が存在する。このCD25陽性CD4陽性制御性T細胞は転写因子Foxp3を特異的に発現している。*FOXP3*遺伝子は制御性T細胞の発生および機能におけるマスター遺伝子であると考えられている。MCNSの再発時の検討で抑制性T細胞の細胞数自体は正常と変わらないものの，T細胞の増殖を抑える能力がMCNS再発時の制御性T細胞では低下していることが報告されている[16]。また*FOXP3*遺伝子の変異を原因とするX染色体伴性劣性遺伝疾患のIPEX(immune dysregulation, polyendocrinopathy, enteropathy, X-linked, X染色体連鎖免疫制御異常多発性内分泌障害消化器病)症候群では，I型糖尿病が80%，自己免疫性甲状腺炎が70%，そのほか溶血性貧血，血小板減少性紫斑病など様々な自己免疫疾患が生後3年以内に発症するが，一部の症例はネフローゼ症候群を合併することが報告されており[17]，MCNSと制御性T細胞の関連が強く示唆される。

ネフローゼにおけるT細胞分画の変化の検討は始まったばかりといえる。

MCNS患者のリンパ球におけるエピゲノムの変化の検討も行われている。これまでMCNSにおけるヒストンメチル化[18]やDNAのメチル化[19]の変化が報告されているが，現状ではこれが疾患発症の元となるリンパ球機能の変化と関連しているのかについてのデータはない。ステロイドはエピジェネティックな変化を誘導することから，MCNSにおけるステロイド感受性のメカニズムを理解する上でこの分野は今後重要な領域となると予測される。

特発性ネフローゼ症候群とB細胞機能の関わり

一般的な免疫応答の流れとしては，樹状細胞が異物を認識すると，まずT細胞が活性化され，次にB細胞が活性化される。活性化されたB細胞は，プラズマ細胞に分化し，抗体を産生する。現在B細胞は自己抗体産生に加え，その抗原提示能，共刺激分子発現能，サイトカイン産生能など多彩な機能を営むエフェクターB細胞として機能していると考えられている。B細胞は樹状細胞，T細胞，マクロファージ・好中球などの免疫・炎症担当細胞との相互作用を通じて様々な病態へ深く関与している。

MCNSにおけるB細胞の機能についてはT細胞に比べ研究が極めて乏しいが，臨床的にはB細胞抗原であるCD20に対するヒト型のモノクローナル抗体であるリツキシマブが頻回再発型ネフローゼ症候群に対して有効であること[20]，すなわちB細胞を枯渇させることがMCNSの治療となることが明らかとなった。しかしこの効果がリツキシマブによるB細胞に対する作用なのか，B細胞を介したT細胞機能の変化によるものかは明らかではない。一方でリツキシマブはacid sphingomyelinase-like phosphodiesterase 3b(SMPDL-3b)という糸球体上皮細胞に発現する蛋白と結合する。ネフローゼ患者血清は培養糸球体上皮細胞のSMPDL-3bの発現量を低下させ，その下流で細胞骨格の変化を惹起して濾過バリアー機能を低下させるが，リツキシマブはSMPDL-3bの発現量を上昇させ，患者血清による変化を抑制する[21]。これはリツキシマブが免疫細胞を介さずに糸球体上皮細胞に直接作用して蛋白尿抑制作用を持つ可能性を示唆するものであるが，このメカニズムがリツキシマブの臨床的効果にどれほど関与しているのかは現時点では不明である。

その他の循環因子

MCNSと関連するその他の血中因子候補の一つとしてHemopexinが挙げられる。Hemopexinはヘムの代謝に関わる酵素で，ラットにHemopexinを投与することにより可逆性の蛋白尿が惹起される[22]。Hemopexin活性はMCNS患者で増加しており[23]，Hemopexinはin vitroでNephrinを介して糸球体上皮細胞の細胞骨格に作用することから[24]，MCNSの一つのファクターである可能性がある。しかしこの報告は症例数が少なく，一般的な事象なのかどうかについては不明である。

もう一つの血中因子候補はAngiopoetin-like 4(Angptl4)である。2011年にChughらはMCNS患者の血中にAngptl4が増加していることを見出した。患者の糸球体においてもAngptl4の発現が上皮細胞で増強しており，マウスで糸球体上皮細胞特異的にAngptl4を強発現させると蛋白尿を生ずることから，Angptl4の上昇がネフローゼの原因の一つなのではないかと注目を集めた[25]。しかし，その可能性は現在では否定的と考えられる。それはその後の解析で肝臓においてAngptl4を発現させたマウスでは蛋白尿を呈しないことや，血中のAngptl4は糸球体内皮細胞に働きかけ，むしろ蛋白尿を低下させる効果があること[26]が判明したからである。興味深いことにAngptl4濃度は血中アルブミンが低下することにより上昇するが，Angptl4はリポ蛋白リパーゼ(LPL)の活性を抑制する作用を持つため，それによりトリグリセリドからFFAへの変換が抑制され，高脂血症をきたす[26]。そのためAngptl4はネフローゼにおける高脂血症の一端を担っている可能性が指摘されている。

ネフローゼ症候群の液性因子として2011年にマイアミ大学のReiserのグループはFSGS患者血清，特に再発時の血清で可溶性ウロキナーゼ受容体(suPAR：soluble urokinase receptor)の濃度上昇がみられることを報告した[27]。suPARはウロキナーゼ受容体からGPI-アンカー部が切断されて可溶性となる血中循環蛋白である[28]。大規模なコホートにおいても巣状糸球体硬化症患者で血清中のsuPAR濃度の上昇が確認され[29]，またこの蛋白をマウスに投与することにより蛋白尿が生じることなどから，suPARが糸球体硬化症の原因因子の一つ，さらにはFSGSとMCNSの病態の違いを説明する因子ではないかと考えられた。しかし多数の別のグループによる検討によりsuPARは腎機能低下をきたす他の疾患でも上昇し，suPAR濃度は糸球体濾過量と負の相関を示すことが判明し，腎機能との相関以上のsuPAR濃度上昇の意