Table 2 Receiver operating characteristic (ROC) analysis of clinical parameters as predictors of intractable NS or FSGS

Clinical parameters	AUC-ROC	Cut-off value	Sensitivity (=specificity)	*p*	*n*
The diagnostic performance of differentiating intractable NS from non-intractable NS					
2M s-suPAR	0.913	3,373 (pg/mL)	0.762	0.002	27
Δ2M s-suPAR	0.881	−189 (pg/mL)	0.833	0.005	27
2M u-suPAR	0.776			0.066	22
Δ2M u-suPAR	0.906	−127 (pg/mgCr)	0.800	0.007	22
2M UP	0.968	0.97 (g/gCr)	0.952	0.001	27
Δ2M UP	0.833	−6.97 (g/gCr)	0.762	0.014	27
2M eGFR	0.730			0.091	27
Δ2M eGFR	0.579			0.560	27
The diagnostic performance of differentiating FSGS from MCNS					
2Ms-suPAR	0.698			0.186	16
Δ2M s-suPAR	0.905	−268 (pg/mL)	0.857	0.007	16
2M u-suPAR	0.694			0.225	14
Δ2M u-suPAR	0.816	−718 (pg/mgCr)	0.714	0.048	14
2M UP	0.675			0.244	16
Δ2M UP	0.508			0.958	16
2M eGFR	0.587			0.560	16
Δ2M eGFR	0.651			0.315	16

The cut-off value is at the point where the specificity equal to the sensitivity

NS nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis, *MCNS* minimal change nephrotic syndrome, *s-* serum, *u-* urinary; *2M* at 2 months after therapy, *Δ2M* change during 2 months after therapy, *UP* urinary protein, *AUC-ROC* the area under the curve of receiver operating characteristic analysis

factors that influence vital and renal prognoses in a study on Japanese ANCA-GN, and these were found to be important evaluation items to judge the clinical severity of ANCA-GN. Based on these findings, we assumed that s-suPAR was associated with the clinical severity of ANCA-GN, suggesting that s-suPAR is a useful clinical severity marker of ANCA-GN. In addition, a positive correlation was noted between pretreatment s-suPAR and crescent formation in this study. Baraldi et al. [19] detected the strong expression of β3 integrin on crescent cells, podocytes, and Bowman's capsular epithelial cells in RPGN patients, and suggested the involvement of β3 integrin in the mechanism underlying crescent formation. Thus, it was also assumed that s-suPAR activated β3 integrin on podocytes and Bowman's capsular epithelial cells, and this process was part of the mechanism underlying crescent formation in ANCA-GN as well as crescentic FSGS [4].

Regarding s-suPAR as a clinical marker, we also investigated the time-course of s-suPAR over 2 months of immunosuppressive therapy because s-suPAR over 3,000 pg/mL was previously shown to activate podocyte β3 integrin [1]. Although intractable and non-intractable cases could not be differentiated based on s-suPAR over 3,000 pg/mL before immunosuppressive therapy, which is consistent with previous findings [7], 3,373 pg/mL or higher at 2 months may be used for a marker to judge responses to treatments. These changes in s-suPAR after therapy were not significantly influenced by changes in eGFR or CRP in this study. Moreover, 2M s-suPAR was positively correlated with 2M UP and a cut-off value of 2M s-suPAR to differentiate intractable cases was close to the s-suPAR level (3,000 pg/mL) in the first study [1]. Reiser et al. may have observed this phenomenon in primary FSGS cases treated with immunosuppressive drugs.

In our study, differentiating between FSGS and MCNS was possible based on changes in s-suPAR over 2 months of immunosuppressive therapy. Huang et al. [4] compared s-suPAR before immunosuppressive therapy with that after 78 weeks of therapy on average in primary FSGS patients, and observed a significant decrease in complete remission cases, no change in incomplete remission cases, and a significant elevation in ineffective cases. These findings revealed changes in s-suPAR that corresponded to the responses to treatments. Similar to our results, they could not predict therapeutic responses in FSGS patients based on s-suPAR before immunosuppressive therapy [4]. On the other hand, no significant change was noted in s-suPAR over the 2-month period in this study. Considering that the follow-up period was markedly longer (78 weeks on average) in their study than in ours (8 weeks), the 2-month period may have been too short to observe a reduction in

s-suPAR in FSGS, even for patients showing favorable responses to treatments. On the other hand, s-suPAR rapidly decreased within the 2-month period in MCNS, suggesting that the 2-month s-suPAR reduction rate serves as an index to differentiate MCNS.

We also investigated u-suPAR as a clinical marker in primary NS. Pretreatment u-suPAR was significantly higher in the primary NS and ANCA-GN groups. In contrast to recent findings [5], no significant differences were noted between the primary NS and ANCA-GN groups, or among the disease types of primary NS (FSGS, MCNS, and MN). Since pretreatment u-suPAR positively correlated with UP in all primary NS, similar to previous findings [6], we suspected that u-suPAR may be the only initial indication of UP. However, u-suPAR only decreased after treatments were received by non-intractable NS and MCNS patients, but not by FSGS or MN patients. Thus, we assessed whether changes in u-suPAR could be used to differentiate non-intractable NS from intractable NS or MCNS from FSGS. A ROC analysis revealed that Δ2M u-suPAR was a useful marker for differentiating non-intractable NS from intractable NS or MCNS from FSGS. Huang et al. [5] also reported higher u-suPAR levels in the cellular variant and significant decreases in u-suPAR, even in primary FSGS patients with complete remission. In our study, tip lesions were detected in the majority of FSGS patients. Hence, further investigations are needed to resolve the issue of u-suPAR in MCNS and the different pathological lesions of FSGS such as tip lesions and the cellular variant.

In conclusion, s- or u-suPAR may be useful as an index of treatment responses by patients with primary NS for the differentiation of MCNS from FSGS, but not in pretreatment patients. In addition, our study revealed that s- and u-suPAR were associated with the long-term therapeutic responses of all primary NS patients including those with MCNS, FSGS, MN and MPGN. S- and u-suPAR were significantly decreased in MCNS after therapy and could be used to differentiate MCNS from FSGS. In addition, s-, but not u-suPAR levels before therapy may be useful for judging the clinical severity of and crescent formation in ANCA-GN. The ELISA system used in this study only measured the complete form of suPAR; however, several splicing forms and different glycosylation forms are known to exist. Thus, it is also possible that the molecular size or glycosylation of suPAR differs among these diseases, and differences in physiological activity due to these variations in suPAR may lead to differences in the renal histological phenotype. These issues need to be investigated in future studies.

Acknowledgments The authors gratefully acknowledge the help and assistance of their colleagues at the Division of Nephrology. This study was supported, in part, by a Grant-in-Aid for Progressive Renal Disease Research from the Ministry of Health, Labour, and Welfare of Japan and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (HY, (C) No. 25461237; (B) No. 24406029, No. 25305028).

Conflict of interest None of the authors have any conflicts of interest to disclose regarding this paper.

References

1. Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat Med. 2011;17:952–60.
2. Wei C, Moller CC, Altintas MM, Li J, Schwarz K, Zacchigna S, et al. Modification of kidney barrier function by the urokinase receptor. Nat Med. 2008;14:55–63.
3. Wei C, Trachtman H, Li J, Dong C, Friedman AL, Gassman JJ, et al. Circulating suPAR in two cohorts of primary FSGS. J Am Soc Nephrol. 2012;23:2051–9.
4. Huang J, Liu G, Zhang YM, Cui Z, Wang F, Liu XJ, et al. Plasma soluble urokinase receptor levels are increased but do not distinguish primary from secondary focal segmental glomerulosclerosis. Kidney Int. 2013;84:366–72.
5. Huang J, Liu G, Zhang YM, Cui Z, Wang F, Liu XJ, et al. Urinary soluble urokinase receptor levels are elevated and pathogenic in patients with primary focal segmental glomerulosclerosis. BMC Med. 2014;12:81. doi:10.1186/1741-7015-12-81.
6. Sinha A, Bajpai J, Saini S, Bhatia D, Gupta A, Puraswani M, et al. Serum-soluble urokinase receptor levels do not distinguish focal segmental glomerulosclerosis from other causes of nephrotic syndrome in children. Kidney Int. 2014;85(3):649–58.
7. Meijers B, Maas RJH, Sprangers B, Claes K, Poesen R, Bammens B, et al. The soluble urokinase receptor is not a clinical marker for focal segmental glomerulosclerosis. Kidney Int. 2014;85:636–40.
8. Wada T, Nangaku M, Maruyama S, Imai E, Shoji K, Kato S, et al. A multicenter cross-sectional study of circulating soluble urokinase receptor in Japanese patients with glomerular disease. Kidney Int. 2014;85:641–8.
9. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
10. The Japanese Society of Nephrology. Guidelines for the treatment of nephrotic syndrome. Nihon Jinzo Gakkai Shi. 2011;53:78–122.
11. The Japanese Society of Nephrology. Guidelines for the treatment of rapidly progressive glomerulonephritis. Nihon Jinzo Gakkai Shi. 2011;53:509–55.
12. Maas RJ, Wetzels JF, Deegens JK. Serum-soluble urokinase receptor concentration in primary FSGS. Kidney Int. 2012;81:1043–4.
13. Maas RJ, Deegens JK, Wetzels JF. Serum suPAR in patients with FSGS: trash or treasure? Prediatr Nephrol. 2013;28:1041–8.
14. Zang B, Shi W, Ma J, Sloan A, Faul C, Wei C, et al. The calcineurin-NFAT pathway allows for urokinase receptor-mediated β3 integrin signaling to cause podocyte injury. J. Mol. Med. (Berl.). 2012;90:1407–20.
15. Bock ME, Price H, Gallon L, Langman CB. Serum soluble urokinase-type plasminogen activator receptor levels and idiopathic FSGS in children: A single-center reports. Clin Am Soc Nephrol. 2013;8:1304–11.

16. Backes Y, van der Sluijs KF, Mackie DP, Tacke F, Koch A, Tenhunen JJ, et al. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. Intensive Care Med. 2012;38:1418–28.
17. Koch A, Voigt S, Kruschinski C, Sanson E, Duckers H, Horn A, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit Care. 2011;15:R63.
18. Yilmaz G, Koksal I, Karahan SC, Mentese A. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in systematic inflammatory response syndrome. Clin Biochem. 2011;44:1227–30.
19. Baraldi A, Zambruno G, Furci L, Ballestri M, Tombesi A, Ottani D, et al. β_1 and β_3 integrin upregulation in rapidly progressive glomerulonephritis. Nephrol Dial Transplant. 1995;10:1155–61.

Clin Exp Nephrol
DOI 10.1007/s10157-014-0993-y

ORIGINAL ARTICLE

The influences of larger physical constitutions including obesity on the amount of urine protein excretion in primary glomerulonephritis: research of the Japan Renal Biopsy Registry

Yuriko Yonekura · Shunsuke Goto · Hitoshi Sugiyama · Hiroshi Kitamura · Hitoshi Yokoyama · Shinichi Nishi

Received: 20 August 2013 / Accepted: 22 May 2014

Abstract
Background This study aimed to describe the influences of larger physical constitutions including obesity on the amount of urine protein excretion (AUPE) in primary glomerulonephritis. The distinct effects on the AUPE in various types of glomerulonephritis were evaluated.
Methods Using the database of the Japan Renal Biopsy Registry (J-RBR) from 2007 to 2010, 4060 cases with primary glomerulonephritis including MCNS, FSGS, MN, MPGN, IgAN, and non-IgA were reviewed. The AUPEs were compared between high and low Body Mass Index (BMI) groups, and larger and smaller body surface area (BSA) groups using the indexes of BMI 25.0 kg/m^2 and BSA 1.73 m^2 in all cases and in each histological group. Multivariable analysis was performed to evaluate the predominant contributors to the AUPE.
Results The larger physical constitution groups (BMI $\geq$25.0 kg/m^2 or BSA $\geq$1.73 m^2) had significantly higher AUPEs in all cases with primary glomerulonephritis. When compared in each histological group, the mean AUPEs were significantly higher in the larger physical constitution groups, excluding the FSGS and non-IgA groups. Multiple regression analysis revealed that the significant contributors to the AUPE were BMI and BSA in MCNS and MN, whereas BMI and BSA were not significant and mean blood pressure and serum creatinine were significant in FSGS and non-IgA.
Conclusion Larger physical constitutions including obesity had a significant impact on the increase in the AUPE in primary glomerulonephritis, especially in MCNS and MN. However, FSGS and non-IgA were distinct for having blood pressure and renal dysfunction as possibly the major causes of proteinuria.

Keywords Obesity · Urine protein · Glomerulonephritis · Body mass index · Body surface area

Y. Yonekura · S. Goto · S. Nishi (✉)
Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki, Chuo-ku, Kobe, Hyogo 650-0017, Japan
e-mail: snishi@med.kobe-u.ac.jp

H. Sugiyama
Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, 2-5-1 Shikata, Okayama 700-8558, Japan

H. Kitamura
Department of Pathology, Clinical Research Center, National Hospital Organization Chiba East National Hospital, 673 Nitona, Chiba, Chiba 260-8712, Japan

H. Yokoyama
Department of Nephrology, Kanazawa Medical University School of Medicine, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

Introduction

Multiple mechanisms, such as hyperfiltration, increased glomerular capillary wall tension, and podocyte stress, have been postulated as affecting the amount of urine protein of obese person [1]. Ohashi et al. [2] used bioelectrical impedance analysis to evaluate the correlation between BMI, ambulatory blood pressure, and total body water. These investigators found significant relationships between BMI, proteinuria and hypertension. In glomerulonephritis, Tanaka et al. [3] proved that increased proteinuria is associated with glomerular enlargement and glomerular basement membrane thickening in obese

Published online: 11 June 2014

patients with IgA nephropathy. Woo et al. [4] suspected that the dramatic increase in focal segmental glomerulosclerosis in Asian countries might be related to the increase of the obesity population worldwide. It is thought that larger physical constitutions including obesity affect the glomeruli both physiologically and pathologically, as well as the amount of urine protein excretion (AUPE) in glomerulonephritis. Recently, the average age of patients with glomerulonephritis has been increasing in Japan. Data from the Japan Renal Biopsy Registry (J-RBR) showed that the mean ages of patients with chronic nephritic syndrome and nephrotic syndrome were 42.5 and 51.5 years, respectively [5]. In the middle-aged population, the rate of complications from obesity rises [6]; therefore, attention should be given to the extent of the affect this non-histological factor has on the AUPE. However, unfortunately edematous condition is not perfectly excluded in nephrotic patients. Thus, we determined that the goal of the study was to evaluate the relationships between larger physical constitution including obesity and the AUPE in each histological type of glomerulonephritis.

Materials and methods

Subjects

This was a cross sectional study of a cohort of patients who were registered in the J-RBR from 31 July 2007 to 31 October 2010. During that time period, 10,550 patients $\geq$18 years were registered in the J-RBR. From the data on these patients, information was extracted on pathologically and clinically diagnosed primary glomerulonephritis including minimal change nephrotic syndrome (MCNS), focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), membranoproliferative glomerulonephritis (MPGN), IgA nephropathy (IgAN), and non-IgA type mesangial proliferative glomerulonephritis (non-IgA). Exclusion criteria were: patients with secondary glomerulonephritis, body weight under 30 kg or over 150 kg, height under 140 cm or over 200 cm, serum creatinine (S–Cr) under 0.1 mg/dL or over 20 mg/dL, serum total protein under 1 g/dL or over 12 g/dL, serum albumin over 6 g/dL, and the AUPE over 30 g/day. The AUPE was measured from a 24-h urine collection. Nephrotic syndrome was defined as low albumin (<3.0 g/dL) and AUPE more than 3.5 g/day.

The ethics committee of the Japanese Society of Nephrology comprehensively approved the study, and a local committee of participating centers and their affiliated hospitals individually approved the study. Written informed consent was obtained from the patients at the time of biopsy and at the time they were registered to participate in the study. The J-RBR/J-KDR is registered in the Clinical Trial Registry of UMIN (Registered Number UMIN000000618).

Physical constitutions

BMI and BSA were used to evaluate a patient's body constitutions. According to the definition of obesity by the Japan Society for the Study of Obesity (JASSO), the patients were divided into two groups: BMI $\geq$25 as the high BMI group, and BMI <25 as the low BMI group. The DuBoi's formula ($Wt^{0.425}$(kg) $\times$ $Ht^{0.725}$(cm) $\times$ 0.007184) was used to calculate BSA. The subjects were divided into two groups: BSA $\geq$1.73 m^2 as the high BSA group, and BSA <1.73 m^2 as the low BSA group.

Analysis between AUPE and physical constitution

The mean $\pm$ standard deviation (SD) of the AUPE for each type of glomerulonephritis was calculated. The AUPE was compared between the high and low BMI groups, and between the high and low BSA groups in all patients and in each type of glomerulonephritis. Additionally, the AUPE was compared between patients with or without nephrotic syndrome.

Multivariable analyses were performed to extract significant factors contributing to AUPE. BMI, BSA, age, S–Cr, and mean arterial pressure (MAP = [(2 $\times$ diastolic BP) + systolic BP]/3), were selected as independent variables.

Statistical analyses

Statistical analyses were performed using Dr. SPSS II for Windows (SPSS, Tokyo, Japan). Data were compared using Kruskal–Wallis analysis and Mann–Whitney *U* test. p values <0.05 were considered to indicate statistical significance. Multiple linear regression analysis was performed as a multivariable analysis in this study.

Results

Patient profiles

Among the 10,550 cases registered in the J-RBR, 4060 cases with primary glomerulonephritis were extracted (MCNS, 454 cases; FSGS, 279 cases; MN, 696 cases; MPGN, 105 cases; IgAN, 2354 cases; and non-IgA, 172 cases). The mean age was 47.3 years. The mean BMI and mean BSA were 23.2 kg/m^2 and 1.65 m^2, respectively. Among the 4060 patients with primary glomerulonephritis, 27.6 % of the patients had BMI $\geq$25.0 kg/m^2 and 35.2 %

had BSA $\geq$1.73 m^2. The mean AUPE was 2.50 g/day. A quarter of all cases had proteinuria at the nephrotic level of $\geq$3.5 g/day (Table 1).

In the MN and MPGN groups, the mean ages tend to be older, >60 years. In the IgAN group, the ages tend to be younger, about 40 years. The mean AUPE exceeded the nephrotic level, 3.5/day, in the MCNS, MN, and MPGN groups. The average AUPE in the MCNS group was 7.00 g/day, but 22.5 % of the patients in this group showed the sub-nephrotic range of AUPE at the time of registration. In the IgAN and non-IgA groups, the rates of nephrotic syndrome were lower, <10 %. In the FSGS, MN, and MPGN groups, MAP averages were higher and exceeded 95.0 mmHg (Table 2).

Table 1 Characteristics of all patients (n = 4060)

Age (years)	47.3 ± 17.9
Male (%)	54.4
Body weight (kg)	61.6 ± 12.5
Height (m)	1.63 ± 0.09
BMI (kg/m^2)	23.2 ± 3.80
Rate of BMI $\geq$25 (%)	27.6
BSA (m^2)	1.65 ± 0.19
Rate of BSA $\geq$1.73 (%)	35.2
Proteinuria (g/day)	2.50 ± 3.34
Rate of nephrotic syndrome (%)	25.0
Serum creatinine (mg/dL)	1.04 ± 0.78
Serum albumin (g/dL)	3.40 ± 1.01
Mean arterial pressure (mmHg)	92.5 ± 13.5

Data are shown as mean ± S.D

BMI body mass index, *BSA* body surface area

Comparison between larger and smaller physical constitution groups

In the evaluation of all subjects, the mean AUPE was significantly higher in the high BMI and high BSA groups, respectively (low vs. high BMI 2.21 ± 3.03 vs. 3.27 ± 3.93, $p < 0.001$; low vs. high BSA 2.28 ± 2.99 vs. 2.89 ± 3.88, $p < 0.001$) (Fig. 1). When compared with each histological group, the averages of the AUPE were significantly higher in the high BMI groups than in the low BMI groups, excluding the FSGS and non-IgA groups (Fig. 2). The averages of the AUPE were also significantly higher in the high BSA groups compared with the low BSA groups, excluding the FSGS, MPGN and non-IgA groups (Fig. 3).

Comparison of nephrotic and non-nephrotic cases

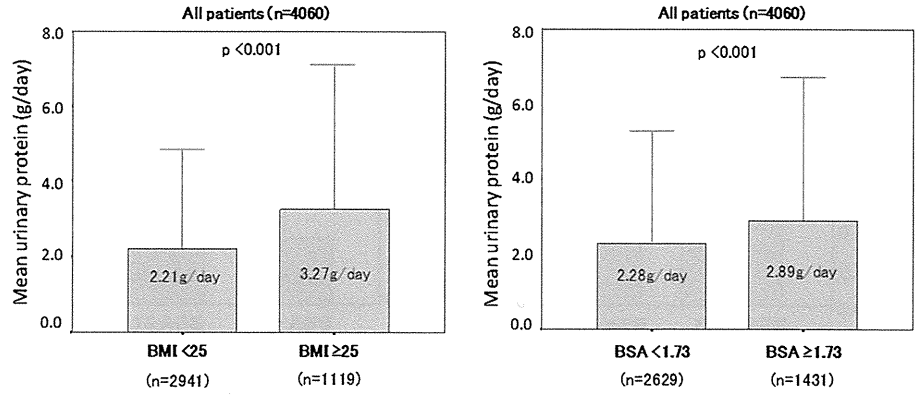
Comparison of the AUPE in the nephrotic patients of each histological group showed significant differences between the MCNS, MN, MPGN, and IgAN groups. The averages of the AUPE were significantly higher in the high BMIgroups (low vs. high BMI, MCNS 8.23 ± 4.18 vs. 9.31 ± 4.64, $p = 0.021$; MN 6.08 ± 3.19 vs. 7.23 ± 3.97, $p = 0.003$; MPGN 5.69 ± 2.18 vs. 8.30 ± 3.27, $p = 0.007$; IgAN 5.00 ± 1.95 vs. 5.94 ± 2.43, $p = 0.015$). However, the FSGS and non-IgA groups did not show significant differences between the high and low BMI groups with nephrotic syndrome (Fig. 4). Comparing the

Table 2 Basic characteristics of patients in each primary histological group

Histological diagnosis	MCNS	FSGS	MN	MPGN	IgAN	Non-IgA	p
Number of Patients	454	279	696	105	2354	172	–
Age (years)	46.5 ± 19.6	49.3 ± 18.2	64.0 ± 11.4	61.6 ± 17.0	41.5 ± 15.5	50.2 ± 16.9	<0.01
Male (%)	57.9	64.2	57.8	56.2	51.9	47.7	–
Body Weight (kg)	63.6 ± 13.1	64.4 ± 14.3	60.6 ± 11.6	58.3 ± 10.8	61.4 ± 12.5	61.0 ± 12.4	<0.01
Height (m)	1.63 ± 0.09	1.63 ± 0.09	1.60 ± 0.09	1.59 ± 0.09	1.63 ± 0.088	1.62 ± 0.09	<0.01
BMI (kg/m^2)	23.8 ± 4.10	24.1 ± 4.57	23.7 ± 3.55	23.1 ± 3.50	22.88 ± 3.68	23.3 ± 3.78	<0.01
Rate of BMI $\geq$25 (%)	31.7	35.8	31.8	21.9	24.7	29.1	–
BSA (m^2)	1.68 ± 0.19	1.69 ± 0.20	1.62 ± 0.18	1.59 ± 0.17	1.66 ± 0.19	1.64 ± 0.18	<0.01
Rate of BSA $\geq$1.73 (%)	40.1	44.4	28.3	21.9	36	33.1	–
AUPE (g/day)	7.00 ± 4.89	3.41 ± 3.13	4.08 ± 3.54	4.10 ± 3.39	1.06 ± 1.38	1.43 ± 2.27	<0.01
Rate of nephrotic syndrome (%)	77.5	38.7	49.3	51.4	5.90	11.0	–
Serum creatinine (mg/dL)	1.06 ± 0.77	1.24 ± 0.96	0.90 ± 0.47	1.45 ± 1.05	1.02 ± 0.74	1.12 ± 1.44	<0.01
Serum albumin (g/dL)	1.92 ± 0.77	3.10 ± 1.06	2.63 ± 0.81	2.87 ± 0.73	3.94 ± 0.55	3.87 ± 0.78	<0.01
Mean arterial pressure (mmHg)	89.6 ± 12.2	95.9 ± 13.7	95.2 ± 13.8	99.6 ± 14.2	91.4 ± 13.3	94.1 ± 13.8	<0.01

Data are shown as mean ± SD

MCNS minimal change nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis, *MN* membranous nephropathy, *MPGN* membranoproliferative glomerulonephritis, *IgAN* IgA nephropathy, *non–IgA* non-IgA glomerulonephritis, *AUPE* amount of urinary protein excretion



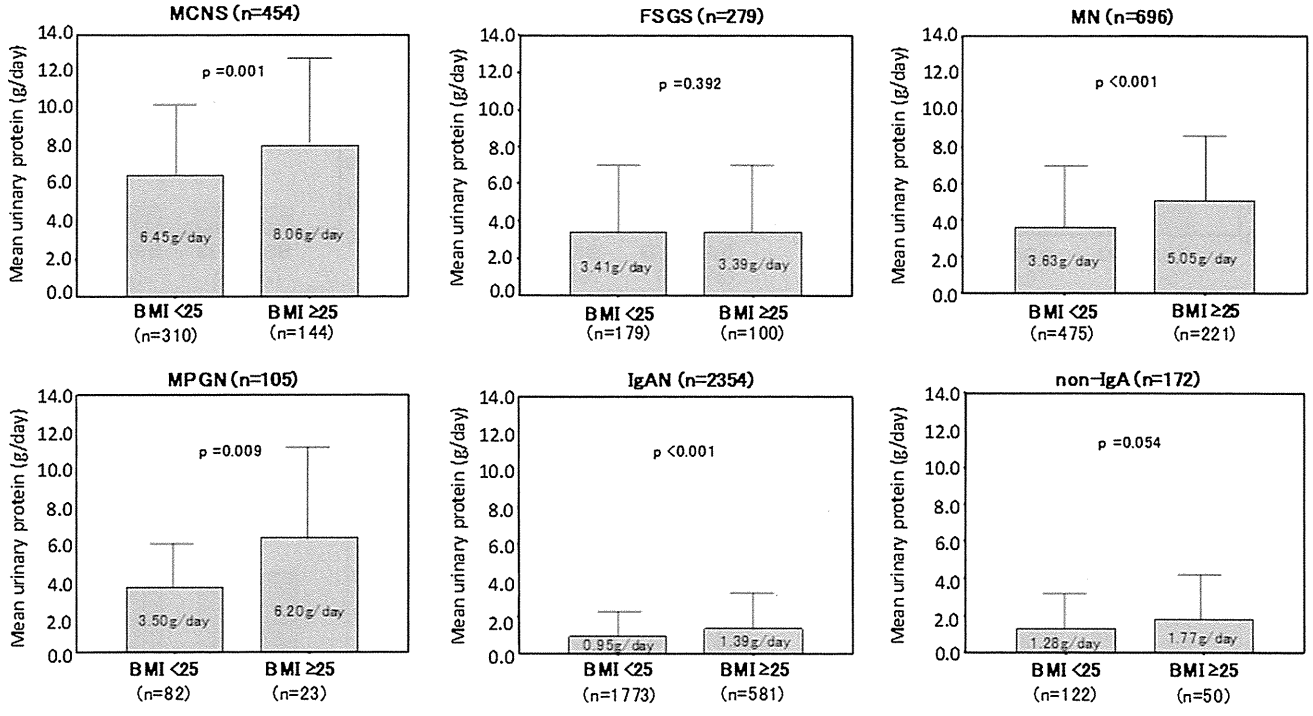


Fig. 1 The comparison amount of urine protein between high and low BMI/BSA groups in all patients. Data are shown as mean ± SD, *BMI* body mass index, *BSA* body surface area

Fig. 2 The comparison amount of urine protein between high and low BMI groups in all patients of each histological group. Data are shown as mean ± SD, *BMI* body mass index, *BSA* body surface area

high and low BSA groups with nephrotic syndrome, significant differences were noted only in the MCNS and MN groups (low vs. high BSA, MCNS 7.62 ± 3.59 vs. 9.94 ± 4.96, $p < 0.001$; MN 6.17 ± 3.19 vs. 7.31 ± 4.13, $p = 0.004$) (Fig. 5).

Comparison of the AUPE in non-nephrotic patients of each histological group showed significant differences in the FSGS and IgAN groups between the high and low BMI groups (low vs. high BMI, FSGS 1.24 ± 0.93 vs. 1.60 ± 0.99, $p = 0.016$; IgAN 0.74 ± 0.72 vs. 0.96 ± 0.77, $p < 0.001$) (Fig. 6). Comparison of the high and low BSA groups with non-nephrotic syndrome, significant differences were noted only in the IgA and non-IgA groups (low vs. high BSA, IgAN 0.75 ± 0.73 vs. 0.87 ± 0.75, $p < 0.001$; non-IgA 0.71 ± 0.86 vs. 0.82 ± 0.71, $p = 0.039$) (Fig. 7).

Multivariable analysis

To evaluate the contributing factors for the AUPE, multiple regression analysis was performed in all, nephrotic and non-nephrotic patients. BMI and BSA were found to be the significant independent contributing factors for the AUPE in all cases, all nephrotic and all non-nephrotic patients, respectively. Nevertheless, in the restricted FSGS group, BMI and BSA were not the significant independent contributing factors for the AUPE even in nephrotic and non-nephrotic cases. Rather, S–Cr and MAP were the

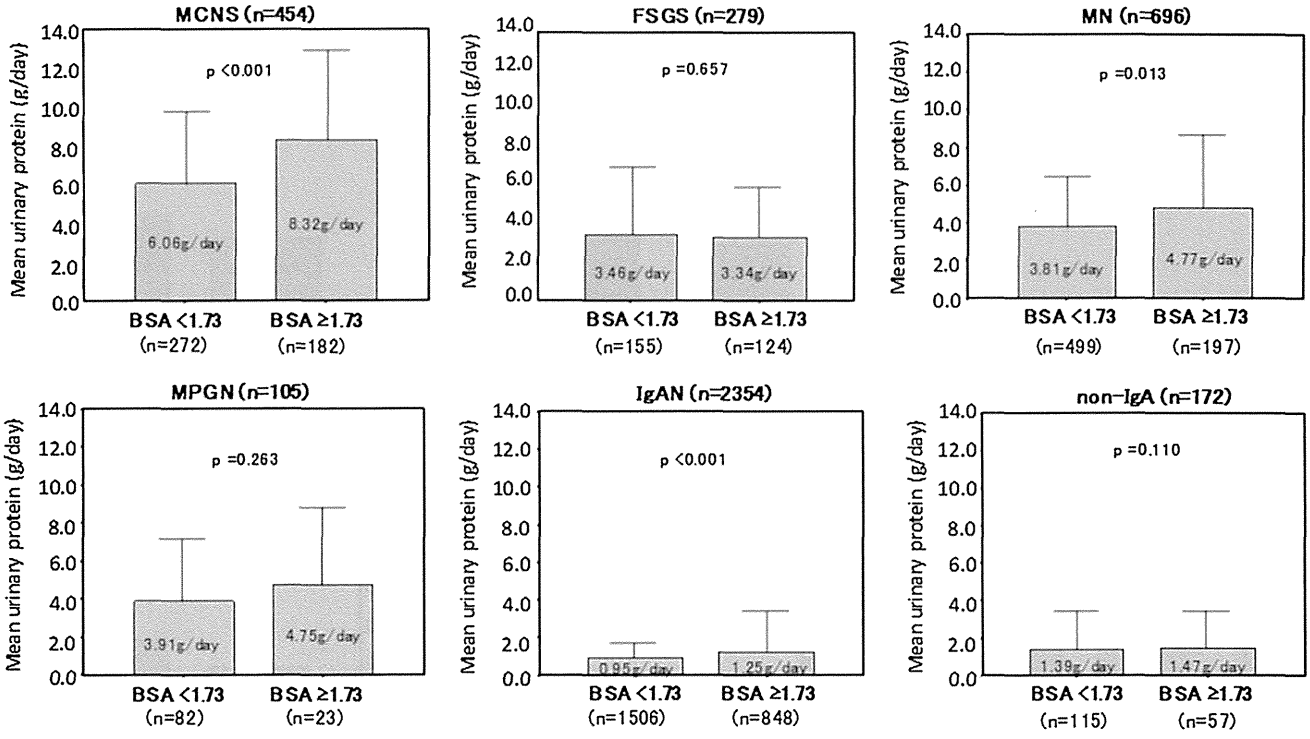


Fig. 3 The comparison amount of urine protein between high and low BSA groups in all patients of each histological group. Data are shown as mean ± SD, *BMI* body mass index, *BSA* body surface area

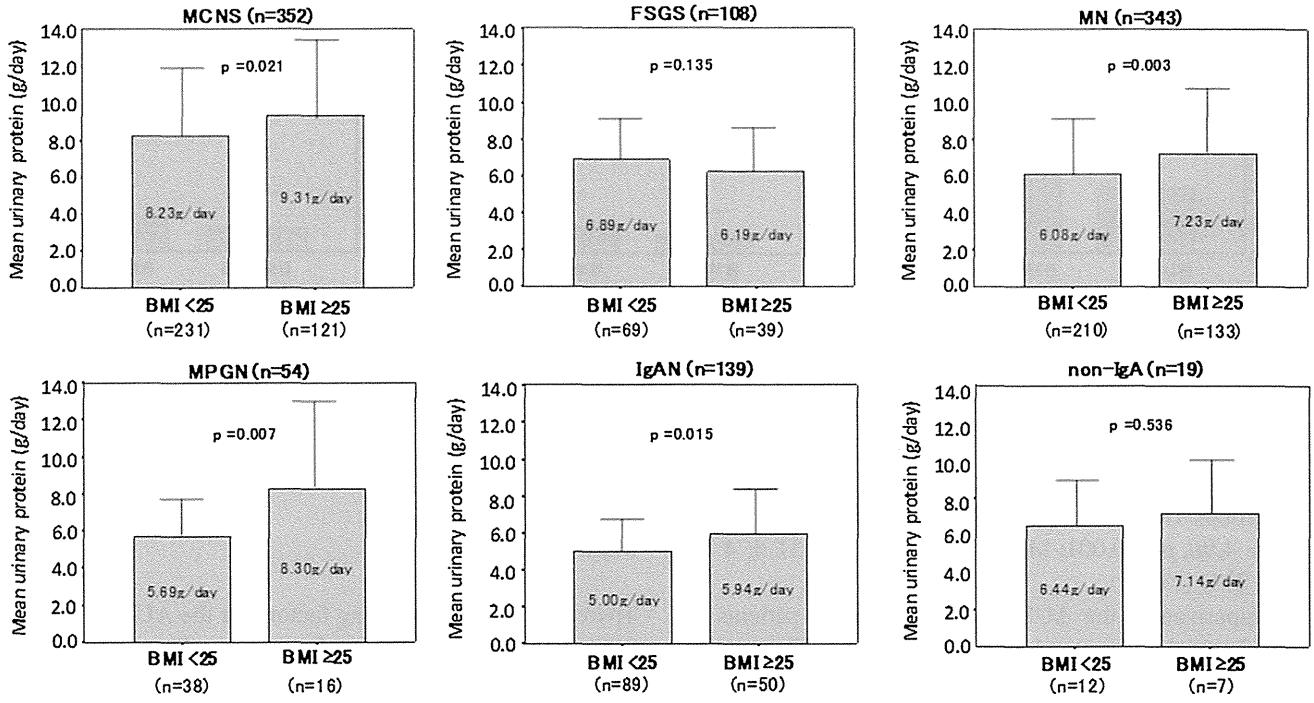


Fig. 4 The comparison amount of urine protein between high and low BMI groups in nephrotic patients of each histological group. Data are shown as mean ± SD, *BMI* body mass index, *BSA* body surface area

significant contributing factors for the AUPE in the FSGS group. Similarly in the analysis of the non-IgAN group, BMI and BSA were not the significant independent contributing factors for the AUPE even in nephrotic and non-nephrotic cases. Only S–Cr was a significant independent factor contributing to the AUPE (Table 3). In other

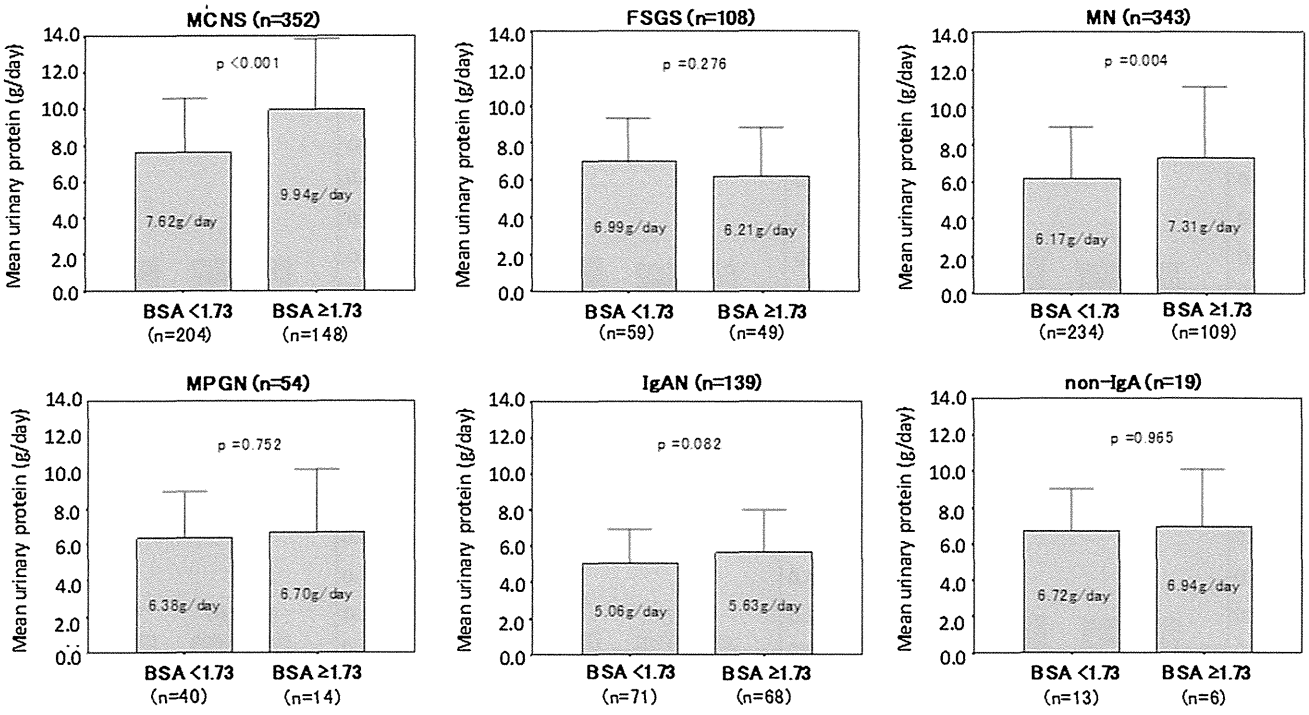


Fig. 5 The comparison amount of urine protein between high and low BSA groups in nephrotic patients of each histological group. Data are shown as mean ± SD, *BMI* body mass index, *BSA* body surface area

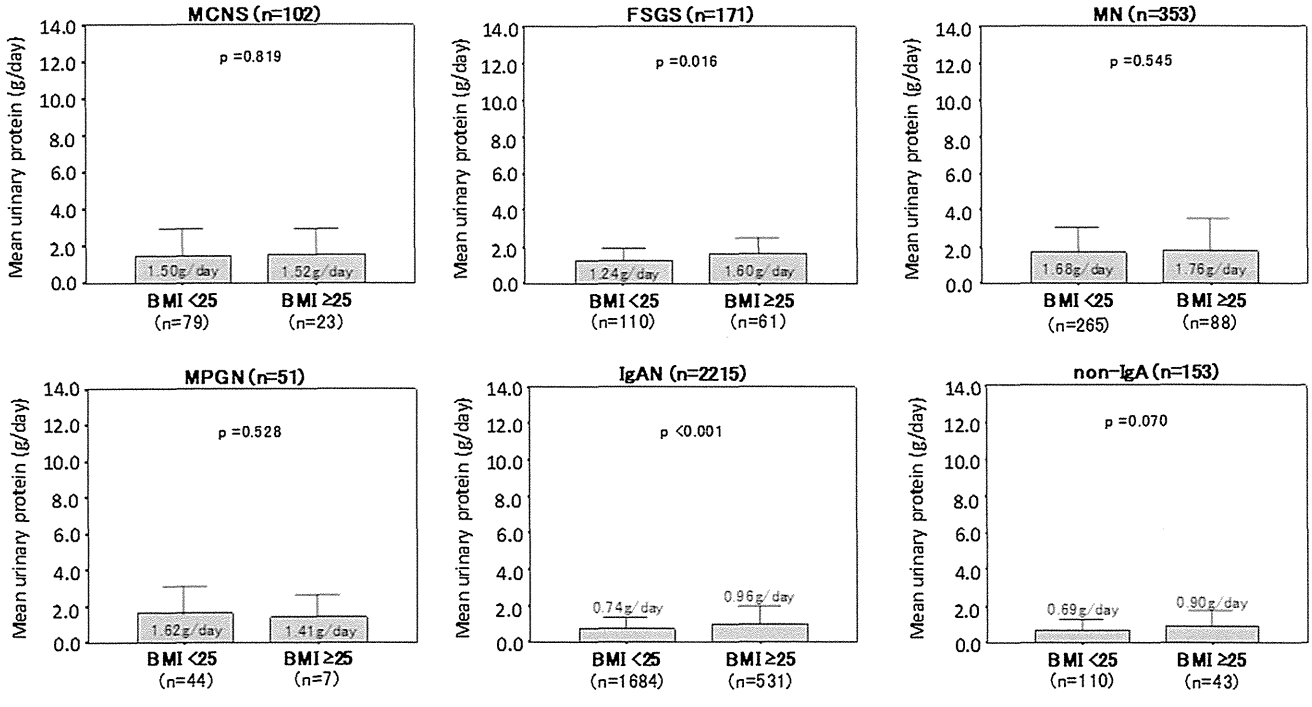


Fig. 6 The comparison amount of urine protein between high and low BMI groups in non-nephrotic patients of each histological group. Data are shown as mean ± SD, *BMI* body mass index, *BSA* body surface area

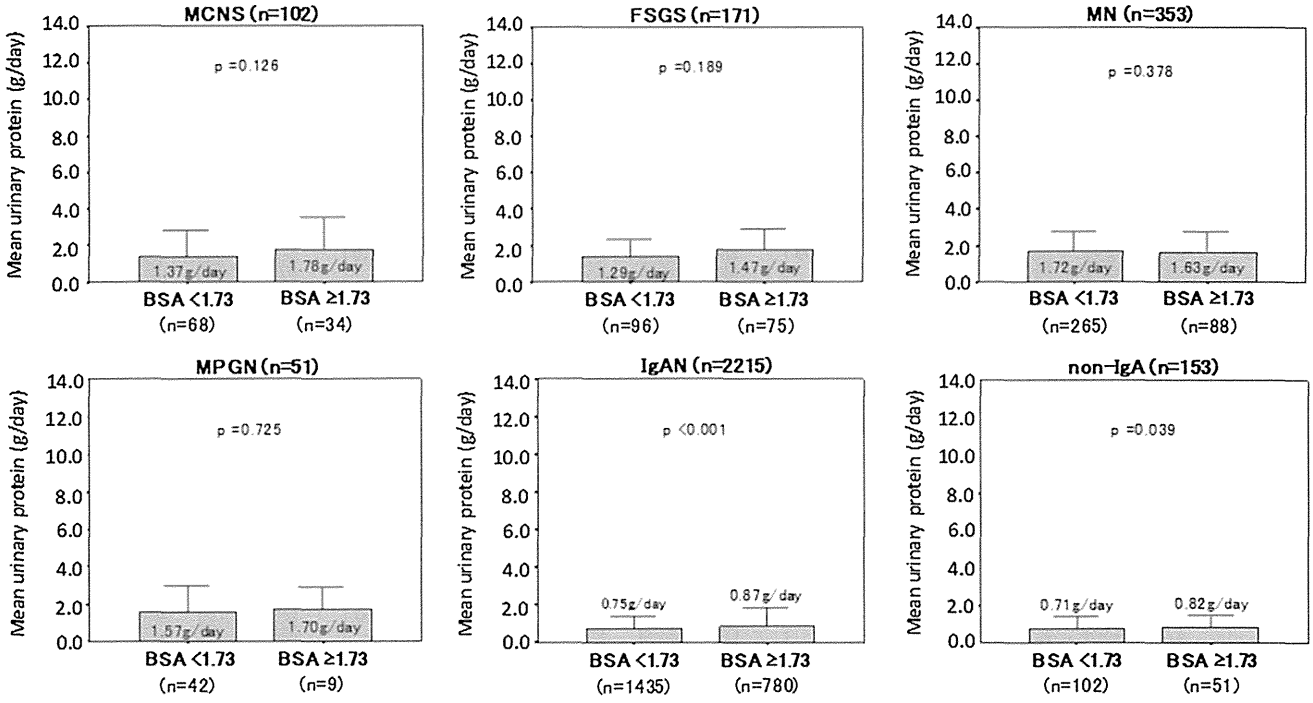


Fig. 7 The comparison amount of urine protein between high and low BSA groups in non-nephrotic patients of each histological group. Data are shown as mean ± SD, *BMI* body mass index, *BSA* body surface area

way BMI and BSA showed the significant impacts on the AUPE in only nephrotic MCNS and MN. Interestingly BMI and BSA of IgAN were significant contributors to the AUPE in nephrotic and non-nephrotic groups.

Discussion

Consistent with the tendency toward an aging society, the average age of patients with primary glomerulonephritis has been rising in recent years. The latest J-RBR report revealed that the average ages of patients with chronic nephritic syndrome and nephrotic syndrome were 42.5 and 51.5 years, respectively [5]. When limited to patients with membranous nephropathy, the average was 62.2 years in another J-RBR data report [7].

In the present study, the mean age of all patients with primary glomerulonephritis was 47.3 years (Table 1). The mean age of each histological group exceeded 40 years (Table 2). The rates of obesity (BMI $\geq$25 kg/m^2) ranged from 21.0 to 35.5 % in the histological groups.

The prevalence of obesity has been increasing in many countries. According to the international definition of obesity, the prevalence of obesity (BMI $\geq$30 kg/m^2) in Japanese adults has been said to be approximately 1–3 % [6], which is quite low compared with other countries. On the other hand, the recent prevalence of being overweight (BMI $\geq$25 kg/m$^{2)}$ that corresponds to the Japanese definition of obesity, was discovered to be approximately 20–30 %, especially in middle-aged people [6].

There has been a focus on obesity as a risk factor for impairment due to chronic kidney disease (CKD) and cardiovascular disease because obesity is an inducer of massive proteinuria [8]. Additionally, in the progression of CKD in obese patients, the pathogenic mechanisms are considered to be hypertension, dysregulated production of adipokines, and inflammation derived from fat accumulation [8, 9]. Therefore, reduction of BMI is the preferential strategy for preventing the progress of CKD [10, 11]. However, the influences of larger physical constitutions including obesity on the AUPE have not been sufficiently evaluated in various types of primary glomerulonephritis.

Obesity-related glomerulopathy has received attention as an independent glomerulopathy characterized by dominant proteinuria with glomerular hypertrophy alone or focal segmental glomerulosclerosis lesions with glomerular hypertrophy [12–14]. Its etiologies have been conjectured to be associated with activation of the renin-angiotensin system, inflammation, and oxidative stress that develops in obese patients [15, 16]. In particular, glomerular hyperfiltration is believed to lead to the characteristic proteinuira [12, 17]. Thus, it is important to consider that proteinuria is produced not only by glomerular damage, but also through the disproportional physical constitution that is found particularly in obese patients.

Table 3 Multiple linear regression analysis for the amount of urine protein

All cases				
	β	Coefficient	95 % CI	*p*
All cases				
Model 1				
Age	0.030	0.163	0.024 to 0.037	<0.01
BMI	0.123	0.142	0.093 to 0.153	<0.01
S–Cr	0.395	0.090	0.245 to 0.546	<0.01
MAP	0.008	0.031	−0.001 to 0.017	0.089
Model 2				
Age	0.036	0.196	0.030–0.043	<0.01
BSA	2.056	0.117	1.447–2.666	<0.01
S–Cr	0.351	0.080	0.198–0.503	<0.01
MAP	0.010	0.039	0.001–0.0019	<0.05
Non–nephrotic cases				
Model 1				
Age	0.011	0.215	0.009–0.013	<0.01
BMI	0.011	0.045	0.002–0.020	<0.05
S–Cr	0.151	0.024	0.104–0.199	<0.01
MAP	0.011	0.162	0.008–0.014	<0.01
Model 2				
Age	0.011	0.218	0.009 to 0.013	<0.01
BSA	−0.031	−0.007	−0.215 to 0.153	0.74
S–Cr	0.153	0.123	0.105 to 0.210	<0.01
MAP	0.012	0.174	0.009 to 0.014	<0.01
Nephrotic cases				
Model 1				
Age	−0.031	−0.152	−0.044 to 0.017	<0.01
BMI	0.106	0.120	0.045 to 0.167	<0.01
S–Cr	0.205	0.048	−0.089 to 0.500	0.171
MAP	−0.021	−0.080	−0.039 to 0.02	<0.05
Model 2				
Age	−0.020	−0.102	−0.035 to 0.006	<0.01
BSA	3.008	0.164	1.675 to 4.341	<0.01
S–Cr	0.152	0.036	−0.143 to 0.447	0.312
MAP	−0.022	−0.085	−0.040 to 0.004	<0.05
FSGS cases				
	β	Coefficient	95 % CI	*p*
All cases				
Model 1				
Age	0.014	0.081	−0.009 to 0.038	0.236
BMI	−0.028	−0.042	−0.119 to 0.062	0.539
S–Cr	0.426	0.140	0.026 to 0.826	<0.05
MAP	0.054	0.231	0.022 to 0.086	<0.01
Model 2				
Age	0.015	0.086	−0.009 to 0.040	0.223
BSA	−0.058	−0.004	−2.178 to 2.062	0.957
S–Cr	0.420	0.138	0.018 to 0.822	<0.05
MAP	0.052	0.223	0.021 to 0.084	<0.01
Non–nephrotic cases				
Model 1				
Age	0.008	0.138	−0.002 to 0.018	0.126
BMI	0.016	0.075	−0.020 to 0.051	0.386
S–Cr	0.159	0.192	0.019 to 0.299	<0.05
MAP	0.015	0.209	0.002 to 0.028	<0.05
Model 2				
Age	0.007	0.132	−0.003 to 0.018	0.158
BSA	0.097	0.020	−0.764 to 0.957	0.824
S–Cr	0.158	0.191	0.017 to 0.299	<0.05
MAP	0.016	0.220	0.003 to 0.029	<0.05
Nephrotic cases				
Model 1				
Age	0.008	0.057	−0.022 to 0.038	0.6
BMI	−0.090	−0.166	−0.210 to 0.031	0.142
S–Cr	0.574	0.192	−0.077 to 1.226	0.083
MAP	0.021	0.110	−0.021 to 0.063	0.324
Model 2				
Age	0.006	0.047	−0.024 to 0.037	0.678
BSA	−1.431	−0.119	−4.134 to 1.272	0.295
S–Cr	0.555	0.185	−0.100 to 1.210	0.095
MAP	0.017	0.089	−0.025 to 0.059	0.419
Non–IgAN cases				
	β	Coefficient	95 % CI	*p*
All cases				
Model 1				
Age	0.024	0.189	0.002 to 0.046	<0.05
BMI	0.051	0.086	−0.053 to 0.155	0.332
S–Cr	0.535	0.181	0.029 to 1.041	<0.05
MAP	0.005	0.035	−0.022 to 0.033	0.699
Model 2				
Age	0.027	0.212	0.004 to 0.050	<0.05
BSA	1.142	0.094	−0.993 to 3.277	0.292
S–Cr	0.513	0.174	0.003 to 1.023	<0.05
MAP	0.007	0.046	−0.020 to 0.034	0.601
Non–nephrotic cases				
Model 1				
Age	−0.005	−0.095	−0.014 to 0.004	0.321

Table 3 continued

Non–IgAN cases				
	β	Coefficient	95 % CI	*p*
BMI	0.036	0.161	–0.005 to 0.077	0.086
S–Cr	0.333	0.264	0.100 to 0.567	<0.01
MAP	0.002	0.036	–0.009 to 0.013	0.705
Model 2				
Age	–0.003	–0.070	–0.013 to 0.006	0.485
BSA	0.463	0.096	–0.448 to 1.374	0.316
S–Cr	0.324	0.257	0.083 to 0.565	<0.0 1
MAP	0.004	0.070	–0.007 to 0.015	0.451
Nephrotic cases				
Model 1				
Age	–0.125	–0.563	–0.349 to 0.099	0.244
BMI	–0.414	–0.606	–1.160 to 0.332	0.244
S–Cr	0.033	0.016	–1.764 to 1.831	0.968
MAP	–0.028	–0.144	–0.200 to 0.144	0.727
Model 2				
Age	–0.099	–0.447	–0.355 to 0.157	0.409
BSA	–4.691	–0.450	–19.248 to 9.865	0.489
S–Cr	–0.026	–0.012	–2.418 to 2.366	0.981
MAP	0.030	–0.156	–0.251 to 0.191	0.769

Model 1: Independent variables are Age, BMI, S-Cr, MAP. Model 2: Independent variables are Age, BSA, S-Cr, MAP

BMI body mass index, *BSA* body surface area, *S–Cr.* serum creatinine, *MAP* mean arterial pressure

It has been reported that the amount of urine protein is affected by obesity [2]. In general, the physical constitution of Japanese people is smaller compared with that of people in the West. The Western definition of obesity is usually BMI of 30 kg/m^2, while the Japanese definition is 25 kg/m^2, as determined by Japan Society for the Study of Obesity (JASSO) [18]. Glomerular filtration rate (GFR) is usually adjusted by BSA at a value of 1.73 m^2 worldwide, because 1.73 m^2 is the average BSA in the general adult population in Western countries. However, the mean BSA of Japanese people seems to be smaller than 1.73 m^2. In chemotherapy for malignancy, anti-cancer agents are usually adjusted by BSA. In the relevant literature, 1.75 m^2 is seen to have been determined as a larger BSA size [19, 20]. Therefore, we set the parameters between larger and smaller physical constitutions as BMI 25 kg/m^2 and BSA 1.73 m^2, respectively.

Both of these indexes of Japanese physical constitutions, BMI $\geq$25 kg/m^2 and BSA $\geq$1.73 m^2, affected the AUPE in the evaluation of all the study patients. The higher BMI and BSA groups had significantly larger AUPEs ($p < 0.001$; $p < 0.001$) (Fig. 1). Interestingly, in the evaluation of each histological group, the FSGS and non-IgA groups did not show any significant differences between larger and

Table 4 Multiple linear regression analysis for the amount of urine protein

MCNS cases				
	β	coefficient	95 % CI	p
All cases				
Model 1				
Age	–0.038	–0.152	–0.063 to –0.12	<0.01
BMI	0.184	0.162	0.063 to 0.306	<0.01
S–Cr	0.395	0.067	–0.219 to 1.009	0.207
MAP	0.027	0.068	–0.016 to 0.070	0.217
Model 2				
Age	–0.018	–0.073	–0.045 to 0.009	0.194
BSA	5.535	0.221	2.775 to 8.295	<0.01
S–Cr	0.212	0.036	–0.409 to 0.834	0.502
MAP	0.023	0.057	–0.020 to 0.065	0.292
Non–nephrotic cases				
Model 1				
Age	0.013	0.206	–0.002 to 0.027	0.09
BMI	0.014	0.040	–0.064 to 0.092	0.723
S–Cr	–0.102	–0.096	–0.350 to 0.146	0.415
MAP	0.016	0.173	–0.005 to 0.037	0.138
Model 2				
Age	0.014	0.231	–0.001 to 0.029	0.061
BSA	0.745	0.106	–0.803 to 2.293	0.341
S–Cr	–0.112	–0.106	–0.359 to 0.134	0.368
MAP	0.015	0.161	–0.006 to 0.036	0.164
Nephrotic cases				
Model 1				
Age	–0.056	–0.253	–0.081 to 0.031	<0.01
BMI	0.087	0.088	–0.031 to 0.204	0.148
S–Cr	0.976	0.160	0.264 to 1.688	<0.05
MAP	0.010	0.028	–0.034 to 0.054	0.654
Model 2				
Age	–0.042	–0.188	–0.069 to 0.014	<0.01
BSA	3.705	0.170	0.917 to 6.494	<0.01
S–Cr	0.775	0.127	0.046 to 1.503	<0.05
MAP	0.003	0.009	–0.040 to 0.047	0.875

MN cases				
	β	Coefficient	95 % CI	*p*
ALL cases				
Model 1				
Age	0.012	0.037	–0.014 to 0.038	0.373
BMI	0.187	0.183	0.103 to 0.271	<0.01
S–Cr	1.608	0.216	1.000 to 2.215	<0.01
MAP	0.010	0.038	–0.011 to 0.030	0.366
Model 2				
Age	0.024	0.075	–0.004 to 0.051	0.089
BSA	2.760	0.143	1.086 to 4.435	<0.01
S–Cr	1.555	0.209	0.938 to 2.172	<0.01

Table 4 continued

MN cases				
	β	Coefficient	95 % CI	*p*
MAP	0.012	0.047	−0.009 to 0.033	0.262
Non–nephrotic cases				
Model 1				
Age	0.021	0.237	0.010 to 0.032	<0.01
BMI	0.009	0.030	−0.027 to 0.044	0.622
S–Cr	0.043	0.014	−0.331 to 0.416	0.822
MAP	0.009	0.117	<0.001 to 0.018	0.054
Model 2				
Age	0.018	0.204	0.006 to 0.031	<0.01
BSA	−0.335	−0.061	−1.074 to 0.405	0.373
S–Cr	0.112	0.037	−0.282 to 0.505	0.576
MAP	0.010	0.130	0.001 to 0.019	<0.05
Nephrotic cases				
Model 1				
Age	−0.018	−0.058	−0.054 to 0.018	0.333
BMI	0.184	0.186	0.067 to 0.301	<0.01
S–Cr	0.787	0.128	0.067 to 1.507	<0.05
MAP	−0.008	−0.035	−0.037 to 0.020	0.563
Model 2				
Age	0.000	<0.001	−0.038 to 0.038	1.00
BSA	3.374	0.181	1.088 to 5.660	<0.01
S–Cr	0.777	0.126	0.055 to 1.498	<0.05
MAP	−0.070	−0.020	−0.036 to 0.021	0.619
IgAN cases				
	β	Coefficient	95 % CI	*p*
ALL cases				
Model 1				
Age	0.004	0.041	0.000–0.008	0.079
BMI	0.031	0.086	0.015–0.047	<0.01
S–Cr	0.384	0.215	0.304–0.464	<0.01
MAP	0.018	0.176	0.013–0.023	<0.01
Model 2				
Age	0.005	0.054	0.001–0.009	<0.05
BSA	0.323	0.045	0.009–0.637	<0.01
S–Cr	0.377	0.211	0.296–0.457	<0.01
MAP	0.019	0.187	0.014–0.024	<0.05
Non–nephrotic cases				
Model 1				
Age	0.001	0.025	−0.001 to 0.004	0.299
BMI	0.013	0.063	0.004 to 0.023	<0.01
S–Cr	0.251	0.228	0.201 to 0.301	<0.01
MAP	0.013	0.218	0.010 to 0.016	<0.01
Model 2				
Age	0.002	0.034	−0.001 to 0.004	0.151
BSA	0.250	0.226	0.199 to 0.300	<0.01

Table 4 continued

IgAN cases				
	β	Coefficient	95 % CI	*p*
S–Cr	0.014	0.233	0.011 to 0.016	<0.01
MAP	0.016	0.004	−0.164 to 0.196	0.863
Nephrotic cases				
Model 1				
Age	0.005	0.044	−0.017 to 0.027	0.662
BMI	0.084	0.211	0.005 to 0.163	<0.05
S–Cr	−0.012	−0.008	−0.292 to 0.269	0.935
MAP	−0.007	−0.058	−0.034 to 0.019	0.584
Model 2				
Age	0.009	0.081	−0.013 to 0.031	0.419
BSA	2.781	0.308	1.003 to 4.559	<0.01
S–Cr	−0.056	−0.041	−0.331 to 0.220	0.689
MAP	−0.006	−0.048	−0.032 to 0.020	0.639

Model 1: Independent variables are Age, BMI, S-Cr, MAP. Model 2: Independent variables are Age, BSA, S-Cr, MAP

BMI body mass index, *BSA* body surface area, *S–Cr.* serum creatinine, *MAP* mean arterial pressure

smaller physical constitutions as evaluated by BMI and BSA (Fig. 2). There were no significant differences in the comparison of MPGN between the larger and smaller BSA groups. In MCNS and MN groups, the main histological damage is in the glomerular capillary walls. Conversely, in the remaining groups, the main histological damage is found both on the glomerular capillary walls and in the mesangial areas. We suspected that this histological difference might produce the distinguished results in the types of glomerular diseases.

We performed detailed examinations to divide the patients into two groups, the nephrotic and non-nephrotic groups, because the nephrotic patients of the larger physical constitution groups may have two conditions: systemic edema and obesity [20]. Patients with MCNS and MN showed significant differences, but even in the evaluation of nephrotic cases, patients with FSGS and non-IgA did not show any significant differences in the AUPE between the larger and smaller BMI or BSA groups.

This result may indicate that the capillary wall damage seen in patients with MCNS and MN is easily affected by hyperfiltration associated with obesity or larger physical constitution. The condition of overfilling, dependent on sodium retention in nephrotic syndrome, may worsen the permeability of the glomerular capillary walls in MCNS and MN [21]. Recently, sodium retention derived from activation of the amiloride-sensitive epithelial sodium channel (ENaC) has attracted attention in nephrotic syndrome [22]. The larger physical condition including systemic edema and obesity might more strongly affect the

AUPE in the condition of increased capillary wall permeability of nephrotic MCNS and MN cases. Conversely, we hypothesized that the complex damage to the glomerular capillary walls and mesangial areas seen in FSGS and non-IgA may produce severe proteinuria that is influenced by factors other than glomerular hyperfiltration and/or overfilling condition, even in patients with obesity or larger physical constitution.

To confirm this hypothesis, we performed multivariable analysis. In the analysis in all cases, BMI and BSA were certainly the significant independent factors contributing to the AUPE (Table 3). On the other hand, BMI and BSA were not the significant contributors to the AUPE in the analysis of FSGS and non-IgA. Even in nephrotic cases, BMI and BSA did not significantly affect AUPE in FSGS and non-IgAN. In these groups, MAP and S–Cr were significant independent factors contributing to the AUPE, respectively (Table 3). The main influencing causes of proteinuria were apparently different between the MCNS/MN group and the FSGS/non-IgA group. In the analysis of the non-nephrotic groups (Fig. 4), the effects of larger physical constitutions on the AUPE seemed to disappear in MCNS and MN. This result supports the fact that larger physical constitutions including obesity easily affect the AUPE in particular nephrotic syndrome that have main histological damages on GBM. Both IgAN and non-IgAN are indeed mesangial proliferative GN, while the BMI and BSA significantly affected the AUPE of IgAN in multivariate analysis (Table 4). Unfortunately, we could not understand this conflicting point. IgAN cases were youngest and their mean blood pressure was lowest in our all cases. From this feature, larger physical condition including edema and obesity might easily affect the glomerular capillary permeability in IgAN compared to non-IgAN.

We noticed some different effects between BMI and BSA in MPGN and IgAN in nephrotic cases (Figs. 4, 5). Originally, BMI parallels to body fat mass and BSA to body volume. From the formula, BMI easily increases a numerical value compared to BSA when body weight increases from the edema. Thus BSA might fail to show significant differences in nephrotic cases of MPGN and FSGS. In non-nephrotic cases FSGS and non-IgAN showed the inverse results (Figs. 6, 7). We could not understand the reason of this.

Woo et al. reported that FSGS has dramatically increased in the last decade in Asian countries [4, 23]. These investigators suspected that socioeconomic factors, including the rising obesity rate, might play a significant role in this phenomenon. The etiologies of FSGS are complicated; among them, hypertension has been of concern as another important cause in animal models and human FSGS [23, 24]. In FSGS, hypertension induces the reduction of nephron mass from arteriosclerosis [25] and podocyte stress due to glomerular hypertension [14]. These multifactorial conditions might lead to the increase in urine protein in FSGS.

Limitations

The limitations of this study relate to the melding of systemic edema and obesity, and the unclear definitions of larger physical constitutions. The patients with nephrotic syndrome were thought to have systemic edema and obesity in the larger constitution groups at the same time. Thus, we could not insist that obesity purely affected the AUPE in this study. We used two types of indexes of physical constitution. BMI is generally used as a marker to determine obesity, but it is unclear whether this marker is a precise marker of body volume. We selected another marker, BSA, which is used to constitutionally correct the glomerular filtration rate; therefore, we thought it might be an appropriate index to evaluate the AUPE to some extent. But we could not make a clear decision on an applicable value of BSA to use to indicate the boundary between larger and smaller physical constitutions.

Conclusion

This study confirmed that the larger physical constitutions including obesity, BMI $\geq$25 kg/m^2 and BSA $\geq$1.73 m^2, have a significant impact on the increase in the AUPE in primary glomerulonephritis and especially in nephrotic patients. At the same time, patients with FSGS and non-IgA did not show the same tendency, rather, systemic blood pressure and renal dysfunction were the major causes of proteinuria in the patients with FSGS and non-IgA. Larger physical constitutions including obesity distinctively affect the AUPE in the different types of primary glomerulonephritis.

Acknowledgments The authors are grateful for the help and support from the J-RBR committee and all of the colleagues who participated in the J-RBR governed by Japanese Society of Nephrology. This study was supported by a grant-in-aid from the Research Group on Progressive Renal Disease from the Ministry of Health, Labor and Welfare, Japan.

Conflict of interest The authors have declared that no Conflict of interest exists.

References

1. Wickman C, Kramer H. Obesity and kidney disease: potential mechanisms. Semin Nephrol. 2013;33:14–22.
2. Ohashi Y, Otani T, Tai R, Okada T, Tanaka K, Tanaka Y, Sakai K, Aikawa A. Associations of proteinuria, fluid volume

imbalance, and body mass index with circadian ambulatory blood pressure in chronic kidney disease patients. Kidney Blood Press Res. 2012;36:231–41.
3. Tanaka M, Yamada S, Iwasaki Y, Sugishita T, Yonemoto S, Tsukamoto T, Fukui S, Takasu K, Muso E. Impact of obesity on IgA nephropathy: comparative ultrastructural study between obese and non-obese patients. Nephron Clin Pract. 2009;112:c71–8.
4. Woo KT, Chan CM, Mooi CY, L-Choong H, Tan HK, Foo M, Lee GS, Anantharaman V, Lim CH, Tan CC, Lee EJ, Chiang GS, Tan PH, Boon TH, Fook-Chong S, Wong KS. The changing pattern of primary glomerulonephritis in Singapore and other countries over the past 3 decades. Clin Nephrol. 2010;74:372–83.
5. Sugiyama H, Yokoyama H, Sato H, Saito T, Kohda Y, Nishi S, Tsuruya K, Kiyomoto H, Iida H, Sasaki T, Higuchi M, Hattori M, Oka K, Kagami S, Kawamura T, Takeda T, Hataya H, Fukasawa Y, Fukatsu A, Morozumi K, Yoshikawa N, Shimizu A, Kitamura H, Yuzawa Y, Matsuo S, Kiyohara Y, Joh K, Nagata M, Taguchi T, Makino H, Committee for Standardization of Renal Pathological Diagnosis and Committee for Kidney Disease Registry, Japanese Society of Nephrology, Japan. Japan renal biopsy registry and Japan kidney disease registry: committee report for 2009 and 2010. Clin Exp Nephrol. 2013;17:155–73.
6. Matsushita Y, Takahashi Y, Mizoue T, Inoue M, Noda M, Tsugane S, JPHC Study Group. Overweight and obesity trends among Japanese adults: a 10-year follow-up of the JPHC Study. Int J Obes. 2008;32:1861–71.
7. Yokoyama H, Taguchi T, Sugiyama H, Sato H, Committee for the Standardization of Renal Pathological Diagnosis and for Renal Biopsy and Disease Registry in the Japanese Society of Nephrology. Membranous nephropathy in Japan: analysis of the Japan Renal Biopsy Registry (J-RBR). Clin Exp Nephrol. 2012;16:557–63.
8. Alicic RZ, Patakoti R, Tuttle KR. Direct and indirect effects of obesity on the kidney. Adv Chronic Kidney Dis. 2013;20:121–7.
9. Kalaitzidis RG, Siamopoulos KC. The role of obesity in kidney disease: recent findings and potential mechanisms. Int Urol Nephrol. 2011;43:771–84.
10. Chagnac A, Weinstein T, Herman M, Hirsh J, Gafter U, Ori Y. The effects of weight loss on renal function in patients with severe obesity. J Am Soc Nephrol. 2003;14:1480–6.
11. Hou CC, Shyu RS, Lee WJ, Ser KH, Lee YC, Chen SC. Improved renal function 12 months after bariatric surgery. Surg Obes Relat Dis. 2013;9:202–6.
12. Kambham N, Markowitz GS, Valeri AM, Lin J, D'Agati VD. Obesity-related glomerulopathy: an emerging epidemic. Kidney Int. 2001;59:1498–509.
13. Hoy WE, Hughson MD, Zimanyi M, Samuel T, Douglas-Denton R, Holden L, Mott S, Bertram JF. Distribution of volumes of individual glomeruli in kidneys at autopsy: association with age, nephron number, birth weight and body mass index. Clin Nephrol. 2010;74:105–12.
14. Darouich S, Goucha R, Jaafoura MH, Zekri S, Ben Maiz H, Kheder A. Clinicopathological characteristics of obesity-associated focal segmental glomerulosclerosis. Ultrastruct Pathol. 2011;35:176–82.
15. Praga M, Morales E. Obesity, proteinuria and progression of renal failure. Curr Opin Nephrol Hypertens. 2006;15:481–6.
16. Tang J, Yan H, Zhuang S. Inflammation and oxidative stress in obesity-related glomerulopathy. Int J Nephrol. 2012;2012:608397.
17. Ezequiel DG, Costa MB, Chaoubah A, de Paula RB. Weight loss improves renal hemodynamics in patients with metabolic syndrome. Bras Nefrol. 2012;34:36–42.
18. Kanazawa M, Yoshiike N, Osaka T, Numba Y, Zimmet P, Inoue S. Criteria and classification of obesity in Japan and Asia-Oceania. Asia Pac J Clin Nutr. 2002;11:S732–7.
19. Harada H, Omura K. Preoperative concurrent chemotherapy with S-1 and radiotherapy for locally advanced squamous cell carcinoma of the oral cavity: phase trial. J Exp Clin Cancer Res. 2010;29:33.
20. Fujita K, Ichikawa W. Yamamoto. Fixed dosing and pharmacokinetics of S-1 in Japanese cancer patients with large body surface areas. Ann Oncol. 2009;20:946–9.
21. Siddall EC, Radhakrishnan J. The pathophysiology of edema formation in the nephrotic syndrome. Kidney Int. 2012;82:635–42.
22. Bockenhauer D. Over- or underfill: not all nephrotic states are created equal. Pediatr Nephrol. 2013;28(8):1153–6.
23. Woo KT, Chiang GS, Pall A, Tan PH, Lau YK, Chin YM. The changing pattern of glomerulonephritis in Singapore over the past two decades. Clin Nephrol. 1999;52:96–102.
24. de Mik SM, Hoogduijn MJ, de Bruin RW, Dor FJ. Pathophysiology and treatment of focal segmental glomerulosclerosis: the role of animal models. BMC Nephrol. 2013;14:74.
25. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. Am J Kidney Dis. 2004;14:368–82.

Clin Exp Nephrol
DOI 10.1007/s10157-014-1064-0

ORIGINAL ARTICLE

Clinicopathological characteristics of M-type phospholipase A2 receptor (PLA2R)-related membranous nephropathy in Japanese

Norifumi Hayashi · Shin'ichi Akiyama · Hiroshi Okuyama · Yuki Matsui · Hiroki Adachi · Hideki Yamaya · Shoichi Maruyama · Enyu Imai · Seiichi Matsuo · Hitoshi Yokoyama

Received: 3 September 2014 / Accepted: 26 November 2014

Abstract
Background Recent studies have suggested that assessments of serum antibodies against M-type phospholipase A2 receptor (PLA2R) and the glomerular expression of PLA2R antigen in biopsy specimens are useful for the diagnosis of primary membranous nephropathy (MN). In this study, we assessed both of them and investigated the clinicopathological characteristics of PLA2R-related Japanese MN.
Methods We retrospectively enrolled 22 primary and 3 secondary Japanese patients whose serum samples and renal specimens were collected before treatment. According to the findings of serum antibodies and antigen in glomeruli, the primary MN patients were classified into PLA2R-related or -unrelated MN. We compared their clinicopathological findings, including IgG subclass staining, and electron microscopic findings, and evaluated the predictors of proteinuria remission.
Results In primary MN, 16 patients (73 %) were classified into the PLA2R-related group, and 6 patients into the PLA2R-unrelated group. There was no significant difference in baseline laboratory data and electron microscopic findings, except for eGFR and serum IgG levels. IgG4-dominant deposition was more common in the related group (63 vs. 0 %). The 10 PLA2R-related patients with dominant IgG4 deposition had a lower rate and prolonged time in remission compared with the 6 PLA2R-related patients with non-dominant IgG4 (log-rank, $p = 0.032$). Furthermore, dominant IgG4 deposition was an unfavorable predictor of remission by multivariable Cox proportional hazard analysis.
Conclusions Assessments of both serum PLA2R antibodies and PLA2R antigen in glomeruli were more sensitive for the diagnosis of PLA2R-related MN, and among affected Japanese patients, those with dominant IgG4 deposition had worse clinical outcomes.

Keywords Electron microscopic findings · IgG subclass staining · Membranous nephropathy · Phospholipase A2 receptor · Western blotting

Introduction

Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults. MN is characterized by immune complex deposition in the subepithelial space of glomerular capillaries. In 10–20 % of patients, MN is associated with an underlying disease, such as autoimmune disease (e.g., systemic lupus erythematosus), infection, drugs and malignancies [1]. When no underlying cause is identified, the disease is classified as primary (idiopathic). It has long been suspected that primary MN is evoked by the in situ formation of immune complexes as circulating antibodies react with a podocyte antigen. In 2009, M-type phospholipase A2 receptor (PLA2R) was identified as a target podocyte antigen in primary MN. Circulating

Electronic supplementary material The online version of this article (doi:10.1007/s10157-014-1064-0) contains supplementary material, which is available to authorized users.

N. Hayashi · H. Okuyama · Y. Matsui · H. Adachi · H. Yamaya · H. Yokoyama (✉)
Division of Nephrology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan
e-mail: h-yoko@kanazawa-med.ac.jp

S. Akiyama · S. Maruyama · E. Imai · S. Matsuo
Division of Nephrology, Department of Internal Medicine, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

Published online: 10 December 2014

autoantibodies against PLA2R were found in 70 % of patients with idiopathic MN, but not in those with secondary MN or other glomerular diseases [2]. The level of circulating anti-PLA2R autoantibody correlated with clinical disease activity and was useful to monitor response to treatment [3–5]. In addition, assessment of the glomerular expression of PLA2R antigen in biopsy specimens was also useful for the diagnosis of primary MN [6, 7]. Svobodova et al. [7] showed that assessment of both circulating anti-PLA2R antibodies and PLA2R antigen in biopsy might better categorize patients into different groups than only assessing anti-PLA2R antibodies, even during active disease.

In this study, we assessed both serum anti-PLA2R antibodies and PLA2R antigen in glomeruli at the same time before treatment and defined the patients with either elevated anti-PLA2R antibody in the serum or enhanced PLA2R in glomeruli as having PLA2R-related MN. We assessed their clinicopathological characteristics and clinical outcomes in comparison with those in PLA2R-unrelated primary MN and secondary MN.

Materials and methods

Patients

We retrospectively enrolled 25 Japanese MN patients who were admitted to Kanazawa Medical University Hospital between 1998 and 2013, and whose serum samples were collected before treatment at the time of renal biopsy. We followed these patients for at least 6 months (median (IQR), 26 [16.8–35.3] months). Diagnosis was confirmed in all patients by percutaneous needle renal biopsy. To detect secondary causes of MN, clinical workup including detailed medical history and physical examination, serological analysis (tumor markers, lupus autoantibodies, hepatitis B and C) and CT scan were conducted. The patients with lupus nephritis were excluded from this study.

In primary MN, we defined the patients who were either serum anti-PLA2R autoantibody- or glomerular PLA2R-positive as the PLA2R-related group and those who were negative in both serum and glomeruli as the PLA2R-unrelated group. Between these two groups, we compared the baseline clinicolaboratory data, pathological characteristics and clinical outcomes, such as remission rate.

We measured urine protein:creatinine ratio (uPCR) and evaluated clinical status according to Japanese clinical categories as follows: the nephrotic state, that is, the presence of heavy proteinuria (greater than 3.5 g/gCre) and hypoalbuminemia (less than 3.0 g/dL); incomplete remission type II, that is, mean daily proteinuria of 1.0–3.5 g/gCre accompanied by an improvement of serum albumin levels (more than 3.0 g/dL); incomplete remission type I, that is, mean daily proteinuria of 0.3–1.0 g/gCre with normal serum albumin levels (more than 3.0 g/dL); and complete remission, that is, daily proteinuria of less than 0.3 g/gCre with normal serum albumin levels. For the purpose of analysis, we defined remission as improvement of clinical status to the point of incomplete remission type I, according to the criterion of good long-term renal outcome in Japan [8]. The patients were treated non-randomly, depending on the judgment of the doctor in charge of each case. The protocol of this study was approved by the Clinical Study Ethics Review Board of Kanazawa Medical University. Prior to the study, verbal/written informed consent was obtained from all patients (Clinical Study Ethics Review Board of Kanazawa Medical University, Approval No. 80). This study was conducted according to the principles of the Declaration of Helsinki.

Western blotting

The anti-PLA2R autoantibodies in the serum were detected by Western blot analysis with cell lysate of HEK293 cells expressing recombinant PLA2R proteins. HEK293 cells were transfected with coding cDNA of the extracellular domain of PLA2R and homogenized in RIPA buffer (Wako Pure Chemicals, Japan) with protease inhibitor cocktail (Roche Applied Science, USA). Then, the cell lysate was mixed with 4× LDS sample buffer (Invitrogen, USA) and heated for 10 min at 70 °C. Equal amounts of the cell lysate were electrophoresed in 4–12 % Bis–Tris polyacrylamide gel (Invitrogen, USA) under non-reducing conditions and transferred to polyvinylidene difluoride membranes (Invitrogen, USA). The membranes were blocked with Blocking-One (Nacalai Tesque, Japan) and incubated with the serum at a dilution of 1:100. After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated mouse anti-human IgG secondary antibody (Abcam) at a dilution of 1:20,000. 5 % Blocking-One in phosphate-buffered saline with 0.05 % Tween 20 (PBST) was used for all primary and secondary antibody dilutions. The immunoreactive bands were visualized by a chemiluminescent technique using ImmunoStar LD (Wako Pure Chemicals, Japan) as an HRP substrate.

Immunohistological staining

Fresh tissue specimens, embedded in OCT compound and frozen in acetone-dry ice mixture, were cut at a thickness of 3 μm on a cryostat. The frozen sections were fixed in 1:1 acetone:methanol and blocked with 10 % goat serum in 0.01 mol/L phosphate-buffered saline (PBS). To detect PLA2R, we used rabbit anti-PLA2R antibodies (Atlas Antibodies) at a dilution of 1:400, followed by fluorescein

isothiocyanate (FITC)-labeled goat anti-rabbit IgG antibodies (Cosmo Bio) at a dilution of 1:640. We detected the IgG subclass in the MN immune deposits with mouse anti-IgG1, -IgG2, -IgG3 and -IgG4 monoclonal antibodies (AbD Serotec), each at a dilution of 1:50, followed by FITC-labeled goat anti-mouse IgG antibodies (Cosmo Bio) at a dilution of 1:160. Immunofluorescence staining intensity was arbitrarily graded on a scale from 0 to 3 (0 negative, 1 weak staining, 2 moderate staining, 3 strong staining) and the median score was calculated (Supplementary Figures S1 and S2).

Electron microscopic examination

The specimens obtained from 24 patients were fixed with glutaraldehyde and osmium tetroxide, embedded in Epon 812, cut into 0.1 μm sections, double-stained with uranyl acetate and lead citrate, and examined with a Hitachi H-7650 electron microscope. For the electron microscopic study, we examined the specimens with emphasis on the phase and synchronicity of subepithelial and intramembranous electron-dense deposits and divided them into two subtypes, namely, homogeneous type with monophasic deposits and heterogeneous type with polyphasic deposits, as previously reported [9, 10]. We also used the Ehrenreich and Churg classification for the ultrastructural staging of MN [11].

Statistical analysis

Continuous measures and ranking scales were summarized using medians (25–75 % interquartile range), whereas categorical measures were summarized using counts and percentages. Mann–Whitney *U* test was used to assess the differences between two groups. Fisher's exact test was utilized to compare proportions. The Kaplan–Meier life-table method and Cox proportional hazard analysis were performed to evaluate predictors of remission of proteinuria. In addition, Cox proportional hazard models were developed with relevance to remission with subsequent addition of sociodemographic (age and sex), clinical variables (eGFR and uPCR), pathological findings (PLA2R, IgG subclass staining and electron microscopic stage), and treatment. *P* values of less than 0.05 were considered statistically significant. The Cox proportional hazard analysis was conducted using Stat Flex, version 6.0 (Artech, Japan), and the others were conducted using SPSS, version 20.0 (Chicago, IL, USA).

Results

PLA2R of primary and secondary MN in Japanese

In this study, 22 primary MN patients and 3 secondary MN patients were enrolled. The causes of secondary MN were malignancy (malignant thymoma, $n = 1$), bucillamine ($n = 1$) and chronic graft versus host disease (GVHD) after peripheral blood stem cell transplantation ($n = 1$).

In secondary MN, no patient had autoantibody in serum and glomerular PLA2R deposits. On the other hand, in 12 primary MN patients (55 %), circulating PLA2R autoantibodies were positive with glomerular PLA2R in 10 patients, but not in 2 patients. Of the 10 primary MN patients with no detectable PLA2R autoantibodies in serum, 4 patients had glomerular PLA2R deposits. According to these findings, 16 patients (73 %) were classified into the PLA2R-related group and 6 patients into the PLA2R-unrelated group.

Clinicopathological characteristics of primary MN related to PLA2R positivity

The clinicolaboratory data and pathological characteristics at baseline are presented in Table 1. There was no significant difference in clinicolaboratory data, except for eGFR and serum IgG levels. The intensity of IgG4 deposits was much higher in the PLA2R-related group (3.0 vs. 0.5, $P = 0.010$). Then, IgG4 pre- or co-dominant patients were more common in the PLA2R-related group (63 vs. 0 %, $P = 0.0152$). There was no significant difference in the electron microscopic findings.

Clinical outcomes of primary MN

The primary MN patients were treated using supportive therapy ($n = 11$, 50 %), corticosteroid alone ($n = 2$, 9.1 %), cyclosporine with steroid ($n = 6$, 27.3 %) or mizoribine with steroid ($n = 3$, 13.6 %). During the follow-up period, no patient developed end-stage renal failure (ESRF). The results of the univariate time-dependent analysis by the Kaplan–Meier method are shown in Fig. 1. Although there was no significant difference in the remission rate and the time to remission between the PLA2R-related and PLA2R-unrelated groups (Fig. 1a), the 10 PLA2R-related patients with dominant IgG4 deposition had a low rate and prolonged time in remission compared with the 6 PLA2R-related patients with non-dominant IgG4 (log-rank, $p = 0.032$, Fig. 1b). The clinical predictors of remission in the multivariable Cox proportional hazard analysis are presented in Table 2. It is revealed that dominant IgG4 deposition was an unfavorable predictor and immunosuppressive therapy was a favorable predictor of remission.

In patients whose serum antibodies were positive at baseline, with the exception of one patient, we assessed this variable again at the end of the follow-up period (Table 3 and Supplementary Figure S3). In 4 of 8 patients (50 %) who achieved remission, the serum antibodies became

Table 1 Clinicolaboratory data and pathological characteristics of primary MN at baseline

	PLA2R-related [$n = 16$]	PLA2R-unrelated [$n = 6$]	P value
Age (years)	64.5 [61–70]	70 [64–74]	0.336
Male/female	12/4	4/2	0.541
Follow-up (months)	26 [13–36]	24 [16–34]	0.914
Serum creatinine (mg/dl)	0.77 [0.65–0.89]	0.96 [0.80–1.41]	0.083
eGFR (ml/min/1.73 m^2)	75.2 [64.7–82.3]	59.3 [28.9–68.6]	0.049*
Serum albumin (g/dl)	2.35 [2.05–3.05]	2.70 [1.90–3.40]	0.684
Serum IgG (mg/dl)	691.5 [517.5–989.5]	1,045.0 [1,038.0–1,079.0]	0.018*
uPCR (g/gCre)	10.0 [5.3–12.8]	5.5 [3.7–8.1]	0.261
Immunohistological staining			
IgG1 score	1.5 [0.0–2.5]	1.5 [1.0–2.0]	0.909
IgG2 score	0.0 [0.0–1.0]	0.0 [0.0–0.0]	0.089
IgG3 score	1.0 [0.0–1.5]	0.0 [0.0–1.0]	0.448
IgG4 score	3.0 [1.0–3.0]	0.5 [0.0–1.0]	0.010*
IgA score	0.0 [0.0–1.0]	0.0 [0.0–1.0]	0.763
C1q score	0.0 [0.0–1.0]	0.5 [0.0–1.0]	0.738
C3 score	3.0 [0.5–3.0]	1.0 [0.0–2.0]	0.143
Electron microscopic findings	PLA2R-related [$n = 15$]	PLA2R-unrelated [$n = 6$]	P value
Ehrenreich–Churg stage	2.0 [1.0–2.5]	1.5 [1.0–2.0]	0.480
Subtype homogeneous	14 [93 %]	5 [83 %]	0.500
Subtype heterogeneous	1 [7 %]	1 [17 %]	

PLA2R M-type phospholipase A2 receptor, *MN* membranous nephropathy, *uPCR* urine protein/creatinine ratio

* P values of less than 0.05

negative. On the other hand, in all patients who did not achieve remission, the serum antibodies were still positive.

Discussion

Although the exact mechanisms and the role of PLA2R in the pathogenesis of primary MN are currently unknown, PLA2R is probably one of the intrinsic glomerular antigens. In this study, we collected serum samples before treatment at the time of renal biopsy, and assessed both serum anti-PLA2R antibodies and glomerular PLA2R deposits. In previous studies, the prevalence of serum anti-PLA2R antibodies by western blotting in patients with primary MN ranged from 69 to 82 % [2, 4, 5, 12, 13] and the prevalence of glomerular PLA2R deposits ranged from 69 to 84 % [6, 7, 14, 15]. In this study, we detected serum anti-PLA2R antibodies in 55 % and glomerular PLA2R deposits in 64 % of patients with primary MN. The reason for the low prevalence in our serum antibody detection is unclear at this time. A larger Japanese study ($n = 96$) by our colleagues showed similar results (50 %, unpublished data). In other Asian countries, on the other hand, the reported prevalence of serum anti-PLA2R antibodies was 82 % in a Chinese study [12] and 69 % in a Korean study [13]. In this study, combined with the results of glomerular PLA2R deposits, the sensitivity of the diagnosis of PLA2R-related MN rose to 73 %.

In previous studies, serum anti-PLA2R antibodies and PLA2R antigen in glomeruli were largely negative in patients with secondary causes of MN including lupus nephritis, HBV, and malignancy-associated MN [12, 15]. We also examined 3 patients with representative cause of secondary MN (1 malignancy, 1 GVHD, and 1 bucillamin) and found that no patient had autoantibody in serum and glomerular PLA2R deposits.

Similar to previous studies [7, 14], we found that 4 patients had no circulating antibodies, although they had PLA2R detected in glomeruli, while 2 patients with circulating antibodies had no detectable PLA2R in glomeruli. The findings of serum negativity and glomerular positivity were explained by the rapid clearance of antibodies from the blood or by the late sampling of patients when proteinuria persisted because of irreversible ultrastructural changes [7, 14]. However, there was no significant difference in proteinuria at baseline and time to remission between the patients with serum negativity and glomerular positivity and the patients with both serum and glomerular positivity in this study. On the other hand, for the patients with circulating antibodies but without detectable PLA2R in glomeruli, it was speculated that the antibodies were not nephritogenic or that epitopes were poorly accessible at the time of renal biopsy [7, 14]. In this study, circulating antibodies were not detected after remission in 2 patients with serum positivity and glomeruli negativity at baseline (Table 3). Finally, neither PLA2R antigen in glomeruli nor

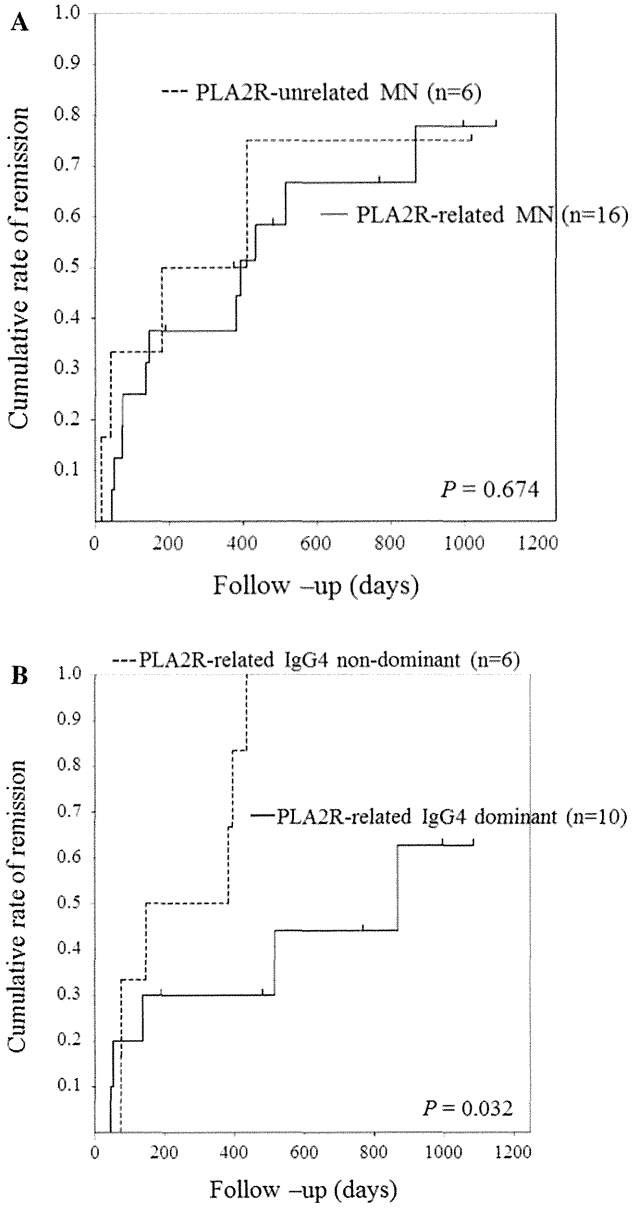


Fig. 1 Kaplan–Meier curves of remission in Japanese membranous nephropathy. **a** There was no significant difference in the remission rate and the time to remission between the PLA2R-related and -unrelated groups. **b** The 10 PLA2R-related patients with dominant IgG4 deposition had a low rate and prolonged time in remission compared with the 6 PLA2R-related patients with non-dominant IgG4 (log-rank, $p = 0.032$)

PLA2R antibody in serum was found in 6 primary MN patients. It is possible that they have an as yet undetected cause of MN or other recently described antigens, such as alpha-enolase, aldose reductase and SOD2 [16].

IgG subtype analysis in glomeruli has been carried out for the discrimination between primary (idiopathic) and secondary MN. IgG4-dominant staining is associated with primary MN, whereas IgG1, IgG2 and IgG3 dominate in the deposits of secondary MN [17–19]. Serum autoantibodies to PLA2R are largely IgG4, and IgG4 could be co-localized with the PLA2R antigen within the glomerular immune deposits of idiopathic MN [2, 3], except for some cases such as monoclonal IgG3-kappa targeting the PLA2R [20]. Consistent with these findings, in our PLA2R-related group, IgG4-dominant deposits in glomeruli were more common than in the PLA2R-unrelated group and secondary MN patients.

Furthermore, IgG4 dominancy was an unfavorable predictor of remission. Hofstra et al. [3] found that spontaneous remissions were less likely to occur in patients with high serum IgG4 antibody titers to PLA2R. Although IgG4 does not activate the classic complement pathway, there are usually abundant deposits of complement, including C4 and C3, in the immune deposits in primary MN. A recent preliminary study reported that degalactosylated anti-PLA2R IgG4 can bind mannan-binding lectin, which suggests the possibility that the lectin pathway of complement might be activated in glomerular IgG4-predominant immune deposits [21]. We speculated that two characteristics in the immune regulation of IgG4 were relevant to the worse remission of proteinuria. One is the finding that the production of IgG4 antibodies seems to be driven by T helper 2 (Th2) cytokine activation in primary MN [22]. The other is its tendency to appear only after prolonged immunization. It usually takes many months of repeated antigen exposure before IgG4 responses become prominent [23]. In this regard, Huang et al. [24] found, in early stages of MN, that IgG1 was the dominant IgG subclass, whereas IgG4 became dominant in later stages. We also found that almost all IgG4-dominant patients (8 of 10) had other IgG subclass deposits to some extent.

Then, we hypothesized that cases of PLA2R-related MN, if caused by immune reactions evoked by repeated exposure to the intrinsic antigen, would have later stages or the heterogeneous type with a different phase of electron-dense deposits. However, there was no significant difference in electron microscopic findings between the PLA2R-related and -unrelated groups. Moreover, early electron-dense deposits such as stage 1 including IgG4 were also observed in the IgG4-predominant or co-dominant group. These findings may suggest that co-localized non-complement-activating IgG4 antibodies and other IgG subclasses such as IgG1, 2 and 3 can augment complement-activating injury, as previously reported in a mouse antibody-mediated allograft rejection model [25].

We found that four patients whose serum antibodies were still positive even after they achieved remission. Among serum positive and negative patients after remission, there was no significant difference about treatment and IgG subclass staining. In previous studies, although there were few atypical patients [4, 7], it was found that anti-PLA2R antibodies disappeared in advance of