day 8 週間）は Barratt やその他の報告で明らかである[10]．しかし，ステロイド依存性ネフローゼ症候群に対する有用性に関しては，2 mg/kg/day 8 週間では無効とされている[11]．また，2 mg/kg/day 12 週間では有効とする報告[12]もあるが，無効とする報告[13]もあり controversial である．また，骨髄抑制，肝機能障害，出血性膀胱炎，性腺障害や催腫瘍性などの副作用に注意する必要がある．特に男性の性腺障害は重大な問題であり，累積投与量が 300 mg/kg を超えると高率に無精子症あるいは乏精子症を起こすので，累積投与量は 200～300 mg/kg 以内にとどめるべきである．

3 ミゾリビン治療

ミゾリビンは日本で開発された代謝拮抗薬であり，小児ミゾリビン研究会による double-blind, placebo-controlled, multicenter trial により，頻回再発型・ステロイド依存性ネフローゼ症候群に対するミゾリビン 4 mg/kg/day 48 週間投与とプラセボ 48 週間投与が比較され，その有効性，安全性が検討された．その結果，登録症例全体では，ミゾリビン群と placebo 群間で再発率に有意な差を認めなかったが，10 歳以下の症例ではミゾリビン群の再発率は placebo 群に比して有意に低かった[14]．しかし，10 歳以下の症例でもミゾリビン治療開始 1 年後の寛解維持率は 40%以下であり，再発抑制という点からは十分な効果は期待できない．一方，副作用としては高尿酸血症が認められたのみであり，その大半はミゾリビンを中止や減量することなく継続投与が可能であった．したがって，ミゾリビンは，その有効性は低いが，副作用が非常に少ないという点では有用である．

4 今後の課題

1．タクロリムス治療

タクロリムスは，シクロスポリンと同じカルシニューリン阻害作用をもつ免疫抑制薬で，シクロスポリンに代わる免疫抑制薬として開発された．現在，タクロリムスは，腎移植後の免疫抑制薬としてアメリカやわが国では第一選択薬となっている．腎移植におけるタクロリムスについて，Cochrane Database of Systematic Review では，100 人の移植患者をシクロスポリンの代わりにタクロリムスで治療すると，12 人の急性拒絶反応と 2 人の移植腎廃絶を回避することができ，副作用でも慢性移植腎障害，多毛や歯肉肥厚がシクロスポリンより少ない点で，タクロリムスはシクロスポリンより優れていると結論づけている[15]．小児腎移植のタクロリムス治療とシクロスポリン治療のランダム化比較試験でも，1 年時の急性拒絶反応がタクロリムス治療群 36.9%，シクロスポリン治療群 59.1%と，タクロリムス治療がシクロスポリン治療より腎移植後の急性拒絶反応を減少すると報告されている[16]．

ネフローゼ症候群では，ステロイド抵抗性を示す小児患者を対象としたランダム化比較試験で完全寛解がタクロリムス治療群 85.7%，シクロスポリン治療群 80.0%と，タクロリムス治療はシクロスポリン治療と同等の効果を示し，寛解後の再発は，タクロリムス治療群はシクロスポリン治療群より少なかったと報告されている[17]．この試験でタクロリムス治療群とシクロスポリン治療群の副作用はそれぞれ腎毒性が 38.0%と 60.0%，多毛が 0.0%と 95.0%，歯肉肥厚

が4.7%と60.0%とタクロリムス治療群はシクロスポリン治療群より少なかったと報告されている．Cochrane Database of Systematic Review によると，北米では副作用の点で，タクロリムスがシクロスポリンより好まれて使用されており，タクロリムスと他の免疫抑制薬とのランダム化比較試験を行うべきであると結論づけている[18]．

以上より小児頻回再発型・ステロイド依存性ネフローゼ症候群を対象とした，シクロスポリンとタクロリムスのランダム化比較試験が早急に必要である．

2．高用量ミゾリビン治療

ミゾリビンは，わが国で開発された免疫抑制薬である．ミゾリビンは腸管で吸収された後，ほとんど代謝を受けずに腎臓から排泄される．他の免疫抑制薬に比較して安全性が高いことが知られている．わが国では，腎移植における拒絶反応の抑制，原発性糸球体疾患を原因とするネフローゼ症候群，ループス腎炎，関節リウマチに適応があるが，小児特発性ネフローゼ症候群（ステロイド感受性，頻回再発型）への適応はない．しかし，実際にはミゾリビンは小児の頻回再発型の治療薬として検討され使用されてきた．

一方，ミゾリビンの作用は濃度依存性であり，小児では，成人と比較して細胞外液量が多く分布容積が大きいこと，ミゾリビンの腸管吸収率が低い可能性があること等から，成人と同等の用量では最高血中濃度が低くなり，成人と同等の最高血中濃度を得るには成人の約2倍量を要すると考えられている．最近，小児の頻回再発型患者に対し，高い最高血中濃度を目指した高用量のミゾリビン投与が検討され，報告されている．これらの報告では，再発回数減少を認めた例のミゾリビン投与2時間後の血中濃度（C2）は約3 μg/ml，ミゾリビン平均投与量は10 mg/kg/日，用法は1日1回投与が主で，ミゾリビンの重篤な薬物有害反応は報告されていない[19]．ミゾリビン10 mg/kg/日の1日1回投与は，安全性が高く再発抑制効果が期待できる可能性があり，ランダム化比較試験の実施が望まれる．

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Original Article

Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome

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Abstract

Background. We previously established a treatment protocol for conventional cyclosporine (Sandimmune, Novartis, Basel, Switzerland) in children with frequently relapsing nephrotic syndrome; ~50% of patients remained relapse free for 2 years, without serious adverse events. Recently, microemulsified cyclosporine (Neoral, Novartis), which has a more stable absorption profile than conventional cyclosporine, has been developed. We tested the hypothesis that microemulsified cyclosporine is at least as effective as conventional cyclosporine.

Methods. To evaluate the safety and efficacy of microemulsified cyclosporine, a prospective, multicentre trial was conducted according to the previously established protocol, using microemulsified cyclosporine instead of conventional cyclosporine. The duration of treatment was 24 months. During the first 6 months, patients received microemulsified cyclosporine in a dose that maintained the trough level between 80 and 100 ng/mL of cyclosporine. For the next 18 months, the dose was adjusted to maintain a level between 60 and 80 ng/mL.

Results. A total of 62 patients (median age, 5.4 years; 48 males, 14 females) were studied. The frequency of relapse decreased from 4.6 ± 1.4 to 0.7 ± 1.5 times per year ($P<0.0001$). The probability of relapse-free survival at Month 24 was 58.1% (95% confidence interval, 45.8–70.3%). The probability of progression (to frequently relapsing nephrotic syndrome)-free survival at Month 24 was 88.5% (95% confidence interval, 80.4–96.5%). Cyclosporine nephrotoxicity was detected in only 8.6% of patients who underwent renal biopsy after 2 years of treatment. Antihypertensive agents were administered to 12.9% of the patients to control hypertension without severe sequelae.

Conclusions. Microemulsified cyclosporine administered according to our treatment protocol is safe and effective in children with frequently relapsing nephrotic syndrome.

Keywords: clinical trial; microemulsified cyclosporine; nephrotic syndrome; paediatric nephrology

Introduction

Managing frequently relapsing nephrotic syndrome (FRNS) in children remains challenging despite progress in treatment. The development of immunosuppressive therapies other than corticosteroids has been enthusiastically attempted to date [1–4] because repeated treatment with corticosteroids can lead to serious adverse events.

Cyclosporine is one treatment of choice for children with FRNS or steroid-dependent nephrotic syndrome [5–10]. For such patients, we have already established a safe and effective protocol for treatment with conventional cyclosporine, Sandimmune (Novartis, Basel, Switzerland), in a prospective, randomized, multicentre trial [11]. With our protocol, the dose of cyclosporine is titrated on the basis of the whole-blood trough level. Approximately 50% of children with FRNS treated according to this protocol are expected to remain relapse free for 2 years, without serious adverse events.

Microemulsified cyclosporine, Neoral (Novartis, Basel, Switzerland) is a newer formulation of cyclosporine, designed to promote stable absorption and improved bioavailability [12–15]. Several small studies have compared safety and efficacy between conventional cyclosporine and microemulsified cyclosporine in children with nephrotic syndrome [16] and recipients of renal transplants [17,18];

microemulsified cyclosporine has consistently been suggested to be more effective, without compromising safety.

Because cyclosporine is stably absorbed after administration of microemulsified cyclosporine, the dose of microemulsified cyclosporine can be titrated on the basis of the area under the concentration-time curve during the first 4 h after treatment (AUC_{0-4h}) or the 2-h post-dose cyclosporine level (C2) in children who receive kidney transplants [19,20]. The clinical efficacy of microemulsified cyclosporine titrated by monitoring AUC_{0-4h} or C2 in patients with nephrotic syndrome is also expected but remains to be confirmed.

We performed a prospective, single-arm, multicentre trial according to our previously established protocol, using microemulsified cyclosporine instead of conventional cyclosporine. The principal aim of this trial was to evaluate the efficacy in terms of relapse-free survival probability and the safety of microemulsified cyclosporine in children with FRNS. The benefits of AUC_{0-4h} and C2 monitoring in this clinical setting were also assessed.

Materials and methods

Patients

The study group comprised children (1–18 years of age) with FRNS who had idiopathic nephrotic syndrome. Patients were excluded if they had any of the following conditions: (i) other renal or systemic forms of nephrotic syndrome diagnosed on the basis of renal biopsy, clinical features or serology; (ii) poorly controlled hypertension; (iii) chronic renal dysfunction (creatinine clearance of ≤60 mL/min/1.73 m^2); (iv) active infectious disease; (v) severe liver dysfunction; (vi) a history of treatment with cyclosporine; or (vii) pregnancy.

The criteria for and definitions of nephrotic syndrome, remission, and relapse were in accordance with the International Study of Kidney Disease in Children [21]. FRNS was defined as two or more relapses of nephrotic syndrome within 6 months after the initial episode, three or more relapses within any 6-month period, or four or more relapses within any 12-month period. Steroid dependence was defined as the occurrence of two consecutive relapses on tapering the steroid dosage or within 14 days after the termination of steroids.

'Ethical Guidelines for Clinical Research', requiring that all protocols for clinical studies are reviewed by an external ethics committee, were issued by the Japanese Ministry of Health, Labour and Welfare in 2003. At the start of our trial (January 2000), the study protocol was approved by the director or other responsible person at each participating centre and was not reviewed by an external review board. Therefore, at the time of the submission for publication, a retrospective approval by an ethical committee was performed. The ethical standards laid down in the Declaration of Helsinki were applied accordingly in the design and execution of this study. Informed consent was obtained from all patients or their parents.

Protocol

The total duration of treatment was 24 months. For the first 6 months, all patients received microemulsified cyclosporine in a dose that maintained a whole-blood trough level between 80 and 100 ng/mL of cyclosporine; for the next 18 months, the dose was adjusted to maintain a trough level between 60 and 80 ng/mL. Maintenance prednisolone was not prescribed. After 2 years of treatment, all patients were scheduled to undergo renal biopsy, and the dose of cyclosporine was tapered by 0.5–1.0 mg/kg per day every week. The concomitant use of drugs other than corticosteroids and immunosuppressants was not restricted. Antihypertensive agents, including angiotensin-converting enzyme inhibitors, and HMG-CoA reductase inhibitors (statins) were also permitted.

Blood analysis (complete blood cell count and blood chemistry) and urine tests (urinalysis and quantitative proteinuria) were performed monthly during follow-up. The trough level of cyclosporine was measured monthly by monoclonal radioimmunoassay. In addition to the trough level, other indices of cyclosporine absorption (i.e. AUC_{0-4h} and C2) were examined at Month 1. For cyclosporine AUC_{0-4h} and C2 sampling, time lags of ±5 mins were allowed. AUC_{0-4h} was calculated by the linear trapezoidal method.

Patients in whom FRNS or steroid-resistant nephrotic syndrome developed during treatment received off-protocol therapy, left to the discretion of the physician in charge.

Corticosteroid treatment

To treat relapses of nephrotic syndrome immediately before study entry, patients received 2 mg/kg/day of prednisolone in three divided doses (maximum dose, 80 mg/day) for 4 weeks, followed by a single dose of 2 mg/kg of prednisolone administered in the morning on alternate days for 2 weeks, 1 mg/kg on alternate days for 2 weeks and 0.5 mg/kg on alternate days for 2 weeks. Patients who had relapses of nephrosis during the study period received 2 mg/kg/day of prednisolone in three divided doses (maximum, 80 mg/day) until remission, followed by a single dose of 2 mg/kg of prednisolone administered in the morning on alternate days for 2 weeks, 1 mg/kg on alternate days for 2 weeks and 0.5 mg/kg on alternate days for 2 weeks.

Histopathological examination

A pathologist at each study centre examined each renal biopsy specimen. An independent investigator at the coordinating centre who was blinded to all patient data also reviewed the histologic sections. Arteriolar changes, tubular atrophy and interstitial fibrosis were graded semi-quantitatively on a scale of 0–3+ as follows: 0, none; 1+, mild; 2+, moderate; and 3+, intense.

Statistical analysis

The primary end point was the probability of relapse-free survival, based on the period until the first relapse. The secondary end point was the probability of progression-free survival, based on the period until the development of FRNS. Survival curves were estimated by the Kaplan–Meier method. Survival curves from our previous study of conventional cyclosporine are included in the figures of this study. Multivariate analyses using Poisson regression were performed to estimate the relations of AUC_{0-4h} or C2 to the incidence of relapse, adjusting for sex, age and steroid dependence. Data were analysed according to the intention-to-treat. A two-sided P-value of <0.05 was considered to indicate statistical significance. All statistical analyses were performed with the use of the software package SAS for Windows, release 9.13 (SAS Institute Inc., Cary, NC, USA).

Clinical trial registration

This study has been registered in a public trials registry, the University Hospital Medical Information Network (UMIN, ID C000000010, http://www.umin.ac.jp/ctr/index.htm).

Results

Data set

Between January 2000 and December 2005, a total of 66 children were enrolled at 21 institutions, and 4 patients were excluded from all analyses. Therefore, 62 children (59 with minimal change nephrotic syndrome and 3 with mesangial proliferative glomerulonephritis; 48 males and 14 females) received treatment and were included in analysis (Figure 1). Their median ages at diagnosis and at study entry were 3.0 years (range, 1.3–14.5) and 5.4 years (range, 1.7–15.3), respectively. The clinical characteristics of the patients at entry are shown in Table 1.

As for concomitant medications, antihypertensive agents were given to eight patients (angiotensin-converting enzyme inhibitors, 4 patients; calcium channel blockers, 3;

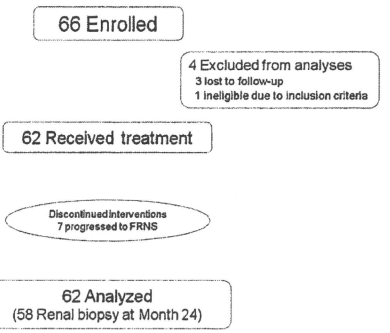

Fig. 1. Flow diagram. FRNS, frequently relapsing nephrotic syndrome.

and both drugs, 1). A HMG-CoA reductase inhibitor (statin) was given to one patient.

Cyclosporine dosage and trough level

The mean dose of cyclosporine required to maintain the whole-blood trough level between 80 and 100 ng/mL during the first 6 months of treatment was 5.1 mg/kg/day. During the next 18 months, the mean dose of cyclosporine required to maintain a trough level between 60 and 80 ng/mL was 4.5 mg/kg/day. The distributions of the trough level are shown in Figure 2.

Decreased frequency of relapses after treatment with cyclosporine

Before treatment, the mean number of relapses was 4.6 ± 1.4 times per year. During the 2 years of treatment with cyclosporine, the mean number of relapses decreased significantly to 0.7 ± 1.5 times per year (paired *t*-test, $P<0.0001$).

Probability of relapse-free and progression-free survival

The estimated relapse rate, defined as the total number of patients who had relapse during the trial divided by the duration of observation for all patients, was 0.28 (95% confidence interval, 0.17–0.39) per year. Figure 3 shows the results of Kaplan–Meier analysis. At Month 24, the

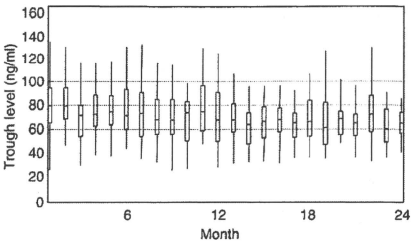

Fig. 2. Median trough level of cyclosporine during study period.

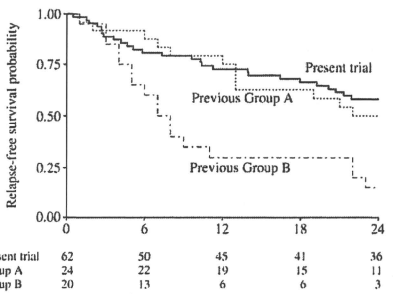

Fig. 3. Comparison with the previous trial [11]. Previous Group A, patients in Group A received conventional cyclosporine according to the same protocol in the previous trial; Previous Group B, patients in Group B received a fixed dose of conventional cyclosporine (2.5 mg/kg) from Month 7 onwards. Most patients in Group B had a trough level <60 ng/mL during this period.

probability of relapse-free survival was 58.1% (95% confidence interval, 45.8–70.3%). In this figure, the probability of relapse-free survival in the present study was compared with that in our previous trial [11]. The probability of relapse-free survival in Group A [24 patients (18 males); median age, 7.3 years], which received conventional cyclosporine according to the same protocol, was 50.0%, while that in Group B [20 patients (17 males); median age, 6.9 years old), which received a fixed dose of conventional cyclosporine (2.5 mg/kg) from Month 7 onwards, was 15.0%.

Table 1. Patient's characteristics

Age (years)	Sex (*n*)		Number of relapses before entry (*n*)				Steroid dependence (*n*)		
	Male	Female	NA	≥2–<4/year	≥4–<6/year	≥6/year	NA	(−)	(+)
All ages	48	14	1	17	32	12	1	29	32
0–<3	6	5	0	1	6	4	0	3	8
≥3–<6	18	8	0	7	15	4	0	13	13
≥6–<10	12	0	1	5	5	1	1	4	7
≥10–<15	10	1	0	4	4	3	0	7	4
≥15	2	0	0	0	2	0	0	2	0

NA, not available.

Table 2. Analysis for predictors of relapse

		Univariate			Multivariate		
		Hazard ratio	95% CI	P	Hazard ratio	95% CI	P
Sex	Male	2.72	0.82–9.07	0.10	1.77	0.33–9.38	0.49
	Female	1.00			1.00		
Age	≥6 years	2.17	1.01–4.70	0.05	1.95	0.69–5.52	0.19
	<6 years	1.00			1.00		
Steroid dependence	Yes	1.01	0.46–2.20	0.99	1.30	0.52–3.26	0.58
	No	1.00			1.00		
AUC_{0-4h}	Each increment of 100-ng × h/mL	0.95	0.88–1.03	0.19	0.95	0.87–1.04	0.23

CI, confidence interval; AUC_{0-4h}, the area under the concentration-time curve during the first 4 h after treatment.

Table 3. Nephrotoxicity of cyclosporine

Age/sex	Relapse during treatment	Progression to FRNS during treatment	AUC_{0-4h} at 1 month (ng × h/mL)	C2 at 1 month (ng/mL)	Mean trough level (ng/mL)	Hypertension	Renal histology
2.9 male	Yes	No	1063	290	91.1	(+)	Mild arteriolar hyalinosis Mild tubular atrophy Mild interstitial fibrosis
3.9 male	No	No	2160	690	92.4	(−)	Mild arteriolar hyalinosis and vacuolation Mild tubular atrophy Mild interstitial fibrosis
5.4 male	No	No	2251	720	72.2	(−)	Mild arteriolar hyperplasia Mild-to-moderate tubular atrophy No interstitial fibrosis
6.2 male	No	No	975	380	60.0	(−)	Mild arteriolar hyalinosis and vacuolation Mild tubular atrophy Mild interstitial fibrosis
10.8 male	Yes	No	619	160	72.0	(+)	Mild arteriolar hyalinosis Mild tubular atrophy Mild interstitial fibrosis

FRNS, frequently relapsing nephrotic syndrome, AUC_{0-4h}, the area under the concentration-time curve during the first 4 h after treatment; C2, the 2-h post-dose cyclosporine level.

The estimated rate of progression to FRNS was 0.06 (0.02–0.11) per year. The probability of progression (to FRNS)-free survival at Month 24 was 88.5% (95% confidence interval, 80.4–96.5%).

Steroid-resistant nephrotic syndrome did not develop in any patient during the trial.

AUC_{0-4h}, C2 and relapse

The mean AUC_{0-4h} at 1 month was 1493.4±681.2 ng × h/mL, and that of C2 was 486.0 ± 203.9 ng/ml. Table 2 shows the results of Poisson regression analysis for AUC_{0-4h}, adjusted for important prognostic factors. None of the four risk factors analysed [AUC_{0-4h} (continuous), sex (male or female), age (≥6 years or <6 years), steroid dependence (yes or no)] were significantly related to relapse. The hazard ratio for AUC_{0-4h} was 0.95 (95% confidence interval, 0.87–1.04; P=0.23) for each 100-ng × h/mL increment.

The results of Poisson regression analysis using C2 in place of AUC_{0-4h} were similar, and the hazard ratio for C2 was 0.86 (95% confidence interval, 0.64–1.15; P=0.30) for each 100-ng/mL increment.

Growth

Before cyclosporine treatment (at study entry), the mean standard deviation (s.d.) score for body height was −0.27 ± 1.01 (n=62); at the end of the trial, the mean s.d. score for body height was 0.33±0.97 (n=58). The s.d. score for height increased significantly from the start to the end of 2-year treatment (paired t-test, P<0.001).

Adverse events

Renal biopsies were performed in 58 patients at the end of 2 years of treatment. The results are shown in Table 3. Mild nephrotoxicity attributed to cyclosporine occurred in 5 (8.6%) of the 58 patients. Other adverse events are shown in Table 4. Hypertension, defined as a requirement for antihypertensive agents during the trial, was detected in 12.9% of the patients. Severe sequelae of hypertension,

Table 4. Adverse events

Adverse events	Number of events (%)
Hypertrichosis	20 (32.3)
Hypertension	8 (12.9)
Gingival hypertrophy	7 (11.3)
Elevation of alkaline phosphatase	5 (8.1)
Herpes zoster	2 (3.2)
Elevation of serum creatinine	1 (1.6)
General fatigue	1 (1.6)

ALP, alkaline phosphatase.

such as encephalopathy, seizures and cardiac dysfunction, were not detected. One patient had a mild elevation of the serum creatinine concentration, which was transient and resolved. No patient had serious adverse events that required discontinuation of the trial.

Discussion

This prospective, open-label, multicentre trial evaluated the safety and efficacy of 2 years of treatment with microemulsified cyclosporine (Neoral) in children with FRNS. Our results showed that microemulsified cyclosporine significantly decreased the frequency of relapse and increased the probability of relapse-free survival, suggesting that treatment with microemulsified cyclosporine is effective for children with FRNS. Renal biopsy was performed after 2 years of treatment and showed that the treatment protocol was safe in terms of nephrotoxicity.

The significant decrease in the frequency of relapse during 2 years of treatment suggested that microemulsified cyclosporine is effective in children with FRNS. The probability of relapse-free survival in the present trial was compared with that in our previous trial, in which conventional cyclosporine was given to children with FRNS [11]. The probability of relapse-free survival in the present trial (58.1% in 2 years) was higher than the lowest target level, which was the upper limit of the 95% confidence interval for the probability of relapse-free survival in Group B [given a fixed dose of 2.5 mg/kg conventional cyclosporine from Month 7 onwards in the previous trial (37.9%), i.e. standard treatment]. On the other hand, better outcomes in terms of probability of relapse-free survival with microemulsified cyclosporine as compared with conventional cyclosporine were not obtained. The dose of cyclosporine did not differ significantly (data not shown). In this regard, microemulsified cyclosporine was not superior to conventional cyclosporine. Further clinical studies are thus needed to confirm the efficacy of microemulsified cyclosporine in children with FRNS.

The results of our study do not allow us to make firm conclusions about whether AUC_{0-4h} and C2 monitoring are clinically useful for titrating the dose of cyclosporine in children with FRNS. AUC_{0-4h} and C2 monitoring have been shown to be a useful method for titrating the dose of cyclosporine, particularly the microemulsified formulation, in adults [22,23] and in children who receive renal transplants [19,20,24]. C2 is the best single time point predictor of AUC_{0-4h}, but the trough level closely correlates with acute rejection [25]. On the other hand, limitations of C2 monitoring in renal transplant recipients have been demonstrated: C2 levels did not predict rejection or toxicity; poor and/or slow absorption were observed in a substantial number of patients; and C2 levels were not dose-proportional [26]. Moreover, in a randomized setting, C2 monitoring was not superior to trough monitoring in terms of graft survival in renal transplant recipients [27]. In the present trial, Poisson regression analysis was used to assess the relations of AUC_{0-4h} and C2 to relapse. The risk of relapse was not dependent on AUC_{0-4h} or C2, probably because the dose of cyclosporine was adjusted on the basis of trough levels, and neither AUC_{0-4h} nor C2 had sufficient variability (or power) to test the relation to relapse. To settle these issues, further studies are required; another new multicentre randomized controlled trial supported by the Ministry of Health, Labour and Welfare, entitled 'Cyclosporine C2 monitoring for frequently relapsing nephrotic syndrome in children: a randomized controlled trial', is now being conducted in Japan to evaluate the safety and efficacy of C2 monitoring for cyclosporine (Neoral).

Improvement in the mean height s.d. score is another encouraging result of our trial. Growth failure is a serious adverse effect of steroids in children. Improvement in the mean height s.d. score is attributed to the steroid-sparing effect of cyclosporine. This effect is an important reason for using immunosuppressants such as cyclosporine in children with FRNS. At the same time, our protocol for the use of prednisolone in this trial appears to be appropriate.

Adverse events associated with cyclosporine were acceptable in our trial. The main adverse events of cyclosporine are nephrotoxicity, neurotoxicity including encephalopathy and seizures, hypertension, gingival hyperplasia, hirsutism, and hypomagnaesemia [10,28–30]. In our trial, five patients (8.6%) had nephrotoxicity, and four (6.9%) had interstitial fibrosis. Fibrosis was mild in all of our patients. However, since irreversibility of interstitial fibrosis has been reported [31] and paediatric patients have a long life expectancy, nephrotoxicity due to cyclosporine should be closely monitored, and renal biopsy should be performed to confirm safety in patients who receive repeated or prolonged treatment with cyclosporine. Hypertension, defined as a requirement for antihypertensive agents, was detected in 12.9% of our patients. Although severe sequelae of hypertension such as seizures did not occur in this study, management of blood pressure is an important concern whenever cyclosporine is administered. No patient had serious adverse events that required the discontinuation of treatment during the trial.

An important limitation of the present trial is the study design: no control group was established. Because of several differences between the present trial and our previous trial, caution should be exercised when comparing the results. The results of the aforementioned randomized controlled trial are awaited to confirm our findings. Our study group was characterized by a significant male preponderance (48 boys and 14 girls), which has also been reported in children with nephrotic syndrome, including frequently relapsing nephrotic syndrome [7,9]. In our previous study, the male:female ratio was also as high as 35:9.

Another concern is missing data, such as the results of renal biopsy after treatment and measurement of AUC_{0-4h} and C2. Some patients refused repeated renal biopsy because of associated risks. AUC_{0-4h} measurement, which requires multiple blood samples, was inconvenient and was occasionally not performed by the physicians in charge; a single C2 measurement might be more practical. Finally, lack of adequate statistical power, particularly on Poisson regression analysis, was also a weakness of the present study.

A major limitation of cyclosporine treatment for children with FRNS is relapse after drug withdrawal. Several studies have evaluated relapse after cyclosporine treatment, albeit the treatment protocols differed from ours [8,32,33]; most patients had relapse of FRNS after the discontinuation of cyclosporine. Such patients require further treatment with cyclosporine or other immunosuppressants. We are continuing to follow up our patients to better define this critical issue.

In conclusion, treatment with microemulsified cyclosporine (Neoral) for 2 years in a dosage that maintains the trough level between 80 and 100 ng/mL for the first 6 months and 60–80 ng/mL for the next 18 months appears to be safe and effective in children with FRNS. Among several immunosuppressants recommended for children with FRNS, e.g. cyclophosphamide, levamisole, chlorambucil and mycophenolate mofetil, microemulsified cyclosporine is considered an important treatment option. Follow-up studies are being conducted to evaluate the risk of relapse after the withdrawal of cyclosporine.

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Patients with Epstein–Fechtner syndromes owing to *MYH9* R702 mutations develop progressive proteinuric renal disease

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Recent linkage analyses of nondiabetic African-American patients with focal segmental glomerulosclerosis (FSGS) have identified *MYH9*, encoding nonmuscle myosin heavy chain IIA (NMMHC-IIA), as a gene having a critical role in this disease. Abnormalities of the *MYH9* locus also underlie rare autosomal dominant diseases such as May–Hegglin anomaly, and Sebastian, Epstein (EPS), and Fechtner (FTNS) syndromes that are characterized by macrothrombocytopenia and cytoplasmic inclusion bodies in granulocytes. Among these diseases, patients with EPS or FTNS develop progressive nephritis and hearing disability. We analyzed clinical features and pathophysiological findings of nine EPS–FTNS patients with *MYH9* mutations at the R702 codon hot spot. Most developed proteinuria and/or hematuria in early infancy and had a rapid progression of renal impairment during adolescence. Renal histopathological findings in one patient showed changes compatible with FSGS. The intensity of immunostaining for NMMHC-IIA in podocytes was decreased in this patient compared with control patients. Thus, *MYH9* R702 mutations display a strict genotype–phenotype correlation, and lead to the rapid deterioration of podocyte structure. Our results highlight the critical role of NMMHC-IIA in the development of FSGS.

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KEYWORDS: Epstein syndrome; FSGS; *MYH9*; NMMHC-IIA; podocyte

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May–Hegglin anomaly and Sebastian syndrome are rare autosomal dominant disorders characterized by thrombocytopenia, giant platelets, and granulocyte cytoplasmic inclusion bodies called Döhle body-like inclusion bodies.[1] There are two related disorders, namely, Fechtner syndrome (FTNS) and Epstein syndrome (EPS), in which progressive hearing disability and nephritis are observed.[1] In FTNS, cataract is also present. Recently, *MYH9*, a gene encoding nonmuscle myosin heavy chain IIA (NMMHC-IIA), has been identified as the causative gene for these four disorders.[2–5] Several mutations in *MYH9* have been identified, and the existence of mutational hot spots in *MYH9*, that is, codons R702, R1165, D1424, E1841, and R1933, has been reported.[6–9]

As mutations in a single gene cause four distinct disorders, a novel nomenclature, *MYH9* disorders, has been proposed.[6,8,10] However, the mechanisms by which mutations in a single gene cause a variety of phenotypes remain to be elucidated.[1] Certain mutations in *MYH9* have been associated with the development of renal phenotypes, and R702 mutation is one of these.[7–10] Nevertheless, detailed information on renal manifestations, renal histology, and prognosis has been lacking.

In addition to macrothrombocytopenic diseases due to *MYH9* mutations, a significant association between the development of idiopathic focal segmental glomerulosclerosis (FSGS) or end-stage renal disease (ESRD) in African-American individuals and single-nucleotide polymorphisms in *MYH9* was identified in 2008.[11,12] This finding shows that NMMHC-IIA is responsible for not only EPS–FTNS, but also FSGS. Structural or functional abnormalities of NMMHC-IIA are considered to be critical in the development of FSGS in the African-American population.

In this study, we analyzed nine cases with R702 mutation in *MYH9*. Most cases with R702 mutation in *MYH9* develop nephritis characterized by proteinuria and/or hematuria in early infancy, and the deterioration of renal function accelerates in early adolescence. Hearing disability also manifests in early infancy and progresses to deafness at approximately 30 years of age. Findings of a serial renal biopsy in one case indicated that the pathological feature underlying this progressive nephritis is FSGS.

RESULTS

Clinical presentation and *MYH9* mutations

The clinical backgrounds of each case are summarized in Table 1. The age at the latest examination ranges from 4 to 33 years. Most of the cases were diagnosed as having idiopathic thrombocytopenic purpura at first presentation.

Genetic analysis revealed R702H mutation in case 1 and R702C mutation in the remaining 8 cases. All the R702 mutations identified in this study were *de novo* mutations. No disease-related family histories were noted.

Urinary abnormalities and development of renal dysfunction

The renal manifestations of nine cases are described in Table 1. Except for case 1, all the other cases developed proteinuria and/or hematuria before 12 years of age. Case 2 developed significant proteinuria (1+; urine protein/gCr = 310 mg/g creatinine (Cr)) as early as 2 years and 7 months of age. It is notable that four cases over 15 years old developed ESRD between 15 and 20 years of age. Distinct from another progressive hereditary nephritis, that is, Alport's syndrome, none of the cases showed macroscopic hematuria at presentation. For cases 6, 7, 8, and 9 who developed ESRD, their serum Cr levels are plotted in Figure 1. Each case progressed to ESRD shortly after the serum Cr level exceeded 1.0 mg/dl. The clinical status at the latest evaluation is described in Table 1 along with age at final evaluation.

Extrarenal manifestations

Among these cases, only case 1 showed cataract (Table 2). A hearing disability was observed in most of the cases by approximately 5 years of age, which progressed rapidly. Over 30 years old, all of the cases in this study became deaf.

Renal histopathological analysis of case 6

Light and electron microscopy findings of serial renal biopsies of specimens from case 6 are shown in Figure 2. The first biopsy was performed at 9 years of age by surgical operation, when the serum Cr level was 0.4 mg/dl. Twenty-four glomeruli were obtained, and mild mesangial cell proliferations and expansion were observed in most glomeruli (Figure 2a). Sclerotic lesions were not observed in any glomeruli, and tubulointerstitial changes were minimal. Immunofluorescence studies using immunoglobulins G, A, M, and C1q, C3, and C4 antibodies showed negative or no significant findings. Electron microscopy of these specimens revealed focal lesions of podocyte foot process effacement (indicated by an arrow in Figure 2c). Focal glomerular basement membrane (GBM) thickening lesions were observed (up to 1000–1500 nm in diameter), whereas most GBM show normal appearance (thickness ranges from 300 to 400 nm). The other GBM abnormalities, such as splitting, attenuation, or reticulation, were not observed. The second biopsy was performed by needle biopsy when the case was 11 years and 9 months of age. Only four glomeruli were obtained; one glomerulus showed global sclerosis, and two of the remaining three glomeruli showed segmental sclerosis.

Table 1 | Clinical backgrounds and renal manifestations in patients with R702 mutation in *MYH9*

Case no.	Sex	Ethnic background	*MYH9* mutation	First clinical diagnosis	Age and urinalysis findings when urine abnormalities were first noted: Age	Proteinuria	Hematuria	Age at latest evaluation and each clinical status: Age	Clinical status	Age at CKD 5 onset	Proteinuria	Hematuria
1	F	Japanese	R702H	ITP		(−)	(−)	4y11m	w.n.l		(−)	(−)
2	F	Japanese	R702C	MHA	2y7m	(1+)	(−)	4y2m	CKD stage 1		(−)	(−)
3	F	Chinese	R702C	ITP	6y8m	(−)	(1+)	6y8m	CKD stage 1		(−)	(1+)
4	M	Japanese	R702C	ITP	5y8m	(2+)	(3+)	12y3m	CKD stage 1		(3+)	(3+)
5	M	Japanese	R702C	ITP	11y8m	(+)	(+/−)	11y8m	CKD stage 1		(+)	(+/−)
6	F	Japanese	R702C	ITP	8y8m	(1+)	(1+)	21y3m	Transplant	15y		
7	M	Japanese	R702C	MHA	9y	(1+)	(1+)	20y7m	Transplant	17y		
8	F	Japanese	R702C	ITP	Unknown		Unknown	33y	PD/HD	16y		
9	F	Japanese	R702C	ITP	5y8m	(+/−)	Unknown	32y	Transplant	20y		

Abbreviations: HD, hemodialysis; HPF, high power fields; ITP, idiopathic thrombocytopenic purpura; MHA, May-Hegglin anomaly; m, month; PD, peritoneal dialysis; y, year.
In the urinalysis, stages of proteinuria and hematuria were defined as follows:
Proteinuria: +/−, 15 mg/dl; +, 30 mg/dl; 2+, 100 mg/dl; 3+. 300 mg/dl or over 300 mg/dl.
Hematuria: +/−, RBC ~5/HPF; 1+, 5-10/HPF; 2+, 10~50/HPF; 3+, >50-100/HPF.

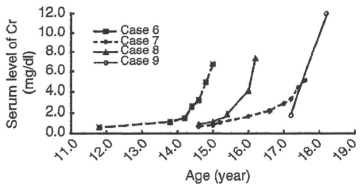

Figure 1 | Rapid progression of renal dysfunction in cases 6, 7, 8, and 9. Serum Cr levels (mg/dl) of cases 6, 7, 8, and 9 are plotted. The rapid deterioration of renal function is apparent in these cases. Each case progressed to end-stage renal disease shortly after Cr levels exceeded 1.0 mg/dl.

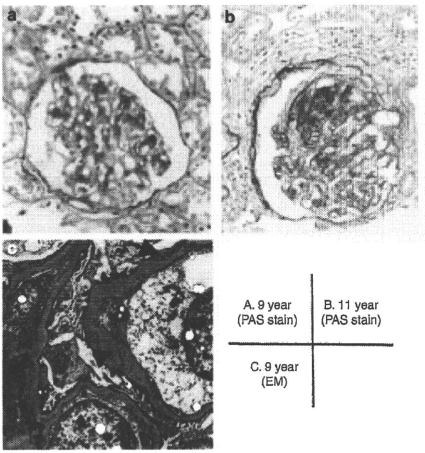

Figure 2 | Histopathological analysis of renal specimen from case 6. Light and electron microscopies of the renal specimen from case 6. (**a**) Periodic acid Schiff (PAS) staining of typical glomerulus and surrounding tubules of the renal specimen of case 6 at 9 years of age (first biopsy). (**b**) Findings of the same case at 11 years of age (second biopsy). This glomerulus shows typical morphological changes compatible with focal segmental glomerulosclerosis. (**c**) Electron microscopy of first biopsy specimen shows a nearly normal appearance of the glomerular basement membrane with focal podocyte foot process effacement (arrow) and focal thickening of GBM (arrowhead).

The glomeruli were not enlarged. Interstitial fibrosis, cellular infiltration, and tubular atrophy were observed around the impaired glomeruli. At the time of the second renal biopsy, the serum Cr level was 0.6 mg/dl, and the estimated glomerular filtrate rate calculated by Schwartz's formula was 107 ml/min per 1.73 m^2. The findings of the second biopsy of case 6 are compatible with the diagnosis of FSGS. Considering an almost normal glomerular filtrate rate at the time of the second renal biopsy, absence of enlarged glomeruli in the kidney specimen, and the subsequent rapid progression to ESRD in this patient, focal segmental sclerosis is considered to be the primary lesion due to *MYH9* mutation rather than a phenomenon secondary to nephron mass reduction.

Immunohistochemical analyses of NMMHC-IIA in control and case 6 kidney samples

Figure 3 shows the immunostaining data of NMMHC-IIA in the glomerulus in normal control and case 6 kidney samples. In the glomeruli of the control sample, the intensity of NMMHC-IIA immunostaining is very strong in podocytes (Figure 3a and b). In the first biopsy specimen from case 6, the intensity of immunostaining of NMMHC-IIA is already significantly decreased (Figure 3c and d). The second biopsy specimen from case 6 (Figure 3e and f) also shows a decreased NMMHC-IIA immunostaining intensity.

In the normal kidney sample, NMMHC-IIA is also expressed in renal tubular cells, particularly those of the distal tubule, Henle's loop, and proximal tubular cells (Figure 4a-d). Endothelial cells of interlobular arteries and arterioles, and peritubular capillaries also express NMMHC-IIA (Figure 4a-c). In case 6, NMMHC-IIA expression in renal tubular cells and endothelial cells did not change significantly (Figure 4e and f).

NMMHC-IIA distribution in peripheral blood neutrophils

The NMMHC-IIA distribution pattern in peripheral blood neutrophils was examined by immunofluorescence analysis. In normal blood neutrophils, NMMHC-IIA distributes diffusely in the cytoplasm (Figure 5, control 1, 2, and 3). In all the cases with *MYH9* R702 mutations, NMMHC-IIA was condensed and localized in the peripheral region of neutrophils (Figure 5, cases 1–9; condensation of NMMHC-IIA is indicated by an arrowhead). This granular pattern (type II) was observed in neutrophils from all the cases with R702 mutations (Figure 5 and Table 2, see Methods and Discussion sections for the definition of NMMHC-IIA distribution patterns in neutrophils).

Effect of ARB/ACEI on urinary abnormalities and renal function in three cases

Three cases, namely cases 2, 4, and 7, were treated with angiotensin receptor blockers (ARB) and/or angiotensin-converting enzyme inhibitors (ACEIs) for progressive nephritis. Figure 6 shows the effect of ARB/ACEI on urinary protein level in case 2. The urinary protein level evaluated in terms of urine protein/Cr was decreased from 500–700 mg/gCr to approximately 100 mg/gCr by administration of 20 mg of valsartan (Figure 6). In cases 4 and 7, the effect of ARB/ACEI was not very conclusive (data not shown). In case 4, other drugs such as cyclosporine A were used simultaneously; therefore, the effect of only ARB/ACEI could not be determined. In case 7, the effect of ARB/ACEI was transient.

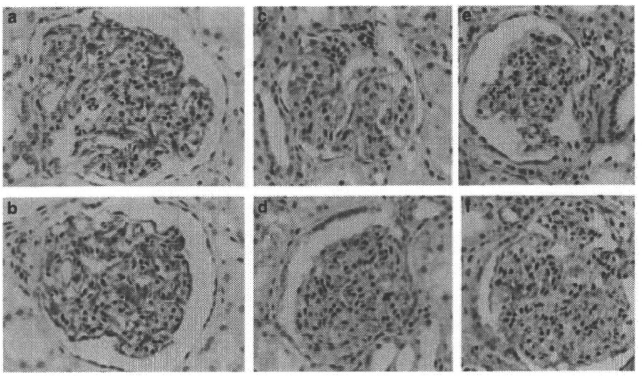

Figure 3 | Expression of NMMHC-IIA in the glomerulus from normal control and case 6 kidney samples. (**a** and **b**) NMMHC-IIA expression in the glomerulus from normal control sample. NMMHC-IIA is strongly expressed in podocytes. (**c** and **d**) First biopsy specimen from case 6. The intensity of immunostaining of NMMHC-IIA is already decreased. (**e** and **f**) Second biopsy specimen from case 6. The intensity of immunostaining of NMMHC-IIA is decreased.

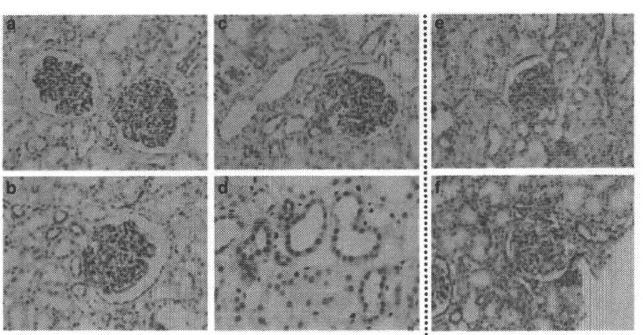

Figure 4 | Expression of NMMHC-IIA in normal control and case 6 kidney samples. (**a–d**) NMMHC-IIA expression in normal control kidney sample. Compared with the strong staining of NMMHC-IIA in podocytes, its expression in other renal tissues is relatively weak. Weak expression of NMMHC-IIA is observed in the distal nephron (**b** and **d**) and loop of Henle (**b**). Endothelial cells of arteries also express NMMHC-HHA (**c**). In the first (**e**) and second (**f**) renal biopsy specimen from case 6, expression of NMMHC-IIA in tubular cells and endocapillary cells is not remarkably changed compared with the controls, whereas decreased staining of podocytes is noted.

DISCUSSION

In this study, we showed that cases with *MYH9* R702 mutation show a very rapid deterioration of renal function with concurrent progressive hearing disability. Proteinuria and/or hematuria was detected in early infancy, and ESRD developed during adolescence. To date, several mutations including S96L, R702C, R702H, R1165C, and D1424 have been associated with the development of nephritis.[7–10] Pecci *et al.*[9] showed that mutations in the motor domain of NMMHC-IIA are associated with severe thrombocytopenia and the development of nephritis and deafness before 40 years of age, whereas patients with mutations in the tail domain not only have a much lower risk of developing such impairments but also significantly higher platelet counts. Heath *et al.*[7] and Dong *et al.*[8] described the development of nephritis in patients with R702 mutations. However, description of the clinical course of nephritis in these reports was very limited. In this study, we examined the precise clinical manifestations in nine patients with *MYH9* R702 mutations, and showed a definite genotype–phenotype correlation in both renal impairment and hearing disability.

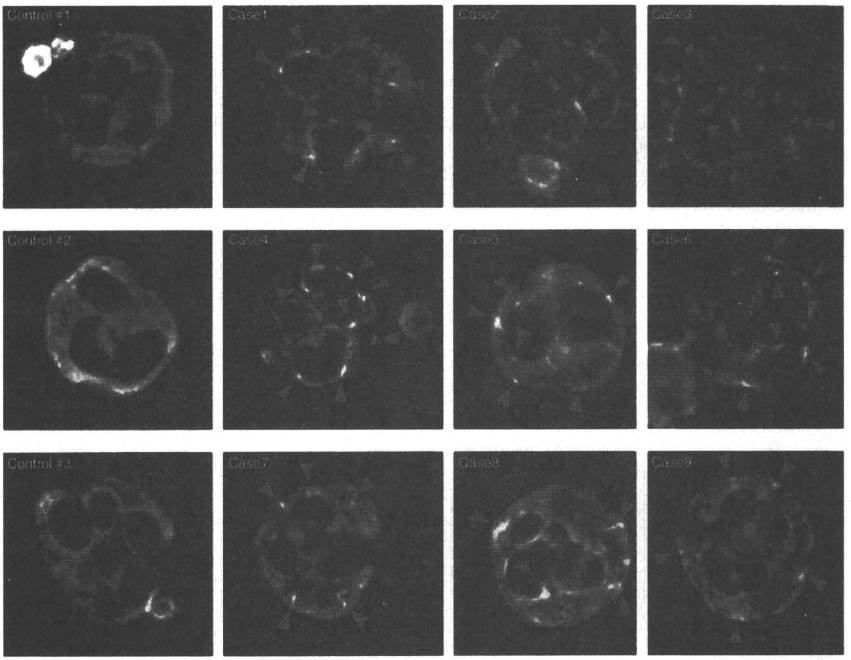

Figure 5 | Distribution of NMMHC-IIA in the cytoplasm of neutrophils in control and cases with R702 mutations. Controls 1, 2, and 3: Normal distribution of NMMHC-IIA in the cytoplasm of neutrophils. NMMHC-IIA is diffusely distributed in the cytoplasm. Cases 1 to 9: In all the cases with *MYH9* R702 mutations, NMMHC-IIA was condensed and localized in a granular pattern in the cytoplasm as indicated by gray arrowheads.

Table 2 | Extrarenal manifestations in each patient

Case no.	Type of cytoplasmic distribution of NMMHC-IIA	Hearing disability: Age of presentation and severity	Hearing disability: Latest hearing level	Cataract
1	II	2y2m: 50 db	NT	+
2	II	(−)	(−)	(−)
3	II	(−)	(−)	(−)
4	II	4y	NT	(−)
5	II	4y	40–50 dB	(−)
6	II	8y10m: 30 dB	40–55 dB	(−)
7	II	10y	70–80 dB	(−)
8	II	9y	Deaf	(−)
9	II	5y	Deaf	(−)

Abbreviations: m, month; NMMHC-IIA, nonmuscle myosin heavy chain IIA; NT, not tested; y, year.
Note: Abnormal distributions of NMMHC-IIA are classified into three types according to the number, size, and shape of accumulated NMMHC-IIA granules (see also the Materials and Methods section).[16] Type II is characterized by the presence of up to 20 circular to oval NMMHC-IIA-positive spots ($\leqslant 1\,\mu m$).

In Epstein–Fechtner syndrome, renal biopsy is principally contraindicated because of the accompanying thrombocytopenia. There have been few reports on the morphological changes of renal histology in Epstein–Fechtner syndrome. Only three reports on renal pathological findings are available in the literature.[13–15] Epstein *et al.*[13] described the renal morphology in a 13-year-old patient with Epstein syndrome. Their study revealed interstitial fibrosis, focal mesangial proliferation, and global sclerotic changes when the serum Cr level was 0.6 mg/dl. A recent genetic study by Heath *et al.*[7] identified *MYH9* R702C mutation in this patient. Moxey-Mims *et al.*[14] performed renal biopsy twice in an African-American female with Fechtner's syndrome. The type of *MYH9* mutation in this patient was not identified in the literature. The first biopsy at 7 years of age showed proliferation of mesangial cells and matrix with alterations in the GBM, such as effacement, thickening, and splitting; the second biopsy at 10 years of age revealed global sclerotic changes in 75% of glomeruli.[14] Moxey-Mims *et al.*[14] concluded that these renal changes are similar to those in Alport syndrome. Ghiggeri *et al.*[15] performed renal biopsy in FTNS patients with D1424H mutation, and electron microscopy showed focal and segmental effacement of podocytes and loss

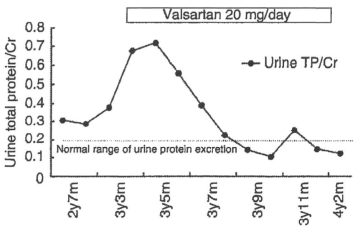

Figure 6 | Effect of valsartan on urinary protein excretion. The effect of 20 mg of valsartan on urinary protein excretion (urine protein g/urine creatinine mg) in case 2 is shown. The start of treatment with valsartan in case 6 was immediately after the age of 3 years (y) and 4 months (m), when the urinary protein level was nearly 0.7 g/g Cr.

of the interpodocyte slit diaphragm. The histopathological changes reported so far could occur in various types of glomerulonephritis. In this study, renal biopsy was performed twice in case 6 with platelet transfusion. The second renal biopsy specimen from case 6 showed that the pathological diagnosis is FSGS. In Epstein–Fechtner's syndrome with R702 mutation, progressive proteinuria and rapid deterioration of renal function are the common characteristics, as shown in this study, and the renal histology in case 6 is compatible with the clinical features of Epstein–Fechtner's syndrome with *MYH9* R702 mutation.

Epstein syndrome has been considered to be a variant of Alport syndrome, because of their very similar phenotypes, except for macrothrombocytopenic purpura, which is observed only in Epstein syndrome. However, the following two lines of evidence make them distinct from each other. The first is the different types of urinary abnormality. As shown in Table 1, proteinuria is equivalent or predominant in patients with Epstein syndrome; in contrast, hematuria is predominant, and macroscopic hematuria is often observed in patients with Alport syndrome much earlier than proteinuria. The second is the differences in the expression of causative genes in the glomeruli. The type IV collagen α5 chain gene responsible for Alport syndrome encodes an essential structural component of GBM; in contrast, NMMHC-IIA encoded by *MYH9* is exclusively expressed in podocytes, renal tubular cells and endothelial cells, as shown in Figure 3. In general, GBM abnormalities could occur secondary to podocyte dysfunction. Taken together, we consider that the alterations of GBM in Epstein syndrome are a phenomenon secondary to the dysfunction NMMHC-IIA molecule in podocytes owing to *MYH9* mutation.

Kunishima *et al.*[16] reported that the abnormal distributions of NMMHC-IIA in blood neutrophils in cases with *MYH9* disorders are classified into three types: type I, NMMHC-IIA is condensed into one or two granular masses; type II, NMMHC-IIA is present as granular masses of up to 20; type III, NMMHC-IIA is diffusely distributed as fine granules throughout the cytoplasm. Kunishima *et al.*[16] also indicated that the type of abnormal distribution of NMMHC-IIA is closely related to the site of the *MYH9* mutation.[16] In all the cases in this study, NMMHC-IIA distribution in neutrophils was of type II (Figure 5 and Table 2).

The abnormal distribution of NMMHC-IIA in Epstein–Fechtner syndrome could be directly associated with the pathogenesis in the kidney. Arrondel *et al.*[17] showed the expression of NMMHC-IIA in podocytes, endocapillary cells, and proximal tubular cells in the human kidney. In this study, we show that NMMHC-IIA is expressed in the glomerulus, tubular cells including the distal tubule, loop of Henle, and after the proximal tubule, endothelial cells of the interlobular arteries and arterioles, and peritubular capillaries. Ghiggeri *et al.*[15] discussed that the aggregation and compartmentation of NMMHC-IIA in podocytes might be associated with podocin dysfunction. In addition, there is another possibility. Two hereditary types of FSGS with an autosomal dominant trait are known, and one is caused by mutations in the gene encoding α-actinin-4 (*ACTN4*).[18] Several studies have indicated the following possible mechanisms: mutations in *ACTN4* increased the ability of binding of mutated α-actinin-4 to actin filaments, alter their intracellular localization, and finally cause FSGS.[19,20] Michaud *et al.*[20] showed that when mutated α-actinin-4 is expressed in cultured podocytes, and the localization of α-actinin-4 changes; that is, aberrant sequestering of α-actinin-4 impairs podocyte spreading and motility and decreases the number of peripheral projections. They suggested that these cytoskeletal derangements may underlie podocyte damage and foot process effacement. Recently, several studies have indicated that NMMHC-II has important roles in cell polarity, cell adhesion, and cell migration.[21] Podocytes are highly differentiated epithelial cells, and are connected to each other through a specific cell–cell adhesion molecule complex, that is, a slit diaphragm, which is crucial for glomerular filtration. NMMHC-IIA is considered to be located at the scaffolding underneath the plasma membrane and in the cytoplasm, and to have a role in maintaining and disassembling the adhesion junction complex.[21] It is plausible that mutated NMMHC-IIA would impair the function and structure of the slit diaphragm, which would result in proteinuria and the development of FSGS.

It has been reported that human immunodeficiency virus-related or hypertension-related FSGS are common in the African-American populations. Recently, two groups have independently identified a highly significant association between the development of FSGS or ESRD and single-nucleotide polymorphisms in *MYH9* in African-American individuals using an admixture-mapping linkage-disequilibrium genome scan method.[11,12] The development of ESRD was associated with hypertension, not with diabetes mellitus. This evidence strongly indicates that NMMHC-IIA is responsible for the development of not only Epstein–Fechtner

syndrome, but also idiopathic FSGS. There is no information on why specific single-nucleotide polymorphisms in *MYH9* increase the susceptibility of African-American individuals to FSGS or ESRD. Clarification of the pathophysiological mechanisms underlying the development of FSGS in Epstein–Fechtner syndrome would provide clues elucidating the molecular mechanisms underlying the development of FSGS.

Just recently, Pecci *et al.*[22] have reported a favorable effect of ACEI and/or ARB on proteinuria in patients genetically diagnosed with Epstein–Fechtner syndrome. In this study, three cases among the nine enrolled were treated with ARB and/or ACEI. In case 2, administration of 20 mg of valsartan markedly reduced the amount of protein in the urine (Figure 3). In cases 4 and 7, ARB and ACEI partially reduced the amount of proteinuria. These findings, as well as those of Pecci *et al.*, suggest the protective effect of ARB and ACEI on the kidney in cases with D1424H and N93K mutations. As the susceptibility to ESRD or FSGS caused by certain single-nucleotide polymorphisms in *MYH9* in African Americans is also associated with hypertension, ARB and ACEI presumably showed a protective effect on the progression of renal dysfunction owing to their direct effects on podocytes. This is another important issue in the elucidation of the mechanisms underlying the development of FSGS related to NMMHC-IIA.

In conclusion, in this study, we showed a rapid deterioration of renal function in cases with *MYH9* R702 mutation, and the pathological findings in one case were consistent with FSGS; apparent changes in NMMHC-IIA expression in podocytes were observed in this patient. Further studies are required to elucidate the possible pathophysiological mechanisms by which podocyte dysfunction occurs because of R702 mutations in *MYH9*, which is cardinal in the development of FSGS in African-American populations. Such studies could provide us clues to the mechanisms underlying the development of idiopathic FSGS.

MATERIALS AND METHODS

Cases

We performed genetic analysis in approximately 100 patients suspected of having *MYH9* disorders. Among those 100 patients, we selected all unrelated patients with R702 mutation and enrolled them in this study (Table 1). Some of the clinical and hematological data of patients 1, 2, 4, and 5 were previously published:[23] except for case 4, there were no symptoms of nephritis in these patients previously reported. The other cases are all new.

Details of clinical data and courses, the treatment for nephritis, the treatment for ESRD, and the latest clinical data were obtained by the attending physician in each case. Ocular abnormalities and hearing disabilities were evaluated by ophthalmologists and otolaryngologists, respectively.

The renal biopsy specimens from case 6 were evaluated by light microscopy and electron microscopy using conventional techniques. Immunohistochemical analysis was carried out as described below.

Genetic analysis of *MYH9* was approved by the institutional review boards of Nagoya Medical Center and each of the hospitals where the patients were enrolled, and was performed after informed consent was obtained from the patients and/or their parents. The IRB of the National Hospital Organization Nagoya Medical Center also approved the publication of the case reports and the obtained experimental data. Mutational analysis of *MYH9* was performed as previously described.[6]

Immunohistochemical analysis of renal specimens from control and case 6 kidney samples

Renal biopsy specimens from normal control and case 6 kidney samples were also subjected to immunohistochemical analysis. This was performed using 3 μm-thick sections of a paraffin-embedded sample. A renal specimen derived from a 51-year-old renal transplantation donor kidney, which was excised from the donor with immediate perfusion (0 h), was used as a control. Each renal section was autoclaved for 15 min at 121 °C in a citrate buffer of pH 6.0. After washing with water and phosphate-buffered saline, each section was incubated with an anti-NMMHC-IIA antibody (BT561, Biomedical Technologies, Stoughton, MA, USA, 1:100) for 2 h at room temperature. After washing, each section was further incubated with a secondary antibody (ENVISION, Dako, Kyoto, Japan) for 20 min. Subsequently, each section was treated with streptavidin-horseradish peroxidase and diaminobenzidine. The sections were then counterstained with hematoxylin.

Immunofluorescence analysis of NMMHC-IIA in peripheral blood neutrophils

Immunofluorescence analysis was performed to evaluate the subcellular localization of NMMHC-IIA in peripheral blood neutrophils as previously described.[16] The cytoplasmic distribution patterns of NMMHC-IIA in neutrophils can be classified according to the number, size, and shape of accumulated NMMHC-IIA granules, into types I, II, and III. Type I comprises one or two large (0.5–2 μm), intensely stained, oval- to spindle-shaped cytoplasmic NMMHC-IIA-positive granules. Type II comprises up to 20 circular to oval cytoplasmic granules ($\leqslant$1 μm). Type III appears as a speckled staining. The pattern of localization correlates with the site of *MYH9* mutation. Mutations in exons 16, 26, and 30 are associated with type II localization.[16]

DISCLOSURE

All the authors declared no competing interests.

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BRIEF REPORT

Long-term remission of nephrotic syndrome with etanercept for concomitant juvenile idiopathic arthritis

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Abstract Etanercept is a tumor necrosis factor (TNF)-α inhibitor that has been applied beneficially for juvenile idiopathic arthritis (JIA). We experienced long-term remission of nephrotic syndrome (NS) in a boy treated with etanercept, which was initially used for concomitant JIA. He developed NS at age 3 years 7 months and had mostly been treated with cyclosporine because of steroid dependency and frequent relapses. Cyclosporine was halted at 10 years 7 months because of nephrotoxicity, and he was subsequently treated with mizoribine. However, he had three relapses in the first year and developed JIA at 11 years 7 months. He was treated with sulfasalazine, methotrexate, and prednisolone, but his arthritis persisted. Etanercept was started at 12 years 3 months. Thereafter, his arthritis went into complete remission. Surprisingly, he has remained relapse-free for both NS and JIA for more than 3 years with etanercept and mizoribine. It is difficult to know whether the NS remission after initiating etanercept was coincidental. However, there are many reports of increased TNF-α or soluble TNF-α receptor in NS relapse. To date, there are two reports of the efficacy of TNF-α inhibitors against NS. It is possible that TNF-α inhibitors may have potential as therapeutic agents for NS.

Keywords Nephrotic syndrome · Etanercept · TNF-α · Juvenile idiopathic arthritis · Children

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Introduction

The etiology of idiopathic nephrotic syndrome (NS) is still unknown. However, it has been speculated that the etiology is strongly associated with abnormal immunity. For a long time, T cells have been assumed to play major roles in its pathogenesis. Recently, however, the anti-CD20 monoclonal antibody rituximab has emerged as a new agent for refractory NS [1]. There have been many reports of its remarkable effects, which shed light on the role of B cells. On the other hand, the roles of cytokines have not been well elucidated. We experienced long-term remission of NS in a 15-year-old boy treated with etanercept, a TNF-α inhibitor, which was initially used for concomitant juvenile idiopathic arthritis (JIA). We consider whether TNF-α inhibitors may have potential as new therapeutic agents for refractory NS based on our observations and some previous reports.

Case report

A boy aged 11 years 7 months developed JIA. He had developed NS at 3 years 7 months. A renal biopsy revealed

minimal changes in the NS. He had mostly been treated with cyclosporine, which was used from 5 years 9 months to 10 years 5 months, because of steroid dependency and frequent relapses. He had one or two relapses per year while being treated with cyclosporine. Cyclosporine treatment was halted when he was 10 years 5 months because of nephrotoxicity proven by a renal biopsy. He was subsequently treated with mizoribine instead of cyclosporine. However, he had three relapses in the first year after mizoribine initiation, and returned to steroid dependency (Fig. 1).

The patient abruptly developed arthritis in the right hip joint at 11 years 7 months, and his NS relapsed simultaneously. The serum C-reactive protein (CRP) level increased to 6.5 mg/dl. Daily prednisolone (PSL) of 40 mg for the NS relapse resolved not only the proteinuria but also the arthritis. However, after 3 months, when PSL had been tapered to 10 mg, he developed arthritis again in the bilateral knee joints, bilateral hip joints and proximal interphalangeal joints of the right fourth toe. Magnetic resonance imaging revealed fluid in the knee joints and synovial thickening. Enthesopathy of the right ankle was also indicated clinically. Rheumatoid factor, anti-cyclic citrullinated peptide antibodies, and anti-nuclear antibodies were all negative. Ophthalmologic abnormalities such as uveitis were absent. Serum complements were normal, and anti dsDNA antibody, pancytopenia and urinary abnormalities were not present. Systemic lupus erythematosus was ruled out by laboratory examinations and clinical manifestations. HLA-B27 was found, and he was diagnosed as having HLA-B27-related JIA.

He was initially treated with naproxen 400 mg, salazosulfapyridine 1,000 mg, and PSL 10 mg. Since mizoribine is known to be effective for both NS and rheumatoid arthritis, mizoribine was continued. However, the patient did not respond to these medications. Additional methotrexate was transiently effective, but could not ameliorate his arthritis. He presented with gait disturbance owing to severe pain in the swollen bilateral knee joints. CRP and erythrocyte sedimentation tests were elevated.

Biweekly etanercept of 25 mg was administered from 12 years 3 months and he improved dramatically within 2 weeks. The swollen and painful joints promptly resolved, and CRP and erythrocyte sedimentation tests decreased to the normal ranges. PSL was successfully discontinued. Salazosulfapyridine was also discontinued at 14 years 3 months. To date, he has been free from recurrence of arthritis for over 3 years with etanercept and mizoribine. Surprisingly, no relapse of NS has been experienced for over 3 years with mizoribine. Thus far, he has not had any severe adverse events for etanercept (Fig. 1).

Discussion

Our experience has raised the possibility that etanercept, a TNF-α inhibitor, may be effective for the prevention of NS relapse. In our case, it is difficult to know whether the NS remission after initiating etanercept was coincidental, because most patients with NS tend to exhibit decreased relapses with growth and puberty. In addition, synergistic effects of etanercept, mizoribine, and salazosulfapyridine may have resulted in the favorable outcome in this patient, although salazosulfapyridine was subsequently discontinued and mizoribine alone was initially ineffective. Regretfully, we did not examine the serum TNF-α or soluble

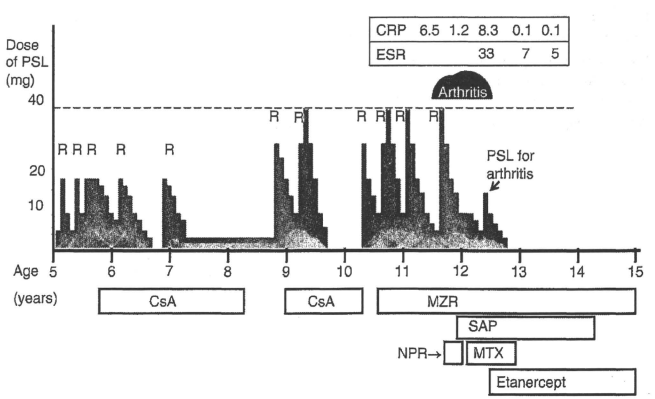

Fig. 1 Clinical course of the patient. *R* relapse; *PSL* prednisolone; *CsA* cyclosporine; *MZR* mizoribine; *SAP* salazosulfapyridine; *MTX* methotrexate; *NPR* naproxen; *CRP* C-reactive protein; *ESR* erythrocyte sedimentation ratio