Comprehensive genetic diagnosis of Japanese patients with severe proteinuria

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Numerous disease-causing gene mutations have been identified in proteinuric diseases, such as nephrotic syndrome and glomerulosclerosis. This report describes the results of comprehensive genetic diagnosis of Japanese patients with severe proteinuria. In addition, the report describes the clinical characteristics of patients with monogenic disease-causing mutations. We conducted comprehensive gene screening of patients who had either congenital nephrotic syndrome, infantile nephrotic syndrome, steroid-resistant nephrotic syndrome, or focal segmental glomerular sclerosis. Using targeted next-generation sequencing, 60 podocyte-related genes were screened in 230 unrelated patients with proteinuria. A retrospective review of clinical data was conducted for these patients. We detected monogenic disease-causing mutations in 30% (69 of 230) of patients among 19 of the screened genes. Common genes with disease-causing mutations were WT1 (25%), NPHS1 (12%), INF2 (12%), TRPC6 (10%), and LAMB2 (9%). With various immunosuppressive or renoprotective therapies, remission of proteinuria in patients with unknown causative mutations was observed in 26% of patients, whereas only 5% of patients with monogenic disease-causing mutations exhibited complete remission. We assessed the genetic backgrounds of Japanese patients with severe proteinuria. The proportion of patients with gene defects was similar to that of other reports, but the disease-causing gene mutation frequency was considerably different.

Idiopathic nephrotic syndrome has an estimated incidence of approximately 2 to 6.5 per 100,000 children per year, depending on ethnic background12. Steroid-resistant nephrotic syndrome (SRNS) occurs in approximately 10%–20% of children with nephrotic syndrome, and is associated with an increased risk of complications due to persistent proteinuria and therapeutic drug side effects. Most patients with SRNS initially exhibit the histological pattern of focal segmental glomerulosclerosis (FSGS), which is the primary glomerular aetiology of end-stage renal disease (ESRD) in children13. Congenital nephrotic syndrome (CNS), which develops at 0–3 months of age, and infantile nephrotic syndrome (INS), which develops at 4–12 months of age, are most commonly associated with gene mutations that encode the structural and regulatory proteins of the glomerular filtration barrier5.

Whole-exome NGS sequencing has revealed new disease-causing gene mutations associated with SRNS. Recently, more than 50 podocyte-related gene mutations have been identified in monogenic forms of CNS/INS/SRNS/FSGS6,8. Genetic aetiologies were identified in 29.5% of patients with SRNS before 25 years of age in a Western population8 and in 28.3% of such patients in Chinese cohort9. The proportion of patients in whom disease-causing gene mutations could be detected decreased with increasing age at the onset of nephrotic syndrome in previous studies10,11. A genetic diagnosis may enable clinicians to begin early management and treatment, such as the discontinuation of immunosuppressant therapy due to its strong side effects, and the initiation of renoprotective drugs. In addition, disease-causing mutations in some genes encoding enzymes of the coenzyme Q10 (CoQ10) pathway can be treated by supplementation with CoQ1012,13. Finally, mutation detection analyses can facilitate the prediction of post-transplant recurrence of nephrotic syndrome14,15.

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Studies of Western and Chinese cohorts of paediatric SRNS patients showed that the results of genetic analyses vary among ethnicities\(^8\). Following the advent of NGS, the discovery of proteinuric disease-causing gene mutations in patients has grown rapidly, although the exact incidence remains unclear. Until now, there have been no large-scale mutation screening studies of Japanese patients with nephrotic syndrome.

In this study, we used targeted NGS for simultaneous sequencing of 60 podocyte-related genes and aimed to clarify the clinical characteristics of Japanese patients with one of the following diseases: CNS, INS, SRNS, FSGS, or asymptomatic proteinuria with likely genetic disease.

**Results**

**Mutations.** We collected samples from facilities throughout Japan (Supplementary Fig. S1). A total of 230 unrelated patients (132 men/boys and 98 women/girls) were included. Their median age at disease onset was 3 years (range, 1 day to 65 years). Of these 230 patients, 10 were diagnosed with CNS, 14 were diagnosed with INS, 101 were diagnosed with SRNS, and 105 were diagnosed with FSGS or asymptomatic proteinuria. We detected disease-causing gene mutations in 69 of 230 unrelated patients (30%). The coverage depths of detected genes are shown in Supplementary Table S1. One of the 69 patients was from a consanguineous family, whereas 15 patients had a positive family history of proteinuria, and 15 patients had a positive family history of renal failure (Table 1).

In the 25 patients harbouring autosomal recessive disease-causing mutations, four homozygous and 21 compound heterozygous mutations were found (Table 2).

We detected the disease-causing gene mutation in 85% of patients with CNS, 53% of patients with INS, 26% of patients with onset age of 1–3 years, 17% of patients with onset age of 4–6 years, 31% of patients with onset age of 7–12 years, 20% of patients with onset age of 13–18 years, and 20% of patients with onset age of 19 years or older (Supplementary Fig. S2). WT1 gene variants were most common, detected in 17 patients; variants in NPHS1 and INF2 were detected in eight patients, variants in TRPC6 were detected in seven patients, and variants in LAMB2 were detected in six patients (Table 3).

Genes with disease-causing mutations within the first year of life were as follows: 37% of mutations in WT1, 26% of mutations in NPHS1, 26% of mutations in LAMB2, and 11% of mutations in other genes. WT1 was also the most frequent gene mutated in individuals with onset of SRNS after the age of 1 year. In 27 (12%) patients, one or several extra-renal abnormalities were reported; these included symptoms suggestive of Denys-Drash syndrome (caused by WT1 gene mutation and characterised by nephropathy, Wilms tumour, and genital abnormalities) and Pierson syndrome (caused by LAMB2 gene mutation and characterised by the occurrence of congenital nephrotic syndrome and ocular anomalies in combination with microcoria).

**Renal prognosis.** Seven patients had progressed to chronic kidney disease (CKD) stage 5 by the time of genetic analysis. The estimated glomerular filtration rate was above 90 ml/min/1.73 m\(^2\) in 108 of 173 evaluable patients (62%); it was 60 to 89 ml/min/1.73 m\(^2\) in 36 patients (21%), 30 to 59 ml/min/1.73 m\(^2\) in 18 patients (10%), and below 30 ml/min/1.73 m\(^2\) in 11 patients (6%) (Table 4). Kaplan–Meier analysis of renal survival showed that patients with genetic proteinuria exhibited faster progression to CKD stage 4 (p < 0.0221; Supplementary Fig. S3). The most common histopathologic diagnosis was FSGS (62%), followed by minor glomerular abnormalities (28%), mesangio proliferative GN (4%), and diffuse mesangial sclerosis (2%) (Table 5).

**Clinical characteristics.** When patients were stratified on the basis of causative mutation detection, patients with mutations in the analysed genes had higher frequencies of family history (p = 0.0004) and younger age (p = 0.024) than patients without mutations in the analysed genes. Patients with mutations had lower frequencies of nephrotic syndrome (p = 0.0421), oedema (p = 0.0018), and remission (p = 0.0104) than patients without mutations (Table 6).

Six parameters (age, sex, family history, oedema, remission, and nephrotic syndrome) were entered in multivariate logistic regression analysis. Patients with mutations had a significantly higher frequency of family history than patients without mutations (OR = 8.85; 95% CI = 2.96–26.48; p < 0.0001). Patients with mutations were approximately six-fold more likely to show absence of oedema (OR = 6.67; 95% CI = 2.03–21.84; p = 0.0017) and absence of remission (OR = 4.67; 95% CI = 1.39–15.69; p = 0.0128), relative to patients without mutations. Patients with mutations had higher odds of younger age than patients without mutations (OR = 0.89; 95% CI = 0.82–0.95; p < 0.0001) (Table 7).

**Discussion**

In this study, we found that 30% (69 of 230) of the patients had a single gene defect in one of 60 currently known podocyte-related genes in the Japanese population. In a previous study\(^9\), genetic diagnoses were established in 526 patients from 183 families (detection rate of 29.3%); four genes were identified as major SRNS genes: NPHS2 (9.9%), NPHS1 (7.34%), WT1 (4.77%), and PLCE1 (2.17%). The highest rate of mutation detection (69.4%) was recorded in the youngest group of patients (0–3 months); this proportion decreased with age. In the PodoNet study\(^15\), genetic disease was identified in 23.6% of patients; the most common mutated genes were NPHS2, WT1, and NPHS1. In that report, the proportion of patients with gene mutations also decreased with age: it was 66% in patients with CNS, whereas it decreased to 15–16% in older children. In the PodoNet study, the distribution of causative genes in patients with CNS was as follows: 40% had mutations in NPHS1, 10.6% had mutations in NPHS2, 8.5% had mutations in WT1, 5.5% had mutations in LAMB2, and 4.7% had mutations in all other genes (combined). In the present study, genetic diagnoses were established in 69 of 230 unrelated patients (30%); the mutation detection rate was similar. Furthermore, common genes were WT1 (25%), NPHS1 (12%), INF2 (12%), TRPC6 (10%), and LAMB2 (9%). In the present study, the distribution of causative genes in patients with CNS was as follows: 36% had mutations in NPHS1, 36% had mutations in LAMB2, and 18% had mutations in WT1. No NPHS2 mutations were detected in our study, which was consistent with the results of a study of Korean children.
| Patient | Gene            | Sex | Age at onset(y) | Category* | ESRD(y) | Histopathologic diagnosis | Family history** | Extra-renal symptom | Age at gene analysis(y) | eGFR (ml/min/1.73 m²) |
|---------|-----------------|-----|-----------------|-----------|---------|---------------------------|-----------------|---------------------|------------------------|-------------------------|
| Neph7   | WT1 (NM_024426.4) | F   | 0.33            | 2         | 0.5     | DMS                       | —               | —                   | 0.75                   | 5                      |
| Neph15  | WT1             | F   | 10              | 4         | —       | MGA                       | —               | —                   | 16                     | 114.4                  |
| Neph33  | WT1             | F   | 3               | 4         | —       | MGA                       | —               | —                   | 9                      | 113.5                  |
| Neph48  | WT1             | M   | 0.5             | 4         | —       | FSGS                      | —               | microcephaly, cryptorchidism | 2                     | 75.82                  |
| Neph52  | WT1             | F   | 6               | 3         | —       | FSGS                      | —               | —                   | 9                      | 84.8                   |
| Neph90  | WT1             | M   | 3               | 3         | 1.2     | FSGS                      | —               | —                   | 12                     | 11.9                   |
| Neph92  | WT1             | F   | 0               | 1         | 0       | —                         | —               | —                   | 0                      | 14                     |
| Neph95  | WT1             | M   | 3               | 4         | 11      | FSGS                      | —               | —                   | 30                     | 33.9                   |
| Neph107 | WT1             | F   | 0.17            | 2         | 0.17    | —                         | —               | —                   | 0.17                   | 10.24                  |
| Neph132 | WT1             | F   | 0.5             | 2         | 0.5     | sclerosis                  | —               | —                   | 1                      | 3                      |
| Neph136 | WT1             | F   | 6               | 4         | —       | MGA                       | —               | —                   | 17                     | 83.4                   |
| Neph137 | WT1             | F   | 2               | 3         | —       | Mesangial proliferative glomerulonephritis | — | — | 5 | 100 |
| Neph154 | WT1             | M   | 3               | 3         | —       | FSGS                      | —               | cryptorchidism, hypoplasia | 14 | 21 |
| Neph171 | WT1             | M   | 9               | 3         | —       | FSGS                      | —               | —                   | 29                     | 25.8                   |
| Neph185 | WT1             | M   | 0.5             | 2         | 2       | DMS                       | —               | —                   | 20                     | 71.1                   |
| Neph197 | WT1             | F   | 3               | 4         | 7       | FSGS                      | —               | —                   | 7                      | 12.5                   |
| Neph208 | WT1             | F   | 0.42            | 2         | 0.42    | —                         | —               | —                   | 0.5                    | 18.76                  |
| Neph27  | NPHS1 (NM_004646.3) | M   | 0               | 1         | —       | —                         | —               | —                   | 0.08                   | 34.1                   |
| Neph69  | NPHS1           | M   | 0.08            | 1         | —       | —                         | —               | —                   | 0.67                   | 32.1                   |
| Neph91  | NPHS1           | F   | 8               | 3         | —       | MGA                       | —               | —                   | 20                     | 244.3                  |
| Neph104 | NPHS1           | F   | 0               | 1         | —       | —                         | 1               | —                   | 0.08                   | —                      |
| Neph106 | NPHS1           | F   | 0               | 1         | —       | —                         | —               | —                   | 0.17                   | 55.5                   |
| Neph113 | NPHS1           | M   | 6               | 3         | —       | MGA                       | —               | —                   | 32                     | 165.4                  |
| Neph192 | NPHS1           | M   | 0.75            | 3         | —       | MGA                       | —               | —                   | 2                      | 92.3                   |
| Neph203 | NPHS1           | F   | 7               | 3         | —       | C1q nephropathy            | 1               | —                   | 10                     | 195                    |
| Neph59  | INF2 (NM_024893.3) | F    | 13              | 4         | —       | Focal glomerular obsolescent | 2 | — | 25 | 113 |
| Neph76  | INF2            | M   | 10              | 4         | 25      | FSGS                      | —               | —                   | 17                     | 7.2                    |
| Neph134 | INF2            | F   | 9               | 4         | —       | FSGS                      | —               | —                   | 15                     | 96.6                   |
| Neph155 | INF2            | M   | 11              | 4         | 14      | FSGS                      | —               | —                   | Charcot-Marie-Tooth disease | 15 | 8.4 |
| Neph177 | INF2            | F   | 30              | 4         | —       | FSGS                      | 2               | —                   | 32                     | 63                     |
| Neph198 | INF2            | F   | 13              | 4         | —       | MGA                       | —               | —                   | 16                     | 115.2                  |
| Neph202 | INF2            | M   | 31              | 4         | —       | FSGS                      | 2               | —                   | 39                     | 12.6                   |
| Neph227 | INF2            | F   | 7               | 3         | 13      | FSGS                      | —               | —                   | 22                     | 53.2                   |
| Neph5   | TRPC6 (NM_004621.5) | F    | 3               | 4         | 6       | FSGS                      | —               | —                   | 14                     | 92.4                   |
| Neph24  | TRPC6           | F   | 4               | 3         | 4       | FSGS                      | —               | —                   | 4                      | 91.6                   |
| Neph39  | TRPC6           | F   | 3               | 3         | 7       | C1q nephropathy            | —               | —                   | 7                      | 8.16                   |
| Neph139 | TRPC6           | F   | 2               | 4         | —       | FSGS                      | —               | —                   | 2                      | 94.8                   |
| Neph149 | TRPC6           | M   | 7               | 3         | —       | MGA                       | —               | —                   | 8                      | 95.17                  |
| Neph176 | TRPC6           | M   | 14              | 4         | —       | FSGS                      | 2               | —                   | 14                     | 140.13                 |
| Neph234 | TRPC6           | F   | 7               | 4         | —       | FSGS                      | 2               | —                   | 7                      | 119.8                  |
| Neph4   | LAMB2 (NM_002292.3) | M    | 0.33            | 2         | —       | FSGS                      | —               | —                   | 4                      | 121                    |
| Neph23  | LAMB2           | F   | 0               | 1         | 0.25    | Diffuse glomerular obsolescence | — | — | 1 | ESRD |
| Neph58  | LAMB2           | M   | 0               | 1         | 0       | —                         | —               | microcoria            | 0                      | 4.1                    |
| Neph87  | LAMB2           | M   | 0.17            | 1         | 0.5     | DMS                       | —               | retinal detachment    | 10                     | 68.9                   |
| Neph89  | LAMB2           | M   | 2               | 3         | —       | sclerosis                  | —               | —                   | 2                      | 93.8                   |
| Neph133 | LAMB2           | F   | 0               | 1         | 0.08    | —                         | —               | choroiditis, chorioretinal atrophy | 6 | 89.9 |
| Neph36  | ADCK4 (NM_024876.3) | F    | 6               | 4         | —       | FSGS                      | —               | —                   | 8                      | 89.7                   |
| Neph56  | ADCK4           | F   | 3               | 4         | —       | FSGS                      | —               | —                   | 4                      | 111.2                  |
| Neph160 | ADCK4           | M   | 3               | 4         | —       | FSGS                      | —               | —                   | 5                      | 60.8                   |

* Continued *
with SRNS (patients with CNS were excluded from that study)\textsuperscript{16}. In China, the most common mutated genes were ADCK4 (6.67\%), NPHS1 (5.83\%), WT1 (5.83\%), and NPHS2 (3.33\%)\textsuperscript{9}. The results of these studies show that there are differences in the types and frequencies of mutations among ethnicities and regions.

The Child Welfare Law, passed in 1961 in Japan, mandated urinary screening for preschool children, typically at 3 years of age. The purpose of urinary screening for preschool children was to prevent progression to ESRD or to improve the quality of life of children who were expected to develop ESRD. This first urinalysis is performed by using dip-and-reagent strips. In our study, we detected the disease-causing gene mutation in 41\% of patients at the age of 3 years; this high detection rate was likely because of the mandatory urine screening for preschool children, which helped to detect the presence of proteinuria and could increase the likelihood that genetic analyses were conducted in affected children.

The treatment of SRNS is a challenging task for nephrologists because of its poor response to immunosuppressive drugs. High-dose steroids, cyclophosphamide, calcineurin inhibitors, mycophenolate mofetil, and rituximab have been used with variable success rates in children. However, complete remission of non-genetic SRNS was observed in 78\% of patients during calcineurin inhibitor therapy\textsuperscript{17}. In contrast, genetic SRNS was associated with a high rate of ESRD development: one patient with genetic SRNS experienced complete remission and 16\% of patients with genetic SRNS experienced partial remission after calcineurin inhibitor therapy\textsuperscript{17}. In our study, complete remission of SRNS without mutations was observed in 26\% of patients during immunosuppressive therapy. However, this proportion does not reflect the natural clinical course of SRNS because most patients with SRNS who do not have mutations will be treated with immunosuppressants, such as repeated steroid pulses or rituximab treatment after genetic analyses, and there is insufficient long-term follow-up data for these types of patients. Complete remission of nephrotic syndrome in patients with mutations was observed in 5\% (2/37) of patients during treatment with immunosuppressive therapies and in one patient during treatment with angiotensin-converting enzyme inhibitors. Notably, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may cause urinary protein reduction and have renoprotective effects. However, a previous study

### Table 1.

Clinical features of 69 Japanese patients with proteinuria for whom disease-causing mutations were identified. *1: Congenital nephrotic syndrome, 2: Infantile nephrotic syndrome, 3: Steroid-resistant nephrotic syndrome, 4: Focal segmental glomerular sclerosis or asymptomatic proteinuria. **1: positive family history of proteinuria, 2: positive family history of renal failure. Abbreviations: DMS, diffuse mesangial sclerosis; ESRD, end-stage renal disease; FSGS, focal segmental glomerular sclerosis; MGA, minor glomerular abnormality.

| Patient | Gene     | Sex | Age at onset(y) | Category* | ESRD(y) | Histopathologic diagnosis | Family history** | Extra-renal symptom | Age at gene analysis(y) | eGFR (ml/min/1.73 m²) |
|---------|----------|-----|-----------------|-----------|---------|--------------------------|-----------------|---------------------|-----------------------|---------------------|
| Neph225 | ADCK4    | F   | 9               | 4         | —       | FSGS                     | L2              | —                   | 11                    | 90.9                |
| Neph19  | NUP107   | M   | 1.67            | 3         | 2.3     | FSGS                     | —               | —                   | 5                     | 6.2                 |
| Neph66  | NUP107   | F   | 3               | 4         | —       | FSGS                     | —               | —                   | 3                     | 129.4               |
| Neph147 | NUP107   | M   | 7               | 4         | —       | FSGS                     | —               | —                   | 8                     | 105.5               |
| Neph68  | LMX1B    | M   | 3               | 3         | —       | —                        | microcephaly    | 5                   | 167.61               |
| Neph77  | LMX1B    | F   | 3               | 3         | —       | —                        | 2               | 19                  | 109                  |
| Neph37  | ACTN4    | M   | 6               | 4         | 13      | FSGS                     | —               | 12                  | 66.7                 |
| Neph146 | ACTN4    | M   | 8               | 3         | —       | FSGS                     | —               | 11                  | 126                  |
| Neph129 | PAX2     | M   | 3               | 4         | —       | FSGS                     | 1               | 10                  | 106.3                |
| Neph230 | PAX2     | M   | 8               | 4         | —       | FSGS                     | —               | 8                   | 47.3                 |
| Neph178 | COL4A5   | F   | 8               | 3         | —       | FSGS                     | 2               | 54                  | 20.6                 |
| Neph204 | COL4A5   | F   | 1               | 4         | —       | non—IgA nephropathy      | 2               | 17                  | 93.3                 |
| Neph97  | CDQ6     | M   | 0.75            | 2         | —       | —                        | 0.92            | 83                  |                       |
| Neph79  | FAT1     | M   | 3               | 4         | —       | MGA                      | —               | 5                   | 141.8                |
| Neph189 | PICE1    | M   | 1               | 3         | 12      | FSGS                     | 1               | 21                  | 70.16                |
| Neph10  | SMARCA1   | M   | 7               | 3         | —       | FSGS                     | —               | 8                   | 64.1                 |
| Neph143 | TTC21B   | M   | 3               | 4         | —       | FSGS                     | situs inversus  | 4                   | 57.1                 |
| Neph216 | MTH9     | M   | 3               | 4         | —       | FSGS                     | —               | 8                   | 100.1                |
| Neph224 | CUBN     | M   | 3               | 4         | —       | MGA                      | —               | 3                   | 112                  |
| Neph236 | LAMA5    | M   | 0.25            | 1         | 1       | —                        | 2               | 13                  | 36.4                 |
| Patient | Gene | genome | amino acids | Mode of Inheritance | Origin of variant | HGMD | reference allele read depth | alternative allele read depth | dbSNP | Japanese frequency |
|---------|------|--------|-------------|--------------------|------------------|-------|--------------------------|-----------------------------|-------|-------------------|
| Nep7    | WTI  | c.1384 C>T | p.Arg462Trp | AD | de novo | reported | 16 | 12 | rs121907900 | No data (ND) |
| Nep15   | WTI  | c.1432 +4 C>T | AD | Not done (ND) | reported | 15 | 19 | rs58776577 | ND |
| Nep33   | WTI  | c.1178 G>A | p.Cys393Tyr | AD | de novo | novel | 4 | 6 | — | ND |
| Nep48   | WTI  | c.1491 T>A | p.Asp497Glu | AD | ND | novel | 175 | 113 | — | ND |
| Nep52   | WTI  | c.1432 +5 G>A | AD | ND | reported | 63 | 50 | rs58776576 | ND |
| Nep60   | WTI  | c.1392 C>A | p.Arg466Glu | AD | de novo | reported | 5 | 8 | — | ND |
| Nep92   | WTI  | c.1300 C>T | p.Arg434Cys | AD | ND (father) | reported | 36 | 42 | rs121907910 | ND |
| Nep95   | WTI  | c.1384 C>T | p.Arg462Trp | AD | ND | reported | 5 | 8 | rs121907900 | ND |
| Nep107  | WTI  | c.1301 G>A | p.Arg434His | AD | de novo | reported | 1 | 3 | rs121907901 | ND |
| Nep132  | WTI  | c.1432 +4 C>T | AD | ND | reported | 163 | 129 | — | ND |
| Nep136  | WTI  | c.1432 +4 C>T | AD | ND | reported | 74 | 46 | rs58776577 | ND |
| Nep137  | WTI  | c.1432 +4 C>T | AD | ND (father) | reported | 45 | 45 | rs58776577 | ND |
| Nep154  | WTI  | c.1231 C>T | p.His441Tyr | AD | de novo | reported | 257 | 216 | — | ND |
| Nep171  | WTI  | c.1348 C>T | p.Pro450Ser | AD | de novo | reported | 99 | 89 | — | ND |
| Nep185  | WTI  | c.1432 +4 C>T | AD | ND (father) | reported | 48 | 50 | rs58776577 | ND |
| Nep197  | WTI  | c.1351 T>A | p.Phe451Ile | AD | de novo | novel | 20 | 22 | — | ND |
| Nep208  | WTI  | c.1334 A>G | p.His445Arg | AD | de novo | reported | 46 | 56 | — | ND |
| Nep27   | NPHS1 (NM_004646.3) | c.1102 C>T | p.Pro368Ser | AR | father (carrier) | mother (carrier) | reported | 50 | 46 | 38683386 | 386833918 | ND |
| Nep69   | NPHS1 | c.869delp.c.1379 G>A | p.Thr291HisTer51 | AR | ND | novel | reported | 224 | 240 | — | ND |
| Nep91   | NPHS1 | c.2515delc.105 G>C | p.Gln839ArgfsTer8 | AR | father (carrier) | mother (carrier) | reported | 29 | 14 | 386833880 | ND |
| Nep104  | NPHS1 | c.2515delc.105 G>C | p.Gln839ArgfsTer8 | AR | ND | reported | 1 | 44 | rs386833918 | ND |
| Nep110  | NPHS1 | c.1135 C>T | p.Arg379Trp | AR | mother (carrier) | father (carrier) | reported | 55 | 44 | 38683387 | 386833918 | ND |
| Nep113  | NPHS1 | c.1379 G>A | p.Arg460Gln | AR | mother (carrier) | father (carrier) | reported | 21 | 22 | 386833880 | 3867600918 | ND |
| Nep192  | NPHS1 | c.2464 G>A | p.Val822Met | AR | parents (carrier) | reported | 31 | 7 | rs267600918 | ND |
| Nep203  | NPHS1 | c.3162_3165dupc.2464 G>A | p.Gly1056PhesTer41 | AR | father (carrier) | mother (carrier) | reported | 281 | 216 | — | ND |
| Nep59   | INF2 (NM_0022489.3) | c.1345 C>T | p.Pro451Leu | AD | father | novel | 177 | 161 | — | ND |
| Nep76   | INF2 | c.333 T>C | p.Phe111Ser | AD | mother | novel | 135 | 160 | — | ND |
| Nep134  | INF2 | c.529 C>T | p.Arg177Cys | AD | de novo | reported | 153 | 172 | — | ND |
| Nep155  | INF2 | c.218 G>T | p.Gly73Val | AD | de novo | novel | 62 | 34 | rs918089359 | ND |
| Nep177  | INF2 | c.653 G>A | p.Arg218Gln | AD | ND | reported | 103 | 127 | rs267607183 | ND |
| Nep198  | INF2 | c.301 T>C | p.Cys101Arg | AD | mother | novel | 117 | 87 | — | ND |
| Nep202  | INF2 | c.1244 >T.c.172 G>A | p.Leu412Phe | p.Glu547Asp | AD | ND | novel | 51 | 76 | 92 | ND |
| Nep227  | INF2 | c.658 G>A | p.Gln220lys | AD | de novo | novel | 128 | 141 | — | ND |
| Nep5    | TRPC6 (NM_004621.5) | c.517 T>G | p.Tyr173Asp | AD | de novo | novel | 8 | 1 | — | ND |
| Nep24   | TRPC6 | c.2645 +1 G>A | AD | de novo | novel | 82 | 66 | — | ND |
| Nep39   | TRPC6 | c.2683 C>T | p.Arg895Cys | AD | de novo | reported | 112 | 72 | rs121434394 | ND |
| Nep139  | TRPC6 | c.225 C>T | p.Arg75Trp | AD | de novo | reported | 356 | 214 | rs689025541 | ND |
| Nep149  | TRPC6 | c.326 G>A | p.Gly109Asp | AD | de novo | novel | 216 | 222 | — | ND |
| Nep176  | TRPC6 | c.2624 A>T | p.Gln875Val | AD | mother | novel | 225 | 226 | — | ND |
| Nep234  | TRPC6 | c.434 A>G | p.His145Arg | AD | father | novel | 46 | 113 | — | ND |
| Nep4    | LAMB2 (NM_002292.3) | c.225delc.2095 G>C | p.Tyr76ThrfsTer36 | AR | mother (carrier) | father (carrier) | reported | 21 | 14 | 26 | rs28634667 | ND |
| Nep23   | LAMB2 | c.482 T>C | p.Leu161Pro | AR | ND | novel | 0 | 10 | — | ND |
| Nep58   | LAMB2 | c.4519 C>T | p.Ala1507Ter | AR | father (carrier) | mother (carrier) | reported | 239 | 195 | 102 | rs974891221 | ND |
| Nep87   | LAMB2 | c.4904_4906delc.250_14_24-20 Adel | p.Thr1635ArgfsTer23 | AR | ND | novel | 269 | 73 | 43 | ND |
| Nep89   | LAMB2 | c.821 T>C | p.Leu274Pro | AR | mother (carrier) | reported | 5 | 8 | — | ND |
| Nep133  | LAMB2 | c.4166 G>A | p.Glu1390Asn | p.Thr1635ArgfsTer23 | AR | mother (carrier) | reported | 137 | 178 | 105 | 152 | rs758539618 | ND |

Continued
showed that more patients reached ESRD when using calcineurin inhibitors; the investigators concluded that calcineurin inhibitors may cause proteinuria reduction, but may negatively influence kidney function. Most patients with nephrotic syndrome who have mutations do not respond to immunosuppressive therapy; however, patients with disease-causing mutations in LMX1B and TRPC6 at least partially respond to therapy. Thus far, there are insufficient data to determine whether this proteinuria reduction has a renoprotective effect, and further studies of extended cohorts are needed.

The identification of gene mutations is important for decision-making in terms of future treatment strategies and predicting prognosis. However, it is not yet feasible to perform genetic testing in all patients with proteinuria. Therefore, it is necessary to consider which patients should undergo genetic testing. In previous reports of patients with SRNS, the likelihood of identifying a genetic mutation was inversely related to age at disease onset and was increased in patients with positive family history and in those with extra-renal manifestations. In our study, we compared patients with and without known causative mutations, among all patients. The results showed that risk factors of genetic disease were younger age, family history, absence of oedema, and absence of remission. Importantly, absence of remission was strongly associated with genetic disease in this study. In a previous report of adult-onset FSGS, secondary FSGS (i.e., resistance to immunosuppression and atypical primary FSGS) was considered for genetic evaluation; our results concur with those of the prior report.

#### Table 2. Mutation genotypes of 69 Japanese patients with proteinuria for whom disease-causing mutations were identified. Abbreviations: AD, autosomal dominant; AR, autosomal recessive; EAS, East Asian; ExAC, Exome Aggregation Consortium; HGMD, Human Gene Mutation Database; HGVD, Human Genetic Variation Database.

| Patient | Gene | Genome | amino acids | Mode of Inheritance | Origin of variant | HGMD | Reference allele read depth | Alternative allele read depth | dbsNP | Japanese frequency |
|---------|------|--------|-------------|--------------------|------------------|------|---------------------------|-----------------------------|-------|--------------------|
| Neph36  | ADCK4| c.737G>A | p.Ser246Asn | AR | mother(carrier) father(carrier) | novel | 0 | 526 | rs200841458 | HGVD:0.002 |
| Neph56  | ADCK4| c.737G>A | p.Ser246Asn | AR | mother(carrier) | reported | 287 | 171 | 281 | 165 | rs200841458 | HGVD:0.002 |
| Neph160 | ADCK4| c.1468C>T | p.Arg490Cys | AR | mother(carrier) | reported | 163 | 260 | 173 | 260 | rs398122978 | HGVD:0.001 |
| Neph225 | ADCK4| c.1468C>T | p.Arg490Cys | AR | mother(carrier) | reported | 91 | 119 | 91 | 159 | rs750037594 | ExAC_EAS:0.001 |
| Neph19  | NUP107 | c.1079_1083del | p.Glu343GluTer6 | AR | mother(carrier) | reported | 1 | 16 | 1 | 8 | rs64321632 | HGVD:0.001 |
| Neph66  | NUP107 | c.1079_1083del | p.Glu343GluTer6 | AR | mother(carrier) | reported | 19 | 17 | 12 | 3 | ND |
| Neph147 | NUP107 | c.2492A>C | p.Arg831Ala | AR | mother(carrier) | reported | 0 | 145 | 0 | 52 | rs64321632 | HGVD:0.001 |
| Neph68  | LMX1B | c.544G>A | p.Asp182Asn | AD | ND | reported | 316 | 249 | rs781341216 | ExAC_EAS:0.001 |
| Neph77  | LMX1B | c.912+1G>A | AD | ND | 120 | 106 | ND |
| Neph129 | PAX2 | c.71G>C | p.Gly244Ala | AD | ND | 28 | 51 | ND |
| Neph230 | PAX2 | c.215A>C | p.Tyr72Ser | AD | de novo | reported | 58 | 59 | ND |
| Neph178 | COL4A5 | c.2475_2483del | p.Pro826_Gly828del | X-linked | ND | reported | 127 | 84 | ND |
| Neph204 | COL4A5 | c.438+5G>A | X-linked | father | reported | 208 | 178 | rs281874739 | ND |
| Neph97  | COQ6 | c.782C>T | p.Gly1827ValfsTer6 | AR | mother(carrier) father(carrier) | reported | 24 | 12 | rs371260604 | ExAC_EAS:0.001 |
| Neph79  | FAT1 | c.12867dup | p.Glu3790ArgfsTer30 | AR | ND | 179 | 125 | 135 | 81 | ND |
| Neph189 | PLCE1 | c.2674_2675dup | p.Trp858ProfsTer3 | AD | parents(carrier) | reported | 293 | 303 | ND |
| Neph10  | SMARCAL1 | c.678_679del | p.Gly192ValfsTer56 | AR | father(carrier) mother(carrier) | reported | 33 | 79 | 26 | 77 | ND |
| Neph143 | TFC21B | c.7178_7179insG | p.Ala2397ThrfsTer29 | AR | mother(carrier) father(carrier) | reported | 19 | 158 | 11 | 148 | rs69548518 | HGVD:0.001 |
| Neph216 | MYH9 | c.2441G>A | p.Arg814Gln | AD | mother | reported | 69 | 79 | rs760924443 | ExAC_EAS:0.001 |
| Neph224 | CUBN | c.10245G>A | p.Tyr3415Ter | AD | mother(carrier) | father(carrier) | reported | 151 | 144 | 68 | 128 | ND |
| Neph236 | LAMA5 | c.2323>C>T | p.Ala775Thr | AR | mother(carrier) father(carrier) | reported | 172 | 160 | 170 | 138 | rs369268267 | ND |

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This study had several limitations. First, this was a cross-sectional retrospective study with a small study population, which limits the generalisability of the findings. Second, the examinations of individual patients relied entirely on the attending clinicians’ decisions, due to the retrospective nature of the study. Finally, treatment details were not accessible for some patients, which limited our ability to make inferences regarding their disease characteristics.

| Causative Gene | N |
|----------------|---|
| WT1            | 17|
| NPHS1          | 8 |
| INF2           | 8 |
| TRPC6          | 7 |
| LAMB2          | 6 |
| ADCK4          | 4 |
| NUP107         | 3 |
| LMX1B          | 2 |
| ACTN4          | 2 |
| PAX2           | 2 |
| COL4A5         | 2 |
| Others (COQ6, FAT1, PLCE1, SMARCAL1, TTC21B, MYH9, CUBN, LAMB5) | 8 |
| Causative mutations not detected | 161|

Table 3. Genes with disease-causing mutations in 230 Japanese patients with proteinuria.

| CKD stage | eGFR (ml/min/1.73 m²) | N (%) |
|-----------|-----------------------|-------|
| 1         | ≥90                   | 108 (62) |
| 2         | 60–89                 | 36 (21) |
| 3         | 30–59                 | 18 (10) |
| 4         | 15–29                 | 8 (5)   |
| 5         | <15                   | 3 (2)   |

Table 4. Numbers of proteinuric patients stratified by renal function stage. Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

| Histopathologic diagnosis | N (%) |
|---------------------------|-------|
| FSGS                      | 124 (62) |
| MGA                       | 55 (28) |
| MesPGN                    | 7 (4)   |
| DMS                       | 3 (2)   |
| Others                    | 11 (6)  |

Table 5. Histopathologic diagnoses of 230 Japanese patients with proteinuria. Abbreviations: FSGS, focal segmental glomerular sclerosis; MGA, minor glomerular abnormalities; MesPGN, mesangial proliferative glomerulonephritis; DMS, diffuse mesangial sclerosis.

|                        | Patients with mutations | Patients without mutations | p value |
|------------------------|-------------------------|----------------------------|---------|
| Age*                   | 3 (0.6–7)               | 4 (2–9)                    | 0.024   |
| Male, % (n)            | 55% (38/69)             | 58% (94/161)               | 0.6415  |
| Extra-renal presentation, % (n) | 12% (8/69) | 12% (19/161)               | 0.9643  |
| Family history, % (n)  | 23% (16/69)             | 6.9% (11/160)              | 0.0004  |
| Not nephrotic syndrome, % (n) | 46% (32/69) | 32% (52/161)               | 0.0421  |
| No oedema, % (n)       | 61% (41/67)             | 38% (58/151)               | 0.0018  |
| No remission, % (n)    | 94% (65/69)             | 81% (128/158)              | 0.0104  |
| FSGS, % (n)            | 63% (35/56)             | 62% (89/144)               | 0.9276  |

Table 6. Comparison of clinical phenotype between patients with and without mutations in the analysed genes in Japanese patients. *Median (interquartile range).
 ware (Agilent Technologies) to analyse chromosomal patterns within the microarray profiles. Agilent CytoGenomics software, in accordance with the manufacturer’s instructions. We used Agilent CytoGenomics software, in accordance with the manufacturer’s instructions.

**Methods**

**Patients.** This study protocol was approved by the Institutional Review Board of Kobe University Graduate School of Medicine (IRB approval number 301). Informed consent was obtained from the patients or their family members in this study. The patients were recruited between January 2016 and December 2018. Inclusion in the study was based on fulfilment of one of the following criteria: (i) diagnosis of CNS, which presents within the first 3 months of life; (ii) diagnosis of INS, which presents between 3–12 months of age; (iii) diagnosis of SRNS, which is defined by persistent proteinuria after 4 weeks of daily treatment with 60 mg/m² prednisone; (iv) diagnosis of FSGS or asymptomatic proteinuria. Asymptomatic proteinuria was defined as the absence of extra-renal symptoms or the presence of proteinuria and microhaematuria. (Supplementary Table S2). Details regarding family history and other clinical features were obtained from the referring clinician or the patient’s hospital records. eGFR was calculated for ages 3 month to 18 years using the creatinine based eGFR formula in Japanese child21,22. For the patients aged <3 month, eGFR was calculated using the original Schwartz formula23 as follows: $k \times \frac{\text{body length (cm)}}{\text{serum Cr level (mg/dL)}}$. For this study, the $k$ values was set as 0.45.

**Genetic analysis.** As we previously reported24, genomic DNA was isolated from peripheral blood leukocytes from patients and their family members using the Quick Gene Mini 80 system (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), in accordance with the manufacturer’s instructions. Targeted sequencing using NGS was conducted for genes that are associated with inherited glomerular diseases (Supplementary Table S3). NGS samples were prepared using a HaloPlex target enrichment system kit (Agilent Technologies, Santa Clara, CA, USA), in accordance with the manufacturer’s instructions. All indexed DNA samples were amplified by polymerase chain reaction and sequenced using the MiSeq platform (Illumina, San Diego, CA, USA). Resulting sequence data were analysed (from alignment to categorisation of mutations) using SureCall software (version 4.0, Agilent Technologies). As we previously reported24, pair analysis by SureCall was used to determine copy number changes in experimental samples relative to a reference sample without a copy number change. We conducted an additional custom array comparative genomic hybridisation when the identified exons (more than two exons) in a single patient exhibited deletions that were all consistent with the clinical presentation of the patient.

**Custom array comparative genomic hybridisation.** As we previously reported25, we conducted custom array comparative genomic hybridisation for one patient. We selected the COQ6 gene and constructed probes for it and the regions surrounding it. We used a custom HD-comparative genomic hybridisation microarray, $8 \times 15$ K (Agilent Technologies), in accordance with the manufacturer’s instructions. We used Agilent CytoGenomics software (Agilent Technologies) to analyse chromosomal patterns within the microarray profiles.

**Confirmation of the pathogenicity.** Candidate variants were considered disease-causing mutations when they met at least one of the following criteria: (1) previous identification as a disease-causing mutation in a published paper; (2) predicted truncation (i.e., nonsense, obligatory splice site, or frameshift mutations); (3) for all novel missense variants, in silico testing with MutationTaster (http://mutationtaster.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/), or SIFT (http://sift.jcvi.org/) indicated pathogenicity. In addition to these three criteria, we confirmed the absence of contradictions between familial segregation and symptoms.

**Statistical analysis.** Results are presented as median and interquartile range (IQR). The chi-squared test or Fisher’s exact test was used to compare variables between two groups. The Mann-Whitney U test was used to compare median differences between two experimental groups. Multivariate logistic regression analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) after controlling for potential confounders. Statistical analysis was performed using standard statistical software (JMP version 10 for Windows, SAS Institute, Cary, NC, USA). In all tests, $p < 0.05$ was considered statistically significant.

**Data availability**

The data are not available for public access because of patient privacy concerns, but are available from the corresponding author on reasonable request.

| 95% CI   | Risk factor       | Odds Ratio | P value |
|----------|-------------------|------------|---------|
| 0.82–0.95 | Age               | 0.89       | <0.0001 |
| 0.62–2.31 | Sex (Male)        | 1.2        | 0.5944  |
| 2.96–26.48| Family History   | 8.85       | <0.0001 |
| 2.03–21.84| No oedema         | 6.67       | 0.0017  |
| 1.39–15.69| No remission      | 4.67       | 0.0128  |
| 0.83–8.83 | Not nephrotic syndrome | 2.71   | 0.0872  |

Table 7. Multivariate logistic regression analysis of risk factors for patients with mutations.

In conclusion, we found pathogenic disease-causing gene mutations in Japanese patients with severe proteinuria. Detection of these mutations in podocyte-related genes will be helpful in treatment and prediction of renal outcome.
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Author contributions C.N. designed the study and wrote the initial draft of the manuscript. Ka.N. contributed to the analysis and interpretation of data, and assisted in the preparation of the manuscript, T.Y., T.H., Y.A., S.I. and N.S. interpreted the data. Y.S., K.O.N., H.N. and K.I. critically reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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