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Systematic Review of Clinical Characteristics and Genotype-Phenotype Correlation in *LAMB2*-Associated Disease

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Introduction: Laminin subunit beta-2 *(LAMB2)*-associated disease, termed Pierson syndrome, presents with congenital nephrotic syndrome, ocular symptoms, and neuromuscular symptoms. In recent years, however, the widespread use of next-generation sequencing (NGS) has helped to discover a variety of phenotypes associated with this disease. Therefore, we conducted this systematic review.

Methods: A literature search of patients with *LAMB2* variants was conducted, and 110 patients were investigated, including 12 of our patients. For genotype-phenotype correlation analyses, the extracted data were investigated for pathogenic variant types, the severity of nephropathy, and extrarenal symptoms. Survival analyses were also performed for the onset age of end-stage kidney disease (ESKD).

Results: Among all patients, 81 (78%) presented with congenital nephrotic syndrome, and 52 (55%) developed ESKD within 12 months. The median age at ESKD onset was 6.0 months. Kidney survival analysis showed that patients with biallelic truncating variants had a significantly earlier progression to ESKD than those with other variants (median age 1.2 months vs. 60.0 months, $P < 0.05$). Although the laminin N-terminal domain is functionally important in laminin proteins, and variants in the laminin N-terminal domain are said to result in a severe kidney phenotype such as earlier onset age and worse prognosis, there were no significant differences in onset age of nephropathy and progression to ESKD between patients with nontruncating variants located in the laminin N-terminal domain and those with variants located outside this domain.

Conclusion: This study revealed a diversity of *LAMB2*-associated diseases, characteristics of *LAMB2* nephropathy, and genotype-phenotype correlations.

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KEYWORDS: congenital nephrotic syndrome; end-stage kidney disease; genotype-phenotype correlation; *LAMB2*; laminin; Pierson syndrome

Pierson syndrome (OMIM# 609049) is a rare inherited disease characterized by ocular symptoms, severe neuromuscular symptoms, and congenital nephrotic syndrome (CNS), which histologically shows diffuse mesangial sclerosis.[1] CNS in patients with Pierson syndrome typically progresses rapidly to ESKD within the first year of life. Ocular and neuromuscular symptoms are diverse; the former involves bilateral microcoria, abnormal lens, and retinal abnormality[2]; and the latter involves muscular hypotonia, psychomotor disability, and blindness.[3]

The disease is caused by variations in the *LAMB2* gene, and the mode of inheritance is autosomal recessive. *LAMB2* is located on chromosome 3p21.31 and is composed of 32 exons. Laminins are proteins that have heterotrimeric structures with 1 α-chain, 1 β-chain, and 1 γ-chain, and are essential for the formation and function of basement membranes.[4] The major laminin isoform of the glomerular basement membrane (GBM) is laminin α5β2γ1 (laminin-521), which is abundantly expressed in the GBM, muscular basement membrane of the neuromuscular junction, lamina propria, ciliary body, and lens. Laminin β2, encoded by *LAMB2*, is composed of a laminin N-terminal (LN), laminin EGF-like, and laminin IV domains, a coiled-coil domain,

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and a β loop.[4] The short arms formed by LN, laminin EGF-like, and laminin IV domains assemble and stabilize basement membranes by interacting with other extracellular matrix proteins. In particular, the LN domains mediate self-polymerization.[5] In contrast, the long arm formed by the C-terminal domain is typically involved in cellular interactions.[4] Laminin β2 has a restricted tissue distribution and is enriched in basement membranes of kidney glomeruli, muscles at neuromuscular junctions, intraocular muscles, lens capsules, and the retina.[1]

In recent years, the widespread use of NGS has helped the discovery of pathogenic variants of *LAMB2* in patients with isolated nephropathy, such as CNS without ocular symptoms and steroid-resistant nephrotic syndrome (SRNS) developing after infancy.[6-8] In 2010, Matejas *et al*.[9] showed a genotype-phenotype correlation using box-and-whisker plots with onset age of proteinuria and ESKD as response variables in their systematic review of 51 previously reported cases. They confirmed that patients with biallelic truncating variants have severely affected phenotypes, whereas those with at least 1 allele missense variant had mild phenotypes. In addition, patients with biallelic missense variants located in the LN domain had relatively severe phenotypes.[9] Although the Kaplan–Meier method is commonly used for studying kidney prognosis, no reports have evaluated the time to ESKD for each genotype or phenotype using the Kaplan–Meier method. Furthermore, it has not been evaluated whether variants in the LN domain are statistically correlated with a severe phenotype. The number of patients with variants in the LN domain has doubled since the 2010 report. Therefore, in this study, we conducted a systematic review of 110 patients with previously reported *LAMB2*-associated disease, including 12 patients in our institution. We also uncovered genotype-phenotype correlations between the kidney survival period and different variants.

METHODS

Systematic Search

A systematic search of patients with *LAMB2* variants (NM_002292.3 and NG_008094.1) was conducted using the Human Gene Mutation Database (accessed February 2023) and ClinVar (accessed April 2023). The flowchart of the literature search is shown in Figure 1. First, we identified 187 cases and then added the 12 patients who had been genetically diagnosed with *LAMB2*-associated disease at our institution between April 2016 and February 2023. Mutational analysis of *LAMB2* was performed in our patients by NGS using a custom disease panel and by conventional direct sequencing using the Sanger method. After excluding reports written in languages other than English or reports whose full texts were not available, we investigated the data of all patients with *LAMB2* variants (such as genotypes, ocular abnormalities, neuromuscular symptoms, age of onset of nephropathy, and age of onset of ESKD) (Supplementary Table S1) from each report manually using PubMed. We then excluded 11 patients with no information about nephropathy, 41 with insufficient data, and 22 with only monoallelic variants in *LAMB2*. Moreover, among our 12 patients, we excluded 2 (No. 10 and 11) whose splice site variants generated some transcripts, including both truncating and nontruncating ones.[10] Finally, we analyzed 108 patients with clinical data.[1,2,7-9,11-41]

Genotypes

Truncating variants included nonsense, frameshift, deletion, insertion, rearrangement, and splice site variants. Nontruncating variants included missense and in-frame variants. Each variant's pathogenicity was interpreted based on the American College of Medical Genetics and Genomics and the American Association of Molecular Pathology 2015 guideline (Supplementary Table S2).[42]

In additional analyses among patients with biallelic nontruncating variants, we compared those both

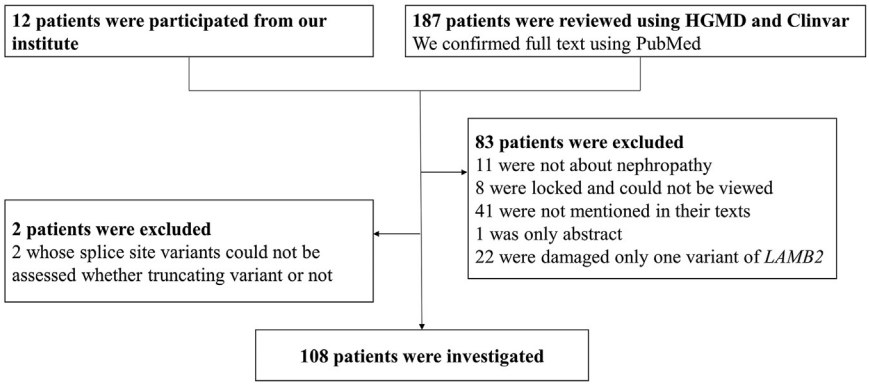

Figure 1. Flowchart of the literature search. HGMD, Human Gene Mutation Database.

Table 1. Clinical characteristics of patients from our institution

Patient No.	Our institution No.	Exon or intron	Variant NM_002292.3 NG_008094.1	Transcript	Observation period (mo)	Age at diagnosis (mo)	Onset of ESKD (mo)	Kidney diagnosis	Treatment	Ocular symptoms	Muscular hypotonia	Psychomotor disability
1.1	Neph4.1	Exon 2 Exon 16	c.225del c.2095G>C	p.Tyr76Thrfs*36 p.Gly699Arg	84	4	-	Proteinuria	RAS-I	-	-	-
1.2	Neph4.2	Exon 2 Exon 16	c.225del c.2095G>C	p.Tyr76Thrfs*36 p.Gly699Arg	123	6	-	SRNS	RAS-I	-	-	-
2	Neph23	Exon 5	c.482T>C Homo	p.Leu161Pro	11 (death)	0	3	CNS	PD	Ny, My	-	DD
3	Neph58	Exon 14 Exon 27	c.1648C>T c.4519C>T	p.Arg550* p.Gln507*	8	0	0.23	CNS	PD	Mc	NA	NA
4	Neph87	Intron 2 Exon 29	c.250-14_-3del c.4904_4905del	abnormal splicing p.Thr1635Argfs*23	120	0.07	6	CNS	KT	RD	NA	NA
5	Neph89	Exon 7	c.821T>C Homo	p.Leu274Pro	30	27	-	SRNS	NA	-	-	Mild DD
6	Neph133	Exon 28 Exon 29	c.4616G>A c.4904_4905del	p.Thr1635Argfs*23[a]	140	0.2	1.5	CNS	KT	Am, RA, RD	-	-
7	Neph267	Exon 28 Exon 29	c.4778C>T c.4904_4905del	p.Ala1593Val p.Thr1635Argfs*23	1	0	0	CNS	PD	Mc	NA	NA
8	Neph520	Exon 20 Exon 30	c.2744del c.5073_5076dup	p.Asp915Alafs*236 p.Gly1693Profs*8	9	0.17	1.23	CNS	PD	Mc	NA	NA
9	Neph524	Exon 27	c.4573C>T Homo	p.Gln1525*	1	0	0	CNS	PD	Mc	NA	PVL
10	A278	Intron 24 Exon 30	c.3797+5G>A c.5073_5076dup	p.Gly1693Profs*8[b]	60	1	-	CNS	RAS-I	-	-	-
11	A813	Intron 24 Exon 30	c.3797+5G>A c.5073_5076dup	p.Gly1693Profs*8[b]	60	38	-	Proteinuria	RAS-I	-	-	-

Am, amblyopia; CNS, congenital nephrotic syndrome; DD, developmental deficit; ESKD, end-stage kidney disease; KT, kidney transplantation; Mc, microcoria; My, myopia; NA, unknown; Ny, nystagmus; PD, peritoneal dialysis; PVL, periventricular leukomalacia; RA, retinal atrophy; RAS-I, renin-angiotensin system inhibitor; RD, retinal detachment; SRNS, steroid-resistant nephrotic syndrome.
[a]The missense variant (c.4616G>A) generated a 44-bp deletion because of aberrant splicing caused by the creation of a novel splice site;
[b]The splicing site variant (c.3797 + 5G>A) generated different size transcripts, including completely normal transcripts, which could be conferred a mild phenotype.

located in the LN domain (residues Ser43 to Asn282; UniProt, Ref NM_002292.3, accessed February 2023) with those both located outside the LN domain because some reports considered the LN domain to be important for the structure of the laminin β2 protein, and even nontruncating variants in the LN domain could lead to a severe phenotype.[5,9]

Phenotypes

The definitions of nephrotic syndrome and proteinuria have been described in each report. The age of onset of nephropathy was defined as the onset of edema or detection of proteinuria. In addition, if a neonate was anuric from birth because of CNS, the age of onset of nephropathy was set to 0 days after birth. Nephrotic syndrome that developed within 3 months was defined as CNS, and nephrotic syndrome that developed after 3 months and ineffective steroid treatment was defined as SRNS. When reports had information on nephrotic-range proteinuria but not on hypoalbuminemia, we defined the cases as proteinuria. The age of onset of ESKD was defined as the age at which the patient was first diagnosed or started on kidney replacement therapy. In this study, we defined severe kidney phenotype as the development of ESKD within 12 months and mild kidney phenotype as the survival of the kidneys for at least 13 months. Neuromuscular symptoms were recorded as muscular hypotonia, psychomotor disability, and blindness according to the classification in the latest review of neuromuscular phenotypes by Wuhl *et al.*[3] In addition to the fact that decreased laminin β2 expression at neuromuscular junction causes muscular hypotonia, these authors also suggest that laminin β2 is involved in the central nervous system and that decreased laminin β2 expression may cause psychomotor disabilities, although the detailed mechanism remains unknown. However, we did not perform semiquantitative assessments of muscular hypotonia and psychomotor disability because most reports did not specify their degrees. We also added blindness to the ocular phenotype because we could not distinguish between neurologic and ocular blindness based on the respective reports.

As for ocular symptoms, microcoria is a well-known symptom of this disorder. However, *LAMB2*-associated disease can cause a variety of symptoms, including amblyopia, anterior segment dysgenesis, lens abnormalities such as cataracts and lenticonus, microphthalmia, myopia, retinal abnormalities such as retinal detachment, and visual impairment, considering the expression sites of laminin β2.[2] When reports had no information on any ocular or neuromuscular symptom, we referred to the data as "unavailable data," and we did not consider that these patients had no symptoms.

Statistical Analysis

The correlation between genotypes and phenotypes was analyzed using Fisher exact test or the Mann–Whitney U test. Survival analyses were performed using the Kaplan–Meier and log-rank tests to investigate any genotype-phenotype correlation between age and ESKD development. The Bonferroni method was used to adjust *P*-values in multiple testing. All statistical analyses were performed using Easy R (EZR), a statistical analysis software,[43] which is a modified version of the R commander designed to add statistical functions frequently used in biostatistics. Associations were considered statistically significant when *P*-values were < 0.05. The survival rate was considered unattained if it had not yet reached 50%.

RESULTS

Genotype and Phenotype

Genetic and phenotypic data were obtained from 108 patients (Tables 1 and 2). The number of patients with biallelic truncating variants was 64 (59%), and that of

Table 2. Clinical characteristics of all patients

Phenotype	Total ($N = 108$)	Biallelic truncating variants ($n = 64$)	All other variants ($n = 44$)	*P* value
Kidney phenotype				
CNS (onset within 3 mo)	81/104 (78%)	56/62 (90%)	25/42 (60%)	< 0.05
SRNS or proteinuria (onset after 3 mo)	23/104 (22%)	6/62 (10%)	17/42 (40%)	
ESKD (total)	66/94 (70%)	47/53 (89%)	19/41 (46%)	< 0.05
ESKD (within 12 mo)	52/94 (55%)	42/55 (79%)	10/41 (24%)	< 0.05
Median age (mo, IQR)				
Onset of nephropathy	1.0 (0.3–3.0)	0.3 (0.1–1.0)	2.0 (0.3–22.5)	< 0.05
Onset of ESKD	2.0 (0.5–12.0)	1.0 (0.2–3.0)	12.0 (4.0–54.0)	< 0.05
Observation period	8.0 (1.3–60.0)	2.6 (1.0–18.3)	18.5 (7.3–87.0)	< 0.05
Extrarenal phenotype				
Ocular symptoms	81/92 (88%)	53/55 (96%)	28/37 (76%)	< 0.05
Neuromuscular symptoms	27/52 (52%)	17/28 (61%)	10/24 (42%)	0.266
Muscular hypotonia	17/52 (33%)	11/28 (39%)	6/24 (25%)	0.376
Psychomotor disability	23/52 (44%)	13/28 (46%)	10/24 (42%)	0.785

CNS, congenital nephrotic syndrome; ESKD, end-stage kidney disease; IQR, interquartile range; SRNS, steroid-resistant nephrotic syndrome.

Table 3. Clinical characteristics of patients with biallelic nontruncating variants located in the LN domain and those with both variants located outside the LN domain

Phenotype	Total ($N = 28$)	In the LN domain ($n = 19$)	Outside the LN domain ($n = 9$)	P value
Kidney phenotype				
CNS (onset within 3 mo)	15/28 (54%)	12/19 (63%)	3/9 (33%)	0.228
SRNS or proteinuria (onset after 3 mo)	13/28 (46%)	7/19 (37%)	6/9 (67%)	
ESKD (total)	9/26 (35%)	4/17 (24%)	5/9 (56%)	0.194
ESKD (within 12 mo)	5/26 (19%)	3/17 (18%)	2/9 (22%)	1.000
Median age (mo, IQR)				
Onset of nephropathy	3.0 (0.3–33.3)	1.0 (0.3–28.5)	18.0 (3.0–120.0)	0.137
Onset of ESKD	12.0 (12.0–60.0)	12.0 (9.8–12.3)	60.0 (12.0–68.4)	0.262
Observation period	33.0 (11.8–93.0)	16.0 (8.0–72.0)	72.0 (42.0–120.0)	0.084
Extrarenal phenotype				
Ocular symptoms	19/22 (86%)	13/16 (81%)	6/6 (100%)	0.532
Neuromuscular symptoms	8/16 (50%)	7/11 (64%)	1/5 (20%)	0.282
Muscular hypotonia	4/16 (25%)	4/11 (36%)	0/5 (0%)	0.245
Psychomotor disability	8/16 (50%)	7/11 (64%)	1/5 (20%)	0.282

CNS, congenital nephrotic syndrome; ESKD, end-stage kidney disease; IQR, interquartile range; LN, laminin N-terminal; SRNS, steroid-resistant nephrotic syndrome.

all patients with other variants (biallelic nontruncating variants or 1 truncating variant with a nontruncating variant) was 44 (41%). Among the patients with biallelic nontruncating variants ($n = 28$), there were 19 (68%) patients who had both variants located in the LN domains (Table 3). When considering variants per family, variances were most commonly found on the Arg246 residue (12/176 variants), including Arg246Trp and Arg246Gln, followed by c.1405 + 1G>A (6/176). The most common domain containing pathologic variants was the LN domain (44/176).

As shown in Table 2, among the 108 patients, the median onset age of nephropathy was 1.0 month; 81 (78%) patients presented with CNS, and 23 (22%) presented with SRNS or proteinuria. The onset age of nephropathy was not described in 4 patients. Patients with biallelic truncating variants had a significantly earlier onset of nephropathy than those with other variants ($P < 0.05$). The median onset age of nephropathy was 0.3 months in patients with biallelic truncating variants and 2.0 months in those with other variants.

Extrarenal features were common; 81 (88%) patients had ocular symptoms, and 27 (52%) had neuromuscular symptoms. Patients with biallelic truncating variants were significantly more likely to have ocular symptoms than those with other variants (96% vs. 76%, respectively; $P < 0.05$). There were no significant differences in the proportions of patients with neuromuscular symptoms between these groups ($P = 0.266$). When the neuromuscular symptoms were divided into subcategories (muscular hypotonia and psychomotor disability), again, no significant differences were found between the groups ($P = 0.376$ and $P = 0.785$, respectively).

Among all patients with described kidney, ocular, and neuromuscular symptoms, 23 (44%) had both CNS and ocular and neuromuscular symptoms, widely known as typical Pierson syndrome. In contrast, 4 patients had only SRNS or proteinuria, 2 patients had only CNS, and for 1 patient, the onset of nephropathy was not described (Supplementary Table S3). Among the 4 patients with only SRNS or proteinuria, 2 patients had a truncating variant with a nontruncating variant, and the other 2 patients had a truncating variant with another variant generating different size transcripts. In these patients, hematuria and proteinuria were detected by chance urinalysis or examination for slowly developing edema. Proteinuria in these patients was responsive to renin-angiotensin system inhibitor (RASI) treatment, and they did not develop ESKD. However, the remaining 3 patients with only CNS or nephropathy developed ESKD.

Age at ESKD Development

After excluding 14 patients whose kidney phenotypes were not sufficiently described, 94 patients, including 66 who developed ESKD, were analyzed for kidney survival using Kaplan–Meier and log-rank tests. The median age at ESKD development was 6.0 months (log-rank test); patients with biallelic truncating variants ($n = 53$) had a significantly earlier progression to ESKD than those with other variants ($n = 41$) (median age 1.2 vs. 60.0 months, $P < 0.05$) (Figure 2). Subanalyses showed that there were no significant differences in the age of onset of ESKD between patients with 1 truncating variant and another nontruncating variant and those with biallelic nontruncating variants (median age 20.0 vs. 72.0 months, $P = 0.252$) (Supplementary Figure S1). In addition, patients with neuromuscular symptoms ($n = 27$) had a significantly earlier progression to ESKD than those without ($n = 24$) (median age 3.0

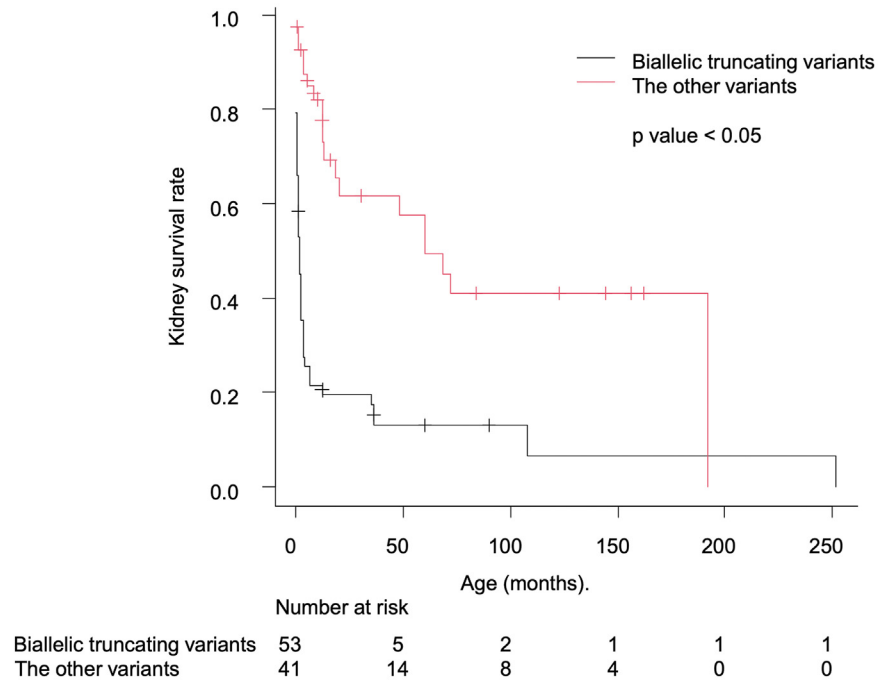

Figure 2. Kidney survival analysis between patients with biallelic truncating variants and those with other variants. The solid black line indicates patients with biallelic truncating variants ($n = 53$); the median age at end-stage kidney disease development is 1.2 months (log-rank test). The solid red line indicates patients with other variants ($n = 41$); the median age of end-stage kidney disease development is 60.0 months. The black line group has a significantly earlier progression to end-stage kidney disease than the red line group ($P < 0.05$).

months vs. 68.4 months, $P < 0.05$) (Figure 3a). However, there were no significant differences in progression to ESKD between patients with ocular symptoms ($n = 74$) and those without ($n = 9$) (median age 3.0 vs. 48.0 months, $P = 0.135$) (Figure 3b).

LN domain

We also analyzed the differences between patients with biallelic nontruncating variants located in the LN domain ($n = 19$) and those located outside the LN domain ($n = 9$) (Table 3). There were no cases with 1

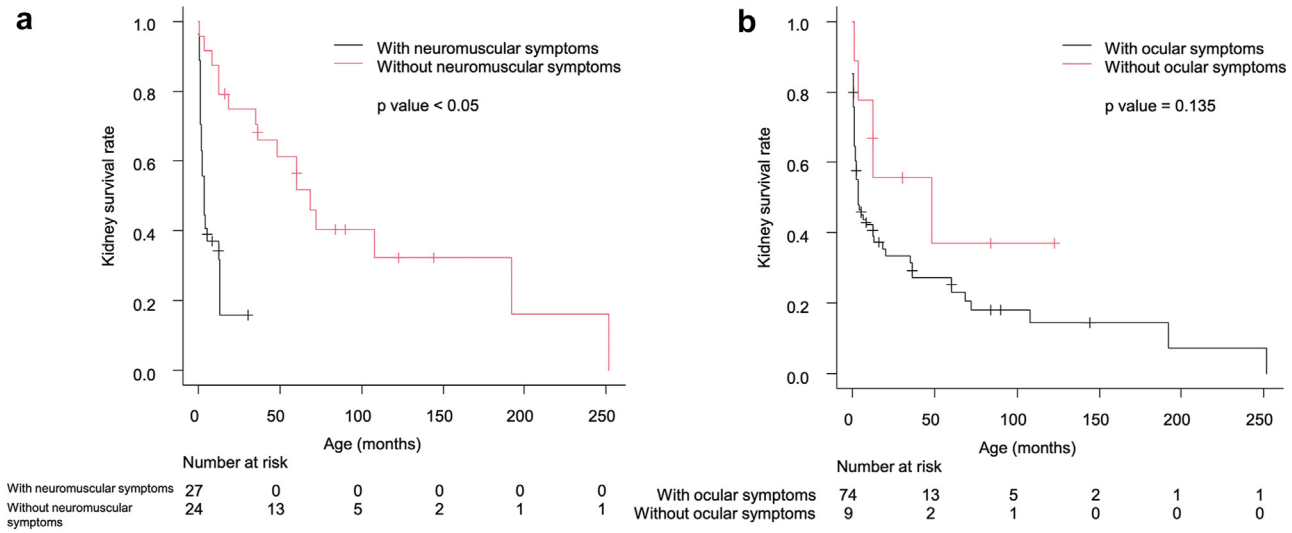

Figure 3. Kidney survival analysis between patients with neuromuscular symptoms and those without neuromuscular symptoms (left), and between patients with ocular symptoms and those without ocular symptoms (right). The left side (a) shows a solid black line indicating patients with neuromuscular symptoms ($n = 27$); the median age at end-stage kidney disease development is 3.0 months (log-rank test). The solid red line indicates patients without neuromuscular symptoms ($n = 24$); the median age of end-stage kidney disease development is 68.4 months. The black line group has a significantly earlier progression to end-stage kidney disease than the red line group ($P < 0.05$). The right side (b) shows a solid black line indicating patients with ocular symptoms ($n = 74$); the median age at end-stage kidney disease development is 3.0 months. The solid red line indicates patients without ocular symptoms ($n = 9$); the median age at end-stage kidney disease development is 48.0 months. The progression to end-stage kidney disease does not significantly differ between the black line and red line groups ($P = 0.135$).

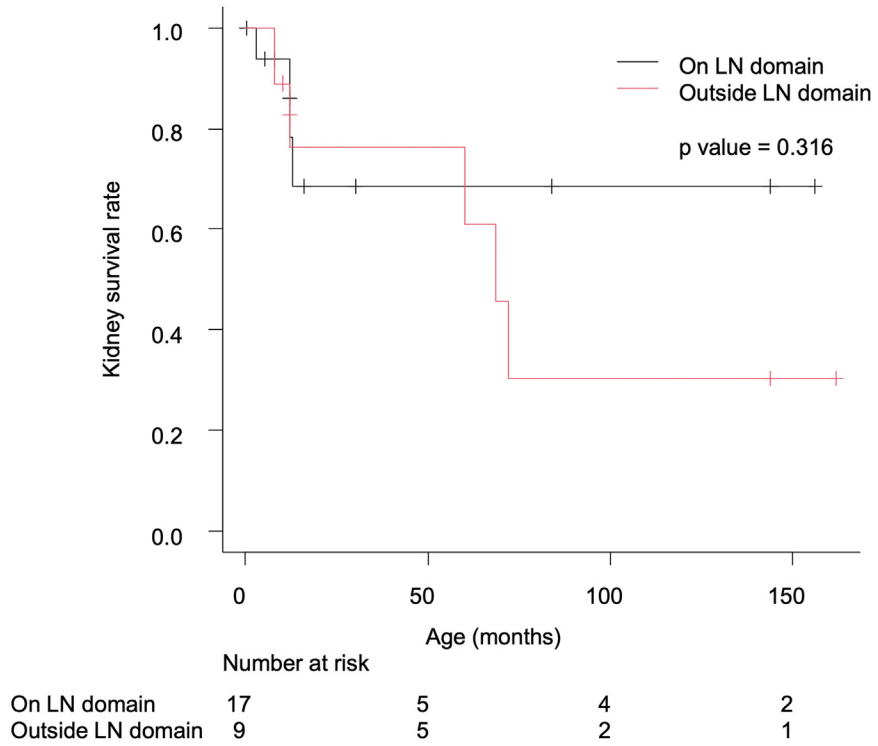

Figure 4. Kidney survival analysis between patients with biallelic nontruncating variants located in the laminin N-terminal domain and those with both variants located outside the laminin N-terminal domain. The solid black line indicates patients with variants both located in the laminin N-terminal domain ($n = 17$); the median age at end-stage kidney disease development is not available because the kidney survival rate does not reach 50%. The solid red line indicates patients with variants located outside the LN domain ($n = 9$); the median age at end-stage kidney disease development is 68.4 months (log-rank test). There are no significant differences in the progression to end-stage kidney disease between the black line group and the red line group ($P = 0.316$).

nontruncating variant in the LN domain and 1 nontruncating variant outside the LN domain. Among them, the median onset age of nephropathy was 3.0 months; 15 (54%) patients presented with CNS, and 13 (46%) patients presented with SRNS or proteinuria. There were no significant differences in the onset age of nephropathy between patients with variants located in the LN domain and those with variants located outside the LN domain ($P = 0.137$) (Table 3). Five (19%) patients progressed to ESKD within 12 months. In the analysis of kidney survival of patients with variants in or outside the LN domain, there were no significant differences in progression to ESKD between patients with variants located in the LN domain and those with variants located outside the LN domain (median age unreached vs. 68.4 months, $P = 0.316$) (Figure 4). With regard to ocular and neuromuscular symptoms, the proportions of patients with variants located in the LN domain and those of patients with variants located outside the LN domain were not significantly different ($P = 0.532$ and $P = 0.282$, respectively).

DISCUSSION

LAMB2-associated diseases typically present with CNS, ocular symptoms and neuromuscular symptoms.[1] In particular, CNS in *LAMB2*-associated disease tends to progress rapidly to ESKD within the first year of life.[9] In this study, we reconfirmed genotype-phenotype correlations for age-developing ESKD with a significantly larger number of patients, and we first showed them using the Kaplan–Meier method. In addition, we obtained negative results regarding the actual clinical impact of the variants in the LN domain.

LAMB2 nephropathy typically presents as CNS and leads to a rapid decline in kidney function. Our study showed that patients with biallelic truncating variants had an earlier onset of nephropathy (median age 0.3 months vs. 2.0 months, $P < 0.05$) (Table 2) and developed ESKD earlier (median age 1.3 months vs. 60.0 months, $P < 0.05$) (Figure 2) than those with other variants. However, a few patients did not follow this genotype-phenotype correlation; some patients with biallelic truncating variants had mild phenotypes (kidneys surviving more than 13 months), and a few patients with other variants had severe phenotypes (ESKD within 12 months) (Figure 5). Using molecular studies, including transcript analysis, Minamikawa *et al.*[10] uncovered a potential reason for 3 of our patients (No. 6, 10, and 11) not following this genotype-phenotype correlation; these patients may carry other missense variants that are known to cause abnormal

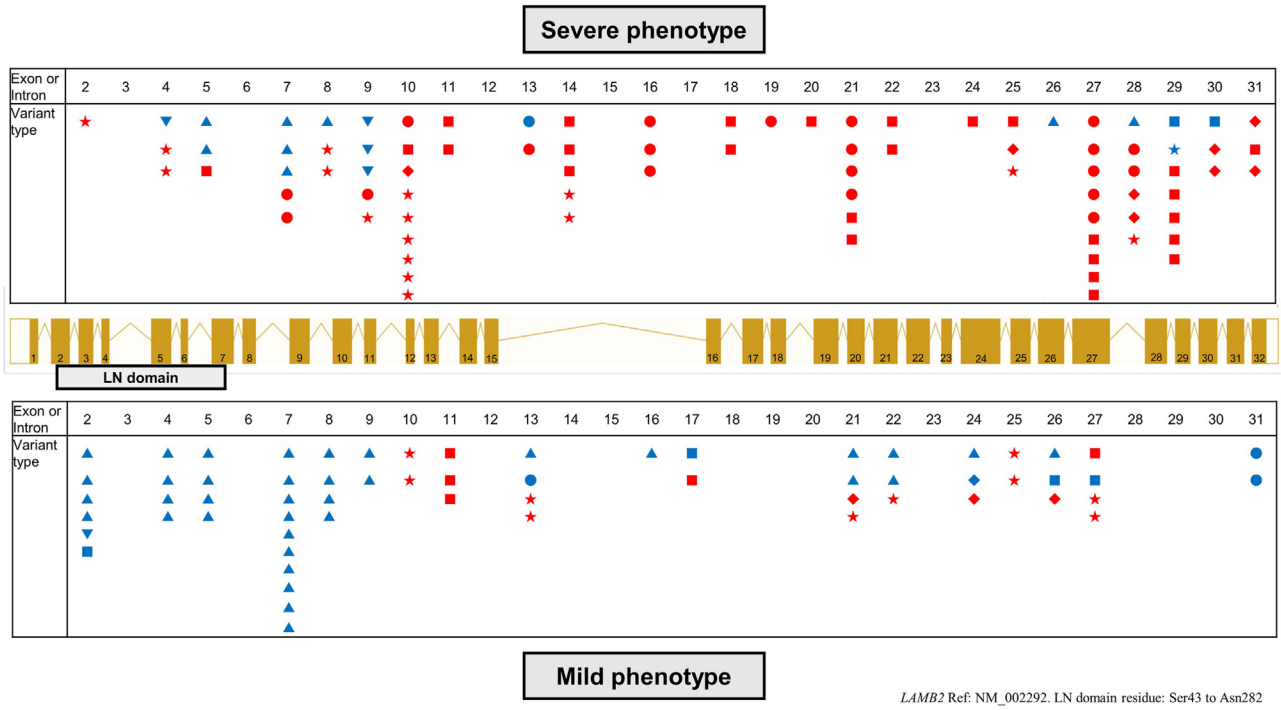

Figure 5. Exon and intron structure of *LAMB2* with geometric shapes indicating relative positions of different types of variants. The horizontal line in the center is the transcript of *LAMB2* (NM_002292.3). Each yellow square represents an exon, and each solid yellow line represents an intron. The gray square shows the position of the laminin N-terminal domain (residue Ser43 to Asn282). The white squares are lined from top to bottom with exons 1, 2, etc. Patients with the severe phenotype (who developed end-stage kidney disease within 12 months) are shown in the upper part, and those with the mild phenotype (whose kidneys survived at least 13 months) in the lower part. Each geometric shape represents a variant type. ▲ indicates missense variants, ▼ indicates in-frame variants because of deletion or insertion, ● indicates nonsense variants, ■ indicates frameshift variants because of deletion, ◆ indicates frameshift variants because of insertion, and ★ indicates splice site variants. Red-colored shapes indicate the variants of patients with biallelic truncating variants, and blue-colored shapes indicate the variants of patients with other types of variants. Most patients follow genotype-phenotype correlations, but a few patients have mild phenotypes regardless of the presence of biallelic truncating variants.

splicing. However, these missense variants have not yet been studied in detail.

Many patients with *LAMB2* variants presented a typical Pierson syndrome phenotype. However, *LAMB2* variants were detected with NGS in 1% to 9% of several SRNS and proteinuria cohorts.[6,32,34,37] In a few reports, some patients had isolated SRNS or proteinuria (i.e., without ocular or neuromuscular symptoms). These patients did not develop ESKD, and proteinuria responded to RAS-I treatment (Supplementary Table S3). Because such patients exist, we should consider the possible involvement of *LAMB2* variants not only in patients presenting with CNS or Pierson syndrome but also in those with isolated SRNS or proteinuria. In addition, considering the high prevalence of ocular symptoms in *LAMB2*-associated disease, these patients should be referred to an ophthalmologist. In the present study, patients with biallelic truncating variants had a higher proportion of ocular symptoms than those with biallelic other variants (96% vs. 76%, $P < 0.05$) (Table 2), whereas no significant difference in the proportion of neuromuscular symptoms was found between these groups (61% vs. 42%, $P = 0.266$) (Table 2). Unfortunately, information on neuromuscular symptoms was unavailable for about half of the patients, and reports that included such information did not provide enough data to perform semiquantitative or quantitative assessments to determine developmental quotient or manual muscle testing levels. Therefore, biallelic truncating variants can predict the presence of ocular symptoms but not the presence or absence of neuromuscular symptoms.

As previously reported,[5,9] nontruncating variants clearly cluster in the LN domain. The LN domain has an important function in the polymerization of laminin, which forms the mesh structure of the GBM.[5] A variant in the LN domain mutates laminin β2 protein and prevents the polymerization of laminin, resulting in a large-pore GBM that is permeable to macromolecules, such as albumin.[5] Surprisingly, our statistical analysis showed no significant differences in the onset of nephropathy and progression to ESKD between patients with biallelic nontruncating variants located in the LN domain and those with variants located outside the LN domain (Table 3 and Figure 4). Similar nonsignificant results were obtained for the proportions of patients with ocular

or neuromuscular symptoms (Table 3). Although the LN domain is an important component of laminin β2 function, dysfunction of the LN domain does not always lead to a severe phenotype. The reason why variants in the LN domain do not cause severe phenotypes remains unclear.

No curative therapies have been developed yet for GBM-associated diseases. However, in patients with CNS, RAS-I can be somewhat effective in reducing proteinuria.[44,45] RAS-I treatment can prolong kidney survival in patients with Alport syndrome, a disease of the same GBM.[46] Although there are no similar trials on the prognosis of *LAMB2*-associated disease, RAS-I may be effective, considering that our patients (No. 1.1, 1.2, 10, and 11) also had a reduction in proteinuria levels and maintained kidney function with RAS-I therapy. Alternatively, kidney transplantation is indicated for patients with hereditary kidney disease, such as hereditary CNS,[45] and also *LAMB2*-associated disease.[47] Two of our patients (No. 4 and 6) received kidney transplantation at the age of 3 years, and their chronic kidney disease stage was maintained at 2 without recurrence of nephrotic syndrome. Regular follow-ups are needed because a report suggests that muscular hypotonia may progress[3] and secondary glaucoma may develop because of lens abnormality, amblyopia, or sudden retinal detachment.[2]

This study has some limitations. First, this study is a systematic review of previously published data, and so, does not include more recent unpublished cases. Considering that NGS has been used widely in recent years, the cases included in this study mostly involved genetic testing; that is, the patients tended to have typical or severe symptoms. As a result, cases with mild symptoms, for example, those with isolated proteinuria or isolated SRNS, might be underrepresented. Physicians should consider this first limitation that *LAMB2*-associated disease may in fact be milder than the result in this study when discussing the life plans of their patients with *LAMB2*-associated disease. Once genetic testing becomes more widely accessible, *LAMB2*-associated disease should be reevaluated.

Second, a few patients did not show a genotype-phenotype correlation. Minamikawa *et al.*[10] suggested that splicing abnormalities were one of the possible causes; however, because they did not evaluate all their patients, their point is not generalizable. Third, variants in the LN domain yielded negative results in terms of clinical impact. We were unable to provide explanations for this result; however, as stated in the first limitation, an association may be found if the number of mild cases increases because of further caseload growth. Fourth, there was no description of extrarenal symptoms in some patients, and it was unclear whether they had any extrarenal symptoms or whether their extrarenal symptoms had been assessed. For example, if neuromuscular symptoms were changed from "no available data" to "no symptoms," patients with biallelic truncating variants would have had a higher proportion of neuromuscular symptoms than those with other variants. Finally, we were unable to evaluate long-term prognosis, such as the efficacy of RAS-I treatment, beyond that of our patients; therefore, prospective studies, including the effectiveness evaluation of RAS-I treatment and the follow-up of neuromuscular and ocular symptoms, are needed.

Conclusion

This study revealed the diversity of *LAMB2*-associated diseases, characteristics of *LAMB2* nephropathy, and genotype-phenotype correlations. Although many patients have a typical phenotype of Pierson syndrome, there are also patients with CNS without any ocular or neuromuscular symptoms and some with isolated SRNS or proteinuria. Genetic testing is useful for predicting kidney prognosis. Biallelic truncating variants lead to severe phenotypes. Conversely, other variants may lead to milder phenotypes, although a few patients contradict this theory. The LN domain is an important component of GBM function; however, our study showed for the first time that variants in the LN domain do not correlate with a severe phenotype.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

Conceptualization was done by RS and KN. Data curation and formal analysis were done by RS, NS, and KN. The investigation was conducted by NS, YI, HK, YT, EO, AK, SI, SI, CN, TY, TH, and KN. The methodology was by RS, NS, and KN. Supervision was by KN. Writing of the original draft was done by RS and KN. Review and editing were done by all authors. All authors read and approved the final manuscript

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Kidney survival analysis between patients with biallelic truncating variants, those with biallelic nontruncating variants, and those with other variants.

Table S1. List of information about each patient.

Table S2. Interpretation of pathogenicity of the variants.
Table S3. List of detailed information about patients with only nephropathy (without ocular and neuromuscular symptoms).

REFERENCES

1. Zenker M, Aigner T, Wendler O, et al. Human laminin beta2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. *Hum Mol Genet*. 2004;13:2625–2632. https://doi.org/10.1093/hmg/ddh284
2. Bredrup C, Matejas V, Barrow M, et al. Ophthalmological aspects of Pierson syndrome. *Am J Ophthalmol*. 2008;146:602–611. https://doi.org/10.1016/j.ajo.2008.05.039
3. Wuhl E, Kogan J, Zurowska A, et al. Neurodevelopmental deficits in Pierson (microcoria-congenital nephrosis) syndrome. *Am J Med Genet A*. 2007;143:311–319. https://doi.org/10.1002/ajmg.a.31564
4. Aumailley M. The laminin family. *Cell Adh Migr*. 2013;7:48–55. https://doi.org/10.4161/cam.22826
5. Kikkawa Y, Hashimoto T, Takizawa K, et al. Laminin beta2 variants associated with isolated nephropathy that impact matrix regulation. *JCI Insight*. 2021;6:e145908. https://doi.org/10.1172/jci.insight.145908
6. Nagano C, Yamamura T, Horinouchi T, et al. Comprehensive genetic diagnosis of Japanese patients with severe proteinuria. *Sci Rep*. 2020;10:270. https://doi.org/10.1038/s41598-019-57149-5
7. Abid A, Shahid S, Shakoor M, Lanewala AA, Hashmi S, Khaliq S. Screening of the LAMB2, WT1, NPHS1, and NPHS2 Genes in Pediatric Nephrotic Syndrome. *Front Genet*. 2018;9:214. https://doi.org/10.3389/fgene.2018.00214
8. Joshi A, Sinha A, Sharma A, et al. Next-generation sequencing for congenital nephrotic syndrome: a multicenter cross-sectional study from India. *Indian Pediatr*. 2021;58:445–451. https://doi.org/10.1007/s13312-021-2215-5
9. Matejas V, Hinkes B, Alkandari F, et al. Mutations in the human laminin beta2 (LAMB2) gene and the associated phenotypic spectrum. *Hum Mutat*. 2010;31:992–1002. https://doi.org/10.1002/humu.21304
10. Minamikawa S, Miwa S, Inagaki T, et al. Molecular mechanisms determining severity in patients with Pierson syndrome. *J Hum Genet*. 2020;65:355–362. https://doi.org/10.1038/s10038-019-0715-0
11. Hasselbacher K, Wiggins RC, Matejas V, et al. Recessive missense mutations in LAMB2 expand the clinical spectrum of LAMB2-associated disorders. *Kidney Int*. 2006;70:1008–1012. https://doi.org/10.1038/sj.ki.5001679
12. Matejas V, Al-Gazali L, Amirlak I, Zenker M. A syndrome comprising childhood-onset glomerular kidney disease and ocular abnormalities with progressive loss of vision is caused by mutated LAMB2. *Nephrol Dial Transplant*. 2006;21:3283–3286. https://doi.org/10.1093/ndt/gfl463
13. Zurowska A, Zaluska-Lesniewska I, Zenker M. LAMB2 gene mutation as a cause of congenital nephrotic syndrome with distinct eye abnormalities and hypotonia. Article in Polish. *Przegl Lek*. 2006;63(suppl 3):37–39.
14. Choi HJ, Lee BH, Kang JH, et al. Variable phenotype of Pierson syndrome. *Pediatr Nephrol*. 2008;23:995–1000. https://doi.org/10.1007/s00467-008-0748-7
15. Maselli RA, Ng JJ, Anderson JA, et al. Mutations in LAMB2 causing a severe form of synaptic congenital myasthenic syndrome. *J Med Genet*. 2009;46:203–208. https://doi.org/10.1136/jmg.2008.063693
16. Machuca E, Benoit G, Nevo F, et al. Genotype-phenotype correlations in non-Finnish congenital nephrotic syndrome. *J Am Soc Nephrol*. 2010;21:1209–1217. https://doi.org/10.1681/ASN.2009121309
17. Zhao D, Ding J, Wang F, et al. The first Chinese Pierson syndrome with novel mutations in LAMB2. *Nephrol Dial Transplant*. 2010;25:776–778. https://doi.org/10.1093/ndt/gfp563
18. Mohney BG, Pulido JS, Lindor NM, et al. A novel mutation of LAMB2 in a multigenerational Mennonite family reveals a new phenotypic variant of pierson syndrome. *Ophthalmology*. 2011;118:1137–1144. https://doi.org/10.1016/j.ophtha.2010.10.009
19. Aydin B, Ipek MS, Ozaltin F, et al. A novel mutation of laminin beta-2 gene in Pierson syndrome manifested with nephrotic syndrome in the early neonatal period. *Genet Couns*. 2013;24:141–147.
20. Togawa H, Nakanishi K, Mukaiyama H, et al. First Japanese case of Pierson syndrome with mutations in LAMB2. *Pediatr Int*. 2013;55:229–231. https://doi.org/10.1111/j.1442-200X.2012.03629.x
21. Lovric S, Fang H, Vega-Warner V, et al. Rapid detection of monogenic causes of childhood-onset steroid-resistant nephrotic syndrome. *Clin J Am Soc Nephrol*. 2014;9:1109–1116. https://doi.org/10.2215/CJN.09010813
22. Cil O, Besbas N, Duzova A, et al. Genetic abnormalities and prognosis in patients with congenital and infantile nephrotic syndrome. *Pediatr Nephrol*. 2015;30:1279–1287. https://doi.org/10.1007/s00467-015-3058-x
23. Sadowski CE, Lovric S, Ashraf S, et al. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. *J Am Soc Nephrol*. 2015;26:1279–1289. https://doi.org/10.1681/ASN.2014050489
24. Willig LK, Petrikin JE, Smith LD, et al. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. *Lancet Respir Med*. 2015;3:377–387. https://doi.org/10.1016/S2213-2600(15)00139-3
25. Zemrani B, Cachat F, Bonny O, et al. A novel LAMB2 gene mutation associated with a severe phenotype in a neonate with Pierson syndrome. *Eur J Med Res*. 2016;21:19. https://doi.org/10.1186/s40001-016-0215-z
26. Peña-González L, Guerra-García P, Sánchez-Calvín MT, Delgado-Ledesma F, de Alba-Romero C. New genetic mutation associated with Pierson syndrome. *An Pediatr (Barc)*. 2016;85:321–322. https://doi.org/10.1016/j.anpedi.2016.01.025
27. Qiu L, Zhou J. Simultaneous mutations of LAMB2 and NPHP1genes in a Chinese girl with isolated congenital nephrotic syndrome: a case report. *BMC Pediatr*. 2016;16:44. https://doi.org/10.1186/s12887-016-0583-0
28. Falix FA, Bennebroek CA, van der Zwaag B, Lapid-Gortzak R, Florquin S, Oosterveld MJ. A novel mutation of laminin beta2 (LAMB2) in two siblings with renal failure. *Eur J Pediatr*. 2017;176:515–519. https://doi.org/10.1007/s00431-017-2871-6
29. Kino J, Tsukaguchi H, Kimata T, et al. Nephron development and extrarenal features in a child with congenital

nephrotic syndrome caused by null LAMB2 mutations. *BMC Nephrol*. 2017;18:220. https://doi.org/10.1186/s12882-017-0632-4

30. Sen ES, Dean P, Yarram-Smith L, et al. Clinical genetic testing using a custom-designed steroid-resistant nephrotic syndrome gene panel: analysis and recommendations. *J Med Genet*. 2017;54:795–804. https://doi.org/10.1136/jmedgenet-2017-104811
31. Wang F, Zhang Y, Mao J, et al. Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol*. 2017;32:1181–1192. https://doi.org/10.1007/s00467-017-3590-y
32. Warejko JK, Tan W, Daga A, et al. Whole exome sequencing of patients with steroid-resistant nephrotic syndrome. *Clin J Am Soc Nephrol*. 2018;13:53–62. https://doi.org/10.2215/CJN.04120417
33. Arima M, Tsukamoto S, Akiyama R, et al. Ocular findings in a case of Pierson syndrome with a novel mutation in laminin ß2 gene. *J Am Assoc Pediatr Ophthalmol Strabismus*. 2018;22:401–403.e401.
34. Schapiro D, Daga A, Lawson JA, et al. Panel sequencing distinguishes monogenic forms of nephritis from nephrosis in children. *Nephrol Dial Transplant*. 2019;34:474–485. https://doi.org/10.1093/ndt/gfy050
35. Wojcik MH, Schwartz TS, Thiele KE, et al. Infant mortality: the contribution of genetic disorders. *J Perinatol*. 2019;39:1611–1619. https://doi.org/10.1038/s41372-019-0451-5
36. Kulali F, Calkavur S, Basaran C, Serdaroglu E, Kose M, Saka Guvenc M. A new mutation associated with Pierson syndrome. *Arch Argent Pediatr*. 2020;118:e288–e291. https://doi.org/10.5546/aap.2020.eng.e288
37. Park E, Lee C, Kim NKD, et al. Genetic study in Korean pediatric patients with steroid-resistant nephrotic syndrome or focal segmental glomerulosclerosis. *J Clin Med*. 2020;9:2013. https://doi.org/10.3390/jcm9062013
38. Sakuraya K, Nozu K, Murakami H, et al. An extremely mild clinical course in a case with LAMB2-associated nephritis diagnosed with next-generation sequencing. *CEN Case Rep*. 2021;10:359–363. https://doi.org/10.1007/s13730-021-00574-1
39. Zenker M, Pierson M, Jonveaux P, Reis A. Demonstration of two novelLAMB2 mutations in the original Pierson syndrome family reported 42 years ago. *Am J Med Genet A*. 2005;138A:73–74. https://doi.org/10.1002/ajmg.a.30894
40. Mbarek IB, Abroug S, Omezzine A, et al. Novel mutations in steroid-resistant nephrotic syndrome diagnosed in Tunisian children. *Pediatr Nephrol*. 2010;26:241–249. https://doi.org/10.1007/s00467-010-1694-8
41. Kagan M, Cohen AH, Matejas V, Vlangos C, Zenker M. A milder variant of Pierson syndrome. *Pediatr Nephrol*. 2008;23:323–327. https://doi.org/10.1007/s00467-007-0624-x
42. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. https://doi.org/10.1038/gim.2015.30
43. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48:452–458. https://doi.org/10.1038/bmt.2012.244
44. Funk SD, Lin MH, Miner JH. Alport syndrome and Pierson syndrome: diseases of the glomerular basement membrane. *Matrix Biol*. 2018;71-72:250–261. https://doi.org/10.1016/j.matbio.2018.04.008
45. Boyer O, Schaefer F, Haffner D, et al. Management of congenital nephrotic syndrome: consensus recommendations of the ERKNet-ESPN Working Group. *Nat Rev Nephrol*. 2021;17:277–289. https://doi.org/10.1038/s41581-020-00384-1
46. Nozu K, Nakanishi K, Abe Y, et al. A review of clinical characteristics and genetic backgrounds in Alport syndrome. *Clin Exp Nephrol*. 2019;23:158–168. https://doi.org/10.1007/s10157-018-1629-4
47. Guler S, Cimen S, Acott P, Whelan K, Molinari M. Kidney transplantation in a child with Pierson syndrome. *Pediatr Transplant*. 2017;21:10.1111/petr.13076. https://doi.org/10.1111/petr.13076