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【 CASE REPORT 】

A Novel Mutation in *LMX1B* (p.Pro219Ala) Causes Focal Segmental Glomerulosclerosis with Alport Syndrome-like Phenotype

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Abstract:
A 69-year-old woman presented with mild renal dysfunction, proteinuria, and sensorineural hearing loss. A renal biopsy showed focal segmental glomerulosclerosis with thinning of the glomerular basement membrane. There was a positive family history of end-stage kidney disease and hearing loss. Although Alport syndrome was suspected from these features, a genetic test using next-generation sequencer identified a novel missense mutation in *LMX1B*, c.655C>G: p.(Pro219Ala). *In silico* analyses predicted the pathogenicity of the mutation. Thus, the present case was diagnosed as LMX1B-associated nephropathy presenting with Alport syndrome-like phenotype, expanding the disease spectrum of LMX1B nephropathy.

Key words: LMX1B, nail-patella syndrome, Alport syndrome, hereditary nephropathy

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Introduction

Mutations in the *LMX1B* gene cause nail-patella syndrome (NPS), an autosomal-dominant disease characterized by dystrophic nails, hypoplastic patella, and iliac horns (1). LIM homeodomain transcription factor 1-β (LMX1B), encoded by *LMX1B*, contains two LIM-domains (LIM-A and LIM-B), which are involved in protein-protein interactions, and a homeodomain, which interacts with specific DNA elements in target genes (2, 3). LMX1B regulates the expression of glomerular basement membrane (GBM) proteins, such as type III and IV collagen, podocin, and CD2AP. Thus, dysfunctional LMX1B causes glomerular disease (4, 5). The prevalence of nephropathy is 10-40% in patients with NPS (6, 7). The classic findings of nephropathy associated with NPS include progressive renal dysfunction accompanied by urinary abnormalities and focal segmental glomerulosclerosis (FSGS) in light microscopy. Electron microscopy typically demonstrates focal or diffuse irregular thickening of the GBM with electron-lucent areas. Some patients with NPS progress to end-stage kidney disease (ESKD) (8).

Recently, a case of LMX1B Arg246Gln without extrarenal manifestations was described (9). Such a limited renal condition caused by LMX1B mutation is recognized as "LMX1B-associated nephropathy" (9, 10). The development of next-generation sequencing has facilitated the accumulation of novel genetic mutations of LMX1B-associated nephropathy, and families carrying Arg246Gln, Arg246Pro, Arg249Gln, and Ala278Val mutations have been identified (11-16).

Alport syndrome (AS) is a progressive glomerular disease that is characterized by sensorineural hearing loss and ocular abnormalities due to mutations in the *COL4A3, COL4A4*, or

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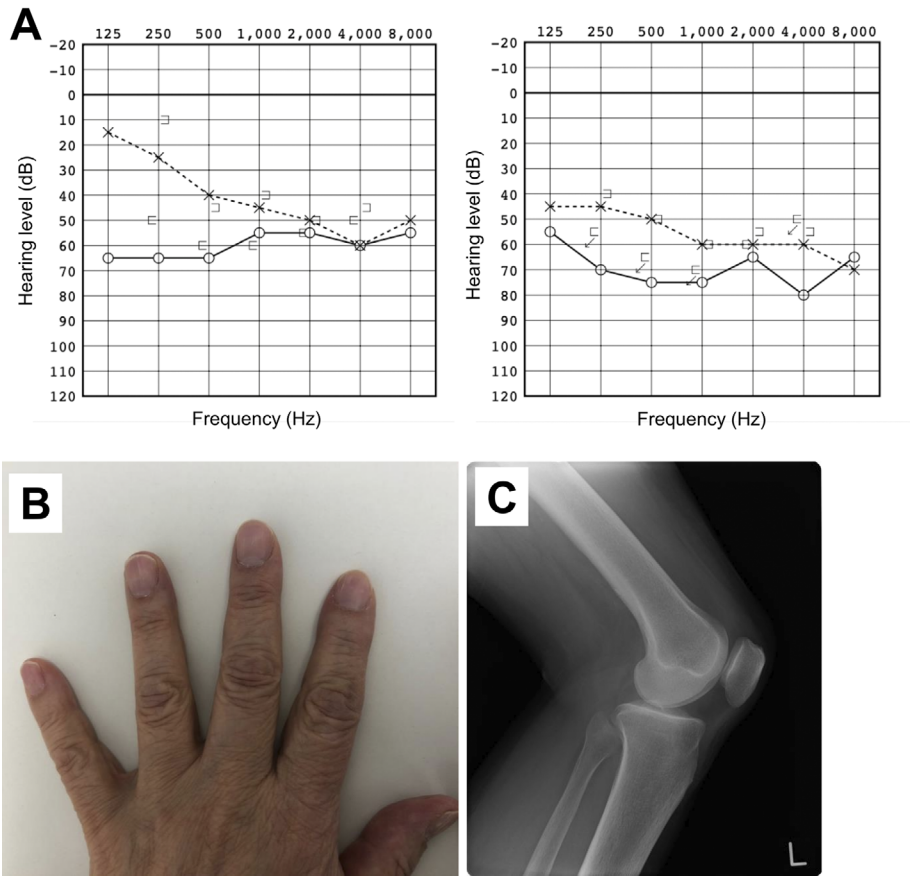

Figure 1. A pure tone audiogram and the findings of the patient's fingers and patellae. A: Pure tone audiograms at 59 years old (left panel) and 69 years old (right panel) suggest progressive sensorineural hearing loss over the 10-year period. B: A photograph of normal fingernails. C: An X-ray image of the patient's left knee.

COL4A5 genes (17). X-linked AS caused by a *COL4A5* mutation is the most frequent, being reported in 80% of all AS. Men are particularly severely affected, and 90% of male patients develop ESKD by 40 years old. Hearing loss is also frequent, affecting 90% of male patients. In contrast, the progression of the disease is slow in women (17-19).

The manifestations of LMX1B-associated nephropathy and AS can overlap because they share abnormalities in type IV collagen. We herein report a case of LMX1B-associated nephropathy harboring a novel missense mutation in the homeodomain of the *LMX1B* gene presenting with AS-like phenotype.

Case Report

A 69-year-old woman with a 10-year history of hypertension had been treated with amlodipine and enalapril. The patient was found to have a urinary abnormality in her teens and diagnosed with progressive sensorineural hearing loss 10 years before her current presentation (Fig. 1A). The patient was referred to our hospital because of a mild elevation of her serum creatinine level to 1.1 mg/dL, with urinary abnormalities (protein+ and blood+/− in urine dipstick). On admission, the patient had a blood pressure of 161/92 mmHg, a body height of 157.2 cm, and a weight of 57 kg. There were no physical abnormalities, including her nails and patellae (Fig. 1B, C). A urinalysis showed a proteinuria level of 4.74 g/g Cre and <4 red blood cells/high-power field. Laboratory tests showed an elevated serum creatinine level of 1.05 mg/dL (eGFR 40 mL/min/1.73 m^2) and blood urea nitrogen (BUN) of 18 mg/dL. Other laboratory findings are shown in Table 1.

A percutaneous renal biopsy was performed. Ten glomeruli were examined microscopically, and four showed global sclerosis. Two glomeruli exhibited segmental glomerulosclerosis; the other glomeruli showed no proliferative lesions (Fig. 2A, B). Tubular atrophy and interstitial fibrotic lesions were observed in 40% of the renal cortex. The immunohistochemistry findings for IgG, IgA, and fibrinogen were all negative in the glomeruli. IgM, C1q, C3c, and C3d were weakly positive in the areas of segmental sclerosis. A diagnosis of FSGS was made based on the light microscopy appearance. Electron microscopy revealed diffuse thinning of the GBM and widening of the subendothelial spaces (Fig. 2C, D).

Regarding her family history, the patient's mother had undergone hemodialysis due to ESKD in her 60s. Of her 4 brothers, a younger brother developed ESKD in his 20s and

Table 1. Laboratory Findings of the Present Case.

Urinalysis			**Biochemistry**		
Dipstick			Blood urea nitrogen	18	mg/dL (8-20)
Protein	2+		Creatinine	1.05	mg/dL (0.46-0.79)
Blood	+/-		eGFR	40	mL/min/1.73 m^2
Protein	4.74	g/g Cre	AST	14	U/L (13-30)
Red blood cell	<4	/high-power field	ALT	11	U/L (7-23)
NAG	27.2	IU/g Cre (0-5.6)	Uric acid	9.7	mg/dL (2.6-5.5)
			Total protein	6.6	g/dL (6.6-8.1)
Blood counts			Albumin	4.0	g/dL (3.7-5.4)
White blood cell	4.4×10^3	/μL	Total cholesterol	190	mg/dL (0-219)
Red blood cell	4.65×10^6	/μL	Triglycerides	124	mg/dL (0-149)
Hemoglobin	13.3	g/dL	C-reactive protein	0.02	mg/dL (0-0.14)
Hematocrit	39.7	%	IgG	989	mg/dL (861-1747)
Platelet	209×10^3	/μL	IgA	141	mg/dL (93-393)
			IgM	79	mg/dL (50-269)
			C3	106	mg/dL (73-138)
			C4	29.6	mg/dL (11-31)
			CH50	60.4	U/mL (31.6-57.6)
			Anti-nuclear antibody	40×	
			Anti-ds DNA antibody	Negative	
			MPO-ANCA	Negative	
			PR3- ANCA	Negative	

Parenthesis indicates the reference values. NAG: N-acetyl-β-D-glucosaminidase, eGFR: estimated glomerular filtration rate, AST: aspartate transaminase, ALT: alanine transaminase, CH50: total complement activity, Anti-ds: Anti-double-stranded, MPO-ANCA: myeloperoxidase anti-neutrophil cytoplasmic antibody, PR3-ANCA: protease-3 anti-neutrophil cytoplasmic antibody

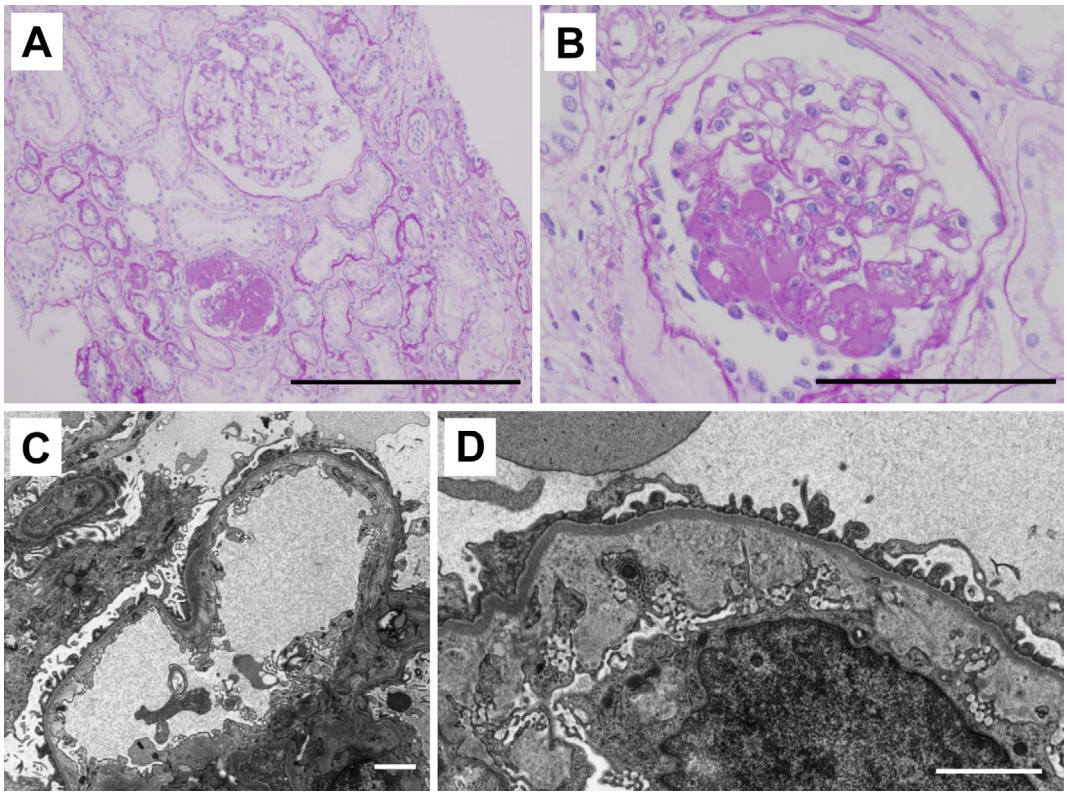

Figure 2. Renal biopsy findings of the case. A, B: Representative photomicrographs of periodic acid-Schiff stain showing an FSGS lesion. Scale bar=500 and 100 μm in panels A and B, respectively. C, D: A thin glomerular basement membrane was observed by electron microscopy. Scale bar=2 μm. FSGS: focal segmental glomerulosclerosis

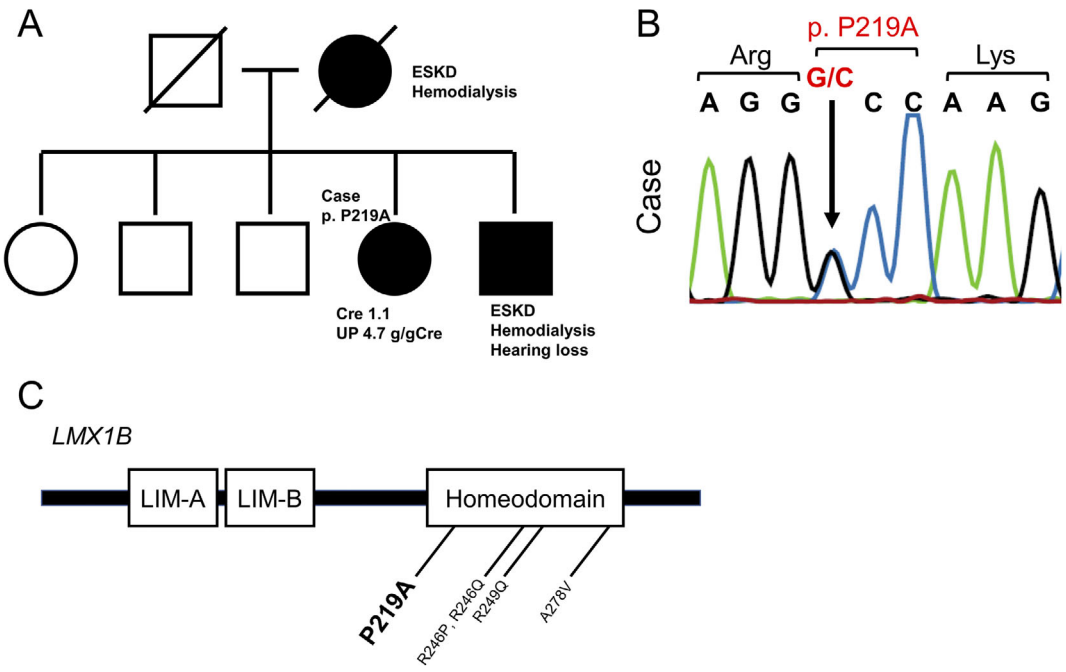

Figure 3. **The genetic analysis and family pedigree in this case. A: The family pedigree. B: The genetic analysis identified a heterozygous mutation of *LMX1B*, c.C655 G (p.Pro219Ala). C: The location of the mutation in the *LMX1B* transcript. Reported mutations of LMX1B-associated nephropathy are also shown. ESKD: end-stage kidney disease, Cre: creatinine, UP: urinary protein, gCre: g creatinine**

was currently on hemodialysis. Furthermore, he had a history of sensorineural hearing loss. Because X-linked AS was suspected based on the patient's family history (Fig. 3A), we screened genomic DNA isolated from the patient's peripheral blood for 166 major inherited kidney disease genes using a next-generation sequencer system (SPEEDIKID) (20). The results of this analysis revealed a novel heterozygous missense mutation in *LMX1B* (NM_002316, c.655C>G), which encodes a proline-to-alanine substitution (p.Pro219Ala), although no mutations were detected in *COL4A5/4A4/4A3*. The mutation was confirmed by Sanger sequencing (Fig. 3B). The minor allele frequency (MAF) of this mutation is 0.0007 in a reference panel of Japanese genomic variations (8.3KJPN) and that in gnomAD (v2.1.1) is 7.3×10^{-6}. The variant is registered in neither the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php) nor ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). *In silico* prediction scores were consistent with pathogenic variants (Table 2), although the clinical interpretation of the genetic variants according to the ACMG/AMP 2015 guideline was "Uncertain significance" (Table 3).

Discussion

We encountered a case of FSGS with hearing loss and a family history of ESKD. X-linked AS was suspected based on her clinical and histological features. However, we identified the novel *LMX1B* mutation c.655C>G (p.Pro219Ala), which was predicted to be pathogenic based on *in silico* analyses. Furthermore, the patient did not have dysplastic nails or hypoplastic patellae. Thus, the patient was diagnosed with LMX1B-associated nephropathy.

The c.655C>G (p.Pro219Ala) mutation is located in the homeodomain of the *LMX1B* gene, with which nephropathy is closely associated. In NPS, missense mutations are clustered in the LIM-A and LIM-B domains (exons 2 and 3, respectively) and the homeodomain encoded by exons 4-6. Interestingly, patients with LMX1B mutations in the homeodomain have more proteinuria than people carrying mutations in the LIM domain (6). In LMX1B-associated nephropathy, all of the reported mutations in the *LMX1B* gene are located in the homeodomains, including cases of hereditary glomerulopathy with an Arg246Gln mutation, hereditary minimal change disease with an Arg246Pro mutation, a large family affected by proteinuria and ESKD with Arg249Gln, and steroid-resistant nephrotic syndrome with a heterozygous Ala278Val mutation (11-16).

The light microscopy observations of nephropathy caused by LMX1B mutation show unremarkable glomerular changes, and most cases are diagnosed as FSGS. On electron microscopy, focal and diffuse irregular thickening of the GBM with electron-lucent areas and irregular depositions of type III collagen fibrils are observed in NPS (8). However, the histological appearances are diverse. For example, two unique families with LMX1B mutations (c.737G>A, p.Arg246Gln) exhibited myelin figures and zebra bodies in their renal biopsy findings, presenting with Fabry disease (16). The current case showed diffuse thinning of the GBM, a finding commonly observed in AS or cases of thin basement membrane nephropathy (21), thus expanding the pathologi-

Table 2. Allele Frequency and Missense Predictions for the Novel Mutation in *LMX1B*.

Score Name	Values	Prediction	Reference
MAF database			
8.3KJPN	0.0007		https://jmorp.megabank.tohoku.ac.jp/202008/
gnomAD (v2.1.1)	7.30E-06		https://gnomad.broadinstitute.org/
Pathogenicity scores			
SIFT_score*1	**0.008**	**Deleterious**	https://sift.bii.a-star.edu.sg/
Polyphen2_HDIV_score*2	**0.989**	**Probably damaging**	http://genetics.bwh.harvard.edu/pph2/
Polyphen2_HVAR_score*3	**0.912**	**Probably damaging**	http://genetics.bwh.harvard.edu/pph2/
MutationTaster_score*4	**1**	**Disease_causing**	http://www.mutationtaster.org/
MetaLR_score*5	**0.887**	**Deleterious**	https://sites.google.com/site/jpopgen/dbNSFP
CADD_phred*6	**26.3**	**-**	https://cadd.gs.washington.edu/
MCAP*7	**0.3905476**	**Deleterious**	http://bejerano.stanford.edu/mcap/
GERP++_RS*8	**4.97**	**-**	http://mendel.stanford.edu/SidowLab/downloads/gerp/
REVEL*9	**0.568**	**-**	https://sites.google.com/site/revelgenomics/

*1 0.0 to 0.05 for deleterious variants. 0.05 to 1.0 for tolerated variants (benign).

*2 Probably damaging (≥0.957), Possibly damaging (0.453≤ ≤0.956), Benign (≤0.452).

*3 Probably damaging (≥0.909), Possibly damaging (0.447≤ ≤0.908), Benign (≤0.446).

*4 The score ranges 0 to 1. Prediction for disease causing is more than 0.5.

*5 The score ranges 0 to 1. The cut-off value between "Tolerated" and "Deleterious" is 0.5.

*6 Higher values are more deleterious.

*7 Scores above 0.025 are considered as "Deleterious" [Jagadeesh, et al. (23)]

*8 The score ranges from a minimum of -12.3 to a maximum of 6.17. Higher values indicate a conserved nucleotide position.

*9 The score ranges from 0 to 1. Higher values are more deleterious.

Table 3. Clinical Interpretation: Uncertain Significance.

PM1 (Moderate)	Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation; Homeodomain
PM2 (Moderate)	Absent from controls (or at an extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
PP3 (Supporting)	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)

cal spectrum of LMX1B-associated nephropathy.

The patient and her brother had a history of sensory hearing loss. Hearing impairment is common and observed in 45.8% of patients with NPS (6). As in AS, LMX1B mutations are associated with abnormality in type IV collagen, which can cause sensory hearing loss.

The patient's younger brother developed ESKD at a relatively young age and was treated with hemodialysis, whereas her mother developed ESKD at an older age, and our patient showed only mild renal dysfunction, suggesting that the men in the patient's family may have been affected more severely than the women. These clinical characteristics are similar to those of X-linked AS (17). However, there were no mutations in the *COL4A5/4A4/4A3* genes on sequencing.

The disease severity of nephropathy caused by LMX1B nephropathy is diverse. For example, in a pair of identical twins with NPS, one developed renal failure, whereas the other showed only proteinuria (8). Furthermore, the renal phenotypes in LMX1B-associated nephropathy differ among distinct generations of a family carrying a c.737G>A mutation (16). Unknown environmental or genetic factors may contribute to the genotype-phenotype correlations in LMX1 B nephropathy. Interestingly, a potential modifier gene variant *PAX2* was recently reported in an LMX1B variant in a patient with NPS with kidney failure, congenital renal hypodysplasia, and vesicoureteral reflux (22). The reason for the differing disease phenotype in the present family is still unclear and requires further examinations.

Identifying this mutation in the present patient's younger brother will help strengthen the causal link of genotype-phenotype correlation. However, one limitation of our study is that we were unable to obtain DNA samples from the brother.

In conclusion, we encountered a case of FSGS caused by a novel *LMX1B* mutation, c.655C>G (p.Pro219Ala) presenting as AS. The findings in this case expand the diversity of LMX1B-associated nephropathy.

Informed consent was obtained from this patient. This study was approved by the institutional Review Board of Tohoku University (2020-1-271).

The authors state that they have no Conflict of Interest (COI).

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