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Experimental Nephrology and Genetics: Case Study of Genetic Interest

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Whole-Exome Sequencing Solved over 2-Decade Kidney Disease Enigma

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LMX1B-associated nephropathy · Genetic focal segmental glomerulosclerosis · Whole-exome sequencing

Abstract
Chronic kidney disease of unknown etiology (CKDu) has been a problem in renal practice as indefinite diagnosis may lead to inappropriate management. Here, we report a 54-year-old father diagnosed with CKDu at 33 years old and his 8-year-old son with steroid-resistant nephrotic syndrome. Using whole-exome sequencing, both were found to be heterozygous for c.737G>A (p.Arg246Gln) in *LMX1B*. The diagnosis of *LMX1B*-associated nephropathy has led to changes in the treatment plan with appropriate genetic counseling. The previously reported cases with this particular mutation were also reviewed. Most children with *LMX1B*-associated nephropathy had nonnephrotic proteinuria with normal renal function. Interestingly, our pediatric case presented with steroid-resistant nephrotic syndrome at 8 years old and progressed to ESRD requiring peritoneal dialysis at the age of 15 years. Our report emphasized the need of genetic testing in CKDu for definite diagnosis leading to precise management.

Introduction

Chronic kidney disease of unknown etiology (CKDu), accounting for approximately 10% of CKD patients [1], remains a clinical challenge for nephrologists. Knowing the cause of CKD is crucial as precise management could be delivered to improve patient outcomes. CKDu patients also face problems when undergoing kidney transplantation. Nephrologists usually encounter diagnostic problems when kidney diseases occur in transplanted kidneys of CKDu patients. Some kidney diseases associated with pathologic findings of FSGS have very high recurrence risk in a transplanted kidney. On the other hand, genetic

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kidney diseases have low recurrence rates after kidney transplantation [2, 3]. Differences in treatment of patients with recurrent diseases in transplanted kidneys compared to those with de novo renal diseases in transplanted kidneys are also noted. Therefore, knowing the causes of CKD is very important considering the entire care pathway of kidney disease. With the advent of high-throughput next-generation sequencing technologies, recent studies showed that about 10% of CKDu were caused by single-gene mutations [4]. Several genes are responsible for kidney diseases, many of which are renal-limited disorders [5]. Applying next-generation sequencing in CKDu will significantly guide and change the management of CKD patients. Our cases demonstrated the benefits of whole-exome sequencing (WES) in CKDu patients. We also extensively reviewed the previously reported cases with the c.737G>A (p.Arg246Gln) mutation in *LMX1B* and emphasized the clinicopathological variability of this particular kidney disease.

Case Report/Case Presentation

In 1999, patient J (II-2) (Fig. 1a), a 33-year-old man who presented to our renal clinic with dyspnea and orthopnea, was diagnosed with CKD stage 5 and pulmonary edema. He received treatment to delay CKD progression without kidney biopsy as imaging showed bilateral small kidney size. Eventually, 8 months after presented to the clinic, he received the initiation of hemodialysis and continued for 3 years before undergoing a deceased donor kidney transplantation. Six months after transplant, he developed nephrotic range proteinuria. The allograft biopsy revealed thickening of glomerular capillary loops compatible with membranous nephropathy. He had been put on angiotensin converting enzyme inhibitors. Currently, he is 54 years of age and has a stable renal function with a proteinuria of 0.6 g per day. His latest serum creatinine level was 1.7 mg/dL, and 24-h urine creatinine clearance was 55 mL/min.

In 2007, a son of patient J (III-2) (Fig. 1a), an 8-year-old boy, was diagnosed with nephrotic syndrome. He had been treated with high-dose corticosteroid for 3 months but did not respond. The kidney biopsy revealed FSGS pattern without cellular proliferation (Fig. 1b). Immunofluorescence staining was negative for IgG, IgM, and IgA. Electron microscopy (EM) showed extensive effacement of the podocyte foot process without electron-dense deposition. He was diagnosed with steroid-resistant primary FSGS and was switched to cyclosporine for 2 years. The proteinuria remained in nephrotic range, and repeated kidney biopsy showed progression of FSGS. His renal function gradually declined, and peritoneal dialysis was initiated when he was 15 years old. Currently, he is 21 years of age and on the waiting list for cadaveric kidney transplantation.

We identified the renal disease in 3 generations in this family (Fig. 1a). Patient I-2 died at the age of 30 years with unidentified kidney disease as comorbidity. Patient II-1 had ESRD at the age of 49 years and subsequently received a kidney transplant. However, he died from a serious infection 7 months after the transplantation.

We investigated the possible genetic defect in this family by WES of patient J (II-2), his wife (II-3), and his affected son (III-2). The analysis revealed that patient J (II-2) and his affected son (III-2) were heterozygous for a previously reported pathogenic missense mutation, c.737G>A (p.Arg246Gln), in the *LMX1B* gene (NM_002316.3). PCR-Sanger sequencing confirmed the presence of this mutation in both patients (Fig. 1c). Notably, II-2 also harbored a synonymous variant, c.726G>C, resulting in the same amino acid (serine) but did not pass it to his son. Re-evaluation for extrarenal manifestations of both patients (II-2 and III-2) revealed normal nails and normal radiographic studies of the elbows, knees, and pelvis.

The WES solved a 21-year enigma of patient J's and his family's kidney disease. He had *LMX1B*-associated nephropathy presenting to us with CKD stage 5. Interestingly, his son, who also harbored the same mutation, presented with steroid-resistant nephrotic syndrome with pathologic findings of FSGS pattern at early age. As genetic FSGS had a very low recurrence rate in transplanted kidney and primary FSGS had about 30% rate of recurrence after kidney transplantation [2, 3], patient J had post-transplant de novo membranous nephropathy in the transplanted kidney together with LMX1B-associated nephropathy in the native kidney.

Discussion

We presented a family with 2 cases of *LMX1B*-associated nephropathy. Both had the same missense mutation, c.737G>A (p.Arg246Gln), in *LMX1B*. Nonetheless, their clinical manifestations were very different, one presenting at 33 years of age and progressing to ESRD in 8 months, while the other presenting at 8 years old and taking >6 years to ESRD. Noted that patient J, the proband, was asymptomatic at the age of 15, while his son already underwent dialysis at this age. *LMX1B* is a gene known to cause nail-patella syndrome (NPS), an autosomal dominant disorder, characterized by nail dysplasia, absent or hypoplastic patellae, elbow abnormality, and iliac horns. Only 20–30% of NPS patients have renal involvement, a major prognostic factor in patients with NPS [6]. NPS patients can develop steroid-resistant nephrotic syndrome with glomerular pattern injury of FSGS [7]. Interestingly, some patients with causative mutations in *LMX1B* may not have extrarenal manifestations as observed in our patients. Genetic testing is therefore required for definite diagnosis that will lead to the appropriate management and genetic counseling. Treatment without genetic testing may lead to misdiagnosis and unnecessary immunosuppressive therapy as patient III-2.

To date, there have been 31 cases of *LMX1B*-associated nephropathy with the p.Arg246Gln in 20 different families, including ours (Table 1). Most (24/31 = 77.4%) are

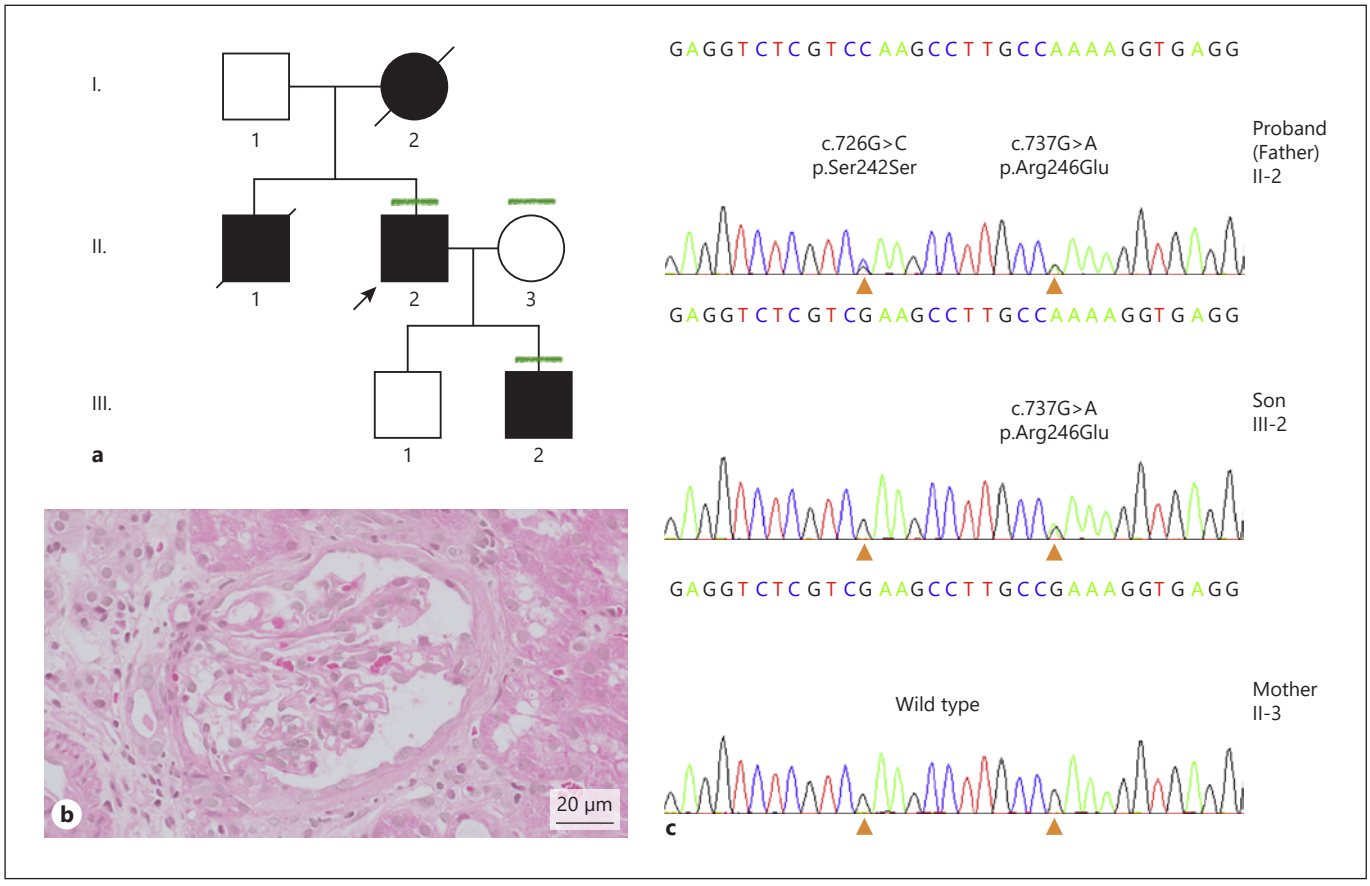

Fig. 1. A family of *LMX1B*-associated nephropathy. **a** Pedigree of a family with *LMX1B*-associated nephropathy. Mutation analysis was performed in patients with green bar. **b** Renal pathology of *LMX1B*-associated nephropathy in patient III-2 showed focal adhesion in the glomerulus, compatible with FSGS pattern. A scale bar in (**b**) equals 20 µm. **c** PCR-Sanger sequencing revealed a missense mutation in the *LMX1B* gene (NM_002316.3, c.737G>A, p.Arg246Glu) in family members II-2 (proband, father) and III-2 (son). It was absent in II-3 (mother). There was a synonymous variant in II-2 (father) (c.726G>C, p.Ser242Ser) which did not pass to his son.

familial cases. The patients were reported across all ethnicities, including White, Hispanic, and Asian, with the majority being female (22/31 = 71.0%). Remarkably, they had varied clinicopathological features as shown in Table 1. None of the *LMX1B*-associated nephropathy cases with the p.Arg246Gln had typical extrarenal manifestations of NPS. Renal presentation can range from nonnephrotic proteinuria with normal renal function to CKD presenting with generalized edema and dyspnea. Among 17 cases with clinical information available, 7 patients (7/17 = 41.2%) were asymptomatic and 10 patients (10/17 = 58.8%) were symptomatic. Clinical diagnosis was reported in 26 patients. Fourteen patients (14/26 = 53.8%) were diagnosed as nonnephrotic proteinuria with normal renal function. Ten patients (10/26 = 38.5%) were diagnosed as steroid-resistant nephrotic syndrome. Two patients (2/26 = 7.7%) were diagnosed as CKD with unknown etiology. All cases were not correctly diagnosed at the first time.

Of those who underwent kidney biopsy (23 cases), 16 patients (16/23 = 69.6%) had FSGS and 3 patients (3/23 = 13.0%) had minimal change disease (MCD) which could be the spectrum of FSGS as some patients with MCD could be unsampling FSGS. Therefore, FSGS spectrum (FSGS plus MCD patients) accounted for 82.6% (19/23). One patient (patient 3) [8] (1/23 = 4.3%) had advanced CKD pathology, and 1 patient (patient 18) [9] (1/23 = 4.3%) was described mesangial proliferative glomerulonephritis without immunofluorescent data in the report (Table 1).

One patient (patient 28) [10] had normal glomerulus by light microscopy and mild foot process effacement of podocyte with positive zebra body by EM. The other case

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Table 1. Clinicopathological data of *LMX1B*-associated nephropathy (c.737G>A, p.Arg246Gln) [8–12, 20–27]

Patient	Family number	Sex	Ethnicity	Age of onset	Familial/ sporadic	Clinical presentation	Clinical diagnosis	Renal pathology	ESRD (Y/N)	Time from onset to ESRD, yr	Age of ESRD, yr	Extrarenal manifestations	Reference
1 (III-2)	1	M	Thai	8	F	B	2	FSGS	Y	6.4	15	None	Our report
2 (II-2)	1	M	Thai	33	F	B	3	NA	Y	0.8	34	None	Our report
3	2	M	European	36	F	na	3	Advanced CKD	Y	22	58	na	[8]
4	2	F	European	22	F	na	1	FSGS	N	–	–	na	[8]
5	2	M	European	6	F	B	2	FSGS	Y	48	54	None	[8]
6	2	F	European	26	F	B	2	FSGS	N	–	–	None	[8]
7	2	M	European	7	F	A	1	na	N	–	–	None	[8]
8	3	F	European	22	F	na	na	FSGS	N	–	–	None	[8]
9	3	F	European	17	F	na	na	FSGS	N	–	–	None	[8]
10	4	F	Japanese	6	S	A	1	MCD	na	–	–	None	[20]
11	5	F	Japanese	6	F	A	1	MCD	Y	32	38	None	[12]
12	5	F	Japanese	13	F	A	1	FSGS	N	–	–	None	[12]
13	5	F	Japanese	1	F	A	1	MCD	N	–	–	None	[12]
14	5	F	Japanese	11	F	A	1	na	N	–	–	None	[12]
15	5	F	Japanese	1	F	A	1	na	N	–	–	None	[12]
16	6	F	White	15	F	B	2	FSGS	N	–	–	None	[21]
17	7	F	Chinese	1	F	na	1	na	N	–	–	None	[9]
18	8	M	Chinese	5	F	na	1	MsPGN	N	–	–	None	[9]
19	9	M	na	21	F	B	2	FSGS	Y	18	39	None	[22]
20	10	F	na	17	S	B	2	FSGS	Y	–	–	None	[23]
21	11	F	Turkish	8	F	na	2	FSGS	Y	1	9	None	[24]
22	12	M	European	4	S	na	2	FSGS	Y	39	43	None	[24]
23	13	F	European	18	S	na	na	FSGS	Y	25	43	None	[24]
24	14	F	East Asian	11	S	na	na	na	na	–	–	na	[25]
25	15	F	European	24	S	na	na	na	na	–	–	na	[25]
26	16	F	Japanese	3	F	B	2	FSGS	N	–	–	None	[26]
27	17	F	Hispanic	3	F	na	1	FSGS	N	–	–	None	[10]
28	18	F	Hispanic	29	F	na	1	FD	N	–	–	None	[10]
29	18	M	Hispanic	9	F	na	1	NA	N	–	–	None	[10]
30	19	F	Hispanic	5	S	B	2	FSGS	Y	30	35	None	[27]
31	20	F	White	58	F	B	1	FD	Y	13	71	None	[11]

Clinical presentation: A = asymptomatic, case detected by program screening or check-up; B = symptomatic such as edema, dyspnea, nephrotic syndrome, or CKD. Clinical diagnosis: 1 = nonnephrotic proteinuria with normal renal function; 2 = steroid-resistant nephrotic syndrome; 3 = CKD or ESRD at presentation. CKD, chronic kidney diseases; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease, F, familial; FD, Fabry disease; FSGS, focal segmental glomerulosclerosis; MCD, minimal change disease, MsPGN, mesangial proliferative glomerulonephritis, N, no; na, not available, S, sporadic; sex M, male; sex F, female; Y, yes; yr, year.

(patient 31) [11] had 30% global sclerosis with focal segmental glomerulosclerosis and capping of podocyte by light microscopy. The EM study showed mild to moderate foot process effacement of podocyte and positive zebra bodies. Zebra body detection in EM was historically considered pathognomonic for Fabry disease. However, in both patients, alpha galactosidase enzyme levels were normal, and genetic analysis revealed no causative mutations in the *GLA* gene. Since both patients also showed no extrarenal manifestation of Fabry disease or NPS, these once again emphasized the importance of genetic testing in renal disease.

Regarding the prognosis, of 28 cases with data available, 9 patients (12/28 = 42.9%) had ESRD. The age of patients who developed ESRD varied from 9 to 71 years. Six patients underwent kidney transplantation and up until now did not have recurrent disease.

Although previous reports suggested that most of the pediatric cases with *LMX1B*-associated nephropathy had nonnephrotic proteinuria with normal renal function [12], our proband presented with advanced CKD at the age of 33, while his son presented with steroid-resistant nephrotic syndrome at the age of 8 years and progressed to ESRD requiring peritoneal dialysis at the age of 15 years. In addition, trio-WES (II-2, II-3, and III-3) did not show any de novo pathogenic or likely pathogenic mutations in 625 genes previously reported to cause kidney diseases [4]. Different severity of the same mutation could be caused by several factors including modifier genes and environmental factors. It has been demonstrated that renal manifestations in NPS could be very different in the same family. A study in 1 pair of identical twins revealed rapidly progressive renal diseases to ESRD in one twin and nonnephrotic proteinuria in the other [13]. Modifier genes may play a substantial role in the phenotypic variation of LMX1B-associated nephropathy as previously demonstrated in other podocyte and glomerular basement membrane genes such as *COL4A3* and *COL4A4* [14]. There have been studies showing that *CLIM2*, *COL4A3*, *COL4A4*, *COL4A5*, *LDB1*, and *PAX2* are the modifier genes of the *LMX1B* mutation [15–18]. However, no additional pathogenic variants were detected in these genes in our study.

With the more widespread use of WES in kidney disease, the causative gene could be identified leading to in-depth insights of disease mechanisms. WES can be diagnostic tools for challenging cases with variable clinical presentations and genetic heterogeneity [19]. Our study emphasized the intrafamilial variability of *LMX1B*-associated nephropathy and the important role of WES in solving kidney disease enigma.

Conclusion

WES successfully identified the genetic defect responsible for unsolved CKD patients. *LMX1B*-associated nephropathy can manifest inter- and intrafamilial variabilities ranging from nonnephrotic proteinuria to ESRD. Notably, the c.737G>A mutation in *LMX1B* could lead to ESRD as early as 9 years of age.

Statement of Ethics

The protocol of this study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University (approval ID: Med Chula IRB 1516/2562).

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Author Contributions

Conceptualization: V.S., K.P., and K.S.; methodology and formal analysis: S.I.; investigation: S.I., C.I., and C.S.; data curation: S.I.; writing – original draft preparation: S.I.; writing – review and editing: A.C., V.S., K.P., and K.S.; supervision: V.S. and K.P.; project administration: K.P.; funding acquisition: K.P. All authors have read and agreed to the published version of the manuscript.

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