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【 CASE REPORT 】

A Perihilar Variant of Focal Segmental Glomerulosclerosis Due to *De novo* Branchio-oto-renal Syndrome

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Abstract:

Branchio-oto-renal syndrome is an autosomal dominant disorder characterized by branchial anomalies, hearing loss, and renal urinary tract malformations. We herein report a 32-year-old Japanese man with a right preauricular pit, bilateral mixed hearing loss, and malposition of the right kidney who presented with proteinuria. The findings of a left kidney biopsy were compatible with a perihilar variant of secondary focal segmental glomerular sclerosis. A trio exome analysis conducted among the patient and his parents failed to identify the causal gene variant, despite a sporadic pattern. His kidney function remained stable for 11 years with an angiotensin II receptor blocker.

Key words: branchio-oto-renal, *de novo*, obesity-related nephropathy, proteinuria, trio analysis

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Introduction

Branchio-oto-renal (BOR) syndrome is a syndrome characterized by three main symptoms of branchial anomalies, hearing loss, and renal urinary tract malformations. It is considered an autosomal dominant disorder, and its estimated prevalence is 1:40,000 (1). BOR syndrome reportedly has variable expressivity and incomplete penetrance between and within families (2). Renal abnormalities were found to be present in approximately 67% of affected individuals (3). Long-term follow-up of a family with BOR syndrome showed that 40% (4 of 10) of the affected members developed end-stage kidney failure (4).

The gene mutation most commonly encountered in BOR syndrome patients is *EYA1*, while less frequently *SIX1*, *SIX5*, or *SALL1* (5, 6). However, mutations in these genes are not always identified in BOR syndrome. Krug et al. reported an identification rate of only 36% (5), and Unzaki et al. reported a rate of 75% (6).

We herein report a case of *de novo* BOR syndrome in which a genetic analysis was performed to search for mutations.

Case Report

A 32-year-old Japanese man was referred for an evaluation of malposition of the right kidney and proteinuria. He had been born one month prematurely and been complicated with intraventricular hemorrhaging in the newborn period. He had had no repetitive respiratory infections or feeding problems of neonates and infants. He had never been found to have any particular abnormalities during school physical examinations. There was no family history of auditory disturbance, kidney disease, or anomalies in appearance.

His height was 158 cm, and his body weight was 86.8 kg, with a body mass index of 34.8. His blood pressure was 113/80 mmHg, and his pulse rate was 65/min. He did not have intellectual disability, motor development delay, digit anomalies, or specific facial features such as coloboma of the eyes, large nose, or Kabuki-like appearance. Although he did not report any auditory disturbance, an audiometric test

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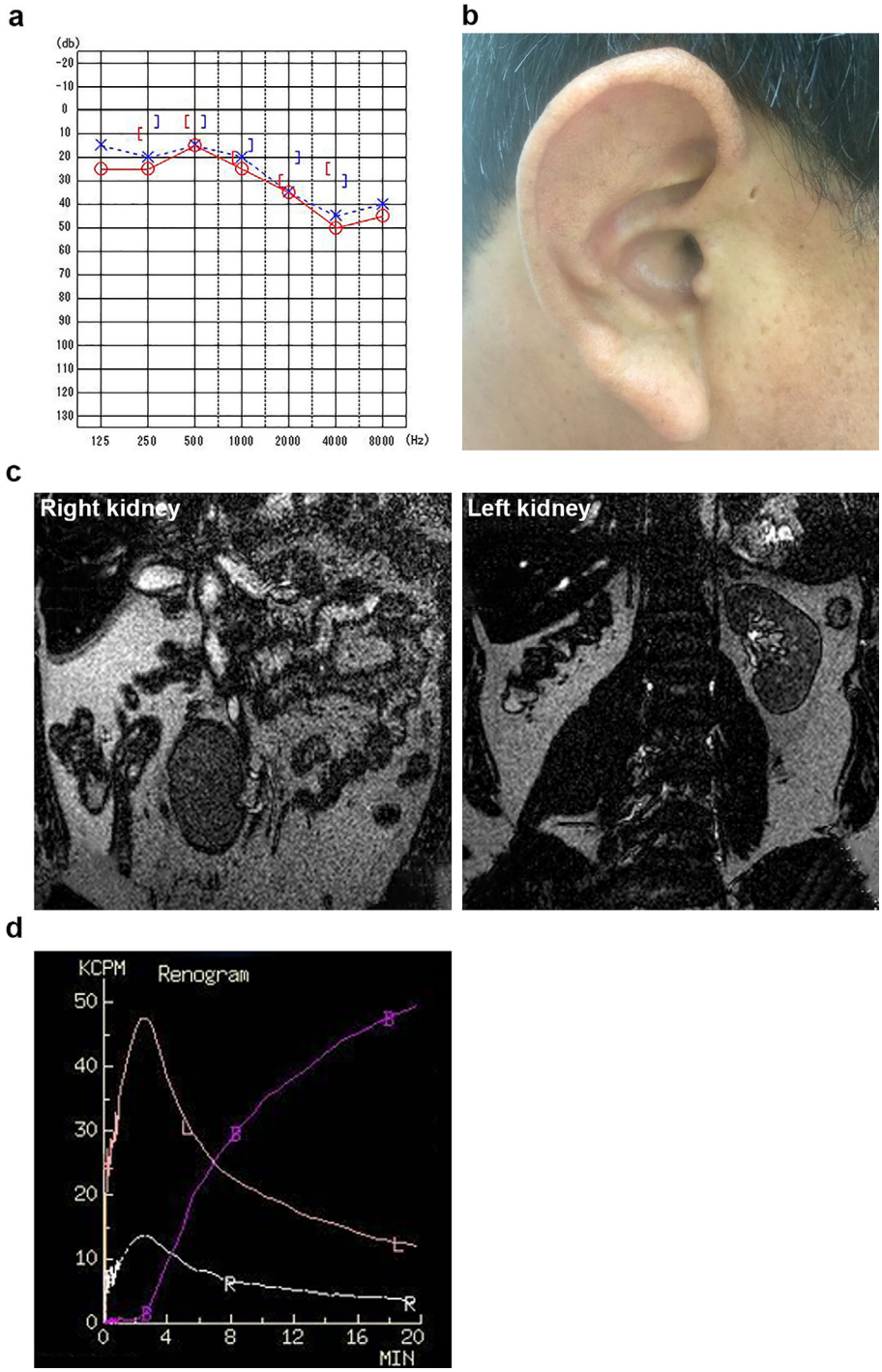

Figure 1. **(a) An audiometric test revealed bilateral mixed hearing loss. (b) There was a pit in front of the right ear. (c) Abdominal magnetic resonance imaging revealed that the right kidney was hypoplastic and abnormally positioned, while the left kidney was normal. (d) ^{99m}Tc-MAG3 scintigraphy showed a decline in the right kidney function.**

revealed bilateral mixed hearing loss (Fig. 1a). A physical examination showed a right preauricular pit (Fig. 1b). The laboratory data are shown in Table 1.

Magnetic resonance imaging (MRI) revealed that the right kidney was hypoplastic and positioned abnormally, while the left kidney was normal (Fig. 1c). ^{99m}Tc-MAG3 scintigraphy showed a decline in the right kidney function (Fig. 1d). A left kidney biopsy revealed no global sclerosis in 11 glomeruli. The diameter of the glomerulus was large at 295.4 μm. Focal mesangial cell proliferation and hyaline arteriolosclerosis were observed on Periodic acid-Schiff staining (Fig. 2a). Immunofluorescence showed the focal weak granular deposition of IgA and IgM. An electron microscopic study showed focal effacement of the podocyte foot processes (Fig. 2b). These findings indicated a perihilar variant of secondary focal segmental glomerular sclerosis caused

Table 1. Laboratory Data.

Urinary examination		Blood chemistry	
pH (4.5-7.5)	6.5	HbA1c (%, 4.7-6.6)	6.7
Protein (g/g·Cr)	0.5	Glu (mg/dL, 70-110)	89
Occult blood	(+/-)	TP (g/dL, 6.5-8.0)	6.9
Glu	(-)	Alb (g/dL, 3.5-5.0)	4.1
β_2MG (μg/L, 5-253)	219	BUN (mg/dL, 8-20)	11
NAG (U/L, 1.0-4.2)	7.1	Cr (mg/dL, 0.60-1.40)	1
		eGFR Cr (mL/min/1.73 m^2)	83.33
Complete blood count		UA (mg/dL, 3.0-8.0)	10.4
WBC (/μL, 4,300-6,900)	10,250	Na (mEq/L, 135-145)	142
RBC (×10^4/μL, 446-515)	557	K (mEq/L, 3.5-5.0)	3.9
Hb (g/dL, 14.0-16.0)	16.3	Cl (mEq/L, 96-109)	105
Ht (%, 43.2-48.6)	47.1	Ca (mg/dL, 8.5-10.5)	10.2
MCV (fL, 91.3-99.3)	84.6	IP (mg/dL, 2.3-4.5)	3.2
Plt (×10^4/μL, 18.0-36.5)	26.6	AST (U/L, 0-40)	42
		ALT (U/L, 0-35)	93
Serology		LDH (U/L, 100-230)	250
ANA	1:40	ALP (U/L, 100-350)	273
MPO-ANCA (U/mL, 0-8.9)	<1.3	γ-GTP (U/L, 0-60)	29
PR3-ANCA (U/mL, 0-3.4)	<1.3	TC (mg/dL, 125-220)	179
Anti-GBM (EU, 0-9)	<10	TG (mg/dL, 35-160)	102
RF (U/mL, 0-20)	3	HDL-C (mg/dL, 40-70)	50.2
ASLO (U/mL, 0-200)	132	CRP (mg/dL, 0-0.3)	0.14
IgG (mg/dL, 800-1,800)	1,280	C3 (mg/dL, 65-141)	156.4
IgA (mg/dL, 80-400)	180	C4 (mg/dL, 13-40)	36.2
IgM (mg/dL, 40-194)	158	CH50 (U/mL, 31-48)	57.6

Alb: albumin, ALP: alkaline phosphatase, ALT: alanine transaminase, ANA: antinuclear antibody, anti-GBM: anti-glomerular basement membrane antibody, ASLO: anti-streptolysin O, AST: aspartate transaminase, β_2MG: β_2-microglobulin, BUN: blood urea nitrogen, C3: complement 3, C4: complement 4, Ca: calcium, CH50: 50% hemolytic complement activity, Cl: chloride, Cr: creatinine, CRP: C-reactive protein, eGFR: estimated glomerular filtration rate, Glu: glucose, γ-GTP: γ-glutamyltranspeptidase, Hb: hemoglobin, HbA1c: hemoglobin A1c, HDL-C: high-density lipoprotein cholesterol, Ht: hematocrit, IgA: immunoglobulin A, IgG: immunoglobulin G, IgM: immunoglobulin M, IP: inorganic phosphate, K: kalium, LDH: lactate dehydrogenase, MCV: mean corpuscular volume, MPO-ANCA: myeloperoxidase antineutrophil cytoplasmic antibody, Na: natrium, NAG: N-acetyl-β-D-glucosaminidase, Plt: platelets, PR3-ANCA: proteinase 3-antineutrophil cytoplasmic antibody, RBC: red blood cells, RF: rheumatoid factor, TC: total cholesterol, TG: triglyceride, TP: total protein, UA: uric acid, WBC: white blood cells

by obesity and unilateral kidney hypoplasia. He started taking angiotensin II receptor blocker after the kidney biopsy. His blood pressure has been stable since then. His estimated glomerular filtration rate and proteinuria remained stable for 11 years (Fig. 3).

While BOR syndrome is suspected of being an autosomal dominant disorder, there was no sign of BOR syndrome in his parents. Therefore, a trio exome analysis was performed among the patient and his parents to search for the *de novo* mutation that caused BOR syndrome in the patient. There were no pathogenic mutations or copy number variants in *EYA1*, *SIX1*, *SIX5*, or *SALL1* in the patient. There were 114 *de novo* mutations in the patient, of which 31 were exonic or at the splice site. Subsequent Sanger sequencing identified 5 synonymous mutations, and no mutations were confirmed in 14 genes. Common, low, and rare variants were denoted by a minor allele frequency of >5%, 1-5%, and <1%, respectively. Pathogenicity was examined in the ClinVar database if the variant category was low or rare. None of the 12 genes were thought to be pathogenic (Table 2). There were two nonsense homozygous genes (*OR1B1* and *KRT37*), neither of which were thought to be pathogenic (Table 2). While there were 17 newly homozygous or compound heterozygous genes, no mutations were confirmed in 4 genes by subsequent Sanger sequencing. Of the 13 genes, 9 were common variants, 3 were low variants, and 1 was a rare variant (Table 2). *FASN* p.Ile1113Val was "Likely benign" in the ClinVar database. *KRT86* p.Arg241Trp and *ZKSCAN2* p.Asp335Glu were "Probably damaging", while *ZKSCAN2* p.Ala454Ser was "Benign" in PolyPhen-2 prediction. *KRT86* p.Arg241Trp was "Deleterious" while *ZKSCAN 2* p.Asp335Glu was "Neutral" in PROVEAN prediction. *KRT86* p.Arg241Trp was "Tolerated" in SIFT prediction.

Finally, to detect any copy number variants in *EYA1*,

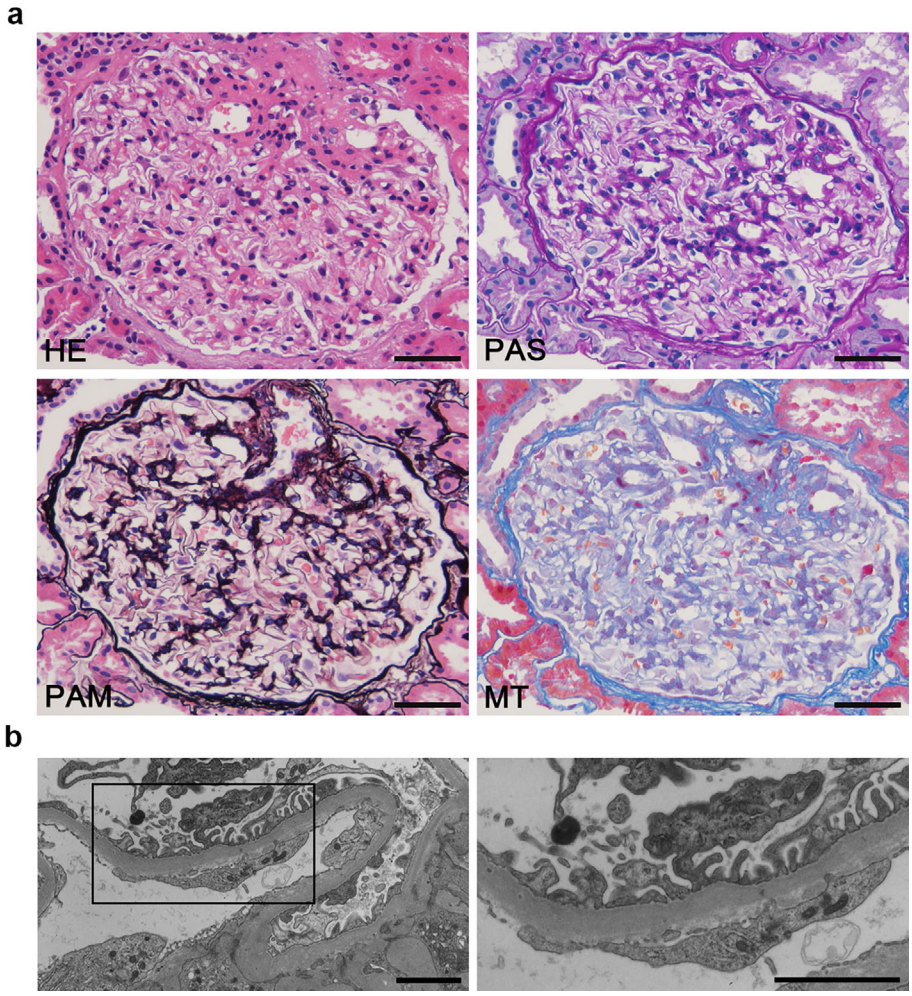

Figure 2. **(a) A light microscopic study. The diameter of the glomerulus was large at 295.4 μm. There was no inflammatory cell infiltration in the glomeruli on HE staining. PAS staining showed focal mesangial cell proliferation and hyaline arteriolosclerosis. There were no spikes or a bubbling appearance of the glomerular basement membranes on PAM staining. There were no immune complex deposits in the glomeruli on MT staining. Bars =50 μm. HE: Hematoxylin and Eosin staining, PAS: Periodic acid-Schiff stain, PAM: Periodic acid-silver-methenamine stain, MT: Masson-trichrome stain. (b) Electron microscopic study. The podocyte foot processes were focally effaced. The right panel is an enlarged picture of the square in the left panel. Bars =2 μm.**

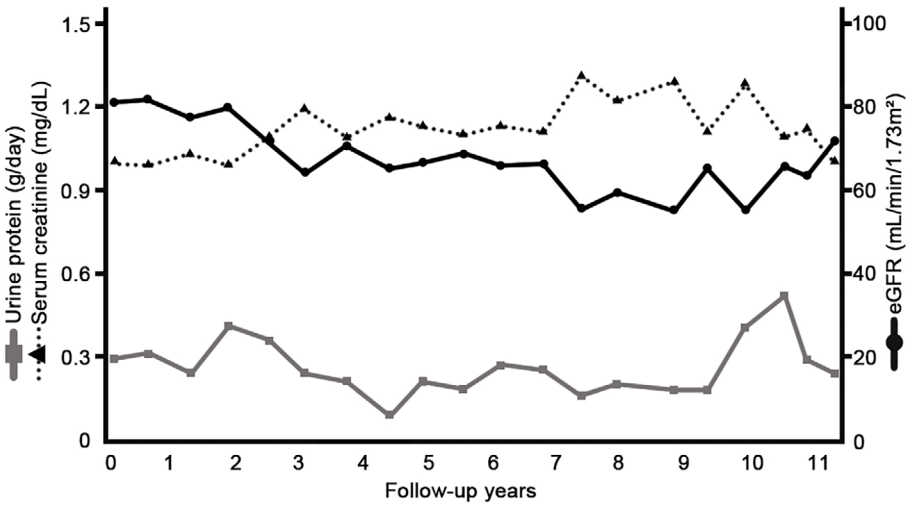

Figure 3. **The clinical course. eGFR: estimated glomerular filtration rate**

Table 2. Results of a Trio Exome Analysis.

Gene	Exome and Sanger sequencing results	Amino acid change	dbSNP	gnomAD	8.3KJPN	Variant category/ Pathogenicity
De novo mutation in exons						
LCE5A	c.119G>A, homo	p.Cys40Tyr	rs2105117	0.550284	0.7341	Common
SPATA3	c.96_122delCCCTGAATCCAC ACCACAGCAGCCTAG, homo	p.Gln48_Gln56del	rs750359768	0.5165	NA	Common
SACM1L	c.1301A>T, homo	p.Tyr434Phe	rs1468542	0.361435	0.2701	Common
PRPF4B	c.247A>G, homo	p.Ile83Val	rs9503893	0.298609	0.8027	Common
IGFBP3	c.95C>G, homo	p.Ala32Gly	rs2854746	0.61758	0.23521	Common
ITIH5	c.1708A>C, hetero	p.Thr570Pro	rs2275069	0.495043	0.7544	Common
DUOXA2	c.298C>G, homo	p.Arg100Gly	rs2576090	0.863151	1	Common
KCNJ12	c.1113C>T, hetero	p.Ser371Arg	rs1612176	0.000007	0.4999	Common
KRTAP9-3	c.58C>A, homo	p.Gln20Lys	rs112082369	0.172332	0.28819	Common
TCF3	c.1475C>T, hetero	p.Ala492Val	rs2074888	0.028454	0.3861	Common
FGF21	c.521T>C, homo	p.Leu174Pro	rs739320	0.739314	0.9999	Common
SIRPB1	c.1087G>C, homo	p.Ala363Pro	rs2243603	0.194994	0.25012	Common
Nonsense homozygous genes						
OR1B1	c.574C>T, homo	p.Arg192Ter	rs1476860	0.247893	0.5983	Common
KRT37	c.703C>T, homo	p.Gln235Ter	rs78158550	NA	0.2939	Common
Newly homozygous or compound heterozygous genes						
HOXD9	c.806_807insGCA, homo	p.Gln269dup	rs56007470	NA	0.40459	Common
KIF1A	c.2721_2723delGGA, homo	p.Glu917del	rs10594016	0.5266	NA	Common
DSP	c.1481A>T, homo	p.Tyr494Phe	rs28763961	0.006181	0.0968	Common
FERD3L	c.234_235insGAA, homo	p.Glu81dup	rs34966908	0.2532	0.1946	Common
PTPRZ1	c.4290_4292delTGA, homo	p.Asp1431del	rs35947407	0.4706	0.0004	Common
ADAMTSL2	c.1090G>A, homo	p.Val364Ile	rs35767802	0.247623	NA	Common
KAT6B	c.3310_3312delGAA, homo	p.Glu1104del	rs71929101	0.3275	NA	Common
DUSP16	c.1090_1098delCCCAGCGTG, homo	p.Pro364_ Val366del	rs59897494	0.2053	0.1414	Common
KRT86	c.721C>T, homo	p.Arg241Trp	rs111429470	0.000685	0.0209	Low/VUS
ZKSCAN2	c.1360G>T, homo	p.Ala454Ser	rs201935635	0.000014	0.0092	Rare/VUS
ZKSCAN2	c.1005T>A, homo	p.Asp335Glu	rs141526402	0.00177	0.0156	Low/VUS
TNK1	c.832G>A, homo	p.Val278Ile	rs55939858	0.004163	0.0748	Common
FASN	c.3337A>G, homo	p.Ile1113Val	rs201182683	0.000057	0.0115	Low/Likely benign

del: deletion, dup: duplication, hetero: heterozygous, homo: homozygous, ins: insertion, NA: not available, VUS: variant of uncertain significance

which was the most strongly suspected causative gene, quantitative polymerase chain reaction (qPCR) of each exon in *EYA1* normalized with *ACTB* or *GAPDH* was performed, providing negative results. Overall, the trio exome analysis failed to identify the causative mutation in the patient.

We therefore focused on searching for a non-allelic homologous recombination around the *EYA1* gene. Long polymerase chain reaction failed to detect any proximal or distal breakpoints.

Discussion

We experienced a case of *de novo* BOR syndrome. Chang et al. proposed clinical criteria for BOR syndrome, which included four major criteria of branchial anomalies, deafness, preauricular pits, and renal anomalies, as well as five minor criteria of external ear anomalies, middle ear anomalies, inner ear anomalies, preauricular tags, and others (2). The present patient met three of the major criteria (deafness, preauricular pits, and renal anomalies), so he was diagnosed with BOR syndrome.

While BOR syndrome is suspected of being an autosomal dominant disorder, the present case seemed to be sporadic, prompting us to perform a genetic analysis among the patient and his parents. However, a trio exome analysis provided negative results. Furthermore, long polymerase chain reaction was performed to search for non-allelic homologous recombination (7), which also provided negative results. These results were considered consistent with those of previous reports, wherein the identification rates of the causative gene in BOR syndrome were 36% and 75% (5, 6). One limitation of the present study was that we did not investigate deep intronic variants. Furthermore, we did not perform a multiplex ligation-dependent probe amplification analysis in *EYA1*, performing qPCR instead. An event at the time of birth might affect the gene expression related to BOR syn-

drome, as our patient was born one month early.

Although the patient had proteinuria with a reduced right kidney function at the first visit, his kidney function did not decline for 11 years. The clinical condition of the present case was thought to be similar to that of patients with unilateral kidney hypoplasia, as ^{99m}Tc-MAG3 scintigraphy showed an almost normal function in the left kidney. A previous report demonstrated that uninephrectomy in army personnel who lost a kidney due to trauma led to few major adverse consequences over 45 years (8). Furthermore, another study showed that the long-term kidney function of donors was stable over 40 years (9).

The results of a left kidney biopsy showed enlarged glomeruli with focal foot process effacement, suggesting compensatory glomerular hyperfiltration. A previous report showed segmental hyalinosis and mesangial proliferation of varying degrees (10). Another report showed a patient with BOR syndrome who had focal glomerulosclerosis (11). In the present case, angiotensin II receptor blocker was initiated to reduce the increased intraglomerular pressure. A reduction in body weight is needed in the present case, since an increased intraglomerular pressure due to obesity has been reported to raise the risk of developing chronic kidney disease (12).

In conclusion, we experienced a case of *de novo* BOR syndrome. The long-term kidney function was stable with conservative therapy despite unilateral kidney hypoplasia.

Consent to participate was obtained for genetic screening of the patient. The genetic analyses were approved by the Institutional Review Board of Mie University Graduate School of Medicine (reference number H2019-113).

Written informed consent was obtained from the patient for the publication of this case report.

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

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