

## Diverse Renal Phenotypes Observed in a Single Family with a Genetic Mutation in Paired Box Protein 2

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### Key Words

Paired box protein 2 mutation · Renal coloboma syndrome · Focal segmental glomerulosclerosis · Congenital anomalies of the kidney and urinary tract · Autophagic dysfunction · Fundoscopic examination

### Abstract

A common renal phenotype of paired box protein 2 (*PAX2*) mutations is renal coloboma syndrome. We report a single family with diverse renal phenotypes associated with *PAX2* mutation. The proband presented steroid-resistant focal segmental glomerulosclerosis with optic coloboma, whereas his two sons showed severe renal hypoplasia with end-stage renal disease, with or without optic coloboma. In all three cases, a heterozygous *PAX2* genetic mutation was identified (exon 2; NM\_003987.3:c.76dupG, p.Val26Glyfs\*28). Based on histopathological findings of the proband, we hypothesized that autophagic dysfunction was associated with the pathophysiology of the focal segmental glomerulosclerosis with *PAX2* mutation. Detailed fundoscopic examination – including the optic disc – might be useful for the diagnosis of renal anomalies associated with *PAX2* mutation.

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## Introduction

The paired box protein 2 (*PAX2*) gene encodes a transcription factor essential for differentiation of the epithelial components of the fetal kidney and ureter. Two intact copies of the gene are required for normal renal development [1]. The most common findings associated with *PAX2* mutations are abnormal renal structure or function (92%), ophthalmological anomalies (77%), and hearing loss (7%) [2]. Mutations in *PAX2* are a common cause of renal coloboma syndrome (RCS) and are usually present early in life. Recently, using in silico analysis and functional data, Barua et al. [3] reported that *PAX2* missense variants may lead to an expanded phenotypic spectrum: not only to congenital anomalies of the kidney and urinary tract (CAKUT), but also to focal segmental glomerulosclerosis (FSGS), through haploinsufficiency and/or dominant negative effects. However, the characteristic clinicopathological features of FSGS associated with *PAX2* mutations are still unknown.

In this report, we describe a single family with diverse renal phenotypes, such as FSGS and CAKUT, associated with *PAX2* mutation.

## Case Reports

Case 1 (the proband), a 27-year-old man, was admitted because of a 7-year history of proteinuria. He had been born at full term and his growth and development had been normal. There was no family history of renal disease. Physical findings were as follows: height 174.0 cm, weight 71.5 kg, body mass index 23.6, and blood pressure 120/80 mm Hg. He did not suffer from deafness. The rest of the physical examination was unremarkable. Urine dipstick analysis was negative for occult blood and 2+ for protein. Urinary protein was 1.6–3.0 g/day. The urinary sediment contained <1 erythrocyte and 3–5 leukocytes per high-power field. Hematocrit was 43.0%, hemoglobin concentration 15.2 g/dl, platelet count 277,000/ $\mu$ l, and leukocyte count 6,200/ $\mu$ l. The serum urea nitrogen level was 15.4 mg/dl, creatinine 1.0 mg/dl, uric acid 7.9 mg/dl, cholesterol 222 mg/dl, total protein 7.2 g/dl, and albumin, 4.9 g/dl. IgG level was 1,070 mg/dl, IgA 139 mg/dl, and IgM 84 mg/dl. Total complement level was 35 IU/l, C3 72 mg/dl, C4 29.5 mg/dl, and C1Q 12.8 mg/dl. Antinuclear antibody was negative. The remaining autoimmune serological findings were within the normal range, and serum viral hepatitis markers were negative. Abdominal computed tomography and ultrasound showed slightly small kidneys with bilateral cysts. Fundoscopic examination by portable ophthalmoscope was normal. On the eighth hospital day, a right renal biopsy was performed. Under light microscopy, ten glomeruli and a localized area of tubular atrophy were found (fig. 1a). One glomerulus was obsolescent and in another there was segmental sclerosis with hyaline deposits near the vascular pole (fig. 1b). Other glomeruli were slightly enlarged and had essentially minor changes, but in several segments a questionable increased mesangium was observed (fig. 1c). By immunofluorescence, no significant deposits of immunoglobulins or complement components were found, except for weak granular mesangial and paramesangial IgM deposits. From the clinical and histological findings, idiopathic FSGS was considered as a tentative diagnosis. Initially he was treated with platelet aggregation inhibitor and low-protein diets in our outpatient clinic, but the treatment was interrupted 3 months after admission. At the age of 31 years, he visited our hospital again to be checked for proteinuria. His urinary protein was 51 mg/dl and 0.8 g/day, his serum urea nitrogen level was 17.3 mg/dl, and creatinine was 1.1 mg/dl. To clarify the indication of prednisolone, a second renal biopsy was performed. Under light microscopy, only five glomeruli were found, but significant histological changes were not observed expect for

focal tubular atrophy with mild interstitial cell infiltration. The patient was treated with 40 mg/day of prednisolone with gradual tapering for 18 months in our outpatient clinic, but the proteinuria was steroid-resistant and remained at about 1.0–1.5 g/day. His renal function gradually worsened. At the age of 52 years, his urinary protein was 64 mg/dl and his serum creatinine level 1.73 mg/dl.

Case 2 (the proband's eldest son) was born by Cesarean section at 39 weeks of gestation when his father, case 1, was 27 years old, with a normal birth weight of 2,880 g but a right pneumothorax and hypoxic ischemic encephalopathy. When he was 2 years old, proteinuria and renal dysfunction were noted. He was suspected to have familial juvenile nephronophthisis. His renal function gradually worsened, and he started continuous ambulatory peritoneal dialysis at the age of 5 years. He received a kidney transplant from a brain-dead donor at the age of 8 years. He is now 26 years old and free from dialysis; his serum creatinine level is 0.80 mg/dl.

Case 3 (the proband's second son) was born by Cesarean section at 41 weeks of gestation when the proband was 28 years old, with a normal birth weight of 3,034 g. From the time of birth, he had proteinuria and renal dysfunction, and ultrasound showed bilateral hypoplastic kidneys. His renal function gradually worsened, and he started continuous ambulatory peritoneal dialysis at the age of 7 years. He received a kidney transplant from his mother at the age of 8 years. After renal transplantation, his renal function gradually worsened because of chronic rejection. He was referred to our hospital to start regular hemodialysis at the age of 23 years. He is now 25 years old and is receiving regular hemodialysis therapy at our hospital.

We initially considered that case 1 was a sporadic case of idiopathic FSGS. However, upon his second son's visit to our hospital, we assumed the existence of a hereditary cause associated with renal hypodysplasia. After obtaining informed consent, we collected DNA from cases 1–3 plus the wife of case 1. Genomic DNA was extracted from whole blood and screened for mutations in *PAX2*. A heterozygous *PAX2* mutation (exon 2; NM\_003987.3:c.76dupG, p.Val26Glyfs\*28) was identified in all three cases, but not in the wife of case 1 (fig. 2). Case 1 did not have a preceding family history of renal disease, suggesting a possible de novo mutation of the *PAX2* gene. In addition, careful ophthalmoscopy revealed bilateral optic nerve atrophy in cases 1 and 3, but case 2 exhibited bilateral glaucomatous cupping (fig. 3).

Taking *PAX2* mutation into account, we performed electron microscopic examination of the first biopsy from case 1. No electron-dense deposits were observed. Foot processes were generally well preserved, but segmental loss of podocytes was observed (fig. 4a). Many podocytes had several vacuoles and some also had large phagosomes (fig. 4b). Several swollen endothelial cells containing vacuoles were also observed (fig. 4c). About half of the mesangial cells had many vacuoles; in some of these vacuoles, degenerating mitochondria and the debris of membranous structures were apparent (fig. 4d). Phagosomes were also occasionally observed in the mesangial cells (fig. 4c, d).

## Discussion

In this study, we report diverse renal phenotypes, such as FSGS and CAKUT, in a single family with *PAX2* mutation. The proband did not have any family history of renal disease, suggesting a possible de novo mutation of *PAX2*. It has been estimated that approximately 50% of *PAX2* mutations occur de novo [2]. Genetic studies have shown that familial FSGS is a disease of podocytes, which are major components of the glomerular filtration barrier [4].

The associated mutated genes can be divided into the following categories: (a) slit diaphragm-associated molecules, (b) podocyte cytoskeleton-related molecules, (c) podocyte transcription factors, and (d) adhesion and extracellular matrix molecules [5].

The *PAX2* gene, which encodes a transcription factor, is expressed in primitive cells of the kidney, ureter, eye, ear, and central nervous system. *PAX2* mutations are particularly associated with RCS, which comprises renal defects and ocular defects affecting the optic nerve and/or the retina [6]. In the present family, case 1 showed steroid-resistant FSGS with optic coloboma. In other recent reports of *PAX2* mutations, Barua et al. [3] reported 24 patients from seven unrelated families with FSGS, and Okumura et al. [7] reported an additional three cases of sporadic FSGS. These findings expanded the phenotypic spectrum associated with *PAX2* mutations.

In case 1, almost half of the mesangial cells had unusually numerous vacuoles; in some of them, degenerating mitochondria and the debris of membranous structures were found. These electron microscopic findings suggested autophagic dysfunction in mesangial cells, although autophagy in the kidney has been attributed to podocytes and tubular cells [8]. Interestingly, in a study by Sooparb et al. [9], using cultured rat renal epithelial cells, chaperone-mediated autophagy contributed to the regulation of growth of tubular kidney cells through degradation of *PAX2*, indicating the possibility that chaperone-mediated autophagy and *PAX2* could play roles in epithelial cell growth. It has been suggested that autophagic dysfunction may also contribute to pathological processes in mesangial and endothelial kidney cells [10].

It should be noted that detailed funduscopy examination – including the optic disc – is useful for the diagnosis of renal defects associated with *PAX2* mutation. Although 25 years ago we could not observe optic disc coloboma using a portable ophthalmoscope, it was detectable by slit lamp microscopy, which is widely used for funduscopy examination today. As in the present cases, *PAX2*-associated CAKUT and FSGS can be identified through a detailed eye examination including the optic disc.

In the present study, all three cases possessed a heterozygous c.76dupG variant mutation (NM\_003987.3:c.76dupG, p.Val26Glyfs\*28) in exon 2. Interestingly, a similar mutation has previously been reported in several patients with RCS [11–13] and is also responsible for the Pax2<sup>1Neu</sup> mutant mouse, an animal model of human RCS [14]. In a study by Barua et al. [3], three of seven *PAX2*-associated FSGS families carried a mutation located in the paired domain near the N-terminus of the protein. These three variants were predicted to affect *PAX2* binding to DNA. In the present family, the mutation (NM\_003987.3:c.76dupG, p.Val26Glyfs\*28) was also located in the paired domain region, but the phenotypes were variable, with anticipation of the renal phenotype. Genotype-phenotype correlations for *PAX2* mutations are not clear [15].

In conclusion, *PAX2* mutation can present as steroid-resistant FSGS; detailed funduscopy including the optic disc is useful for the diagnosis of renal anomalies associated with these mutations. We should be aware that *PAX2* mutations can present not only as CAKUT but also as FSGS. *PAX2* mutation should be listed as a differential diagnosis of FSGS. We hypothesized that autophagic dysfunction was associated with the pathophysiology of FSGS with *PAX2* mutation. If FSGS associated with *PAX2* mutations remains unrecognized, unnecessary and ineffective immunosuppressive agents may be used for such patients. Further studies should be conducted to determine whether renal defects associated with *PAX2*, including FSGS and CAKUT, may be more common than previously thought.

## Acknowledgment

The authors are grateful to Mr. N. Sakamoto, Ms. S. Tsuchida, Ms. M. Yoshinuma, and Ms. M. Igashima (Department of Pathology, Shinrakuen Hospital) for their technical assistance.

## Statement of Ethics

The authors have no ethical conflicts to disclose.

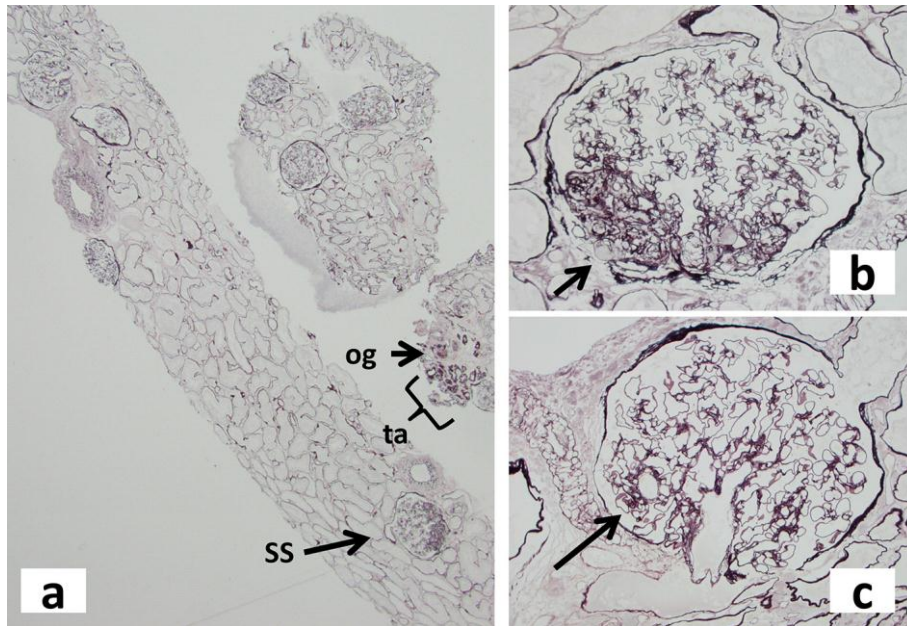
## Disclosure Statement

The authors declare that they have no conflict of interest.

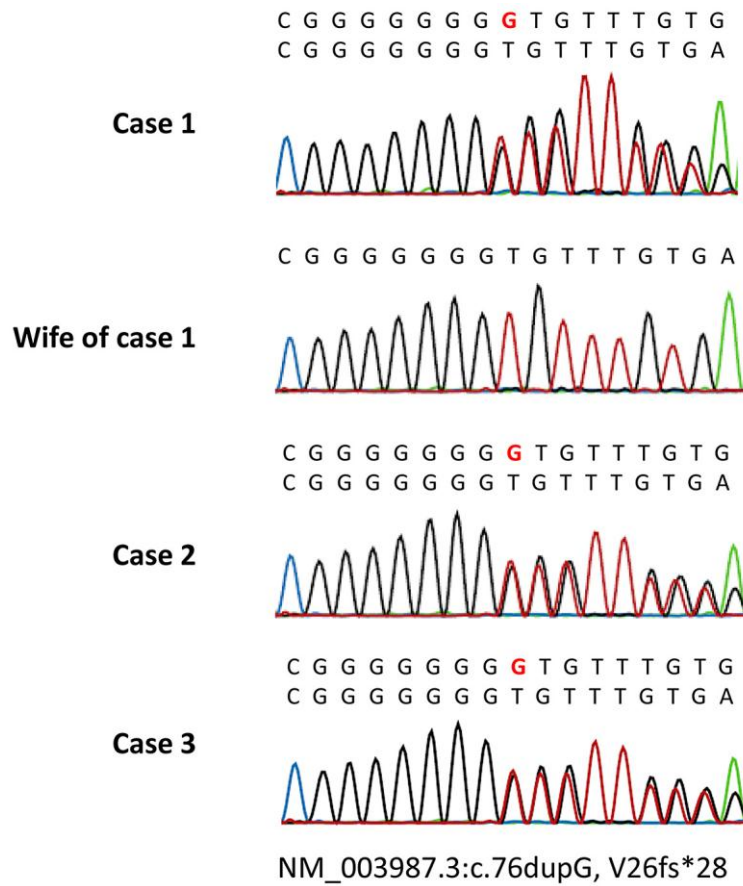
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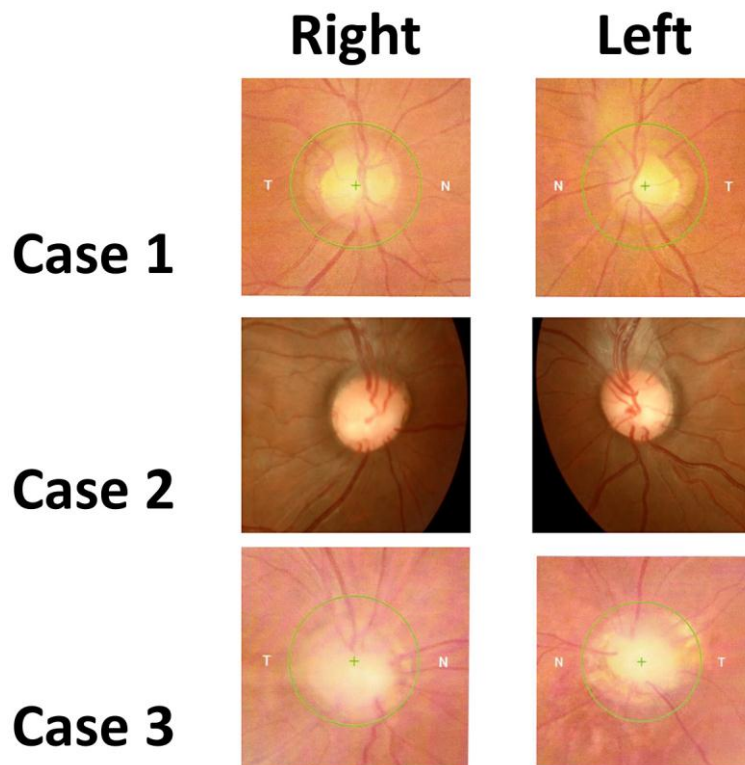
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**Fig. 1.** **a** Glomeruli show essentially minor changes, but one glomerulus has segmental sclerosis (ss) and another glomerulus is obsolescent (og) in a localized area of tubular atrophy (ta). Periodic acid-silver methenamine stain,  $\times 48$ . **b** In this glomerulus segmental sclerosis with hyaline deposits (arrow) near the vascular pole is observed, but other portions show essentially normal appearance. Periodic acid-silver methenamine stain, original magnification  $\times 400$ . **c** This glomerulus appears almost normal, except for a segmental questionable increase in mesangial matrix (arrow). Periodic acid-silver methenamine stain, original magnification  $\times 400$ .

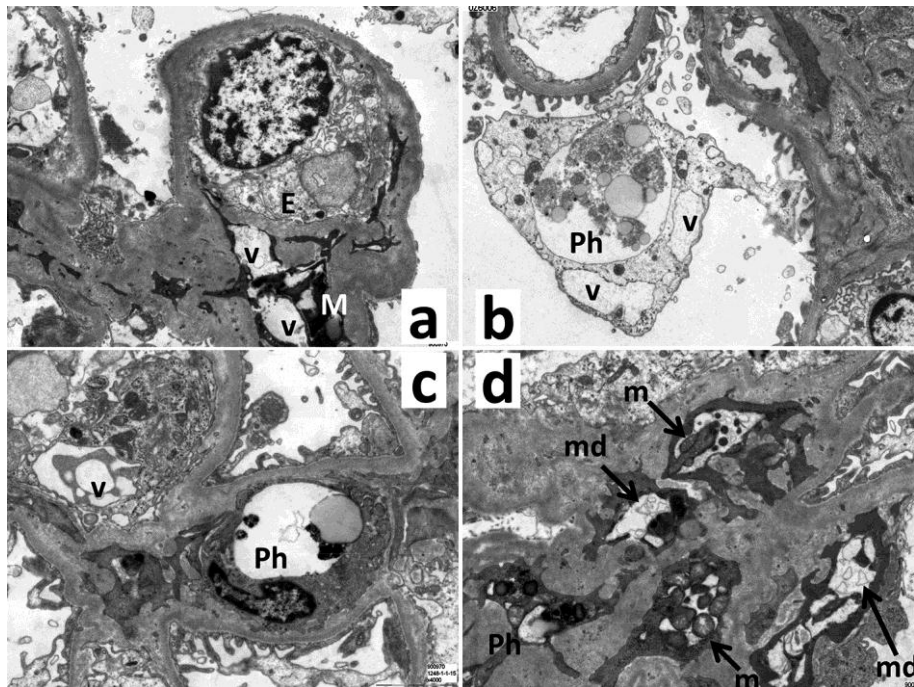


**Fig. 2.** A heterozygous *PAX2* mutation (exon 2; NM\_003987.3:c.76dupG, p.Val26Glyfs\*28) was identified in all three cases, but not in the wife of case 1.



**Fig. 3.** Ophthalmoscopy revealed bilateral optic nerve atrophy in cases 1 and 3, but in case 2 it revealed bilateral glaucomatous cupping.





**Fig. 4.** **a** Segmental loss of podocyte, swollen endothelial cell (E), and vacuoles (v) in the mesangial cell (M) are observed. **b** Foot processes are well preserved; a large phagosome (Ph) and vacuoles (v) are found in the cytoplasm of the podocyte. **c** A large phagosome (Ph) in the mesangial cell and vacuole (v) containing swollen cell are observed. **d** Some mesangial vacuoles contain degenerating mitochondria (arrows m) and membranous debris (arrows md); a lipid-containing phagosome (Ph) is also found in the mesangial cell.