Genotype-phenotype analysis in patients with PAX2 mutations: beyond renal coloboma syndrome

Ji Hyun Kim
Seoul National University Bundang Hospital
Yo Han Ahn (yhahn@snu.ac.kr)
Seoul National University College of Medicine
Yeonji Jang
Uijeongbu Eulji Medical Center, Eulji University School of Medicine
Eujin Park
Korea University Guro Hospital
Hajeong Lee
Seoul National University Hospital
Seong Heon Kim
Seoul National University College of Medicine
Ji Yeon Song
Pusan National University Children's Hospital
Kyoung Hee Han
Jeju National University
Jiwon Jung
University of Ulsan College of Medicine
Joo Hoon Lee
University of Ulsan College of Medicine
Hee Gyung Kang
Seoul National University College of Medicine
Jae Ho Jung
Seoul National University Children’s Hospital, Seoul National University College of Medicine
Hae Il Cheong
Seoul Red Cross Hospital

Article

Keywords:

Posted Date: June 13th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3028260/v1

Additional Declarations: No competing interests reported.

Abstract

PAX2-related disorders encompass renal coloboma syndrome (RCS) and hereditary focal segmental glomerulosclerosis (FSGS)type 7. In this multicenter study on patients with *PAX2* mutations, we explored genotype-phenotype correlations regarding kidney and ocular involvement and long-term clinical outcomes. Among 27 patients with *PAX2* mutations detected from 2004–2022, 19 had RCS, 4 had FSGS, and 4 had isolated congenital anomalies of the kidneys and urinary tract (CAKUT). Based on genotypes, patients were classified into truncating (n=22) and missense (n=5) mutation groups. Truncating mutations were associated with RCS in 81.8% of cases, while missense mutations were linked to FSGS (n=2) and isolated CAKUT (n=2) in 80.0% of cases (*P*=0.034). Fourteen patients developed kidney failure at a median age of 14.5 years, with no difference in kidney survival between the truncating and missense mutation groups. However, mutations in the paired domain of *PAX2* resulted in kidney failure more rapidly than mutations in other sites (*P*=0.025). Regarding ocular manifestations, the truncating mutation group exhibited more common, earlier onset and severe involvement compared to the missense mutation group. Our findings support genotype-phenotype correlations in ophthalmology field and emphasize the impact of the paired domain on kidney outcomes in patients with *PAX2*mutations.

Introduction

Renal-coloboma syndrome (RCS, OMIM 120330) is a rare form of chronic kidney disease (CKD) accompanied by retinal coloboma, which was first described by Weaver et al. in 1988.[1] It is characterized by renal hypodysplasia (RHD) and optic disc anomalies.[2, 3] In 1995, *PAX2* was found to be the causative gene of RCS, as well as vesicoureteral reflux and renal dysplasia.[4] The *PAX2* gene was the first specific gene identified to be associated with congenital anomalies of the kidneys and urinary tract (CAKUT).[4] Currently, *PAX2* mutation is known as the second-most common genetic cause of CAKUT with or without optic disc anomalies.[5–8] In 2014, *PAX2* was found to be associated with adult-onset focal segmental glomerulosclerosis (FSGS) and listed as a causative mutation of FSGS (FSGS7, OMIM616002).[9–11]

PAX2, paired box gene 2, is a transcription factor that plays key roles in development of the kidneys, eyes, ears, and urogenital tract. During kidney development, *PAX2* suppresses apoptosis in the developing ureteric bud; therefore, *PAX2* mutations increase apoptosis during the development of the kidneys and urinary tract, which may underlie the decreased nephron number, hypertrophy of the remaining nephrons, and RHD.[12–14] On the other hand, *PAX2* also suppresses *WT1*, an important transcription factor of podocytes, and its mutation causes congenital nephrotic syndrome and FSGS. A reduction in *PAX2* expression in the visceral epithelium of the future glomeruli of the S-shaped body is accompanied by a marked increase in *WT1* expression,[15] and an animal model demonstrated that *PAX2* re-expression in mature podocytes could be related to glomerular diseases.[16]

With the advent of genetics, recent studies have reported variable and overlapping phenotypes of *PAX2*-related disorders.[17, 18] In addition, some reports have demonstrated that *PAX2* mutations were detected in patients with CKD of unknown etiology.[19–21] However, little data are available regarding the association

between genotype and clinical manifestations in patients with *PAX2* mutations, and previous studies have not provided longitudinal data on the clinical manifestations of *PAX2*-related disorders. In this study, we investigated the genotype-phenotype correlations of renal and ocular involvement, including long-term clinical outcomes, in patients with *PAX2* mutations.

Materials and Methods

Study participants

A total of 27 Korean patients with *PAX2* mutations were enrolled from four pediatric nephrology centers in South Korea between August 2006 and May 2022 (Table 1). Eight patients had *PAX2* mutations identified by Sanger sequencing for RCS with optic nerve coloboma and kidney abnormalities. Twelve patients were confirmed to have *PAX2* mutations by targeted exome sequencing (TES) for CAKUT, steroid-resistant nephrotic syndrome (SRNS), or cystic kidney disease.[22–24] Furthermore, seven patients were found to have *PAX2* mutations by whole exome sequencing (WES) for chronic kidney disease of an unknown cause. All the patients were unrelated, except for one sibling (cases 1 and 2). Thirteen cases have been published in previous studies (Supplementary Table S2).[22, 23, 25, 26] Clinical and laboratory data were obtained through a retrospective review of electronic medical records. This study was approved by the Institutional Review Boards of Seoul National University Hospital (No 2011-048-1171), Seoul National University Bundang Hospital (No B-2104-681-408), Pusan National University Children's Hospital (No. 05-2021-230 (2021 – 0206)), and Jeju National University Hospital (No 2021-01-007). The requirement for informed consent was waived because only retrospective data were used in this study by the the Institutional Review Boards of Seoul National University Hospital (No 2011-048-1171), Seoul National University Bundang Hospital (No B-2104-681-408), Pusan National University Children's Hospital (No. 05-2021-230 (2021 – 0206)), and Jeju National University Hospital (No 2021-01-007). All procedures were performed in accordance with the Declaration of Helsinki.

Table 1
Genotypes and initial presentation of patients with *PAX2* mutations

Case	Sex	cDNA	Protein	Onset age (years)	Initial presentation	Age at last visit (years)
Renal coloboma syndrome						
1	M	c.76dupG	p.Val26Glyfs*28	7.2	Esotropia	35.5
2	M	c.76dupG	p.Val26Glyfs*28	3.8	Nystagmus	32.4
3	M	c.76dupG	p.Val26Glyfs*28	0.2	Microphthalmia	28.6
4	F	c.76dupG	p.Val26Glyfs*28	6.6	Proteinuria	29.7
5	M	c.76dupG	p.Val26Glyfs*28	0.5	Nystagmus	Death at 9.1
6	M	c.310C > T	p.Arg104*	0.3	Nystagmus	17.5
7	M	c.754C > T	p.Arg252*	3.0	Nystagmus	18.8
8	M	c.76dupG	p.Val26Glyfs*28	0.1	CKD	20.5
9	M	c.344G > C	p.Arg115Pro	0.1	CKD	24.4
10	M	c.535_546delinsT	p.Asn179Trpfs*17	0.1	Abnormal prenatal USG	5.9
11	M	c.76dupG	p.Val26Glyfs*28	0.1	CKD	13.1
12	F	c.76dupG	p.Val26Glyfs*28	0.1	Abnormal prenatal USG	6.4
13	M	c.343C > T	p.Arg115*	0.1	Abnormal prenatal USG	11.4
14	F	c.860delA	p.Gln287Argfs*10	10.1	Proteinuria	10.9
15	M	c.754C > T	p.Arg252*	0.3	Nystagmus	13.0
16	M	c.76dupG	p.Val26Glyfs*28	0.1	Abnormal prenatal USG	5.4
17	F	c.76delG	p.Val26Cysfs*3	0.1	Abnormal prenatal USG	16.4
18	F	c.76dupG	p.Val26Glyfs*28	10.2	CKD	43.1
19	M	c.361_373dup	p.Asp125Glyfs*5	4.5	Proteinuria	33.0
Focal segmental glomerulosclerosis						
20	M	c.76dupG	p.Val26Glyfs*28	5.2	Proteinuria	29.4

M, male; F, female; PU, proteinuria; CKD, chronic kidney disease; USG, ultrasonography

The reference sequence of *PAX2* gene is NM_003990.5.

Case	Sex	cDNA	Protein	Onset age (years)	Initial presentation	Age at last visit (years)
Renal coloboma syndrome						
21	M	c.223_226dup	p.Gly76Aspfs*27	13.4	Proteinuria	27.5
22	F	c.74G > A	p.Gly25Glu	7.3	Proteinuria	23.8
23	M	c.419G > A	p.Arg140Gln	7.8	Proteinuria	11.6
Isolated congenital anomalies of the kidney and urinary tract						
24	M	c.686-1G > T	-	9.1	Proteinuria	12.9
25	F	c.832C > T	p.Gln278*	6.1	Proteinuria	11.6
26	F	c.1052G > T	p.Gly351Val	0.1	Proteinuria	1.6
27	M	c.206T > C	p.Leu69Pro	9.1	Proteinuria	31.2
M, male; F, female; PU, proteinuria; CKD, chronic kidney disease; USG, ultrasonography						
The reference sequence of *PAX2* gene is NM_003990.5.						

Genetic analysis

Genomic DNA was extracted from nucleated cells in the peripheral blood or buccal swab samples. All exons of the *PAX2* gene were analyzed by Sanger sequencing in patients with characteristic RCS.[25] Disease-causing genes for CAKUT, SRNS, or cystic kidney disease were selected for TES.[22–24] Exome capture kits, sequencing platforms, and reference human genome sequences were used to perform TES and WES, as previously published (Supplementary Table S3).[22–24, 26] When TES and WES revealed *PAX2* mutations, we confirmed them by Sanger sequencing of the proband and parents, when available. Variants classified as pathogenic or likely pathogenic based on the American College of Medical Genetics and Genomics guidelines were considered disease-causing mutations.[27]

Genotype-phenotype correlation

The clinical phenotypes were categorized as RCS, FSGS, or isolated CAKUT. The diagnosis of CAKUT was made based on kidney imaging studies. Additionally, RHD was defined as abnormally small kidneys with poor corticomedullary differentiation and increased renal parenchymal echogenicity upon kidney imaging, while RCS was defined as CAKUT with ocular involvement. Furthermore, FSGS was defined as compatible pathologic findings of the kidney. Ocular involvement with *PAX2* variants was defined as optic disc dysplasia (abnormal development of the optic disc, such as optic disc excavation or optic disc hypoplasia) with numerous cilioretinal vessels.[28] Fundus photographs were obtained from all patients, and an experienced ophthalmologist reviewed photographs of the fundus of both eyes of each patient. A Snellen chart was used to measure the best corrected visual acuity, which was converted to the logarithm of the minimum angle of resolution (logMAR) value for analysis. Favorable visual acuity was defined as a best visual acuity of 20/40 or more at nadir. Furthermore, visual field testing was performed using Goldmann or

Humphrey visual perimetry, and cross-sectional images of the optic disc and macular area were evaluated using spectral domain optical coherence tomography (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA, USA) when further examination was required.

To evaluate the genotype-phenotype correlation, the patients were grouped as follows: (1) truncating mutation group and missense mutation group according to mutation type in *PAX2* and (2) paired domain group and other sites group according to mutation site in PAX2. Nonsense, frameshifting, and splice site mutations were classified as truncating mutations. Additionally, we evaluated kidney and ocular outcomes according to mutation type and location in the *PAX2* gene.

Statistical analysis

SPSS version 23.0 (Armonk, NY, USA) was used for statistical analysis. Values are presented as median and interquartile range (IQR). Categorical variables were analyzed using Fisher's exact test and continuous variables were compared using the Mann-Whitney U test or Kruskal-Wallis test. The effect on long-term kidney survival rates and the onset of ocular manifestations was analyzed using Kaplan-Meier survival probability estimates and a log-rank test. $P < 0.05$ was considered statistically significant.

Results

Among a total of 27 patients, 19 were diagnosed with RCS, 4 with FSGS, and 4 with isolated CAKUT without ocular manifestations. The median onset age and age at the last follow up were 1.8 (IQR, 0.1–7.0) and 17.0 (IQR, 10.5–27.8) years, retrospectively (Table 1, Supplementary Fig. S1). The most common initial presentation was proteinuria (n = 10), followed by ocular symptoms such as nystagmus and microphthalmia (n = 7), abnormalities on ultrasonography (USG) (n = 5), and CKD (n = 5). The median age at genetic diagnosis was 12.4 (IQR, 8.8–19.0) years.

Kidney manifestations

In the 27 patients, the median age at the onset of kidney manifestations was 6.1 (IQR, 0.1–9.1) years (Table 2). At the time of kidney problem diagnosis, 22 (81.5%) of the 27 patients already had CKD, and 11 were infants. Fourteen (53.8%) patients developed kidney failure at a median age of 14.5 (95% confidence interval, 11.9–17.1) years, which was estimated by Kaplan-Meier analysis.

Table 2
Kidney manifestations in patients with *PAX2* mutations.

Case	Mutation	Onset age at kidney symptoms (years)	Kidney manifestations	USG	Biopsy	Age at CKD (years)	Age at KFRT (years)
Renal coloboma syndrome							
1	c.76dupG	12.0	PU	RHD	FSGS	12.0	12.2
2	c.76dupG	8.0	PU	RHD, horseshoe kidney	ND	8.0	17.5
3	c.76dupG	9.7	PU, CKD	RHD	FSGS	9.7	14.8
4	c.76dupG	6.6	PU	RHD	ND	6.6	8.6
5	c.76dupG	1.0	PU, CKD	RHD	ND	1.0	7.1
6	c.310C > T	1.0	PU, CKD	RHD, CC	ND	1.0	9.5
7	c.754C > T	15.0	PU, CKD	RHD	ND	15.0	NY
8	c.76dupG	0.1	CKD	RHD, CC	ND	0.1	3.2
9	c.344G > C	0.1	CKD	RHD, CC	ND	0.1	10.4
10	c.535_546delinsT	0.1	Abnormal prenatal USG	RHD, CC	ND	0.1	NY
11	c.76dupG	0.1	CKD	RHD, CC	ND	0.1	NY
12	c.76dupG	0.1	Abnormal prenatal USG	RHD, CC	ND	0.1	NY
13	c.343C > T	0.1	Abnormal prenatal USG	RHD, CC	ND	0.1	NY
14	c.860delA	10.1	PU	RHD	ND	10.1	NY
15	c.754C > T	8.5	Abnormal USG	RHD, CC	ND	10.0	NY
16	c.76dupG	0.1	Abnormal prenatal USG	RHD, CC	ND	0.1	NY
17	c.76delG	0.1	Abnormal prenatal USG	RHD, CC	ND	0.1	0.2
18	c.76dupG	10.2	CKD	RHD, CC, HNP	FSGS	10.2	12.9

USG, ultrasonography; CKD, chronic kidney disease; KFRT, kidney failure with replacement therapy; PU, proteinuria; RHD, renal hypodysplasia; CC, cortical cysts; HNP, hydronephrosis; VUR, vesicoureteral reflux; FSGS, focal segmental glomerulosclerosis; ND, not done; NY, not yet

Case	Mutation	Onset age at kidney symptoms (years)	Kidney manifestations	USG	Biopsy	Age at CKD (years)	Age at KFRT (years)
Renal coloboma syndrome							
19	c.361_373dup	4.5	PU	RHD and left VUR	ND	4.5	8.9
Focal segmental glomerulosclerosis							
20	c.76dupG	5.2	PU	No anomaly	FSGS	7.9	15.5
21	c.223_226dup	13.4	PU	No anomaly	FSGS	14.0	NY
22	c.74G > A	7.3	PU	No anomaly	FSGS	7.3	14.5
23	c.419G > A	7.8	PU	No anomaly	FSGS	10.1	NY
Isolated congenital anomalies of the kidney and urinary tract							
24	c.686-1G > T	9.1	PU	RHD	ND	9.1	NY
25	c.832C > T	6.1	PU	RHD	ND	7.0	NY
26	c.1052G > T	0.1	CKD	RHD, CC	ND	0.1	NY
27	c.206T > C	9.1	PU	RHD, CC	ND	9.1	9.7

USG, ultrasonography; CKD, chronic kidney disease; KFRT, kidney failure with replacement therapy; PU, proteinuria; RHD, renal hypodysplasia; CC, cortical cysts; HNP, hydronephrosis; VUR, vesicoureteral reflux; FSGS, focal segmental glomerulosclerosis; ND, not done; NY, not yet

Twenty-three patients with RCS and isolated CAKUT were diagnosed with hypodysplastic kidneys, cortical cysts, hydronephrosis, or horseshoe kidney on USG for the following reasons: proteinuria and/or CKD found incidentally during school screening or routine laboratory findings during hospitalization (n = 17), and abnormalities on USG through prenatal USG (n = 5) or incidental USG (n = 1). Proteinuria was found at the time of diagnosis of kidney manifestations in all RCS and isolated CAKUT patients, except in case 15, whose eGFR was 87 mL/min/m^2 at the last follow-up of 13.0 years. Kidney biopsy, conducted in three patients (cases 1, 3, and 18) at a median age of 10.2 (IQR, 10.0–12.6) years, revealed findings compatible with FSGS. All RCS and isolated CAKUT patients developed CKD during childhood at a median age of 4.5 (IQR, 0.1–9.4) years, and 11 of them required kidney replacement therapy at a median age of 15.0 (IQR, 14.5–15.5) years.

Four patients with FSGS initially presented as incidentally found asymptomatic proteinuria at school screening or routine laboratory screening at the median age of 7.6 (IQR, 6.3–10.6) years. At the time of FSGS diagnosis, there were no abnormalities on kidney USG. Additionally, proteinuria did not respond to

corticosteroids and/or calcineurin inhibitors. Kidney biopsy revealed 5.9–62.5% sclerotic glomerular lesions with tubular atrophy and interstitial fibrosis at a median age of 8.7 (IQR, 6.8–10.9).

Ocular manifestations

Visual acuity ranged from 20/20 to no light perception (Supplementary Table S1). Forty-one eyes (70.7%) had ≥ 20/40 visual acuity. Ophthalmic abnormalities, such as congenital nystagmus, congenital strabismus, microphthalmia, and amblyopia, were the first manifestations of PAX2 related disease in seven patients, while ocular involvement in the other patients was identified through a workup after the diagnosis of kidney problems.

Four (14.8%) patients had normal ocular findings, while nineteen (70.4%) patients with *PAX2* mutations had optic disc excavation and numerous cilioretinal vessels, which are the most typical ocular findings in *PAX2* related disorders. Although optic disc excavation was the most common optic disc abnormality observed, various optic disc dysplasias, such as optic disc segmental hypoplasia, peripapillary atrophy, peripapillary retinoschisis, and rudimentary optic disc, were also identified (Fig. 1). Most patients with optic disc dysplasia also demonstrated anomalous retinal vasculature, numerous cilioretinal vessels, and rudimentary central retinal vessels (Fig. 2). Interestingly, twenty-five eyes had additional ocular abnormalities combined with optic disc dysplasia as follows: retinal detachment (2 eyes), microphthalmia (1 eye), diffuse chorioretinal atrophy (7 eyes), retinal agenesis (1 eye), persistent hyperplastic primary vitreous (1 eye), retinoschisis (5 eyes), visual field defect (6 eyes), and corneal dystrophy (2 eyes) (Fig. 2).

Genotype-phenotype correlation

Fourteen different *PAX2* mutations were detected, including 11 known mutations and 3 novel mutations (Supplementary Fig. S2). Mutation screening of the parents was performed in 13 patients. While three cases (cases 15, 24, and 26) inherited their *PAX2* mutations from one of their parents, who were symptomatic with kidney and/or ocular manifestations, eight patients were confirmed to have *de novo* mutations. In the remaining two patients, the familial cases 1 and 2, mutations were not detected in their parents, suggesting the presence of germline mosaicism. [25] Additionally, none of the 14 patients without family screening had a positive family history of *PAX2* mutation-related phenotypes.

The c.76dupG mutation was common in 11 (40.7%) patients: 10 with RCS and 1 with FSGS. Twenty (74.1%) patients had mutations in the paired domain of *PAX2*, while seven patients had mutations at other sites (two in the homeodomain, one in the transactivation domain, and four in non-functional sites). There were no statistical differences in sex, age at onset, initial presentation, age at kidney manifestations, and developmental delay between the truncating mutation group (n = 22) and the missense mutation group (n = 5) (Table 3). While 18/22 (81.8%) patients with truncating mutations presented with RCS, 4/5 (80.0%) patients with missense mutations presented with FSGS (n = 2) or isolated CAKUT (n = 2) (P = 0.03). Kidney survival did not differ between the truncating and missense mutation groups (Fig. 3A, log-rank test, P = 0.40). In contrast, patients with paired domain mutations progressed more rapidly than those with other site mutations (Fig. 3B, log-rank test, P = 0.03).

Table 3
Comparison of patient phenotypes according to the genotype of *PAX2* mutations

	Truncating (n = 22)	Missense (n = 5)	*P* value
Sex, male:female	16:6	3:2	0.62
Age at onset, years	1.8 (0.1–6.6)	7.0 (0.1–7.8)	0.73
Age at genetic diagnosis, years	12.4 (8.8–16.5)	19.0 (10.9–23.1)	0.50
Age at the last follow up, years	17.0 (11.4–29.4)	23.8 (11.6–24.4)	0.88
Initial manifestations			0.28
Ocular symptoms	7 (31.8)	0	
Kidney symptoms	15 (68.2)	5 (100.0)	
Clinical phenotype			0.03
Renal coloboma syndrome	18 (81.8)	1 (20.0)	
Focal segmental glomerulosclerosis	2 (9.1)	2 (40.0)	
Isolated CAKUT	2 (9.1)	2 (40.0)	
Developmental delay	4 (18.2)	0	0.56
Kidney manifestations			
Age at kidney manifestations, years	5.7 (0.1–9.7)	7.0 (0.1–7.8)	0.68
Proteinuria at initial diagnosis	21 (95.5)	5 (100.0)	0.9
CKD at initial diagnosis	18 (81.8)	4 (80.0)	0.9
Median age at the onset of CKD, years (95% CI)[a]	6.6 (0.0–14.5)	5.1 (0.0-15.8)	0.27
Median age at kidney failure, years (95% CI)[a]	14.8 (10.1–19.5)	10.4 (7.3–13.5)	0.40

Values are expressed as numbers (%) and medians (interquartile range), except for visual acuity.

CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; CI, confidence interval; LP, light perception.

[a]It is estimated by Kaplan-Meier analysis.

[b]Ocular manifestations were analyzed separately in both eyes of patients.

[c]Case 15 could not count cilioretinal vessels due to severe retinal anomalies.

[d]Snellen visual acuity was observed in 42 and 10 eyes in the truncating and missense groups, respectively.

	Truncating (n = 22)	Missense (n = 5)	*P* value
Ocular manifestations[b]	44 eyes	10 eyes	
Optic disc anomaly			< 0.001
Anomalous optic disc	42 (95.5)	1 (10.0)	
Normal optic disc	2 (4.5)	9 (90.0)	
Central retinal artery			0.02
Absence	30 (68.2)	2 (20.0)	
Partial	6 (13.6)	3 (30.0)	
Complete	8 (18.2)	5 (50.0)	
Number of cilioretinal vessel	8.0 (10–7.5)[c]	7.5 (8.0–6.2)	0.05
Presence of retinopathy	16 (38.1)	0 (0.0)	0.01
Visual acuity[d]**, mean (maximum-minimum)**	20/22.5 (20/20–LP)	20/20 (20/20–20/30)	0.04
Visual outcome ≥ 20/40[d]	31/42 (72.5)	10/10 (100)	0.10
Median age at the diagnosis of ocular involvement, years (95% CI)[a]	7.2 (2.3–12.1)	21.4 (18.2–24.6)	0.04

Values are expressed as numbers (%) and medians (interquartile range), except for visual acuity.

CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; CI, confidence interval; LP, light perception.

[a]It is estimated by Kaplan-Meier analysis.

[b]Ocular manifestations were analyzed separately in both eyes of patients.

[c]Case 15 could not count cilioretinal vessels due to severe retinal anomalies.

[d]Snellen visual acuity was observed in 42 and 10 eyes in the truncating and missense groups, respectively.

Severe optic disc anomaly, the presence of absence of a central retinal vasculature, and the coexisting retinopathy were significantly more common in the truncating mutation group than in the missense mutation group (Table 3). Visual acuity was lower in the truncating mutation group than that in the missense mutation group. Furthermore, tathe truncating mutation group was diagnosed with ocular manifestations significantly earlier than the missense mutation group (Fig. 4A, log-rank test, $P = 0.04$).

However, there were no statistical differences in the age at diagnosis of ocular problems according to the mutation sites of the *PAX2* gene (Fig. 4B, log-rank test, $P = 0.58$).

Discussion

To our knowledge, this is the first study to evaluate the genotype-phenotype correlation in patients with *PAX2* mutations, using longitudinal outcome data and detailed ocular examination. Their clinical phenotypes were diverse, with overlapping kidney and ocular manifestations. We found that truncating mutations in *PAX2* were associated with ocular involvement and the severity of this problem. Additionally, kidney outcomes correlated with the mutation site in the *PAX2* gene.

The PAX2 transcription factor plays a critical role in optic nerve differentiation during eye development. Since Savell and Cook's report on "isolated colobomas" [29], some terminologies of this disease have been confused. Investigations by Parsa et al have confirmed that many individuals in the family described by Savell and Cook may have suffered from optic disc excavation instead of optic disc coloboma.[28] They suggested a change of terminology from renal-coloboma syndrome to papillorenal syndrome.[28, 30] Our study demonstrated that optic disc excavation with numerous cilioretinal vessels was the most common finding of *PAX2*-related disorders. In addition, these patients can also have various ocular abnormalities beyond optic disc anomalies, such as retinal detachment, retinoschisis, chorioretinal atrophy, corneal dystrophy, and microphthalmia. However, these terms to date fall short of an inclusive definition due to no evidence of optic disc coloboma and the ocular abnormalities were not limited to the optic.[28] Therefore, we suggest developing more inclusive terminology for renal-ocular abnormalities related to *PAX2* variants.

There was an association between the severity of ocular abnormalities, visual acuity, and the genotype of the *PAX2* variant. To date, genetic analysis of *PAX2*-related disorders has not shown a relationship between mutation type and ocular involvement.[17, 18, 31] In this study, patients with FSGS caused by truncating mutations in *PAX2* had optic nerve abnormalities, as well as those with RCS. This result is in line with previous studies reporting that ocular abnormalities were observed in patients with FSGS caused by truncating mutations of *PAX2*, but not in patients with FSGS caused by missense mutations of *PAX2*.[9–11] Patients with truncating mutations in the *PAX2* gene were diagnosed with ocular involvement significantly earlier than patients with missense mutations. Additionally, the visual outcome was worse in the truncating mutation group than in the missense mutation group.

In our study, 24/27 (88.9%) patients with *PAX2* mutations had optic disc abnormalities before or after kidney manifestations, which is similar to a previous report.[31] Seven patients initially visited hospitals with eye-related symptoms, while others had subtle changes only noted after a detailed funduscopic examination. Some cases of retinal complications occurred during the follow-up period. Therefore, it is necessary to have periodic eye examinations, it can be helpful to prevent further vision impairment. In addition, typical ocular manifestations could provide useful clues for differential diagnosis in pediatric patients with SRNS/FSGS or CAKUT. [32, 33] In contrast, in this study, some patients underwent detailed fundus examination after identifying genetic abnormalities, similar to previous studies.[34, 35] Recently,

genetic screening using TES or WES has become widely used in clinical practice. It is possible to refine phenotypes based on genetic marker data, which is called reverse phenotyping.[36, 37] This approach can provide the correct diagnosis and motivate surveillance for previously unrecognized clinical manifestations and potential future complications. In addition, *PAX2* mutations were detected in recent studies that performed genetic diagnoses of patients with CKD of unknown origin.[19, 21]

Interestingly, *PAX2* mutations could result in both glomerulopathy and congenital kidney anomalies.[35] All of our patients with RCS and isolated RHD, except one (case 15), had proteinuria when their sonographic abnormalities were discovered. Additionally, in three patients with RCS, the kidney pathologic findings showed glomerulosclerosis. Previous studies showed that some RCS patients who underwent kidney biopsy exhibited FSGS.[10, 17] Moreover, Deng et al. revealed that all patients with *PAX2* mutations had both RHD and proteinuria, and 6/10 patients had nephrotic-range proteinuria.[17] These findings suggest that CAKUT caused by *PAX2* mutations might be combined with glomerulosclerosis, which develops proteinuria and facilitates CKD progression. A genetic analysis in CAKUT patients showed that patients with *PAX2* mutations developed kidney failure more rapidly than those with other gene mutations.[7] In this study, most patients with RCS and isolated CAKUT had CKD at initial renal presentation and progressed to kidney failure more rapidly compared to general CAKUT patients, for which a previous study reported a median of 31 years.[38] It could be associated with proteinuria, which is an independent risk factor for CKD progression in patients with non-glomerulopathy.[39] Therefore, patients with CAKUT and proteinuria need to check for *PAX2* mutations and undergo ophthalmologic examinations, especially when accompanied by childhood-onset CKD.

In this study, the timing of kidney replacement therapy was associated with the mutation location in the *PAX2* gene, but not with mutation types. Previous studies have not found any differences in kidney outcomes according to genetic variations.[17, 18, 31] However, a recently published study, which reviewed 234 reported cases with *PAX2* mutations, showed that most *PAX2* missense variants in the paired domain are highly associated with kidney and ocular development.[40] The PAX2 protein is an essential transcriptional factor for kidney development, and the paired domain of *PAX2* is a highly conserved DNA-binding domain which imparts positive or negative transcriptional regulation, depending on the cellular context and the availability of cofactors.[41] PAX2 also potentially plays a role in kidney repair and regeneration after acute kidney injury.[42] A study using induced pluripotent stem cells in patients with mutations in the paired domain of *PAX2* identified three genes (*PBX2*, *POSTN*, and *ITGA9*) that are regulated by *PAX2*.[43] Among these, *POSTN* is a mediator of the mechanism controlling kidney repair following AKI.[44] Therefore, mutations affecting the paired domain in the *PAX2* gene may be related to CKD progression, with the low ability of kidney repair caused by the loss of stability of the domain.

Our study had several limitations. First, the study population was relatively small for general conclusions. Second, trio samples were not available for all patients; therefore, the assessment of penetrance or segregation was insufficient. Nonetheless, to our knowledge, this is the first report which performed a genotype-phenotype analysis in patients with *PAX2* mutations, as well as long-term clinical outcomes in

this patient cohort. In addition, detailed ophthalmological problems were found in patients with *PAX2* mutations, which explains the development of ophthalmic abnormalities.

Conclusions

The clinical phenotypes of *PAX2* mutations, including RCS, FSGS, and isolated CAKUT, are heterogeneous. While truncating mutations of *PAX2* were associated with severe ocular involvement not confined to the optic disc, mutations in the paired domain were related to poor long-term kidney outcomes.

Declarations

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualisation: H.I.C. and Y.H.A.; formal analysis: Y.H.A. and J.H.J.; funding acquisition: H.G.K.; investigation: J.H.K., Y.J., E.P., S.H.K. and J.Y.S.; methodology and writing (original draft): J.H.K., Y.H.A., and J.H.J.; resources: J.H.K., Y.J., H.L., K.H.H., J.J., J.H.L., H.G.K., Y.H.A. and J.H.J.; supervision: Y.H.A., J.H.J. and H.I.C.; writing (review and editing): J.H.K., Y.H.A., Y.J., E.P., H.L., S.H.K., J.Y.S., K.H.H., J.J., J.H.L., H.G.K., J.H.J. and H.I.C.; guarantor: Y.H.A. and J.H.J. All authors reviewed the manuscript.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020R1A2C1100974 to H.G.K.), a cooperative research fund from the Korean Nephrology Research Foundation (2021), and grants 2019-ER7304-00, 2019-ER7304-01, 2019-ER7304-02, and 2022-ER0703-00 to H.G.K. awarded by the Korea Disease Control and Prevention Agency.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Additional information

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Weaver RG, Cashwell LF, Lorentz W, Whiteman D, Geisinger KR, Ball M. Optic nerve coloboma associated with renal disease. Am J Med Genet. 1988;29(3): 597–605.
2. Nishimoto K, Iijima K, Shirakawa T, et al. PAX2 gene mutation in a family with isolated renal hypoplasia. J Am Soc Nephrol. 2001;12(8): 1769–1772.
3. Benetti E, Artifoni L, Salviati L, et al. Renal hypoplasia without optic coloboma associated with PAX2 gene deletion. Nephrol Dial Transplant. 2007;22(7): 2076–2078.
4. Sanyanusin P, Schimmenti LA, McNoe LA, et al. Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. Nat Genet. 1995;9(4): 358–364.
5. Thomas R, Sanna-Cherchi S, Warady BA, Furth SL, Kaskel FJ, Gharavi AG. HNF1B and PAX2 mutations are a common cause of renal hypodysplasia in the CKiD cohort. Pediatr Nephrol. 2011;26(6): 897–903.
6. Hwang DY, Dworschak GC, Kohl S, et al. Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract. Kidney Int. 2014;85(6): 1429–1433.
7. Ishiwa S, Sato M, Morisada N, et al. Association between the clinical presentation of congenital anomalies of the kidney and urinary tract (CAKUT) and gene mutations: an analysis of 66 patients at a single institution. Pediatr Nephrol. 2019;34(8): 1457–1464.
8. Nicolaou N, Pulit SL, Nijman IJ, et al. Prioritization and burden analysis of rare variants in 208 candidate genes suggest they do not play a major role in CAKUT. Kidney Int. 2016;89(2): 476–486.
9. Barua M, Stellacci E, Stella L, et al. Mutations in PAX2 associate with adult-onset FSGS. J Am Soc Nephrol. 2014;25(9): 1942–1953.
10. Okumura T, Furuichi K, Higashide T, et al. Association of PAX2 and Other Gene Mutations with the Clinical Manifestations of Renal Coloboma Syndrome. PLoS One. 2015;10(11): e0142843.
11. Vivante A, Chacham OS, Shril S, et al. Dominant PAX2 mutations may cause steroid-resistant nephrotic syndrome and FSGS in children. Pediatr Nephrol. 2019;34(9): 1607–1613.
12. Porteous S, Torban E, Cho NP, et al. Primary renal hypoplasia in humans and mice with PAX2 mutations: evidence of increased apoptosis in fetal kidneys of Pax2(1Neu) +/- mutant mice. Hum Mol Genet. 2000;9(1): 1–11.
13. Dziarmaga A, Clark P, Stayner C, et al. Ureteric bud apoptosis and renal hypoplasia in transgenic PAX2-Bax fetal mice mimics the renal-coloboma syndrome. J Am Soc Nephrol. 2003;14(11): 2767–2774.
14. Dziarmaga A, Eccles M, Goodyer P. Suppression of ureteric bud apoptosis rescues nephron endowment and adult renal function in Pax2 mutant mice. J Am Soc Nephrol. 2006;17(6): 1568–1575.
15. Yang Y, Jeanpierre C, Dressler GR, Lacoste M, Niaudet P, Gubler MC. WT1 and PAX-2 podocyte expression in Denys-Drash syndrome and isolated diffuse mesangial sclerosis. Am J Pathol. 1999;154(1): 181–192.
16. Wagner KD, Wagner N, Guo JK, et al. An inducible mouse model for PAX2-dependent glomerular disease: insights into a complex pathogenesis. Curr Biol. 2006;16(8): 793–800.
17. Deng H, Zhang Y, Xiao H, et al. Diverse phenotypes in children with PAX2-related disorder. Mol Genet Genomic Med. 2019;7(6): e701.

18. Rossanti R, Morisada N, Nozu K, et al. Clinical and genetic variability of PAX2-related disorder in the Japanese population. J Hum Genet. 2020;65(6): 541–549.
19. Ottlewski I, Munch J, Wagner T, et al. Value of renal gene panel diagnostics in adults waiting for kidney transplantation due to undetermined end-stage renal disease. Kidney Int. 2019;96(1): 222–230.
20. Bullich G, Domingo-Gallego A, Vargas I, et al. A kidney-disease gene panel allows a comprehensive genetic diagnosis of cystic and glomerular inherited kidney diseases. Kidney Int. 2018;94(2): 363–371.
21. Hays T, Groopman EE, Gharavi AG. Genetic testing for kidney disease of unknown etiology. Kidney Int. 2020;98(3): 590–600.
22. Ahn YH, Lee C, Kim NKD, et al. Targeted Exome Sequencing Provided Comprehensive Genetic Diagnosis of Congenital Anomalies of the Kidney and Urinary Tract. J Clin Med. 2020;9(3): 751.
23. 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(6): 2013.
24. Park HC, Ryu H, Kim YC, et al. Genetic identification of inherited cystic kidney diseases for implementing precision medicine: a study protocol for a 3-year prospective multicenter cohort study. BMC Nephrol. 2021;22(1): 2.
25. Cheong HI, Cho HY, Kim JH, Yu YS, Ha IS, Choi Y. A clinico-genetic study of renal coloboma syndrome in children. Pediatr Nephrol. 2007;22(9): 1283–1289.
26. Jung J, Lee JH, Park YS, et al. Ultra-rare renal diseases diagnosed with whole-exome sequencing: Utility in diagnosis and management. BMC Med Genomics. 2021;14(1): 177.
27. 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(5): 405–424.
28. Parsa CF, Silva ED, Sundin OH, et al. Redefining papillorenal syndrome: an underdiagnosed cause of ocular and renal morbidity. Ophthalmology. 2001;108(4): 738–749.
29. Savell J, Cook JR. Optic nerve colobomas of autosomal-dominant heredity. Arch Ophthalmol. 1976;94(3): 395–400.
30. Parsa CF, Parsa A. Diagnosing papillorenal syndrome: see the optic papilla. Pediatr Nephrol. 2008;23(10): 1893–1894.
31. Bower M, Salomon R, Allanson J, et al. Update of PAX2 mutations in renal coloboma syndrome and establishment of a locus-specific database. Hum Mutat. 2012;33(3): 457–466.
32. Izzedine H, Bodaghi B, Launay-Vacher V, Deray G. Eye and kidney: from clinical findings to genetic explanations. J Am Soc Nephrol. 2003;14(2): 516–529.
33. Preston R, Stuart HM, Lennon R. Genetic testing in steroid-resistant nephrotic syndrome: why, who, when and how? Pediatr Nephrol. 2019;34(2): 195–210.
34. Bekheirnia MR, Bekheirnia N, Bainbridge MN, et al. Whole-exome sequencing in the molecular diagnosis of individuals with congenital anomalies of the kidney and urinary tract and identification of a new causative gene. Genet Med. 2017;19(4): 412–420.

35. Iwafuchi Y, Morioka T, Morita T, et al. Diverse Renal Phenotypes Observed in a Single Family with a Genetic Mutation in Paired Box Protein 2. Case Rep Nephrol Dial. 2016;6(1): 61–69.
36. Schulze TG, McMahon FJ. Defining the phenotype in human genetic studies: forward genetics and reverse phenotyping. Hum Hered. 2004;58(3–4): 131–138.
37. Landini S, Mazzinghi B, Becherucci F, et al. Reverse Phenotyping after Whole-Exome Sequencing in Steroid-Resistant Nephrotic Syndrome. Clin J Am Soc Nephrol. 2020;15(1): 89–100.
38. Wuhl E, van Stralen KJ, Verrina E, et al. Timing and outcome of renal replacement therapy in patients with congenital malformations of the kidney and urinary tract. Clin J Am Soc Nephrol. 2013;8(1): 67–74.
39. Warady BA, Abraham AG, Schwartz GJ, et al. Predictors of Rapid Progression of Glomerular and Nonglomerular Kidney Disease in Children and Adolescents: The Chronic Kidney Disease in Children (CKiD) Cohort. Am J Kidney Dis. 2015;65(6): 878–888.
40. Yang X, Li Y, Fang Y, et al. Phenotypic spectrum and genetics of PAX2-related disorder in the Chinese cohort. BMC Med Genomics. 2021;14(1): 250.
41. Grimley E, Dressler GR. Are Pax proteins potential therapeutic targets in kidney disease and cancer? Kidney Int. 2018;94(2): 259–267.
42. Maeshima A, Maeshima K, Nojima Y, Kojima I. Involvement of Pax-2 in the action of activin A on tubular cell regeneration. J Am Soc Nephrol. 2002;13(12): 2850–2859.
43. Yamamura Y, Furuichi K, Murakawa Y, et al. Identification of candidate PAX2-regulated genes implicated in human kidney development. Sci Rep. 2021;11(1): 9123.
44. Kormann R, Kavvadas P, Placier S, et al. Periostin Promotes Cell Proliferation and Macrophage Polarization to Drive Repair after AKI. J Am Soc Nephrol. 2020;31(1): 85–100.

Figures

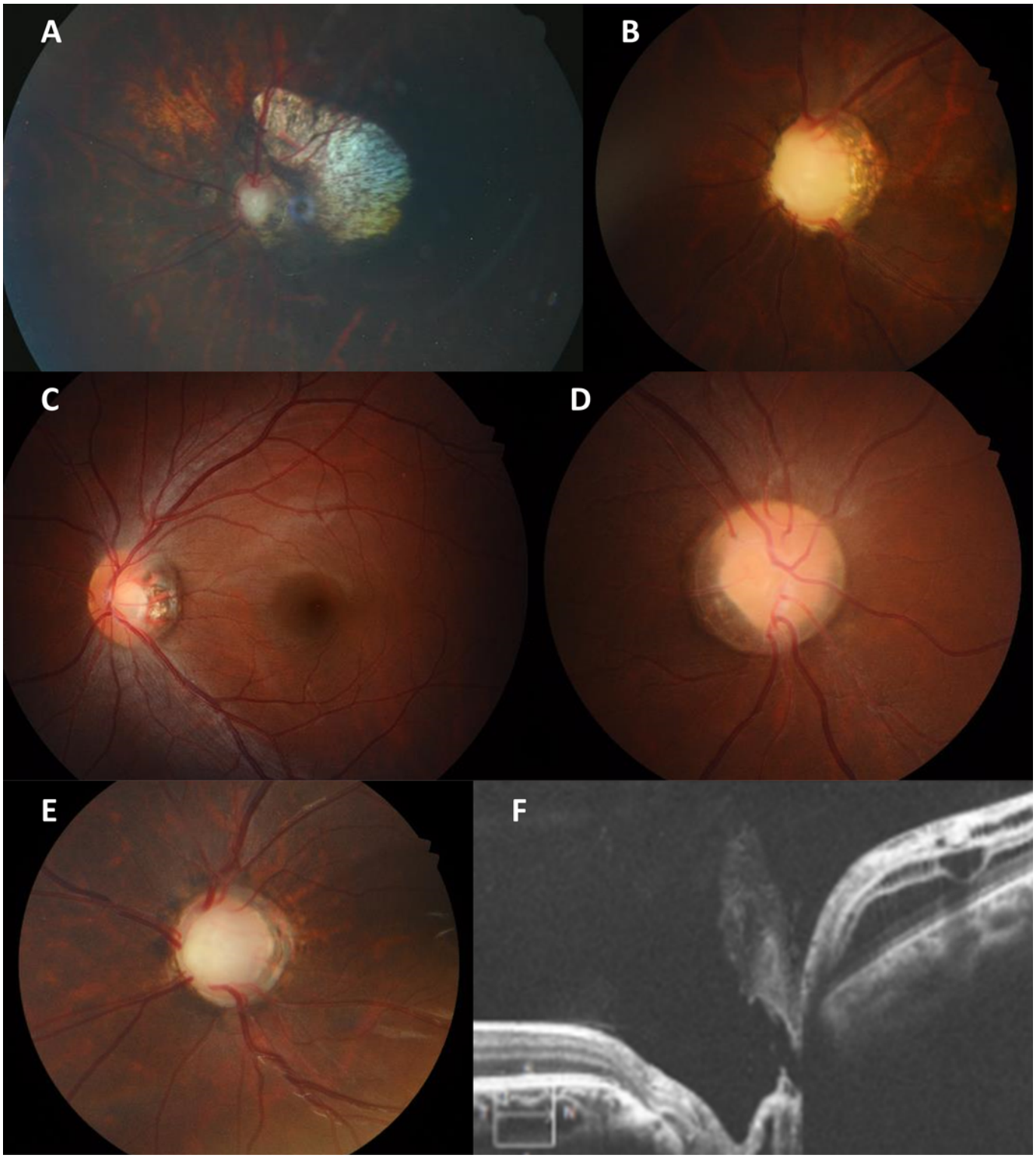

Figure 1

Optic disc abnormalities in patients with *PAX2* mutations

(A) Hypoplastic optic disc with peripapillary atrophy in case 3; (B) excavated optic disc with numerous cilioretinal vessels and absence of a central retinal vessel in case 1; (C) segmental hypoplasia of the optic disc in case 21; (D) optic disc segmental hypoplasia and numerous cilioretinal vessels in case 20; (E)

peripapillary retinoschisis and vitreous strands on the optic disc in case 6; (F) vitreous strands on optic disc in case 21.

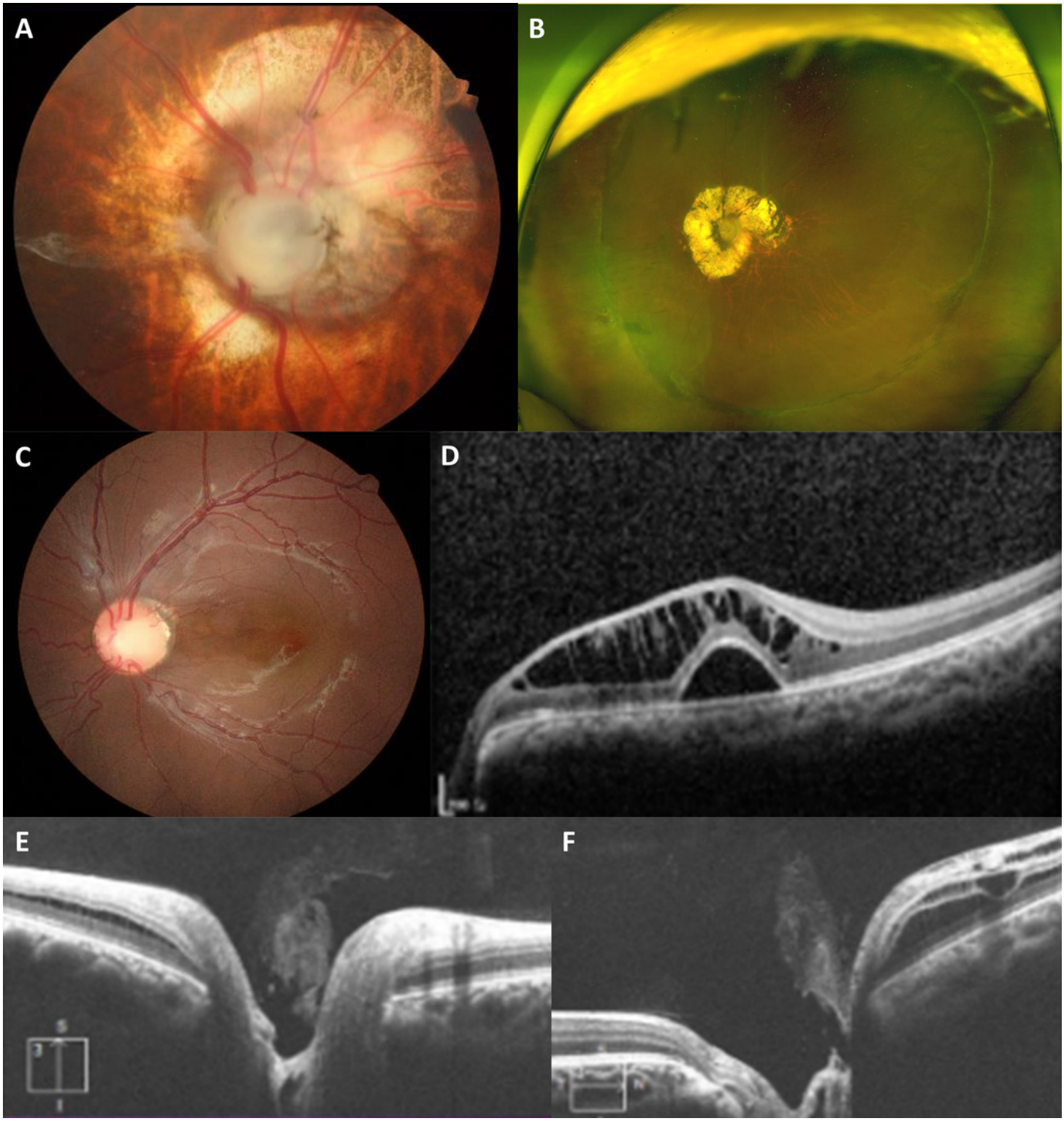

Figure 2

Retinal abnormalities in patients with *PAX2* mutations

(A) Severe chorioretinal atrophy with optic disc dysplasia in case 6; (B) post encircling surgery state due to retinal detachment in case 2; (C) retinoschisis on the macular area in case 11; (D) optical coherence tomography image of retinoschisis and subfoveal retinal detachment in case 11; (E, F) peripapillary retinal schitic change in case 21.

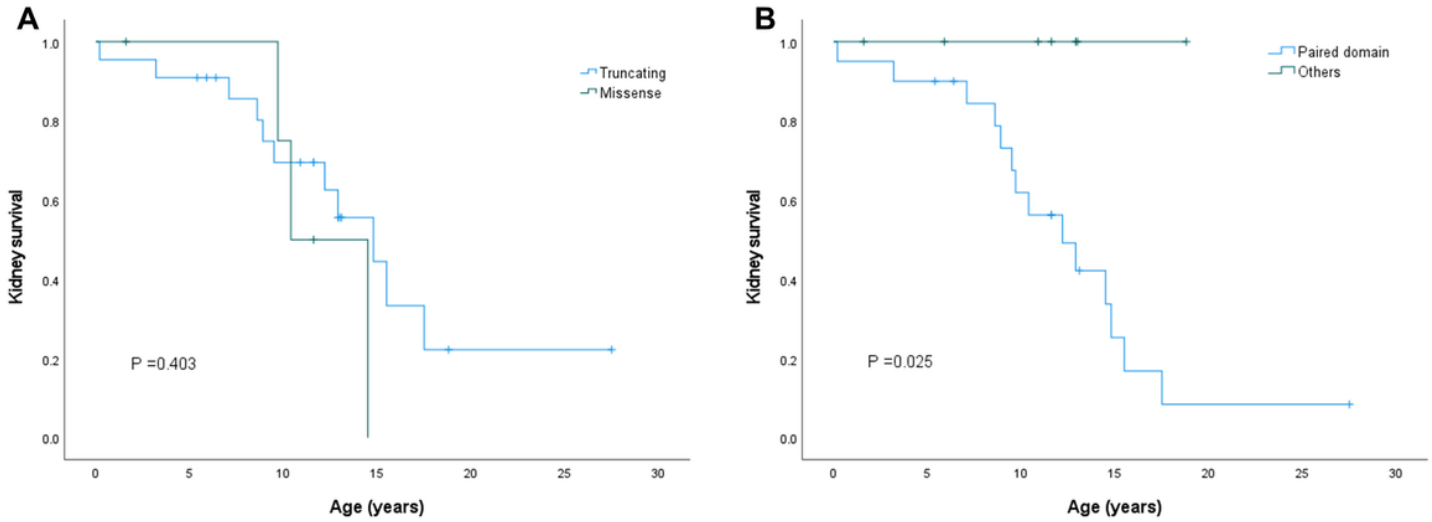

Figure 3

A) Kidney survival analysis by mutation type in the *PAX2* gene. B) Kidney survival analysis by mutation site in the *PAX2* gene.

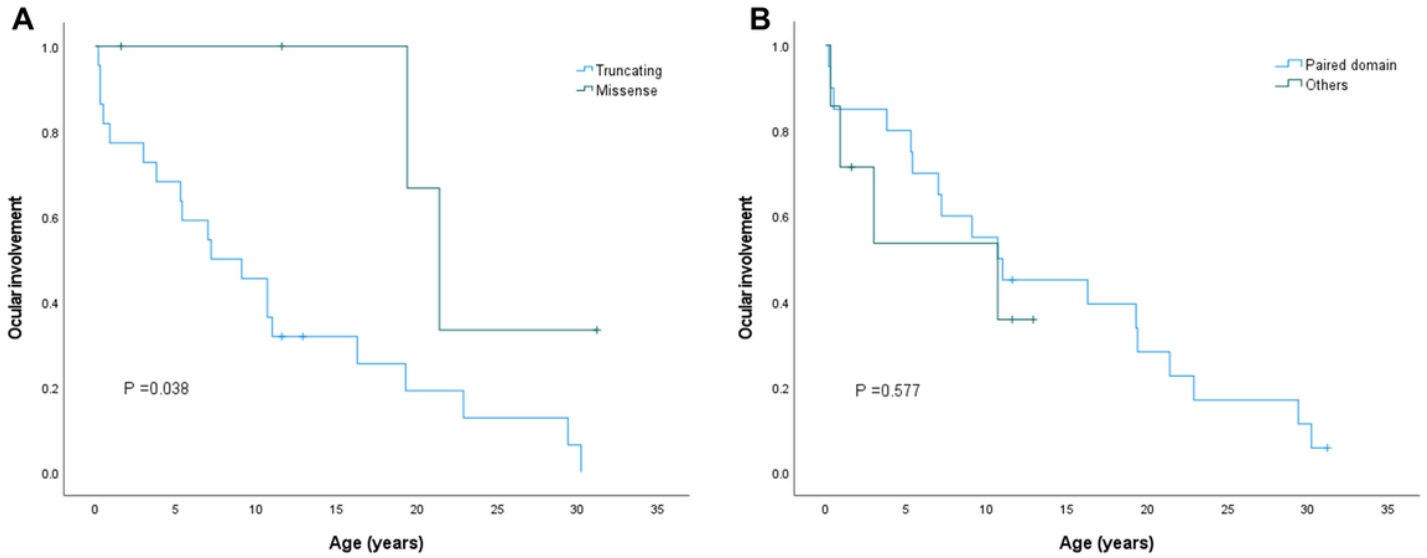

Figure 4

A) Ocular involvement-free survival analysis by mutation type in the *PAX2*gene. B) Ocular involvement-free survival analysis by mutation site in the *PAX2* gene.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarymaterialSci.Reportsfinal.pdf