Clinical and genetic variability of PAX2-related disorder in the Japanese population

Rossanti, Rini ; Morisada, Naoya ; Nozu, Kandai ; Kamei, Koichi ; Horinouchi, Tomoko ; Yamamura, Tomohiko ; Minamikawa, Shogo ; Fujimura…

(Citation)
Journal of Human Genetics,65:541-549

(Issue Date)
2020-06

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90007069

[Article]

Clinical and genetic variability of *PAX2*-related disorder in the Japanese population

Rini Rossanti[1], Naoya Morisada[1,2], Kandai Nozu[1], Koichi Kamei[3], Tomoko Horinouchi[1], Tomohiko Yamamura[1], Shogo Minamikawa[1,4], Junya Fujimura[1,5], China Nagano[1], Nana Sakakibara[1], Takeshi Ninchoji[1], Hiroshi Kaito[1,6], Shuichi Ito[3,7], Ryojiro Tanaka[4,6], Kazumoto Iijima[1]

[1] Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan

[2] Department of Clinical Genetics, Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan, 1-6-7, Minatojimaminami-machi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan

[3] Division of Nephrology and Rheumatology, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan

[4] Department of Emergency and General Medicine, Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan, 1-6-7, Minatojimaminami-machi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan

[5] Department of Pediatrics, Kakogawa City Hospital, Kakogawa, Japan, 439, Honmachi, Kakogawa, Hyogo, 675-8611, Japan

[6] Department of Nephrology, Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan, 1-6-7, Minatojimaminami-machi, Chuo-ku, Kobe, Hyogo, 650-0047, Japan

[7] Department of Pediatrics, Yokohama City University, 3-9, Fukuura, Kanazawa-ku, Yokohama, 236-0004, Japan

Corresponding author: Naoya Morisada, M.D., Ph.D.,
Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo, Kobe, Hyogo 6500017, Japan.
Fax: +81-78-382-6099; Tel.:+81-78-382-6090
E-mail: morisada@med.kobe-u.ac.jp

Conflict of interest K.I. has received grant support from Daiichi Sankyo Co., Ltd. and Zenyaku Kogyo Co., Ltd.

Abstract

Pathogenic variants of paired box gene 2 (*PAX2*) cause autosomal dominant *PAX2*-related disorder, which includes renal coloboma syndrome (RCS). Patients with *PAX2*-related disorder present with renal and ophthalmological pathologies, as well as with other abnormalities, including developmental problems and hearing loss. We sequenced *PAX2* in 457 patients with congenital anomalies of the kidney and urinary tract or with renal dysfunction of unknown cause and identified 19 different pathogenic variants in 38 patients from 30 families (6.5%). Thirty-four patients had renal hypodysplasia or chronic kidney disease of unknown cause, and three had focal segmental glomerulosclerosis. Although no obvious genotype–phenotype correlation was observed, six of the seven patients who developed end-stage renal disease in childhood had truncating variants. Twenty-three patients had ocular disabilities, mostly optic disc coloboma. Non-renal and non-ophthalmological manifestations included developmental disorder, electrolyte abnormality, and gonadal abnormalities. Two unrelated patients had congenital cystic adenomatoid malformations in their lungs. Six of ten probands with *PAX2* mutation identified by next-generation sequencing did not show typical RCS manifestations. We conclude that *PAX2*-related disorder has a variable clinical presentation and can be diagnosed by next-generation sequencing even in the absence of typical RCS manifestations.

Keywords: *PAX2*-related disorder, renal coloboma syndrome, next-generation sequencing, congenital cystic adenomatoid malformation

Introduction

Autosomal dominant *PAX2*-related disorder is caused by the pathogenic variants of paired box gene 2 (*PAX2*).[1] These variants were first identified in 1995 in a family with an autosomal-dominant syndrome characterized by optic nerve abnormalities, renal hypoplasia, mild proteinuria, and vesicoureteral reflux.[2, 3] *PAX2*-related disorder is often referred to as renal coloboma syndrome (RCS) or papillorenal syndrome (OMIM #120330). *PAX* genes derive their name from the paired box DNA sequence motif, a conserved 128 amino acid domain in the amino-terminal portion of the protein.[4] Nine members of the *PAX* gene family have been described in humans, and they have been divided into four groups, depending upon the presence or absence of the following four domains: paired domain, octapeptide domain, homeodomain, and transactivation domain.

Sequencing and restriction mapping of these clones showed that human *PAX2*, which is located on 10q24.31, is composed of 11 exons, spanning approximately 70 kb.[5] *PAX2* has a crucial role in kidney development[6,7] and is also expressed in the optic vesicle and, later, in the retina.[8] More than 90% of patients with *PAX2*-related disorder show various renal problems, including renal hypodysplasia (RHD), focal segmental glomerulosclerosis (FSGS), and vesicoureteral reflux (VUR).[9] Ophthalmological abnormalities, mainly optic disc coloboma, are often present in patients with *PAX2*-related disorder. *PAX2* encodes a transcription factor that mediates the development of the kidneys, eyes, ears, and genital tract;[10] thus, *PAX2*-related disorder may have non-renal and non-ophthalmological symptoms, such as sensorineural hearing loss, neurodevelopmental disorders, soft skin, and joint laxity.[9] Recently, skeletal deformity, congenital heart defect, bone anomalies, including metatarsal macrosomia, have also

been reported.[11] However, the details of clinical phenotypes and their relationship to genotypes in *PAX2*-related disorder are still unknown.

In this study, we analyzed *PAX2* pathogenic variants in patients clinically diagnosed with congenital anomalies of the kidney and urinary tract (CAKUT), cystic kidneys, or renal dysfunction of unknown cause with or without any ocular abnormalities. We used direct sequencing, next-generation sequencing (NGS), and array comparative genomic hybridization to correlate the symptoms of *PAX2*-related disorder with clinical manifestations in the Japanese population.

Methods

Subjects

We analyzed the *PAX2* sequence for patients at Japanese hospitals with clinically diagnosed renal disorders with few abnormalities on urinalysis; CAKUT, polycystic kidney disease, or renal dysfunction of unknown cause in the period from September 2010 to May 2019. Patients with nephrotic syndrome or massive hematuria were excluded. The definition of CAKUT includes RHD, multicystic dysplastic kidney (MCDK), hydronephrosis, VUR, or renal agenesis. Details regarding the renal disease and other clinical features were obtained from the referring clinician or from the hospital records of patients.

Ethical considerations

All procedures involving human participants performed in this study were in accordance with the ethical standards of the Institutional Review Board of the Kobe University Graduate School of Medicine (IRB approvals No. 65 and No. 301) and with the 1964

Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patients or their parents.

Genetic analysis

DNA was isolated from a peripheral blood sample using a QuickGene Mini 80 system (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) in accordance with the manufacturer's instructions. Direct sequencing or targeted sequencing using NGS of genes responsible for the inherited disease was performed. For NGS, we used a HaloPlex (Agilent Technologies, Santa Clara, CA) or a TruSight One target enrichment system kit (Illumina, San Diego, CA) in accordance with the manufacturer's instructions and sequencing was performed using MiSeq platform (Illumina). HaloPlex was used for targeted sequencing of 91 (version 1, Supplementary Table 1), 172 (version 3, Supplementary Table 2), 159 (version 5, Supplementary Table 3), and 181 genes (version 7, Supplementary Table 4) associated with CAKUT or cystic kidneys as cataloged in OMIM (http://www.omim.org/) or PubMed (http://www.ncbi.nlm.nih.gov/pubmed) databases.

Data were analyzed by SureCall 4.0 (Agilent Technologies), a software for end-to-end NGS data analysis. cDNA reference number of *PAX2* in this study was NM_003987.4. Pathogenicity predictions were performed in accordance with the American College of Medical Genetics (ACMG) guidelines.[12] Several websites, including CADD (https://cadd.gs.washington.edu/), PROVEAN (http://provean.jcvi.org/index.php), SIFT (https://sift.bii.a-star.edu.sg/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.pathogenic varianttaster.org/) were used to predict variant pathogenicity (Supplementary Table 5).

Pair analysis by SureCall was used to ascertain changes in copy number relative to a reference. The changes in copy number were confirmed by aCGH.[13]

Statistical analysis

JMP version 10 for Windows (SAS Institute, Cary, NC) was used for statistical analysis. Data are represented as the median and confidence interval. Statistical analyses were performed using the Kruskal–Wallis test and by calculating Pearson's correlation coefficient (r). All statistical analyses were conducted with a significance level of $\alpha = 0.05$ ($P < 0.05$).

Results

Characteristics of patients

We analyzed 457 probands and their relatives (Supplementary Table 6). Twenty-five probands were analyzed only by direct sequencing, and 432 were analyzed by NGS. We identified the causative genes for CAKUT (such as *HNF1B*,[14] *EYA1*,[15] and *SALL1*), nephronophthisis (such as *WDR19*[16] and *OFD1*[17]), and polycystic kidney disease, in 161 probands. *PAX2* pathogenic variants were identified in 38 patients from 30 families (18 familial cases from 10 families and 20 sporadic cases) in 457 probands (6.5%). Nineteen probands were identified only by direct sequencing, and 11 were identified by NGS. The 19 patients identified by direct sequencing showed the typical RCS phenotype except for two patients (SC149, SC549). None of the patients with pathogenic *PAX2* variant had any other renal disease related to the causative gene mutations. The age at which the patients were diagnosed ranged between 1 month and

54 years, with the male/female ratio being 10:9 (Table 1). The detailed phenotypes and genotypes of the patients are shown in Table 2.

Functional abnormalities and underlying renal disease

Most cases, except for the patient's family member (mother of SC472) identified by the segregation analysis, had chronic kidney disease of unknown cause with renal insufficiency (Tables 1, 2). Eleven patients were considered to have RHD and 23 patients presented with renal insufficiency of unknown cause. Five patients had cystic kidneys, and one (SC30) had MCDK. VUR was found in two cases (SC15 sister and SC149). Bilateral hydronephrosis with renal dysfunction was confirmed in one case (SC274). Three cases (SC56 father, SC111, and SC521) presented with FSGS as a pathological finding. Seven patients (SC30, SC56 and his brother, SC287, SC315, SC415, and SC573) developed end-stage renal disease (ESRD) in childhood (13.2%).

Ophthalmological findings

In this cohort, 23/38 (60.5%) patients had ophthalmological abnormalities. Of the remaining 15 patients, 12 had a normal ophthalmological exam, whereas ophthalmological examination was not performed in three patients, most often due to the lack of clinical complaints about visual problems. Optic nerve abnormalities, for example, optic disc coloboma, were noted in 21 cases. Additional findings were amblyopia, exotropia, retinopathy of prematurity, and nystagmus (Tables 1, 2).

Extrarenal manifestations

A wide range of non-renal, non-ophthalmological manifestations of *PAX2*-related disorder was revealed by our study. Developmental abnormalities including autism or language delay were found in four patients (SC30, SC274, SC472, and SC573). Growth abnormality (short stature) was found in three patients (SC274, SC300, and SC468). There were three patients with facial malformation including micrognathia (SC274 and SC622) or low-set ears (SC590). Congenital heart or vascular abnormalities were revealed in two patients (pulmonary stenosis in SC239 and small ventricular septal defect in SC415). We also found electrolyte imbalance (hypocalcemia in SC239 and hyponatremia in SC351), scoliosis (SC10), gonadal abnormalities (retractile testis in SC274, polycystic ovarian disease in SC287), unilateral kidney teratoma (SC287), and median cervical cyst (SC573). Congenital pulmonary abnormality (congenital cystic adenomatoid malformation, CCAM) was observed in two unrelated patients (SC30, SC315). To the best of our knowledge, this is the first observation of CCAM as a complication in *PAX2*-related disorder. In seven cases (18.4%), no extrarenal symptoms, including ophthalmological findings, were observed (Tables 1, 2).

PAX2 pathogenic variant incidence

We identified 19 distinct *PAX2* pathogenic variants in 38 individuals from 30 families. Among them, the 10q24 deletion, including whole *PAX2* gene,[13] was found in two patients from one family, whereas other *PAX2* variants were revealed in 37 patients. The majority of pathogenic variants in this gene are expected to result in a significant truncation of PAX2 protein through a shift in the reading frame (17 families), missense variants (6), nonsense variants (5), or disruption of a conserved splice site (1). Among the 19 different heterozygous pathogenic variants, seven (36.8%) have not been

previously registered in HGMD (http://www.hgmd.cf.ac.uk), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), or ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) (Fig. 1, Table 2).

Genotype-phenotype correlations

Individuals with normal kidney structure tended to have a pathogenic variant in the paired domain region ($P < 0.05$). Although no clear genotype-phenotype correlation in terms of type and site of pathogenic variant emerged from this study, six of the seven patients who developed ESRD in their early period of life had truncating variants (nonsense or frameshift variants), and another one had a splice site mutation.

Discussion

We studied 38 patients from 30 families with *PAX2*-related disorder who were identified by direct sequencing or NGS. The latter comprehensive method is useful for determining the gene responsible when patients do not exhibit the typical phenotype of the target disorder. The use of NGS in our study enabled the characterization of a wide variety of clinical phenotypes in individuals with bona fide *PAX2*-related disorder. Okumura et al. reported that approximately half of the patients clinically diagnosed with RCS had *PAX2* mutations.[18] To the best of our knowledge, we are the first to report on the various phenotypes in Japanese patients with *PAX2* mutation; some of the patients had no ophthalmologic abnormalities.

PAX2 expression is crucial for the formation of kidneys.[6,7] *PAX2* dysregulation correlates with a multitude of various congenital abnormalities and pathological conditions. RHD is characterized by the reduced number and hypertrophy of nephrons.

In similarity with the most common findings of another study of patients with *PAX2*-related disorder,[9] renal insufficiency with RHD, including renal dysfunction of unknown cause (34 patients, 89.4%), was the most frequent renal disease in the present study. Other renal disorders, including MCDK, VUR, and FSGS, were observed in our cohort as in previous reports.[9]

PAX2 expression is first observed in the optic sulcus during normal eye development, followed by the expression throughout the optic vesicle.[19] The incidence of optic disc coloboma varied between each eye of the individuals in a family, although patients had the same *PAX2* pathogenic variant, such as in the family of patient SC47 in the present study. Dysplasia of the optic nerve was the main ophthalmological finding of *PAX2*-related disorder. In a previous study,[9] 72% of the patients showed optic nerve abnormalities, and we have identified 23 patients with some ocular abnormalities (60.5%) in the present study. Eighteen patients had bilateral optic disc coloboma and three had unilateral coloboma. No cases with iris coloboma were observed in our study, which was in accordance with the findings of a previous report.[1] Ophthalmological abnormalities did not consistently correlate with the genotype.

In individuals with *PAX2*-related disorder, a broad variety of non-renal and non-ophthalmological manifestations has been recorded.[1] The *PAX2* gene is expressed in many tissues besides the kidney and eye, including the otic vesicle, genitourinary tract, pancreas, cerebellum, hypothalamus, and midbrain/hindbrain boundaries.[9] Some non-renal and non-ophthalmological findings in our cohort are concordant with the pattern of *PAX2* expression, e.g., in patient SC274 with retractile testis. Four patients had mild developmental disorders. This is a known manifestation of *PAX2*-related disorder.[1] We believe that the causal association between developmental disorders and *PAX2* variants

is unlikely. In the present study, two unrelated patients, both of whom had developed ESRD at an early age, had CCAM, a rare congenital lung malformation characterized by cysts of various sizes as a result of abnormal fetal bronchial development.[20] CCAM has never been reported to be associated with *PAX2*-related disorder. Although the activity of several genes, including *FGF10* or *DICER1*, may affect CCAM pathogenesis,[21] the precise mechanism of CCAM development is still unknown. Further investigations will be needed to clarify the association between *PAX2* and CCAM. Hearing loss is a frequent complication in *PAX2*-related disorder.[1] Although not all the patients received a hearing test, none among them reported that hearing loss interfered with their daily lives.

Seven novel variants out of 19 different pathogenic variants were identified in this study. Four novel variants (c.51del, c.89G>T, c.212G>T, and c.432del) were identified in cases with typical phenotypes of *PAX2* pathogenic variants. Two variants (c.143del and c.89del) were identified in individuals with non-renal and non-ophthalmological findings, such as short stature and low set ear, respectively. The c.497-2A>G variant was identified in one individual with MCDK, autism, and CCAM without any abnormal ophthalmological findings.

There were three recurring pathogenic variants in this cohort: c.76dup, c.76del, and c.310C>T. Furthermore, the frequently described recurring pathogenic variant, c.76dup, was identified in 14 cases from 10 families (33.3%). Clinical symptoms of the patients with c.76dup were variable (Tables 2, 3). Other recurring pathogenic variants were c.310C>T, which was identified in one family and one individual case, and c.76del, which was identified in two individual cases. To date, analyses of all reports do not show a consistent genotype–phenotype correlation in *PAX2* related disorder. There is

currently no definite evidence that the location of a pathogenic variant (paired domain, octapeptide domain, partial homeodomain, or transactivation domain) or its type (missense variant, nonsense variant, or gene deletion) consistently predicts the clinical phenotype.[9]

In the present study, we identified *PAX2* pathogenic variants using NGS in 10 patients (SC30, SC62, SC239, SC287, SC351, SC390, SC468, SC553, SC590, and SC607). Six of these patients had no ophthalmological anomalies, and one patient (SC62) was diagnosed with optic disc coloboma after genetic diagnosis. As we previously reported, patient SC390 displayed a typical RCS phenotype; however, direct sequencing did not identify *PAX2* mutations. Using NGS, we identified whole gene deletions of *PAX2*.[13] Patient SC607 was diagnosed with nephronophthisis-complicated retinopathy by the primary physician. We were able to identify *PAX2* mutations in this patient using NGS. Previously, we reported about a patient with chronic renal insufficiency and bilateral optic disc edema diagnosed with RCS. *PAX2* mutations were not identified in this patient. Using NGS, the patient was diagnosed with nephronophthisis related ciliopathy owing to compound heterozygous *WDR35* mutations.[22] Nephronophthisis is an autosomal recessive disorder, whereas *PAX2*-related disorder is autosomal dominant. Precise diagnoses affect the prognoses of patients and the ability to prescribe appropriate genetic counseling.

PAX2 mutations are a major contributor to CAKUT; however, other contributing pathogenic gene variants include *HNF1B*, *EYA1*, and *SALL1*. Patients with these gene mutations occasionally have pathognomonic extra-renal symptoms.[14,15,23] Therefore, CAKUT patients may be distinguished by the presence of an extra-renal phenotype. However, if they do not possess the extra-renal phenotype, NGS could be useful for

297 identifying the responsible genes. In our study, the patients with *PAX2* mutations,
298 analyzed using NGS, did not have other pathogenic gene variants contributing to their
299 renal disorders.

300 Our study had some limitations: 14 individual cases did not undergo segregation
301 analysis. We could not completely follow up with all patients regarding their clinical
302 courses after genetic analysis.

303 In conclusion, we found that our patients showed considerable variation in clinical
304 manifestations of the *PAX2*-related disorder. Our findings may help in identifying more
305 individuals with pathogenic variants of *PAX2*. An accurate genetic diagnosis at an early
306 stage of the disorder is crucial for the preservation of kidney function, optimization of
307 genetic counseling, and improvement of the quality of life of patients.

308

309 **Acknowledgments**

310 The authors wish to thank all the patients, their social guardians, and primary doctors.
311 We are profoundly grateful to Ms. Akemi Shono, Mrs. Tetsuko Yamanouchi, Mrs.
312 Yoshimi Nozu, and Mrs. Ming Juan Ye (Kobe University) for their excellent technical
313 assistance.

314 Data for patients SC10, SC56, SC111, SC390, and SC456 were published
315 elsewhere.[14, 24-27] Data for patients SC239, SC274, and SC468 have been reported in
316 Japanese journals.[28-30]

317 The following doctors provided patient samples for this study: Takeshi Futatani
318 (Toyama Prefectural Central Hospital), Yoshimitsu Gotoh (Japanese Red Cross Nagoya
319 Daini hospital), Yuko Hamasaki (Toho University), Ken Hatae (Japanese Red Cross
320 Fukuoka Hospital), Atsuko Iida (Tokyo Women's Medical University Medical Center

East), Yoichi Iwafuchi (Niigata Koseiren Sanjo General Hospital), Yuko Kajiho (The University of Tokyo), Chieko Matsumura (National Chiba Higashi Hospital), Koji Nagatani (Uwajima City Hospital), Tomoka Hase (Wakayama Medical University), Masashi Nishida (Kyoto Prefectural University of Medicine), Shunsuke Noda (Nagano Red Cross Hospital), Akifumi Ohtsuka (Saga University), Shin-ichi Okada (Tottori University), Mika Okutsu (National Center for Child and Development), Koji Sakuraya, Shuichiro Fujinaga (Saitama Children's Medical Center), Noriko Sugawara (Tohoku University), Hironori Takahashi (Asahikawa Medical University), Masaki Yamamoto (Seirei Hamamatsu Hospital), and Masato Yasui (Fukuyama City Hospital).

This work was supported by the Health Labor Sciences Research Grant for the Research on Measures for Intractable Diseases (H24-nanchi-ippan-041 to K.I.; H29-nanchi-ippan-039 to N.M.) and Japan Society for the Promotion of Science (KAKENHI Grant Number JP15K09261 and 18K08243 to N.M.).

Conflict of interest

K.I. has received grant support from Daiichi Sankyo Co., Ltd. and Zenyaku Kogyo Co., Ltd.

References

1. Bower MA, Schimmenti LA, Eccles MR. PAX2-Related Disorder. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1451/ (accessed on Oct. 20/2019)
2. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont ME, Sullivan MJ, et al. Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. Nat Genet. 1995;9:358–64.
3. Nishimoto K, Iijima K, Shirakawa T, Kitagawa K, Satomura K, Nakamura H, et al. PAX2 gene mutation in a family with isolated renal hypoplasia. J Am Soc Nephrol. 2001;12:1769–72.
4. Treisman J, Harris E, Desplan C. The paired box encodes a second DNA-binding domain in the paired homeo domain protein. Genes Devel. 1991;5:594–604.
5. Sanyanusin P, Norrish JH, Ward TA, Nebel A, McNoe LA, Eccles MR. Genomic structure of the human PAX2 gene. Genomics. 1996;35:258–61.
6. Torres M, Gomez-Pardo E, Dressler G, Gruss P. Pax-2 controls multiple steps of urogenital development. Development. 1995;121:4057–65.
7. Favor J, Sandulache R, Neuhauser-Klaus A, Pretsch W, Chatterjee B, Senft E, et al. The mouse Pax2(1Neu) mutation is identical to a human PAX2 mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye, and kidney. Proc Natl Acad Sci. 1996;93:13870–5.
8. Tellier A-L, Ameil J, Salomon R, Jolly D, Delezoide A-L, Auge J, et al. PAX2 expression during early human development and its mutation in renal hypoplasia with or without coloboma. Am J Hum Genet. 1998;Suppl 63,A7.

9. Bower M, Salomon R, Allanson J, Antignac C, Benedicenti F, Benetti E, et al. Update of PAX2 mutations in renal coloboma syndrome and establishment of a locus specific database. Hum Mutat. 2012;33:457–66.
10. Dziarmaga A, Quinlan J, Goodyer P. Renal hypoplasia: lessons from Pax2. Pediatr Nephrol. 2006;21:26–31.
11. Deng H, Zhang Y, Xiao H, Yao Y, Liu X, Su B, et al. Diverse phenotypes in children with PAX2-related disorder. Mol Genet Genomic Med. 2019;7:e701.
12. Sue Richards, Nazneen Aziz, Sherri Bale, David Bick, Soma Das, Julie Gastier-Foster, et al. On behalf of the ACMG Laboratory Quality Assurance Committee. 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;17:405–24.
13. Nagano C, Nozu K, Morisada N, Yazawa M, Ichikawa D, Numasawa K, et al. Detection of copy number variations by pair analysis using next-generation sequencing data in inherited kidney diseases. Clin Exp Nephrol. 2018;22:881–8.
14. Nagano C, Morisada N, Nozu K, Kamei K, Tanaka R, Kanda S, et al. Clinical Characteristics of *HNF1B*-related disorders in a Japanese population. Clin Exp Nephrol. 2019;23:1119–29.
15. Unzaki A, Morisada N, Nozu K, Ye MJ, Ito S, Matsunaga T, et al. Clinically diverse phenotypes and genotypes of patients with branchio-oto-renal syndrome. J Hum Genet. 2018;63:647–56.

16. Yoshikawa T, Kamei K, Nagata H, Saida K, Sato M, Ogura M, et al. Diversity of renal phenotypes in patients with WDR19 mutations: Two case reports. Nephrology (Carlton). 2017;22:566–71.
17. Sakakibara N, Morisada N, Nozu K, Nagatani K, Ohta T, Shimizu J, et al. Clinical spectrum of male patients with OFD1 mutations. J Hum Genet. 2019;64:3–9.
18. Okumura T, Furuichi K, Higashide T, Sakurai M, Hashimoto S, Shinozaki Y, et al. Association of PAX2 and other gene mutations with the clinical manifestations of renal coloboma syndrome. PLoS One. 2015;10:e0142843.
19. Nornes HO, Dressler GR, Knapik EW, Deutsch U, Gruss P. Spatially and temporally restricted expression of Pax2 during murine neurogenesis. Development. 1990;109:797–809.
20. Boucherat O, Jeannotte L, Hadchouel A, Delacourt C, Benachi A. Pathomechanisms of congenital cystic lung diseases: focus on congenital cystic adenomatoid malformation and pleuropulmonary blastoma. Paediatr Respir Rev. 2016;19:62–8.
21. Annunziata F, Bush A, Borgia F, Raimondi F, Montella S, Poeta M, et al. Congenital lung malformations: unresolved issues and unanswered questions. Front Pediatr. 2019;7:239.
22. Yamamura T, Morisada N, Nozu K, Minamikawa S, Ishimori S, Toyoshima D, et al. Rare renal ciliopathies in non-consanguineous families that were identified by targeted resequencing. Clin Exp Nephrol. 2017;21:136–42.
23. Kohlhase J. Townes-Brocks Syndrome. 2007 Jan 24 [Updated 2016 Jan 14]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet].

Seattle (WA): University of Washington, Seattle; 1993-2020. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1445/

24. Ishiwa S, Sato M, Morisada N, Nishi K, Kanamori T, Okutsu M, 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;34:1457–64.
25. Ohtsubo H, Morisada N, Kaito H, Nagatani K, Nakanishi K, Iijima K. Alport-like glomerular basement membrane changes with renal-coloboma syndrome. Pediatr Nephrol. 2012;27:1189–92.
26. Iwafuchi Y, Morioka T, Morita T, Yanagihara T, Oyama Y, Morisada N, et al. Diverse renal phenotypes observed in a single family with a genetic mutation in Paired Box Protein 2. Case Rep Nephrol Dial. 2016;6:61–9.
27. Saida K, Kamei K, Morisada N, Ogura M, Ogata K, Matsuoka K, et al. A novel truncating PAX2 mutation in a boy with renal coloboma syndrome with focal segmental glomerulosclerosis causing rapid progression to end-stage kidney disease. CEN Case Rep. 2020;9:19–23.
28. Hatae K, Keida Y, Hinokiyama M, Kuroki R, Kurokawa M, Morisada N, et al. Pseudo-Bartter syndrome in an infant with renal hypo/dysplasia: PAX2 mutation identified by next-generation sequencing led to the diagnosis, renal coloboma syndrome. Jpn J Pediatr Nephrol. 2017;30:54–9 (in Japanese).
29. Iida A, Hoshika S, Kaneko K, Kato F, Sugihara S. An infant case of renal coloboma syndrome with novel PAX2 gene mutation. J Tokyo Wom Med Univ. 2017;87:E98–E101 (in Japanese).

30. Sakuraya K, Fujinaga S, Nozu K, Morisada N, Iijima K. A girl of non-syndromic CAKUT with a novel PAX2 mutation. J Jpn Soc Pediatr Ren Fail. 2019;39:108–10 (in Japanese).

436 **Figure legend**

437 Figure 1. The gene structure of *PAX2* (NM_003987.4) and locations of the various

438 mutations identified in this study.

439

Table 1 Characteristics of patients

		Patients (n = 38)
Age at diagnosis		
Median (months)		66
Range		1 month–54 years
Gender		
Male		20
Female		18
Kidney disease		Patients
Renal hypodysplasia or renal insufficiency of unknown cause		34
Cystic kidney disease (including multicystic dysplastic kidney)		5
Focal segmental glomerulosclerosis		3
Vesicoureteral reflux		2
Hydronephrosis		1
Urinary lithiasis		1
Ocular disease		Patients
Optic disc coloboma		21
bilateral		18
unilateral		3
Others (amblyopia, exotropia, retinopathy of prematurity, nystagmus)		1 case of each
No ocular abnormality		12
Non-renal and non-ophthalmological diseases		Patients
Developmental disorder	Autism	2
	Mild intellectual disability	1
	Speech delay	1
Pulmonary	Congenital cystic adenomatoid malformation	2
Electrolyte	Hypocalcemia	1
	Hyponatremia	1
Gonadal abnormalities	Retractile testis	1
	Polycystic ovarian disease	1
Growth	Short stature	3
Facial malformation	Micrognathia	2
	Low set ears	1
Congenital heart or vascular anomalies	Small ventricular septal defect	1
	Pulmonary stenosis	1
Others	Scoliosis	1
	Teratoma	1
	Median cervical cyst	1

Table 2 Clinical manifestations and genotypes of the patients in the study

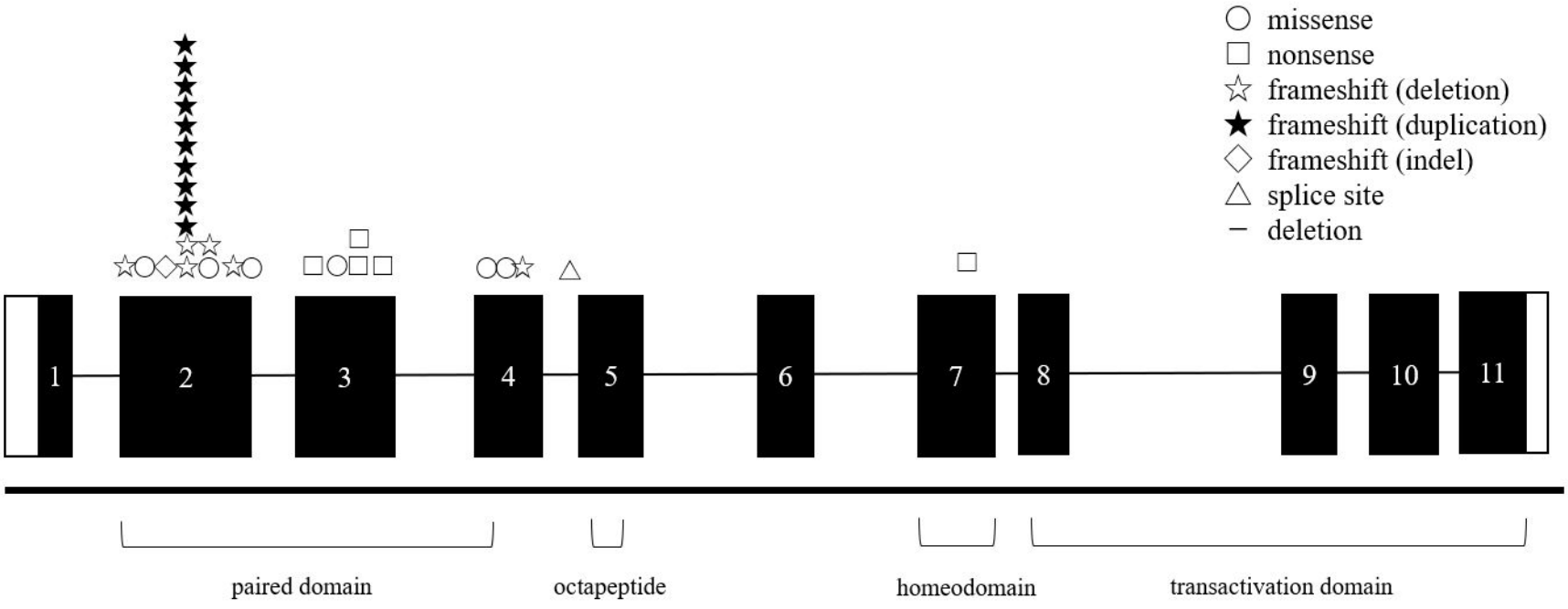
Family	Patient	Age at diagnosis	Gender	FH	Kidney disease	eGFR	RRT (years)	Ocular symptoms	Other symptoms	cDNA	Amino acids	Mutation	HGMD	dbSNP or ClinVar	ACMG interpretation			Method	HPx	Inheritance	Ref
															Category	Evidence of pathogenicity	Classification				
1	SC10	13	F	+	CKD	59.1	-	b. optic disc coloboma	scoliosis	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PP1, PP4, PP5	Very strong 3 Supporting	Pathogenic	DS		Father[1]	25
	Sister	15	F		CKD, VUR	NA	-	b. optic disc coloboma	-									DS			
2	SC30	2	M	-	r. MCDK, l. RHD	NA	7, RT	-	autism, CCAM	c.497-2A>G	-	splice site	-	-	PVS1 PM2 PP4	Very strong 1 Moderate 1 Supporting	Likely pathogenic	TS1		unknown	
3	SC32	5m	F	-	b. RHD	22.4	-	b. optic disc coloboma	-	c.432del	p.Gln144HisfsTer15	frameshift	-	-	PVS1 PM2, PM4	Very strong 2 Moderate	Pathogenic	DS		unknown	
4	SC47	2m	M	+	b. RHD	19.4	-	b. optic disc coloboma	-	c.310C>T	p.Arg104Ter	nonsense	CM076374	-	PVS1 PM2 PP1, PP4, PP5	Very strong 1 Moderate 3 Supporting	Pathogenic	DS		Mother	
	Mother	NA	F		b. RHD	NA	-	-	-									DS		unknown	
	Brother	2m	M		b. RHD	NA	-	NA	-									DS		Mother	
5	SC56	25	M	+	CKD	7.1	7, HD	-	-	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PP1, PP4, PP5	Very strong 3 Supporting	Pathogenic	TS1		Father	26
	Father	51	M		FSGS	NA	-	-	-									DS		unknown	
	Brother	26	M		CKD	NA	8, RT	-	-									DS		Father	
6	SC62	1	M	-	CKD	NA	-	b. optic disc coloboma	-	c.89G>T	p.Gly30Val	missense	-	-	PM1, PM2 PP3, PP4	2 Moderate 2 Supporting	Likely pathogenic	TS1		unknown	
7	SC111	6	M	-	FSGS	29.4	-	b. optic disc coloboma	-	c.70-72delinsA	p.Gly24ArgfsTer29	frameshift	CX194156	-	PVS1 PM2 PP4, PP5	Very strong 1 Moderate 2 Supporting	Pathogenic	DS		unknown	24, 27
8	SC114	3	M	-	CKD	NA	-	b. optic disc coloboma	-	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PS2 PP4, PP5	Very strong 1 Strong 2 Supporting	Pathogenic	DS		*de novo*	
9	SC149	9	M	-	CKD, b. VUR	32.8	-	-	-	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PS2 PP4, PP5	Very strong 1 Strong 2 Supporting	Pathogenic	DS		*de novo*	
10	SC183	14	M	-	CKD	65.4	-	b. optic disc coloboma	-	c.239C>T	p.Pro80Leu	missense	CM148848	RCV000549890.2	PM1, PM2 PP1, PP3, PP4, PP5	2 Moderate 4 Supporting	Likely pathogenic	DS		uniparental[2]	
11	SC184	2	F	-	CKD	38	-	r. optic disc coloboma	-	c.51del	p.His17QGlnfsTer4	frameshift	-	-	PVS1 PS2 PM2 PP4	Very strong 1 Strong 1 Moderate 1 Supporting	Pathogenic	DS		*de novo*	

12	SC239	2m	F	-	CKD	NA	-	-	hypocalcemia, pulmonary artery stenosis	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PP4, PP5	Very strong 2 Supporting	Pathogenic	HPx	1	unknown	28
13	SC252	9	F	-	CKD	46.7	-	b. optic disc coloboma	-	c.76del	p.Val26CysfsTer3	frameshift	CD992538	rs75462234	PVS1 PP4, PP5	Vary strong 2 Supporting	Pathogenic	DS		unknown	
14	SC274	4	M	-	CKD, b hydronephrosis	52.8	-	b. optic disc coloboma	retractile testis, mild ID, micrognathia, short stature	c.220G>T	p.Glu74Ter	nonsense	-	RSV000681874.1	PVS1 PM2 PP3, PP4, PP5	Very strong 1 Moderate 3 Supportive	Pathogenic	DS		unknown	29
15	SC287	1	F	-	r. RHD, l. teratoma	21	3, PD	retinopathy of prematurity	polycystic ovarian disease	c.76del	p.Val26CysfsTer3	frameshift	CD992538	rs75462234	PVS1 PS2 PP4, PP5	Very strong 1 Strong 2 Supportive	Pathogenic	HPx	3	*de novo*	
16	SC300	5	M	+	CKD	72	-	b. optic disc coloboma	short stature	c.143del	p.Gly48ValfsTer35	frameshift	-	-	PVS1 PM2 PP1, PP4	Very strong 1 Moderate 2 Supportive	Pathogenic	DS		Father	
17	SC315	6m	M	-	CKD	NA	2, RT	b. optic disc coloboma	l. CCAM	c.310C>T	p.Arg104Ter	nonsense	CM076374	-	PVS1 PM2 PP4	Very strong 1 Moderate 1 Supporting	Pathogenic	DS		unknown	
18	SC351	1	M	-	CKD	47.6	-	-	hyponatremia	c.418C>T	p.Arg140Trp	missense	CM1815853	rs1217241110	PS2 PM1, PM2 PP3, PP4, PP5	1 strong 2 Moderate 1 Supporting	Pathogenic	HPx	3	*de novo*	
19	SC390	30	F	+	CKD, cystic kidney	34.4	-	r. amblyopia	-	arr[GRCh37] 10q24.2q24.32(100315515_103049862)x1		deletion	CG122659		PVS1 PP1, PP5	Very strong 2 Supporting	Pathogenic	HPx, aCGH	3	Mother	14
	Mother	54	F		CKD, cystic kidney	62.8	-	NA	NA											unknown	
20	SC415	26	F	-	CKD	40.2	7, RT	b. optic disc coloboma, exotropia	small ventricular septal defect	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PP4, PP5	Very strong 2 Supporting	Pathogenic	DS		unknown	
21	SC456	14	F	-	b. RHD	17.1	-	l. optic disc coloboma	-	c.832C>T	p.Gln278Ter	nonsense	CM194157	-	PVS1 PM2 PP3, PP4, PP5	Very strong 1 Moderate 3 Supporting	Pathogenic	DS		unknown	24
22	SC468	3	F	+	CKD, small cystic kidney	65.2	-	-	short stature	c.70G>C	p.Gly24Arg	missense	CM194583	-	PM1, PM2 PP1, PP3, PP4, PP5	2 Moderate 4 Supporting	Likely pathogenic	HPx	5	Mother	30
	Mother	32	F		CKD	NA	-	NA	-									DS		unknown	
23	SC472	2	M	+	CKD	56.3	-	b. optic disc coloboma	mild delay in language development	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1	Very strong	Pathogenic	DS		Mother	
	Mother	NA	F		NA	NA	-	b. optic disc coloboma	urinary lithiasis						PP1, PP4, PP5	3 Supporting		DS		Mother[1]	
24	SC521	14	F	-	FSGS	40	-	-	-	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PP4, PP5	Very strong 2 Supporting	Pathogenic	DS		unknown	

25	SC553	12	M	-	b. RHD	18	-	b. optic disc coloboma	-	c.419G>A	p.Arg140Gln	missense	CM068671	rs865906227	PM1, PM2 PP3, PP4, PP5	2 Moderate 3 Supporting	Likely pathogenic	DS		Mother	
26	SC573	10	M	-	b. RHD, RTA	24.9	11, RT	-	autism, median cervical cyst	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PP4, PP5	Very strong 2 Supporting	Pathogenic	HPx	7	unknown	
27	SC590	1	F	-	b. RHD	43	-	-	low set ears	c.89del	p.Gly30AlafsTer8	frameshift	-	-	PVS1 PS2 PP4	Very strong 1 Strong 1 Supporting	Pathogenic	HPx	7	*de novo*	
28	SC605	2m	F	-	b. RHD	21.2	-	b. optic disc coloboma	pulmonary hypertension, cerebellar hemorrhage	c.212G>T	p.Arg71Met	missense	-	-	PM1, PM2, PM3 PP3, PP4	3 Strong 2 Moderate	Likely pathogenic	DS		unknown	
29	SC607	13	M	-	CKD, cystic kidney	66.9	-	l. optic disc coloboma, nystagmus	hyperuricemia	c.76dup	p.Val26GlyfsTer28	frameshift	CI951965	rs75462234	PVS1 PP4, PP5	Very strong 2 Supporting	Pathogenic	HPx	7	Mother[1]	
30	SC622	1m	M	-	CKD	23.7	-	b. optic disc coloboma, r. orbital cyst	micrognathia	c.343C>T	p.Arg115Ter	nonsense	CM034444	-	PVS1 PM2 PP3, PP4, PP5	Very strong 1 moderate 3 Supporting	Pathogenic	DS		unknown	

[1] the patient's parent was affected with the same disorder; however, genetic analyses were not performed. [2]"uniparental" indicates that the variant was inherited from father or mother.

ACMG, American College of Medical Genetics; b. bilateral; CCAM, congenital cystic adenomatoid malformation; CKD, chronic kidney disease (means renal dysfunction of unknown cause); DS, direct sequencing; eGFR, estimated glomerular filtration rate; F, female; FH, family history; FSGS, focal segmental glomerulosclerosis; HD, hemodialysis; HGMD, Human Genome Mutation Database; HPx, HaloPlex; ID, intellectual disability; l., left; M, male; MCDK, multicystic dysplastic kidney; NA, not available; PD, peritoneal dialysis; PM, moderate evidence of pathogenicity; PP, Supportive evidence of pathogenicity; PS, Strong evidence of pathogenicity; Pt, patient; Ref, references; PVS, very strong evidence of pathogenicity; RHD, renal hypodysplasia; RRT, renal replacement therapy; RT, renal transplantation; RTA, renal tubular acidosis; TS1, TruSight One Sequencing Panel; VUR, vesicoureteral reflux.


missense
nonsense
frameshift (deletion)
frameshift (duplication)
frameshift (indel)
splice site
deletion
1
2
3
4
5
6
7
8
9
10
11
paired domain
octapeptide
homeodomain
transactivation domain

Supplementary Table 1: The gene list constructed using HaloPlex (91 genes).

ACE	*CITED1*	*GATA3*	*NPHP4*	*PAX2*	*SPRY1*	*ZEB2*
ACTN4	*CTDNEP1*	*GDNF*	*IQCB1*	*PAX8*	*TNXB*	
AGT	*CXCL12*	*HOXA13*	*CEP290*	*PKD1*	*TP53*	
AGTR1	*CXCR4*	*HNF1B*	*GLIS2*	*PKD2*	*TRAP1*	
AHI1	*DSTYK*	*HOXD11*	*RPGLIP1L*	*PKHD1*	*TRPC6*	
ANLN	*EP300*	*IFN2*	*NEK8*	*REN*	*UMOD*	
APOL1	*EYA1*	*KAL1*	*SDCCAG8*	*RET*	*UPK3A*	
BMP2	*FAT3*	*LMX1B*	*TMEM67*	*ROBO2*	*VANGL2*	
BMP4	*FAT4*	*MDM2*	*TTC21B*	*ROR1*	*WNT4*	
BMP7	*FOXD1*	*MI2B (CHD4)*	*WDR19*	*ROR2*	*WNT5A*	
CC2D2A	*FGF2*	*MUC1*	*XNF423*	*SALL1*	*WNT7A*	
CD2AP	*FGF9*	*MYO1E*	*CEP164*	*SIX1*	*WNT7B*	
CDC5L	*FGF20*	*NPHP1*	*ANKS6*	*SIX2*	*WNT9B*	
CHD1L	*FZD4*	*INVS*	*IFT172*	*SIX5*	*WNT11*	
CHRM3	*FZD8*	*NPHP3*	*OFD1*	*SOX17*	*WT1*	

Supplementary Table 2: The gene list constructed using HaloPlex (172 genes).

ACE	*B9D1*	*CD2AP*	*CTDNEP1*	*FOXD1*	*IFN2*	*LMNA*	*NUP107*	*RET*	*TBX1*	*TSC1*	*WNT7B*
ACTN4	*B9D2*	*CDC5L*	*CXCL12*	*FRAS1*	*IFT27*	*LMX1B*	*NUP133*	*ROBO2*	*TBX18*	*TSC2*	*WNT9B*
ADCK4	*BBIP1*	*CENPF*	*CXCR4*	*FREM1*	*IFT43*	*LZTFL1*	*NXF5*	*ROR1*	*TCTN2*	*TTC8*	*WNT11*
AGT	*BBS1*	*CEP41*	*DCDC2*	*FREM2*	*IFT81*	*MDM2*	*OFD1*	*ROR2*	*TCTN3*	*TTC21B*	*WT1*
AGTR1	*BBS2*	*CEP83*	*DDX59*	*FZD4*	*IFT122*	*MKKS*	*OSR1*	*RPGLIP1L*	*TMEM67*	*UMOD*	*XPNPEP3*
AGTR2	*BBS4*	*CEP104*	*DSTYK*	*FZD8*	*IFT140*	*MKS1*	*PAX2*	*SALL1*	*TMEM138*	*UPK3A*	*ZEB2*
AHI1	*BBS5*	*CEP120*	*DYNC2H1*	*GANAB*	*IFT172*	*MUC1*	*PAX8*	*SDCCAG8*	*TMEM216*	*VANGL2*	*ZNF423*
ALG13	*BBS7*	*CEP164*	*EP300*	*GATA3*	*INPP5E*	*MYO1E*	*PDE6D*	*SIX1*	*TMEM231*	*WDPCP*	
ALMS1	*BBS10*	*CEP290*	*EYA1*	*GDNF*	*INVS*	*NEK1*	*PKD1*	*SIX2*	*TMEM237*	*WDR19*	
ANKS3	*BBS12*	*CHD1L*	*FAT3*	*GLIS2*	*IQCB1*	*NEK8*	*PKD2*	*SIX5*	*TNXB*	*WDR34*	
ANKS6	*BMP2*	*CHD4*	*FAT4*	*GREM1*	*ITGA8*	*NODAL*	*PKHD1*	*SLIT2*	*TP53*	*WDR35*	
ANLN	*BMP4*	*CHRM3*	*FGF2*	*GRIP1*	*KAL1*	*NPHP1*	*PLCE1*	*SOX17*	*TRAF3IP1*	*WDR60*	
APOL1	*BMP7*	*CITED1*	*FGF9*	*HOXA13*	*KIAA0586*	*NPHP3*	*PODXL*	*SPRY1*	*TRAP1*	*WNT4*	
ARL6	*C5orf42*	*COQ6*	*FGF20*	*HOXD11*	*KIF14*	*NPHP4*	*PTHB1*	*SRGAP1*	*TRIM32*	*WNT5A*	
ARL13B	*CC2D2A*	*CSPP1*	*FGFR2*	*HNF1B*	*LAMB2*	*NPHS2*	*REN*	*TBC1D1*	*TRPC6*	*WNT7A*	

Supplementary Table 3: The gene list constructed using HaloPlex (159 genes).

ACE	*BBS1*	*CEP41*	*DYNC2H1*	*GANAB*	*IFT80*	*LMNA*	*PDE6D*	*SIX1*	*TMEM107*	*WDPCP*
AGT	*BBS2*	*CEP83*	*DZIP1L*	*GATA3*	*IFT81*	*LMX1B*	*PIBF1*	*SIX2*	*TMEM138*	*WDR19*
AGTR1	*BBS4*	*CEP104*	*EP300*	*GDNF*	*IFT122*	*LZTFL1*	*PKD1*	*SIX5*	*TMEM216*	*WDR34*
AGTR2	*BBS5*	*CEP120*	*EVC*	*GLIS2*	*IFT140*	*MKKS*	*PKD2*	*SLIT2*	*TMEM231*	*WDR35*
AHI1	*BBS7*	*CEP164*	*EVC2*	*GLIS3*	*IFT172*	*MKS1*	*PKHD1*	*SOX17*	*TMEM237*	*WDR60*
ALG9	*BBS10*	*CEP290*	*EXOC4*	*GRIP1*	*INPP5E*	*MUC1*	*PTHB1*	*SPRY1*	*TNXB*	*WNT4*
ALMS1	*BBS12*	*CHD1L*	*EXOC8*	*GRLF1*	*INVS*	*NEK1*	*REN*	*SRGAP1*	*TRAF3IP1*	*WT1*
ANKS3	*C2CD3*	*CHD4*	*EYA1*	*HNF1B*	*IQCB1*	*NEK8*	*RET*	*TBC1D1*	*TRIM32*	*XPNPEP3*
ANKS6	*C5orf42*	*CHRM3*	*FAN1*	*HOXA13*	*ITGA8*	*NPHP1*	*ROBO2*	*TBC1D32*	*TSC1*	*ZNF423*
ARL6	*C21orf2*	*CITED1*	*FGF9*	*HPRT1*	*JAG1*	*NPHP3*	*RPGLIP1L*	*TBX1*	*TSC2*	
ARL13B	*CC2D2A*	*CSPP1*	*FGF20*	*HYLS1*	*KAL1*	*NPHP4*	*SALL1*	*TBX18*	*TTBK2*	
ATXN10	*CCDC28B*	*CTDNEP1*	*FGFR2*	*ICK*	*KIAA0586*	*OFD1*	*SARS2*	*TCTN1*	*TTC8*	
B9D1	*CDC5L*	*DCDC2*	*FRAS1*	*IFN2*	*KIF7*	*PAX2*	*SCLT1*	*TCTN2*	*TTC21B*	
B9D2	*CENPF*	*DDX59*	*FREM1*	*IFT27*	*KIF14*	*PAX8*	*SDCCAG8*	*TCTN3*	*UMOD*	
BBIP1	*CEP19*	*DSTYK*	*FREM2*	*IFT43*	*LIFR*	*PBX1*	*SEC61A1*	*TMEM67*	*VANGL2*	

Supplementary Table 4: The gene list constructed using HaloPlex (181 genes).

ACE	*BBS1*	*CEP83*	*EP300*	*GFRA1*	*IFT80*	*LRIG2*	*PIBF1*	*SOX11*	*TNXB*	*ZNF423*
ACTG2	*BBS2*	*CEP104*	*EVC*	*GLIS2*	*IFT81*	*LRP5*	*PKD1*	*SOX17*	*TRAF3IP1*	
AGT	*BBS4*	*CEP120*	*EVC2*	*GLIS3*	*IFT122*	*LZTFL1*	*PKD2*	*SPRY1*	*TRIM32*	
AGTR1	*BBS5*	*CEP164*	*EXOC4*	*GPC3*	*IFT140*	*MAPKBP1*	*PKHD1*	*SRGAP1*	*TSC1*	
AGTR2	*BBS7*	*CEP290*	*EXOC8*	*GREB1L*	*IFT172*	*MKKS*	*REN*	*SUFU*	*TSC2*	
AHI1	*BBS9*	*CHD1L*	*EYA1*	*GREM1*	*INPP5E*	*MKS1*	*RET*	*TBC1D32*	*TTC8*	
ALG8	*BBS10*	*CHD4*	*FAN1*	*GRIP1*	*INTU*	*MUC1*	*ROBO2*	*TBX1*	*TTC21B*	
ALG9	*BBS12*	*CHD7*	*FGF20*	*HNF1B*	*INVS*	*NEK1*	*RPGRIP1L*	*TBX18*	*UMOD*	
ALMS1	*BICC1*	*CHRM3*	*FGFR1*	*HOXA13*	*IQCB1*	*NEK8*	*SALL1*	*TCTEX1D2*	*UPK3A*	
ANKS6	*C2CD3*	*CITED1*	*FGFR2*	*HPRT1*	*ITGA8*	*NOTCH2*	*SARS2*	*TCTN1*	*VANGL2*	
ARL3	*C5orf42*	*CRB2*	*FRAS1*	*HPSE2*	*JAG1*	*NPHP1*	*SCLT1*	*TCTN2*	*WDPCP*	
ARL6	*C8orf37*	*CSPP1*	*FREM1*	*HYLS1*	*KAL1*	*NPHP3*	*SDCCAG8*	*TCTN3*	*WDR19*	
ARL13B	*CC2D2A*	*DCDC2*	*FREM2*	*INF2*	*KIAA0556*	*NPHP4*	*SEC61A1*	*TMEM67*	*WDR34*	
ARMC9	*CCDC28B*	*DDX59*	*GANAB*	*IFT27*	*KIAA0586*	*OFD1*	*SIX1*	*TMEM107*	*WDR35*	
ATXN10	*CDC5L*	*DNAJB11*	*GATA3*	*IFT43*	*KIAA0753*	*PAX2*	*SIX2*	*TMEM138*	*WDR60*	
B9D1	*CDKN1C*	*DSTYK*	*GDF11*	*IFT52*	*KIF7*	*PAX8*	*SIX5*	*TMEM216*	*WNT4*	
B9D2	*CENPF*	*DYNC2H1*	*GDNF*	*IFT57*	*KIF14*	*PBX1*	*SLIT2*	*TMEM231*	*WT1*	
BBIP1	*CEP41*	*DZIP1L*	*GEN1*	*IFT74*	*LMX1B*	*PDE6D*	*SOX9*	*TMEM237*	*XPNPEP3*	

Supplementary Table 5: The *in-silico* evaluations for missense variants in the study

Family	Patient	CADD	PROVEAN	SIFT	Polyphen2	Mutation Taster
6	SC62	28.5	D	D	D	D
10	SC183	34	D	D	P	D
18	SC351	35	D	D	D	D
22	SC468	32	D	D	D	D
	Mother					
25	SC553	35	D	D	D	D
28	SC605	29	D	D	D	D

Abbreviations: CADD, Combined Annotation Dependent Depletion; D, damaging or deleterious. PROVEAN, Protein Variation Effect Analyzer.

Supplementary Table 6: Profiles (gender, age, and type of renal disorder) of the probands in this study.

Gender	Age	Disease	No. of Patients	Total
Male	child (-18 years)	CAKUT	131	263
		Others	79	
	adult (+18 years)	CAKUT	17	
		Others	36	
Female	child (-18 years)	CAKUT	62	189
		Others	88	
	adult (+18 years)	CAKUT	10	
		Others	29	
Unknown	child (-18 years)	CAKUT	1	5
		Others	4	
	adult (+18 years)	CAKUT	0	
		Others	0	
Total				457