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Dominant *PAX2* mutations may cause steroid resistant nephrotic syndrome and FSGS in children

Asaf Vivante^{1,2}, Orna Chacham³, Shirlee Shril¹, Ruth Schreiber⁴, Neveen A. Soliman⁵, Irene Koneth⁶, Mario Schiffer⁷, Yair Anikster⁸, Friedhelm Hildebrandt¹

¹Department of Medicine, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA ²Talpiot Medical Leadership Program, Department of Pediatrics B and Pediatric Nephrology unit, Sheba Medical Center, Tel-Hashomer and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel. ³Metabolic Clinic, Pediatric Division, Soroka Medical Center, Ben-Gurion University, Beer Sheva, Israel. ⁴Pediatric Nephrology Clinic, Pediatric Division, Soroka University Medical Center, Faculty of Health Sciences, Ben-Gurion University, Beer Sheva 84101, Israel. ⁵Department of Pediatrics, Kasr Al Ainy School of Medicine, Cairo University, Cairo, Egypt and Egyptian Group for Orphan Renal Diseases (EGORD), Cairo, Egypt. ⁶Department of Nephrology and transplantation, Kantonsspital St Gallen, CH-9007 St. Gallen, Switzerland ⁷Department of Nephrology, Hannover Medical School, Hannover, Germany. ⁸Metabolic Disease Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel-Hashomer and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

Abstract

BACKGROUND—Heterozygous *PAX2* mutations cause renal coloboma syndrome (RCS) [OMIM# 120330]. RCS is a renal syndromic disease encompassing retinal coloboma and sensorineural hearing loss. Recently a causative role for *PAX2* was reported in adult-onset nephrotic syndrome secondary to focal segmental glomerulosclerosis (FSGS). However, the prevalence of *PAX2* mutations among large cohort of children with steroid resistant nephrotic syndrome (SRNS) and FSGS have not been systematically studied.

METHODS—We employed whole exome sequencing (WES) to reveal the percentage of SRNS cases explained by monogenic mutations in known genes of SRNS/FSGS. As *PAX2* mutations are not an established cause of childhood FSGS we have evaluated a cohort of 215 unrelated families with SRNS, in whom no underlying genetic etiology had been previously established.

RESULTS—Using WES, we identified 3 novel causative heterozygous *PAX2* mutations in 3 out of the 215 families studied (1.3%). All 3 cases were detected in families with more than one affected and compatible with an autosomal dominant mode of inheritance (3/57 familial cases

Correspondence should be addressed to: Friedhelm Hildebrandt, M.D., Division of Nephrology, Department of Medicine, Boston Children's Hospital, 300 Longwood Avenue, Boston, Massachusetts 02115, Phone: +1 617-355-6129; Fax: +1 617-730-0365, friedhelm.hildebrandt@childrens.harvard.edu. Asaf Vivante MD, PhD, Pediatric Department B and Pediatric Nephrology Unit, Edmond and Lily Safra, Children's Hospital, Sackler Faculty of Medicine, Sheba Medical Center, Tel Hashomer, Ramat Gan 5265601, Israel, asafvivante@gmail.com or asaf.vivante@sheba.health.gov.il.

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(5.2%). The clinical diagnosis in three out of four pediatric index patients was done incidentally during routine medical evaluation.

CONCLUSIONS—Our findings demonstrate high frequency of *PAX2* mutations in familial form of SRNS (5.2%) and further expand the phenotypic spectrum of *PAX2* heterozygous mutations to include autosomal dominant childhood-onset FSGS. These results highlight the importance of including *PAX2* in the list of genes known to cause FSGS in children.

Keywords

Congenital anomalies of the kidneys and urinary tract (CAKUT); FSGS; SRNS and *PAX2*

Introduction

PAX2 (Paired Box gene 2) is a transcription factor that plays a central role during early embryonic kidney development. Heterozygous mutations in *PAX2* were first identified in patients with renal coloboma syndrome (also known as “papillorenal syndrome”) which involves renal hypodysplasia, optic nerve abnormalities and deafness [1]. Nonetheless, *PAX2* heterozygous mutations can lead to variable kidney phenotypes across the morphologic continuum of CAKUT (congenital anomalies of the kidney and urinary tract) [2]. Furthermore, pathogenic mutations can lead to isolated CAKUT without optic nerve or hearing abnormalities or with only subtle features of CAKUT [2] [3].

In the absence of a CAKUT phenotype, there is some data suggesting a causative role for *PAX2* mutations among individuals with nephrotic syndrome or nephrotic range proteinuria and focal segmental glomerulosclerosis (FSGS) [4, 5]. These observations have been documented exclusively for adult-onset nephrotic syndrome secondary to FSGS [4], however, the prevalence of *PAX2* mutations among large cohort of children with steroid resistant nephrotic syndrome (SRNS) and FSGS have not been systematically studied.

In the present study, following identification of a child with coloboma, nephrotic range proteinuria and renal histology showing FSGS and in whom we detected a *de novo* pathogenic mutation in *PAX2*, we hypothesized that *PAX2* mutations may be relatively prevalent among children diagnosed with steroid resistant nephrotic syndrome/FSGS and who have no molecular genetic diagnosis. Accordingly, we analyzed whole exome sequencing data from 215 different families with steroid resistant nephrotic syndrome in whom no causative mutation in a known SRNS gene has been previously identified [6–8]. In 57 families there was more than one affected individual. Using this strategy we identified *PAX2* mutations in 3 out of the 215 families studied (1.3%). All 3 cases were detected in families with more than one affected individual (3/57 families (5.2%)). In all 3 cases we established a specific etiologic genetic diagnosis that was not made on the basis of the patient’s clinical findings.

Methods

Study Participants

The study was approved by the Institutional Review Board of the University of Michigan and Boston Children's Hospital. From April of 1998 to June of 2016, patients were enrolled after obtaining informed consent [9]. Inclusion criteria were: onset of symptoms before 25 years and a clinical diagnosis of steroid-resistant nephrotic syndrome (e.g. proteinuria, hypoalbuminemia, and edema) or nephrotic range proteinuria with kidney histology of FSGS or diffuse mesangial sclerosis [9]. Excluded from the study were patients with one of the following: (1) non-nephrotic range proteinuria or isolated hematuria; (2) patients with steroid sensitive nephrotic syndrome; (3) patients with steroid dependent nephrotic syndrome; (3) patients with acute glomerulonephritis; (4) patients older than 25 years at nephrotic syndrome onset and (5) patients with known monogenic etiology. Mutations in the following 32 genes known to be mutated in steroid resistant nephrotic syndrome were excluded by whole exome sequencing (WES) [9] before this study: *ACTN4*, *ADCK4*, *ARHGDI1*, *CD2AP*, *COQ2*, *COQ6*, *CRB2*, *CUBN*, *DGKE*, *FAT1*, *INF2*, *ITGA3*, *KANK1*, *KANK2*, *KANK4*, *LAMB2*, *LMX1B*, *MYO1E*, *NPHS1*, *NPHS2*, *NUP205*, *NUP93*, *PDSS2*, *PLCE1*, *SGPL1*, *SMARCAL1*, *TRPC6*, *TTC21B*, *WDR73*, *WT1*, *XPO5*, *AGXT*, *COL4A3*, *COL4A4*, *COL4A5*, *CLCN5*, *CTNS*, *FN1*, *GLA*, *LRP2*, *MEVF*, and *OCRL*.

Description of Cohort

Our original cohort included 300 families with SRNS. Onset of SRNS in our cohort ranged from birth to 24 years of age. Approximately 55% of cases had onset of SRNS before 6 years of age [9]. We examined each family's pedigree for consanguinity and evaluated WES data for homozygosity as well as for one of the 32 SRNS causing genes noted above. We previously identified known SRNS causing genes in 74 families and mutations in genes causing SRNS phenocopy in 11 families [9]. These families were excluded from the current study. We reviewed each individual for extra-renal manifestations, including cardiac defects, microcephaly, and limb abnormalities. Twenty seven percent of patients in our cohort had extra-renal manifestations. The cohort's most common clinical diagnosis was SRNS in two thirds of the cohort compared to those who were diagnosed with congenital nephrotic syndrome, infantile nephrotic syndrome, or those with biopsy evidence of FSGS or DMS (diffuse mesangial sclerosis) but were not clinically nephrotic [9]. Overall our cohort included clinical data as well as WES data from a total of 215 families with steroid resistance nephrotic syndrome without any known monogenic etiology.

Whole-exome Sequencing and Variant Calling

Whole exome sequencing (WES) was performed using genomic DNA isolated from blood lymphocytes and later processed using Agilent SureSelect human exome capture arrays (Life Technologies™) with next generation sequencing on an Illumina™ sequencing platform at the Broad Institute (Cambridge MA). Sequence reads were mapped to the human reference genome assembly (NCBI build 37/hg19 www.genome.ucsc.edu) using CLC Genomics Workbench (version 6.5.1) software (CLC bio, Aarhus, Denmark) as previously described [10]. Mutation calling for known steroid resistance nephrotic syndrome genes has

been previously described [9]. As noted above these families were excluded in the current study. WES Trio analysis for the index family was performed as previously reported [11].

PAX2 variant calling

Following WES, *PAX2* genetic variants were first filtered to retain only non-synonymous and splice variants. Second, filtering was performed to retain only alleles with a minor allele frequency (MAF) of zero. MAF was estimated using combined datasets incorporating all available data from the 1,000 Genomes Project, the Exome Variant Server (EVS) project, dbSNP142, and the Exome Aggregation Consortium (ExAC). Third, observed sequence variants were analyzed using the UCSC Human Genome Bioinformatics Browser for the presence of paralogous genes, pseudogenes, or misalignments. Fourth, we scrutinized all variants within the sequence alignments of the CLC Genomic Workbench™ software program for poor sequence quality and for the presence of mismatches that indicate potential false alignments. Fifth, we employed web-based programs to assess variants for evolutionary conservation, to predict the impact of disease candidate variants on the encoded protein, and to determine whether these variants represented known disease-causing mutations. All bioinformatics analysis was performed by clinician scientists, with knowledge of the clinical phenotypes and pedigree structure, as well as experience with whole exome sequencing evaluation. Sanger sequencing was performed to confirm the remaining variants in original DNA samples and when available to test for familial segregation of phenotype with genotype.

Results

WES trio analysis identifies an index case of FSGS and PAX2 mutation

We consulted the index family (AN10), a non-consanguineous Bedouin pedigree (Figure 1). The index patient was a female girl born via cesarean section at term. Prenatal ultrasound examination during the second trimester demonstrated microcephaly and mild cardiac hypertrophy. Following birth, the patient was diagnosed with mild hypertrophic cardiomyopathy and later developed hypertension. She started treatment with a nonselective beta blocker/alpha-1 blocker (Carvedilol) and has been well controlled. Post-natal metabolic work-up including urine organic acids, acyl-carnitine profile, plasma carnitine and lactate was unremarkable. In addition, muscle biopsy was normal. Consequently, during infancy her renal function gradually deteriorated (serum creatinine level at age 1y was 0.8–0.9 mg/dL [normal range 0.2–0.5 mg/d]) and she developed nephrotic range proteinuria with urine protein to creatinine ratio (UPC) of 6.7 mg/mg. Renal US demonstrated mildly echogenic kidneys with normal shape and size, without evidence of hydronephrosis or bladder abnormalities. Subsequent renal biopsy demonstrated focal segmental glomerulosclerosis (FSGS) and no features of renal hypodysplasia (Figure 1). The patient did not respond to steroids treatment and subsequently developed end stage renal disease. She required renal replacement therapy at the age of 4 years. At the same time, a routine eye examination revealed bilateral coloboma of the optic nerve.

For this family we performed trio WES analysis. First, we analyzed all known nephrotic syndrome causing genes; this analysis was negative. Next, we performed analysis under

autosomal recessive hypothesis-which also yielded negative results. Finally, we performed *de novo* analysis and detected a previously reported heterozygous truncating mutation in *PAX2* (c.69–70InsG; p.Val26Glyfs*28). We [12] as well as others [13–15] showed that this particular truncating mutation resides in a mutation hot spot region and for which a germline mosaicism inheritance pattern have been suggested previously in three different families [13–15]. Interestingly, previous mouse model carrying this mutation [16], showed abnormal brain development, in addition to the renal, eye and kidney phenotype. This may suggest that our patient's microcephaly may be related to the underline *PAX2* mutation.

Identification of causative mutations in *PAX2* in 3 families with steroid resistant nephrotic syndrome secondary and FSGS

We next analyzed the prevalence of *PAX2* mutations in a cohort of 215 families with steroid resistance nephrotic syndrome. Surprisingly, in 3 families with autosomal dominant familial form of SRNS (Figure 1) we identified 3 different novel *PAX2* mutations. This included two missense mutations affecting a highly conserved amino acid (up to *Danio rerio*) and one obligatory splice mutation. In each family the *PAX2* mutation co-segregated across all affecteds available to us (Table 1). Overall, we identified *PAX2* mutations in 3 out of the 215 families studied (1.3%). All 3 cases were detected in families with more than one affected (3/57 families (5.2%)).

In family A4041 we detected a novel missense mutation affecting the *PAX2* protein paired domain (c.254G>T; p.Gly85Val). In this family, two affected siblings presented during adolescence with nephrotic range proteinuria and impaired GFR. Renal biopsy findings for both siblings showed FSGS (Figure 1). Both failed to respond to steroid treatment. Their father was transplanted at age 40 years and similarly presented with nephrotic range proteinuria and advanced chronic kidney disease. All three affected family members available to us carried the heterozygous *PAX2* mutation. All affecteds had no overt coloboma or hearing impairment or other extra renal or syndromic features. Renal imaging studies as well as renal histology showed no signs of CAKUT.

In family A5089 we detected a novel *PAX2* splice mutation affecting an obligatory splice site, predicted to lead to skip of exon 8 (c.862–1G>A). In this family the index child presented during adolescence with nephrotic range proteinuria (UPC = 8), elevated creatinine and low albumin, which was found incidentally. Renal biopsy showed FSGS. On presentation the patients had severely depressed GFR of 16 ml/min/1.73m² and subsequently required renal replacement therapy. As a result she was not treated with a trail of steroids. The patient's father had at the age of 55 years, normal renal function and nephrotic range proteinuria. The affecteds had neither extra renal or syndromic features nor imaging or histology signs of CAKUT.

In family A5281 we detected a novel missense mutation affecting the *PAX2* protein paired domain (c.275G>T; p.Thr92Met). In this family the index patient presented incidentally with hypertension which was diagnosed on routine examination. He was found to have nephrotic range proteinuria and his renal biopsy was remarkable for FSGS. Interestingly, his mother as well as his maternal grandfather had end stage renal disease and required renal replacement

therapy during adulthood. Unfortunately, for both, DNA and renal biopsy findings were not available to us.

Discussion

In the current study, we examined a group of 215 unrelated families with steroid resistance nephrotic syndrome for the presence of heterozygous *PAX2* disease-causing mutations. We identified three different novel *PAX2* mutations which co-segregated across all affected individuals. All variants were novel and were predicted to be deleterious (Table 1). Specifically, two variants affect the *PAX2* protein paired domain and one affect an obligatory splice site leading to exon skipping. In all patients, CAKUT phenotype was excluded as well as disease causing mutations in known SRNS genes. None of the *PAX2* disease-causing mutations was suspected on clinical grounds prior to the current study, and affected patients were not clinically distinguished from other steroid resistant nephrotic syndrome patients.

Our study highlights several important conclusions: First, we expanded the phenotypic spectrum associated with *PAX2* mutations and further established it to include autosomal dominant childhood-onset FSGS. Second, utilizing a large cohort of patients with SRNS allowed us to determine *PAX2* mutation frequency (5.2% in multiplex families with SRNS). Third, the diagnosis of *PAX2* related SRNS may inform clinical practice as those patients fail to respond to steroid treatment. In addition, it may trigger clinician to look for subtle extra renal findings such as coloboma or hearing impairment. Fourth, in 3 out of the 4 index children studied, the clinical diagnosis was made incidentally. This can explain previous observation ascribing *PAX2* mutations to adult onset FSGS. It also emphasizes the importance of urinalyses screening programs for children. Finally, this study highlights the potential benefits of clarifying diagnosis in SRNS through WES to avoid unnecessary invasive procedures and treatments, especially given that *PAX2* mutations may account for a significant proportion of families designated to have autosomal dominant form of SRNS.

In summary, we identified *PAX2* mutations in 3 out of the 215 families studied (1.3%). All 3 cases were detected in families with more than one affected individuals with SRNS/FSGS (3/57 families (5.2%)). In all 3 cases we established a specific etiologic genetic diagnosis that was not made on the basis of the patient's clinical findings. Our results further establish the role of *PAX2* among children with SRNS/FSGS.

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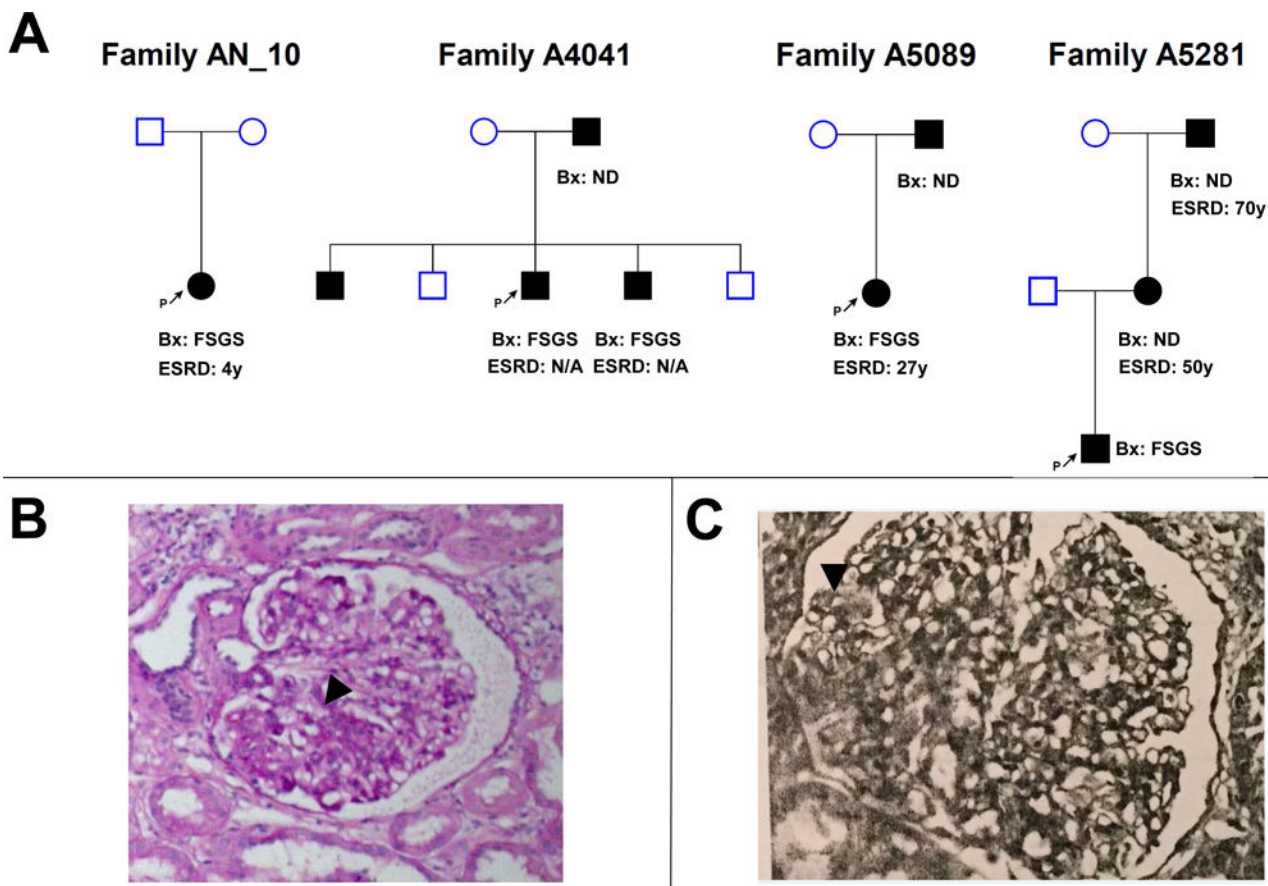


Figure 1. Pedigree structures and representative renal histology findings in patients with steroid resistant nephrotic syndrome and *PAX2* mutations.

(A) Pedigree structure of index family (AN_10) and three families with FSGS and *PAX2* mutations (A4041, A5089, and A5281).

(B) Pathologic findings in renal biopsy specimen from affected individual in family AN_10 showing segmental glomerulosclerosis (black arrow head).

(C) Pathologic findings in renal biopsy specimen from affected proband in family A4041 showing segmental glomerulosclerosis (black arrow head).

Bx, Biopsy; ESRD, end stage renal disease; N/A, not available; ND, not determined.

Table 1.

Four different heterozygous *PAX2* mutations detected in 4 different families with SRNS/FSGS

Family -Individual	Ancestry	Nucleotide alteration	Alteration in coding sequence ^a	Zygosity	Continuous AA sequence Conservation	SIFT/PP2	Presenting symptoms (Age)	Renal Histology	eGFR at presentation /ESRD age	Extra-renal phenotype
AN10 -21 (index family)	Arab	c.69-70InsG ^b	p.Val26Glyfs*28	Het	<i>Truncating</i>	Deleterious/ 1 (reported)	Elevated creatinine and proteinuria (2y)	FSGS	ESRD at age 4y	Coloboma, Cardiomyopathy, microcephaly
A4041 -11	Egypt	c.254G>T	p.Gly85Val	Het	<i>Danio rerio</i>	Deleterious/ 1 (novel)	Proteinuria and elevated Creatinine	n/a	ESRD at age 39y	-
A4041 -23	Egypt	c.254G>T	p.Gly85Val	Het	<i>Danio rerio</i>	Deleterious/ 1 (novel)	Proteinuria and elevated Creatinine (16y)	FSGS	63 ml/min/1.73m ²	-
A4041 -24	Egypt	c.254G>T	p.Gly85Val	Het	<i>Danio rerio</i>	Deleterious/ 1 (novel)	Edema, proteinuria and elevated Creatinine (14y)	FSGS	50 ml/min/1.73m ²	-
A5089 -11	Europe	c.862-1G>A	Splice mutation	Het	<i>Splice</i>	Obligatory splice <i>b</i> (novel)	Proteinuria and normal Creatinine	n/a	n/a	-
A5089 -21	Europe	c.862-1G>A ^c	Splice mutation	Het	<i>Splice</i>	Obligatory splice (novel)	Edema, proteinuria and elevated Creatinine (20)	FSGS	ESRD at age 27	-
A5281	Europe	c.275C>T	p.Thr92Met	Het	<i>Danio rerio</i>	Deleterious/ 1 (novel)	HTN found on screening (18)	FSGS	n/a ^d	Cryptorchidism

^aAll mutations had a PolyPhen2 (PP2) and SIFT score of functional 'deleteriousness' (<http://genetics.bwh.harvard.edu/pph2>; <http://sift.jcvi.org/>)

^bThis mutation has been previously reported as disease causing.

^cThis mutation is predicted to change conserved donor site and to be deleterious using 3 different in silico splice prediction software (MaxEnt, NNSPLICE and HSF).

^dPatients' mother and maternal grandfather went to ESRD at age 50y and 70 y respectively
ESRD, end stage renal disease; FSGS, focal segmental glomerulosclerosis; Het, heterozygous; HTN, hypertension; n/a, not available; pt. patient.