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PAX2/Renal Coloboma Syndrome Expresses Extreme Intrafamilial Phenotypic Variability

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Keywords
Renal coloboma syndrome · PAX2 · Phenotypic variability · Epigenetics

Abstract
Renal coloboma syndrome (RCS) is a disease characterized by kidney and ocular anomalies (kidney hypodysplasia and coloboma). RCS is caused, in half of the cases, by mutations in the paired box 2 (PAX2) gene, a critical organogenesis transcriptional factor. We report the case of a newborn with kidney hypodysplasia in a negative parental context where mother and father were phenotypically unaffected at the initial evaluation. The maternal family presented an important history of kidney disease with undefined diagnosis. Molecular characterization identified a PAX2 variant, classified as likely pathogenic. This variant segregates with the disease, and it was also found in the newborn, explaining his severe symptoms. It is noteworthy that the mother shows the same PAX2 variant, with an apparently negative kidney phenotype, displaying the possibility of an extreme variable expressivity of the disease. This feature suggests extreme caution in segregation analysis and family counseling of PAX2 pedigrees.
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Introduction

Renal coloboma syndrome (RCS) or autosomal dominant papillorenal syndrome (OMIM # 120330) is a disorder characterized by both ocular and renal anomalies, including renal dysplasia and coloboma [1, 2]. Less common findings include high-frequency hearing loss and central nervous system (CNS) anomalies [2, 3].

Affected patients may show eye alterations as optic nerve coloboma or morning glory anomaly, which can lead to the loss of visual acuity up to complete blindness in severe cases [4]. However, the excavation of the optic nerve and retinal vascular abnormalities can be mild, and they may lead to the mistaken diagnosis of normal-tension glaucoma or an isolated congenital anomaly [5].

Renal malformations mainly consist of small kidneys with signs of dysplasia at ultrasound scan (hypodysplasia)

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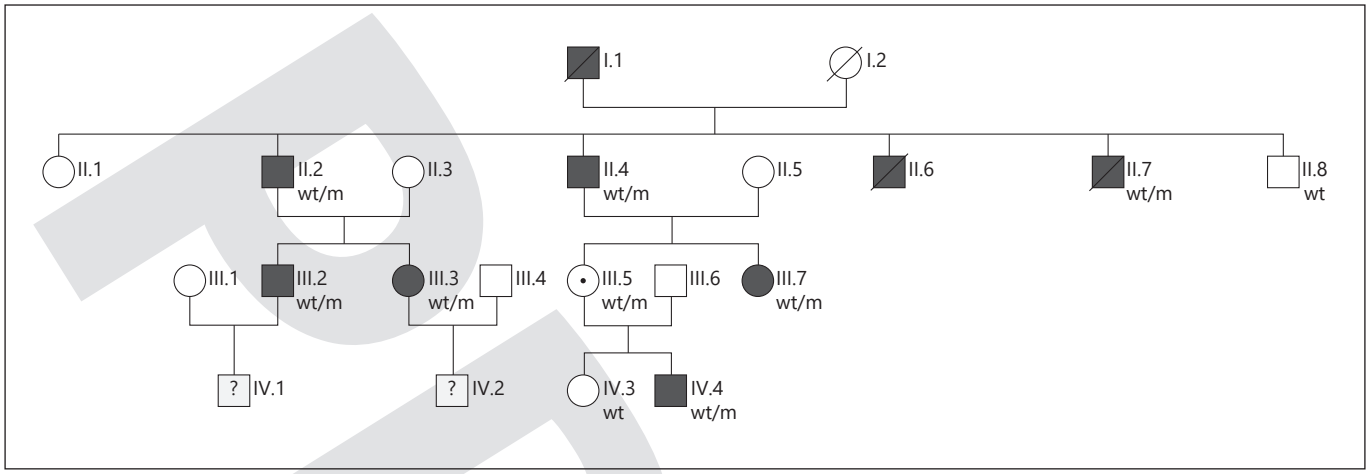

Fig. 1. Pedigree of the family. Affected individuals are indicated in black. The apparently phenotipically unaffected variant carrier is indicated with a black point. The number indicates the generation and pedigree position. wt, PAX2 wild type; m, PAX2: c.410+5G>A.

[3, 4]. Literature reports describe a wide spectrum of congenital renal phenotypes, such as multicystic dysplastic kidney [6] and autosomal dominant adult-onset focal segmental glomerulosclerosis [7]. The most frequent clinical symptoms include hypertension, proteinuria, vesicoureteral reflux, and renal failure [8]. Histologically, kidneys can show focal glomerulosclerosis [7] and compensatory hypertrophic glomeruli (oligomeganephronia) [9].

RCS is associated with paired box 2 (PAX2) mutation in half of the cases. PAX factors have a crucial role in cell fate, early patterning, and organogenesis [10, 11]. The expression of PAX genes is temporally and spatially restricted during the development of various organs such as CNS, kidney, and main sense organs [12, 13].

The involvement of PAX2 during embryo development might explain the inter- and intrafamilial phenotypic heterogeneity reported in RCS [14, 15]. This variability was also replicated in PAX2 mouse models, particularly in the heterozygous *Krd* mutant mice, which showed high kidney phenotype variability [16].

In this study, we report a case of RCS in a family with a form of extreme variable expressivity. The expressivity was so mild in the variant carrying mother to be confused as an unaffected subject at the initial evaluation, highlighting the difficulty of the segregation analysis and familial counseling in the context of a PAX2 pedigree.

Case Presentation

A newborn (Fig. 1, patient IV.4) was affected by severe renal failure and renal hypodysplasia at birth. Parents were both clinically healthy, with no signs of kidney disease. The mother's family history reported cases of nephropathy and kidney failure (Fig. 1) without a conclusive diagnosis.

Until the birth of her child, the mother was considered clinically unaffected by the genetic condition occurring in the family: she presented a bipolar normal kidney length (9.4 cm right, 9.1 cm left) for a woman of 160 cm height and the clinical exams (creatinine 0.96 mg/dL, no urine abnormalities) indicate a low risk of chronic kidney disease [17].

The male infant was born at 38 weeks of gestation (birth weight: 3615 g). Prenatal ultrasound evaluations were reported as normal. A weight loss of more than 10% was observed during the first few days of life. At 1 month of life, birth weight had not been regained (3,250 g) yet, and anorexia with vomiting was reported. Laboratory tests revealed increased creatinine (Cr 0.79 mg/dL), hyponatremia and hypochloremia (Na 131 mEq/L, Cl 93 mEq/L), reduced osmolality (269 mOsm/L), normal protidemia (6.4 g/dL), and albuminemia (41.1 g/L). Arterial blood gas values were within the normal range. Urine analysis provided the following results: specific gravity 1,004, albumin 30 mg/dL, and hemoglobin 0.2 mg/dL (RBCs 102/mL). Additional analyses provided the following: urinary protein/creatinine ratio 3.9 (uPr/uCr, mg/mg), fractional excretion of sodium 1.76%, tubular reab-

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Table 1. Clinical features and PAX2 variants for 13 members of the family

	Age, years	Kidney function (mL/min/1.73 m^2)	Hypodysplastic kidney	Cystic kidney	Kidney biopsy	Coloboma/ocular abnormalities	Auditory abnormalities	PAX2 variants
I.1	Deceased (45 years)	NA	NA	NA	No	NA	NA	NA
II.1	NA	NA	No	No	No	NA	NA	NA
II.2	70	Transplant (43 years)	NA*	Yes (bilateral)	Focal segmental glomerulosclerosis	NA	NA	c.410+5G>A
II.4	68	18	NA*	No	No	Optic disk excavation, negative for coloboma	Bilateral sensorineural hearing loss	c.410+5G>A
II.6	Deceased	NA	NA	NA	No	NA	NA	NA
II.7	65	41	NA	NA	No	NA	NA	c.410+5G>A
II.8	62	60	NA	NA	No	NA	NA	No
III.2	48	Transplant (47 years)	NA*	Yes (right kidney)	Mesangial glomerulonephritis	High ocular pressure, optic disk pit	Right sensorineural hearing loss	c.410+5G>A
III.3	46	Transplant (31 years)	NA*	Yes (parapyelic, right kidney)	Mesangial glomerulonephritis	No	NA	c.410+5G>A
III.5	36	78	No	No	No	Small temporal coloboma	No	c.410+5G>A
III.7	29	Transplant (27 years)	Yes	No	Focal segmental glomerulosclerosis	No	No	c.410+5G>A
IV.3	4	166°	No	No	No	NA	No	No
IV.4	0	31°	Yes	Yes, microcysts	No	No	No	c.410+5G>A

NA, not a vailable. °Kidney function calculated accordind to the revised Schwartz equation for glomerular filtration rate in pediatric subjects. *Ultrasound scans performed in the adult kidney (hyperechogenicity, small kidneys, and poor cortical-medulla differentiation).

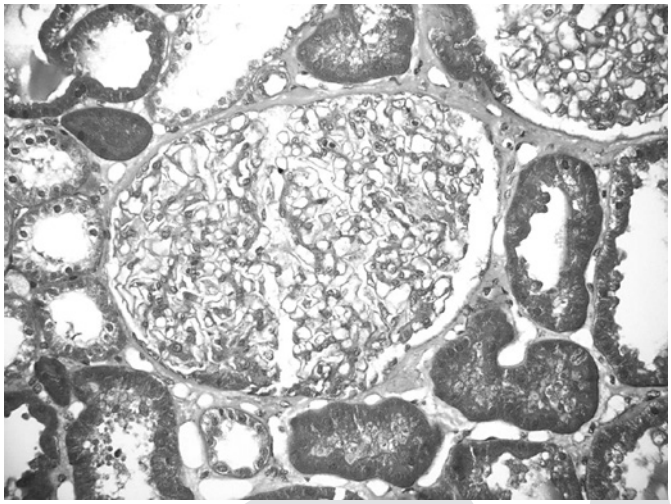

Fig. 2. Kidney biopsy of patient III.7, the image shows a hypertrophic glomeruli (×40 of magnification, Masson trichrome).

sorption of phosphorous 52.2%, and alpha-1-microglobulin 61 mg/L (n.v. <12 mg/L).

Failure to thrive may be a usual presentation of renal hypodysplasia, due to hyperfiltration and low concentration ability, caused by the reduced number of nephrons, primitive tubules, and interstitial fibrosis. For this reason, to minimize weight loss, treatment with NaCl (2 mEq 3 times daily) and captopril (0.3 mg 3 times daily) was started, and additional water was supplemented to infant formula.

At the last follow-up (8 months of age), the patient showed normal ponderal and length growth, with improved renal function (creatinine 0.42 mg/dL, urea 21 mg/dL) and slightly reduced proteinuria (urine analysis: specific gravity 1,005, pH 6, albumin absent, hemoglobin absent, uPr/uCr: 1.1). The kidneys appeared hypoplastic on ultrasound (right kidney 39 mm and left kidney 36 mm), with diffuse cortical hyperechogenicity; some hyperechoic foci were also visible, likely due to microcysts (max 2 mm). The gallbladder was distended, with an endoluminal gallstone of 5–6 mm. Therapy was thus modified to NaCl 2 mEq 3 times daily, captopril 0.3 mg 3 times daily, and ursodeoxycholic acid 24 mg twice daily. The ophthalmological evaluation was unremarkable: normal visual acuity and ocular fundus.

To investigate the etiopathogenesis, the family was clinically and genetically characterized (Table 1). Information regarding the medical history of thirteen members was available: 9 affected and 4 healthy. The relatives' overall workup, performed during a time span of over 20 years, consisted of clinical exams, ultrasound scans, cystourethrogram (two subjects), ophthalmic examination (six subjects), and, in four cases, renal biopsy. Nine subjects developed kidney failure, two of these died (patients I.1 and II.6, Fig. 1); of the remaining seven patients, four received kidney transplants (II.2, III.2, III.3, III.7) between the second and fourth decades of life (37 ± 9.5 years) and three (II.4, II.7, IV.4) have been on conservative treatment. Patient III.7 experienced an early failure of the kidney transplant, and she is currently on hemodialysis. In two cases (III.7, IV.4), ultrasound exams were performed in pediatric age. The results clearly suggested a condition of hypodysplasia. Of the remaining five individuals, four (II.2, II.4, III.2, III.3) underwent ultrasound exams in adult age, reporting renal hyperechogenicity, small kidneys, and poor cortical-medullary differentiation. No ultrasound documentation was received for one subject (II.7). In four cases, multicystic kidneys were reported. Four kidney biopsies were performed. Two showed a picture of focal segmental glomerulosclerosis (subjects II.2, III.7), while the other two a mesangial glomerulonephritis (subjects III.2, III.3). Patient III.7 also showed hypertrophic glomeruli (Fig. 2). Subject III.5 (newborn's mother) presented normal renal function and normal-size kidneys without cysts.

Ophthalmic examinations described a diagnosis of glaucoma or papillary excavation in 2 patients (II.4, III.2), also associated with sensorineural hearing loss. Subject III.5 underwent a first ophthalmic examination before the birth of her child with a negative result. However, after the genetic diagnosis, a specific opthalmic revaluation requested for suspicion of RCS revealed a small right temporal coloboma.

Methods

DNA Analysis

A genetic analysis was made on 10 family subjects. All subjects gave written informed consent in compliance with the Declaration of Helsinki. The III.7 subject was analyzed by Next-Generation Sequencing with the platform MiSeqDx System (Illumina, San Diego, CA, USA). The Nephropathies Solution V_3 (NES, SOPHiA Genetics) Kit, containing 44 genes related to Mendelian kidney diseases, was used (online suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000525022). Sequencing data were processed for single nucleotide variants, indels, and copy number variations via the SOPHiA DDM platform. The pathogenic and likely pathogenic variants (scored according to the American College of Medical Genetics and Genomics [18]) were confirmed by the Sanger technique, platform Applied Biosystems® 3500xL. The segregation of likely pathogenic and pathogenic variants was analyzed by Sanger sequencing.

Table 2. Splicing tools' prediction scores for variant PAX2: c.410+5G>A

Algorithm/matrix	Reference	Variant	Wild type	Mutant	Variation	Threshold
HSF donor site	CAGGTGAGC	CAGGTGAAC	94.23	84.66	−10.16%	<−10% [20]
MaxEnt donor site	CAGGTGAGC	CAGGTGAAC	9.6	5.56	−42.08%	<−30% [20]
AdaBoost	CAGGTGAGC	CAGGTGAAC	NA	0.9994	NA	>0.6 [21]
Random forest	CAGGTGAGC	CAGGTGAAC	NA	0.9060	NA	>0.6 [21]

The algorithms calculated the variation between wild-type and mutant sequences. NA, not applicable; HSF, Human Splice Finder.

Results

The molecular analysis disclosed a heterozygous variant in an intronic region of PAX2: c.410+5G>A (NM_000278.4), previously described in an unrelated Italian patient affected by RCS [19], and no other pathogenic or likely pathogenic variants were found in the proband (subject III.7, online suppl. Table S2). The variant is predicted to have a significant impact on splicing (Table 2), Human Splice Finder indicates a variation between wild type and mutant of −10.16%, and this variation predicts a broken splice site (MaxEnt scores: −42.08%, with a threshold of −30%) [20]. AdaBoost score is 0.9994 and Random Forest 0.9060, with a range between 0 and 1 (high probability) and a threshold of 0.6 [21]. Furthermore, CADD score is 22.9 (according to this predictor, a value above 20 is defined "harmful") [22].

Regarding this variant, Negrisolo et al. [19] suggested a possible effect of exon 3 skipping due to breaking in the canonical splice site. Exon 3 is part of the paired domain involved in the DNA binding of the protein. The variant was defined as likely pathogenic (class 4) according to the ACMG guidelines [18].

The variant segregated with the kidney disease status of the family members, except for the subject III.5, the newborn's mother, that presented normal renal function. The late discovery of a small coloboma in the apparently negative subject III.5 finally confirmed a complete segregation of the likely pathogenic (class 4) variant of PAX2, albeit with extreme kidney phenotypic variability.

Discussion

RCS is characterized by both ocular and renal anomalies, mainly consisting of renal dysplasia and coloboma [1, 2]. In half of the cases, pathogenic variants of the PAX2 gene were found. PAX2 is a transcriptional factor with a central role during the organogenesis of different organs, particularly the kidney, urinary tract, and CNS. The main clinical renal picture of RCS is characterized by hypodysplasia and renal dysfunction. Under ultrasound examination, the kidney usually shows hypoplasia with cysts or signs of tissue mal-differentiation and enhanced echogenicity. These features are reported with high inter- and intrafamilial variability [14, 15, 18]. Iatropoulos et al. [14] reported a clear example of this potential intrafamilial variability: two monozygotic twin sisters carrying the same PAX2 variant, the same genetic background, but different renal and ocular disease expression. One sister underwent kidney transplantation at the age of 23 years, but never presented ocular anomalies. The other showed renal failure that rapidly resolved, but she lost left visual acuity secondary to coloboma at the age of two.

In this study, we report a case of extreme intrafamilial phenotypic variability: a newborn with chronic renal failure and renal hypodysplasia at birth in an apparently negative parental context. Subsequent molecular examination revealed that the affected newborn and the phenotypically unaffected mother are both carriers of the same familial pathogenic variant (PAX2: c.410+5G>A). It is noteworthy that her renal function and morphology are unremarkable; she was reported as negative in a previous ophthalmic examination and a small coloboma was reported only after a specific new evaluation for this suspect. Despite a previous description of this variant in another unrelated Italian patient [19] with a similar kidney phenotype but without family history, the extreme heterogenicity of our case is unprecedented.

In our report, the PAX2 variant expresses different renal and ocular phenotypes within the same family, both in terms of severity of renal failure and impairment of sense organs. Renal phenotype is highly heterogeneous for unilateral or bilateral cysts and histological features (focal segmental glomerulosclerosis and mesangial glomerulonephritis). This heterogeneity shows the highest

Table 3. Potential molecular mechanisms explaining the high variability of the family phenotype

Hypothesis	Brief description	Ref.
Genetic background: modifier genes	Genetic background modulates the expression of a specific gene	[24]
Epigenetics	DNA methylation (and other modifications) that modulate transcriptional and post-transcriptional regulation	[25]
Germline mosaicism (likely not applicable in this family)	A postzygotic mutation occurring in an unaffected parent	[26]

level in subject III.5, who presents normal renal function, negative renal ultrasound examination, and a small coloboma. None of the other members showed retinal coloboma which is typical but not constantly associated with RCS. Nevertheless, further investigations confirmed a diagnosis of glaucoma and papilla excavation in two patients that could be in line with the ocular abnormalities found in other RCS cases [14, 19, 23].

Although the cause of RCS syndrome can be identified in the PAX2 variant, which is shared by all the affected members, the high variability of the phenotype is difficult to explain (see Table 3). Other genes not examined with NES genetic panel could influence the expression and the effect of PAX2 [24]. Besides, studies on monozygotic twins revealed how genetically identical individuals could exhibit differences in phenotype, showing the possible contribution of epigenetics in the definition of phenotype [25]. Mosaicism can be invoked as a possible cause of incomplete penetrance, but this hypothesis is hardly plausible in our family. Indeed, subject III.5 inherited the pathogenic variant from her father (II.4), virtually excluding the possibility of a postzygotic mutation. Even if the hypothesis of modifier genes or epigenetic control of gene expression might be the most suggestive, unfortunately, these theories are difficult to prove with experimental evidence.

In conclusion, this report highlights the difficulty of a correct interpretation of PAX2 gene segregation because of variable penetrance and phenotypic heterogeneity. Subject III.5 was completely asymptomatic and considered unaffected due to the negativity of her clinical picture; the other family members, carriers of the same variant, developed chronic renal failure and they received renal replacement therapy early in their clinical history. Owing to her health status and to her previous healthy child, she sought no formal genetic counseling for the new pregnancy. A higher awareness of the large phenotypic variability of this condition and a genetic test would have better supported the family in making an informed decision about the planning of a new pregnancy. For example, alternative reproductive technologies (preimplantation diagnosis) or adoption could have been considered. Therefore, this case must suggest extreme caution in segregation analysis and familial counseling in the context of a PAX2 pedigree.

Statement of Ethics

The paper is exempt from Ethical Committee Approval because it is a simple case report, we have patients' consents and the report contains nothing which might be considered a risk to patient privacy. Written informed consent was obtained from the patients for publication of this case report and any accompanying images. Patients signed informed consent authorizing the use of anonymized results and anonymized clinical data for scientific purposes. Newborn's consent for publication was obtained by the parent.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conception and drafting the article: Silvia Giovanella and Riccardo Magistroni. Analysis and interpretation of data: Silvia Giovanella, Andrea Pasini, Riccardo Magistroni, and Enrico Tagliafico. Revising the article: Giulia Ligabue, Giacomo Mori, and Francesca Testa. All authors approved the draft.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

PROOF

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