EXPERT REVIEWS

# Genetic and developmental basis of renal coloboma (papillorenal) syndrome

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Renal coloboma syndrome, also known as papillorenal syndrome, is characterized by optic nerve anomalies and kidney hypodysplasia. Autosomal dominant mutations in the gene encoding the paired box transcription factor PAX2 can be identified in nearly half of all patients with this phenotype. The primary ophthalmologic findings include congenital central retinal vasculature absence associated with abnormalities in retinal blood vessel patterning and deeply excavated optic discs. Other published findings include optic nerve hypoplasia, optic nerve cyst, optic nerve pits, retinal coloboma, microphthalmia and scleral staphyloma. Visual acuity ranges from normal to severe impairment. Up to one third of affected patients will develop end-stage renal disease. Mouse and zebrafish with *Pax2/pax2a* mutations provide developmentally based explanations for the observed phenotypic observations in affected patients.

The clinical presentation of optic nerve anomalies associated with renal hypodysplasia should alert the clinician to the possibility that a patient may have renal coloboma syndrome, a condition also known as papillorenal syndrome (OMIM#120330). The optic nerve findings could be described as a 'dysplasia', characterized by absent central vessels with the emergence of vessels from the periphery of the optic nerve papilla. The optic nerve findings in this condition have been referred to as 'coloboma' and a fraction of affected patients have clinical evidence of optic fissure closure defects. Mutations in the gene *PAX2* are currently the only known genetic basis for renal coloboma (papillorenal) syndrome and are found in half of patients. Animal models have been instructive in demonstrating that *PAX2* is critical for optic fissure closure and vascular routing in the retina.

The co-occurrence of optic nerve anomalies in patients who developed end stage renal disease was first recognized 30 years ago in two separate reports by Reiger *et al.* [1] and Karcher *et al.* [2]. Affected family members had optic nerve abnormalities described as 'morning glory anomaly' with deeply excavated optic nerves, abnormal passage of the optic vessels at the periphery of the disc rather than its center and a central glial tuft [1,2]. A total of 10 years later, Weaver *et al.* reported a case of two brothers, both with optic nerve colobomas, interstitial nephritis and renal failure [3]. Weaver noted the similarity of the optic nerve colobomas in his patients with the 'morning glory anomaly', previously reported by Karcher *et al.* [1]. Weaver and coauthors called this condition renal coloboma syndrome. The following year, Bron *et al.* reported a family with an autosomal dominant condition, whose affected members exhibited optic disc dysplasia, microphthalmia, morning glory anomaly, optic nerve glial remnants, central serous retinopathy and renal failure [4]. Bron *et al.* reviewed a number of conditions where eye and kidney findings predominated. These authors suggested the name papillorenal syndrome to describe a variety of conditions where optic nerve and kidney abnormalities are observed [4].

Sanyanusin *et al.* provided the first evidence that renal coloboma (papillorenal) syndrome was caused by autosomal dominant mutations in the transcription factor gene *PAX2*. In this report, a father and three children were described to have optic disc dysplasia/optic nerve coloboma and renal hypodysplasia [5,6]. Following this report, Sanyanusin *et al.* published a second article where they identified a *PAX2* mutation in the previously

discussed family described by Weaver *et al.* [7], confirming that renal coloboma syndrome resulted from mutations in *PAX2*. In 2001, Parsa *et al.* reported two unrelated families with a clinically identical condition that was called papillorenal syndrome based on the observation that no evidence of optic fissure closure defects were noted and that the eye findings consisted of vacant discs with emergence of cilioretinal vessels from the periphery of the optic disc [8]. Mutations in *PAX2* were not identified in these families [8]. However, the clinical similarities suggest that both conditions were the same.

In this review, issues of nomenclature and clinical findings of renal coloboma syndrome (papillorenal syndrome) will be discussed. We will examine evidence from *Pax2/pax2* animal models in an effort to understand how developmental defects contribute to the clinical observations in this condition. About half of individuals who have renal coloboma (papillorenal) syndrome have point mutations in the coding exons of *PAX2*. Therefore, it is very likely that other genetic mechanisms also cause renal coloboma (papillorenal) syndrome.

## Nomenclature

Difference in terms to describe the present condition (i.e., renal coloboma syndrome or papillorenal syndrome) are a continuing source of confusion. In many reports, where individuals were identified to have mutations in *PAX2*, the eye findings have been described as optic nerve colobomas [3,6,9–21], sometimes along with retinal colobomas [10,11,15,19]. Parsa *et al.* noted that anomalous optic nerves characterized by absent central vessels but the emergence of vessels from the periphery of the optic nerve papilla should be considered a 'dysplastic' optic nerve rather than a coloboma, as optic fissure closure defects are not typically observed, and thus recommended the appellation 'papillorenal syndrome' to broadly describe optic nerve abnormalities [8].

Both literature and online resources recognize usage of both names. Online Mendelian Inheritance in Man (OMIM) lists papillorenal syndrome as the primary appellation. A search of PubMed (November 7, 2008), using the search term 'renal coloboma syndrome' identified 138 citations while a search using the term 'papillorenal syndrome' identified 13 citations. From the more frequent use of renal coloboma syndrome in the medical literature, it would suggest that renal coloboma syndrome is the more commonly recognized term. For the remainder of this review, 'renal coloboma syndrome' and 'papillorenal syndrome' will be used to denote the same condition.

## Ocular phenotypes in renal coloboma (papillorenal) syndrome

Published clinical observations of ocular phenotypes from individuals with renal coloboma (papillorenal) syndrome and mutations in *PAX2* include: optic nerve dysplasia, optic nerve coloboma, retinal coloboma, cystic dilatation of the optic nerve, optic nerve hypoplasia and microphthalmia [3,8,11–14,19,20,22–28,101]. FIGURE 1 shows retinal photographs from individuals with renal coloboma (papillorenal) syndrome.

In reviewing the ocular phenotypes of 85 patients with confirmed point mutations in the coding exons of *PAX2*, 46 were described to have optic nerve colobomas [3,6,9–21], 20 were described with optic disc dysplasia [9,11,14,16,19,23,24], five were described with optic disc hypoplasia/atrophy [9,10,14,16,24], six were described with optic nerve pits [9,19,24], six were described with retinal colobomas [10,11,15,19], four were described with morning glory-like anomalies [11,19,29], three had scleral staphyloma [6,15,29], two had optic nerve cyst [10,15], three had microphthalmia [10,11,18] and five had macular anomalies [20,23,24,31]. A single patient with bilateral anomalous discs had foveal hypoplasia and pigmented macular atrophy [30]. In this group of patients with *PAX2* mutations, eight had normal eye examinations [14,16,20,21,29]

In patients without a mutation in *PAX2*, the optic disc abnormalities were comprised of absent central vessels with cilioretinal vessels emerging from the disc margins [8]. Other findings included a low serous retinal detachment and a scarcity of vessels nasal to the disc. Similar observations were made by Nguyen and Riordan-Eva [28], in the patient described by Chen *et al.* [27] and in eight patients described by Dureau *et al.* [19]. These findings overlap with the clinical findings in patients in whom a *PAX2* mutation has been identified.
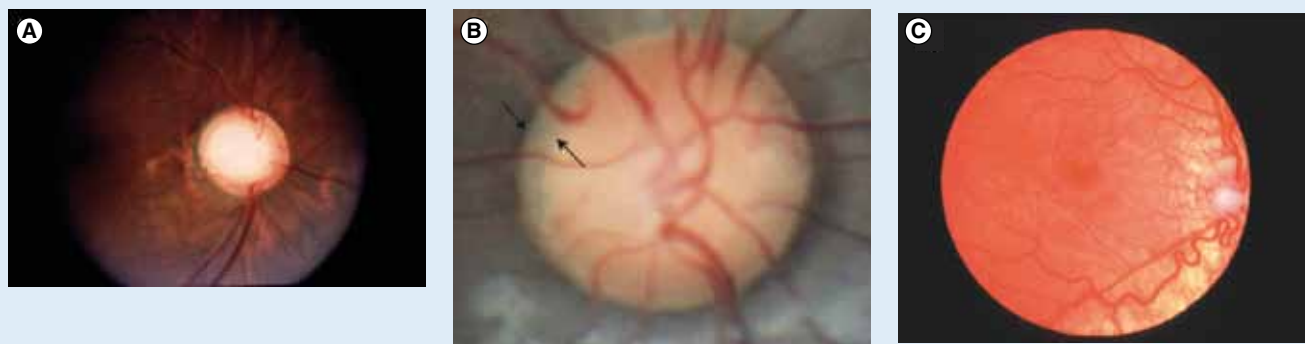


**Figure 1. Fundoscopic photos from three different patients with autosomal dominant mutations in *PAX2*. Note abnormal passage of retinal vessels from the optic nerve head. (A)** This is a photograph of the right fundus from a patient with the *PAX2* mutation c.77dupG [15]**. (B)** This is a photograph of the left fundus from a patient with the *PAX2* mutation c.59delT. The arrow denotes the anomalous portion of the optic nerve head [12]**. (C)** This is a photograph of the right fundus from a patient with the *PAX2* mutation p.Arg115X [23].

Visual acuity was reviewed in 54 studied patients (108 eyes) with mutations in *PAX2*. Visual acuity ranged from normal to light/color perception [23]. A total of 39 eyes were recorded to have normal vision. Ten eyes were reported to have visual acuity to count fingers and two patients had only light and color perception. Only one eye in one patient lacked light perception [10]. Myopia was frequently reported. Studies to determine the long-term visual prognosis have not been performed; however, retinal detachments have been reported [24,29].

Asymmetry of ocular findings has been reported in affected patients. Examples include a patient with a large left optic nerve coloboma and optic pit [19], left normal fundus and right optic nerve atrophy [14], and left optic nerve hypoplasia and right coloboma [16].

## Kidney phenotypes of renal coloboma (papillorenal) syndrome

The most common phenotype in patients with renal coloboma (papillorenal) syndrome who have confirmed mutations in *PAX2* is renal hypodysplasia [16,32]. A number of histologic abnormalities have been noted in affected kidneys, including an overall decrease in nephron number with nephron hypertrophy (called oligomeganephronia) [14,24], focal segmental glomerulosclerosis [10], interstitial fibrosis and tubular atrophy [3], and multicystic dysplastic kidney [16,17].

In a review of 29 patients, 17 had small kidney size and one had an absent kidney [9]. From examination by ultrasonography, the kidneys are described as small and echogenic with poor cortiomedullary differentiation [6]. Horseshoe kidney has been reported in one family [21].

In patients who have ocular findings of renal coloboma (papillorenal) syndrome, underlying renal pathology can be determined by the presence of renal tubular acidosis [12], failure to thrive [6], hypertension and proteinuria [6,10].

Up to a third of patients with renal coloboma (papillorenal) syndrome will develop end stage kidney disease [101]. The age of onset is variable even within the same family. Absent renal tissue has been observed in an affected fetus with oligohydramnios [29] and renal failure has been observed at birth [6,29]. In the same families, individuals with later onset of renal failure have been observed. The latest reported onset of renal failure has been in the sixth decade [23].

Vesicoureteral reflux and associated abnormalities, such as hydorureter and hydronephrosis, have been described in a number of affected individuals [6,17,24,29]. Spontaneous remission of vesicoureteral reflux has been reported in this condition [29]. Furthermore, ureteral pelvic junction obstruction has been reported [16].

## Hearing loss & renal coloboma (papillorenal) syndrome

Hearing loss has been reported in eight out of 85 patients with mutations in *PAX2*, suggesting a frequency of 10%. When hearing loss has been identified, it has been confirmed by audiogram as high frequency sensorineural hearing loss [6,9,10,16,17,23].

## Prevalence

The true population prevalence of renal coloboma (papillorenal) syndrome remains unknown; however, studies have been performed to determine the prevalence of *PAX2* mutations among groups of patients with renal hypodysplasia. Nishmoto *et al.* surveyed 20 patients, ascertained primarily for renal hypodysplasia, and found that two patients in this cohort had mutations in *PAX2* [14]. A recent series of 100 patients (99 unrelated probands) with renal hypodysplasia from the European Multicenter Effect of Strict Blood Pressure Control and angiotensin-converting enzyme inhibition on Progression in chronic renal failure Pediatric Patients Study (ESCAPE), were studied to determine the genetic basis of renal hypodysplasia. In this study, four different *PAX2* mutations in seven affected individuals from six families were identified. When these patients were examined for eye abnormalities, five out of seven were found to have subtle eye malformations, described as coloboma or dysplasia that had previously escaped detection [16]. Based on this study, the prevalence of *PAX2* mutations in children with renal hypodysplasia is approximately 6%.

Only one study has been performed to identify the prevalence of *PAX2* mutations in 100 patients with optic fissure closure defects, such as uveoretinal coloboma and patients with optic nerve anomalies. One patient in this group was identified to have a mutation in *PAX2*. This patient had optic nerve dysplasia and renal disease [13].

There are more than 80 published cases of renal coloboma (papillorenal) syndrome associated with *PAX2* mutations (see Table 1). It is estimated that mutations in *PAX2* can be identified in 50% of patients suffering from renal coloboma (papillorenal) syndrome [8,19]. This finding suggests that there are other genetic mechanisms underlying renal coloboma (papillorenal) syndrome. One possible mechanism is that another gene (locus) causes the syndrome. In order identify these other loci, linkage studies in families without *PAX2* mutation can be performed. In the event that the syndrome does not map to 10q24, then genes that map to a newly identified locus would be candidate genes that may cause this syndrome. The second possible cause is that there may be yet unidentified mutations at the *PAX2* locus, such as large deletions, noncoding mutations such as deep intronic mutations or promoter mutations. This is an area of active investigation.

## PAX2 & the paired box family of transcription factors

PAX2 belongs to of a well-known family of transcription factors, first identified in *Drosophilia* and found to be highly conserved from flies to mammals [33]. In mammals, including both mouse and human, there are nine *PAX/Pax* genes, encoding transcription factors characterized by the paired domain, a highly conserved bipartite helix-loop-helix DNA binding domain [34]. The paired box family of transcription factors share similar protein structures characterized by an N-terminal paired domain, a regulatory octapeptide domain, a full or truncated homeodomain and a proline-and serine-rich carboxy-terminal domain (Figure 2) [22,35].

The *PAX2* gene maps to human chromosome 10q24. It is encoded by 12 exons spanning a genomic region of 84,367 nucleotides from postion 102,495,322 to postion 102,579,688, accroding to the most recent build of the human genome (hg18) [102]. Exons 2, 3 and 4 encode the paired domain. Exon 5 encodes the negative regulatory octapeptide domain. Exon 7 encodes a truncated homeodomain (in contrast to PAX6, which has a full homeodomain). Exons 8, 9 and 10 encode the proline, serine, threonine-rich transactivation
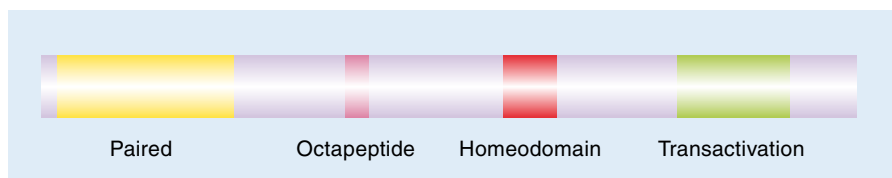
**Figure 2. Schematic diagram depicting the human PAX2 protein.** The amino terminal domain depicted in yellow represents the paired box DNA binding domain, the domain depicted in purple represents the octapeptide domain, the domain depicted in pink represents the truncated homeodomain and the domain depicted in green represents the proline, serine, threonine rich transactivation domain.

domain. There are at least five separate isoforms of *PAX2* mRNA that result from alternative splicing of the 6th and 10th exon and/or alternative reading frames of the last two exons [36].

### *PAX2* mutations

A variety of sequence variants (mutations) leading to renal coloboma (papillorenal) syndrome have been reported. The majority of mutations occur in exons 2, 3 and 4, which encode the paired domain and are frameshift or nonsense mutations leading to a null allele. As the paired domain is highly conserved, most missense mutations in this domain would be expected to interfere with the protein's secondary structure, preventing DNA binding. Frameshift, nonsense and missense mutations have also been reported in exons 7, 8 and 9. Few genotype–phenotype correlations have been made. Although imperfect and with few examples to date, mutations occuring in exons 7, 8 and 9 result in phenotypes that are more likely to be associated with milder ocular phenotypes [9]. **Table 1** lists the reported mutations.

The most commonly reported recurring mutation in *PAX2* is insG619, now described as c.77dupG in the updated numbering system from the start of the ATG rather than the start of exon 1. The mutation c.77dupG has been reported more than 20 times [7,9–11,15,16,18,20,29] and occurs within a string of seven guanines (CTCGGGGGGGTGT) at the 5' end of exon 2, which is the beginning of the region encoding the paired domain. This mutation has been identified in patients of European, African and Asian origin [7,15,18], suggesting that it does not result from a founder effect. This mutation has been identified in affected children but not in their unaffected parents, demonstrating that this mutation can occur *de novo*. It has also been identified in affected children and in a gonsosomal mosaic state in an unaffected parent [11]. Additionally, mutations that lead to a single deletion within this string of guanines have been identified (c.77delG [15]), as well as expansions to nine guanines (c.77dupGG [11]). This evidence strongly suggests that this string of seven guanines is a mutation hot spot and may occur from a DNA polymerase slippage event during replication and not from a founder effect.

Two chromosomal abnormalities have been reported in patients with renal coloboma (papillorenal) syndrome. The first was identified in a child with renal coloboma (papillorenal) syndrome who had a reciprocal translocation between chromosomes 10q24 and 13q12–14. This translocation breakpoint occurred between exons 3 and 4 and resulted in absent expression of the *PAX2* gene [37]. The second was a large deletion at 10q24, which included *PAX2* as well as 90 other genes. Interestingly, this patient had renal disease but normal ocular findings [20].

### Pax2 expression during development

Most of our understanding of the role of PAX2 in eye development comes from vertebrate animal models, particularly mouse and zebrafish. In the developing mouse, Pax2 expression occurs in the kidney, midbrain/hindbrain, individual cells within the dorsal and ventral spinal column, the otic vesicle (developing ear), and the developing eye. In mouse eye development, Pax2 expression begins in the optic sulcus, followed by expression in the optic vesicle. As the vesicle forms into the optic cup and stalk, Pax2 expression becomes restricted to the ventral cup and throughout the stalk [38,39]. Pax2-expressing cells reside ventrally at the edge of the optic fissure at embryonic day 11.5 and in a few remaining cells in the optic cup at embryonic day 12.5 when the fissure closes. Upon closure of the optic fissure, Pax2 expression remains in individual cells of the optic stalk and in a cuff of cells surrounding the optic stalk as it exits the inner layer of the optic cup.

In the developing mouse kidney, Pax2 is initially expressed in the pronephric duct, which is the earliest epithelial structure of the developing kidney. This is followed by expression in the mesonephric tubles and, ultimately, in the condensing mesenchyme at the ureteric bud tips. As the mesenchyme undergoes epithelial conversion, Pax2 expression is suppressed (reviewed in [40]). Pax2 is not expressed in the postnatal kidney.

Furthermore, Pax2 is expressed in the developing brain at the midbrain/hindbrain boundary, in cells of the spinal cord, the cerebellum and in the otic vesicle [39]. Recent studies also show Pax2 expression in forebrain development, particularly in cells of the hypothalamus and eminentia thalami [41].

A number of *Pax2* mutant mouse models exist; all alleles of which lead to null expression or impaired functioning of the paired domain. These include the transgene-induced deletion model *Kidney Retinal Defects* (*Krd*) mouse [42,43], a *Pax2*-knockout model [44], and the *Pax2*[1Neu] [45] and *Pax2*[A220G] models [46]. The *Pax2*[1Neu] model has an insertion of a guanine residue in *Pax2*, at position c.77dupG (formerly insG619). This mutation is identical to the most common mutation observed in affected patients. The *Pax2*[A220G] mutation has also been previously described in a human patient [24].

All homozygous mutant (*Pax2*^{-/-}) mice die before birth and most are anephric [42–46]. Phenotypic anomalies found in homozygous embryos include midbrain/hindbrain malformations and a high rate of cranial neural tube closure defects that vary with background strain. Cochleae are absent. The ocular phenotype is characterized by failure of optic fissure closure with failed basement membrane dissolution, evidenced by persistent laminin immunoreactivity at the edges of the optic fissure [42–46].

In heterozygous *Pax2* knock-out mice, optic nerve dysplasia with abnormal appearing vessels emerging from the periphery of the optic dics is observed. Retinae are thinned and rosettes are visible [42,44–46].

## Table 1. *PAX2* mutations

| Exon | Mutation: original description | Mutation: current numbering from ATG | Mutation type | Protein domain | Ref. |
|------|-------------------------------|--------------------------------------|---------------|----------------|------|
| 2 | delT602 | c.59delT | Frameshift | Paired | [12] |
| 2 | delT611 | c.68delT | Frameshift | Paired | [13] |
| 2 | insG619 | c.77dupG | Frameshift | Paired | [7,9–11, 15,16,18,20,32] |
| 2 | insGG619 | c.77dupGG | Frameshift | Paired | [29] |
| 2 | delG619 | c.77delG | Frameshift | Paired | [15] |
| 2 | 635-640 delGGCCCC | c.92_97 delGGCCCC | Frameshift | Paired | [16] |
| 2 | del658-663 | c.115_120del CAGCGC | Frameshift | Paired | [14] |
| 2 | del674-695 | c.131_152del | Frameshift | Paired | [10] |
| 2 | G755C | c.212G>C | p.Arg71Thr (missense) | Paired | [20] |
| 2 | del682-691 | c.139_148delCAGGGTGTGC | Frameshift | Paired | [17] |
| 3 | 763insgagacc | c.220_225 dup GAGACC | p.74Glu_75 Thr duplication | Paired | [25] |
| 3 | G769A | c.176G>A | p.Gly76Ser (missense) | Paired | [25] |
| 3 | delG832 | c.289delG | Frameshift | Paired | [29] |
| 3 | | c.310C>T | p.Arg104X (nonsense) | Paired | [18] |
| 3 | C886T | c.343C>T | p.Arg115X (nonsense) | Paired | [24] |
| Intron 3 | A>G954-2 | c.410-2A>G* | Splice site intron 3 | Paired | [16] |
| 4 | G>A961 | c.418G>A | p.Arg140X (nonsense) | Paired | [16] |
| 5 | delC1104 | c.561delC | Frameshift | Octapeptide | [5] |
| 7 | C1228T | c.754C>T | p.Arg229X (nonsense) | Homeodomain | [9] |
| 7 | C1318T | c.775C>T | p.Gln236X (nonsense) | Homeodomain | [27] |
| 8 | | c.911delG | Frameshift | Transactivation domain | [21] |
| 9 | C1566A | c.1023C>A | p.Tyr318X (nonsense) | Transactivation domain | [27] |
| | 46, XY, t(10;13) (q24.3;q12.3) | | | Translocation between exons 3 and 4 | [41] |
| | 46XX, del(10) (q23.2q24.3) | | | Deletion of PAX2 and 90 other genes | [33] |

Heterozygotes do not have optic fissure closure defects but rather anomalous vasculature, as observed in humans with *PAX2* mutations. Renal anomalies in these heterozygous mice include small dysplasic kidneys, renal cysts and lower nephron numbers [9,45,47,48].

In **Figure 3**, eyes from embryonic day 14.5 *Pax2*[1Neu] mice are pictured. **Figure 3A** shows an eye from a heterozygous mutant embryo and **Figure 3B** shows an eye from a homozygous mutant embryo. In whole mount embryo preparations, heterozygous embryos appear similar to wildtype embryos and apparent closure of the optic fissure can be observed (**Figure 3A**). By contrast, in homozygous *Pax2*[1Neu] embryos, the optic fissure has failed to close (**Figure 3B**). Heterozygous

*Pax2*-mutant mice do not have iris colobomas but exhibit optic nerve abnormalities, retinal patterning defects with fewer cells and formation of cellular rosettes [42,43,45]. In heterozygous *Pax2*-mutant mice, the optic fissure does not extend into the optic stalk [47]. Optic nerve cell number is reduced and optic nerve routing is abnormal [46]. The ultimate fate of the Pax2-expressing cells is the astrocyte (glial) population of the optic nerve [49,50].

The ocular phenotype observed in individuals with *PAX2* mutation and in animal models may be partially explained by the developmental pattern of *PAX2/Pax2* expression. In mice, *Pax2* is initially expressed along with *Pax6* throughout the optic
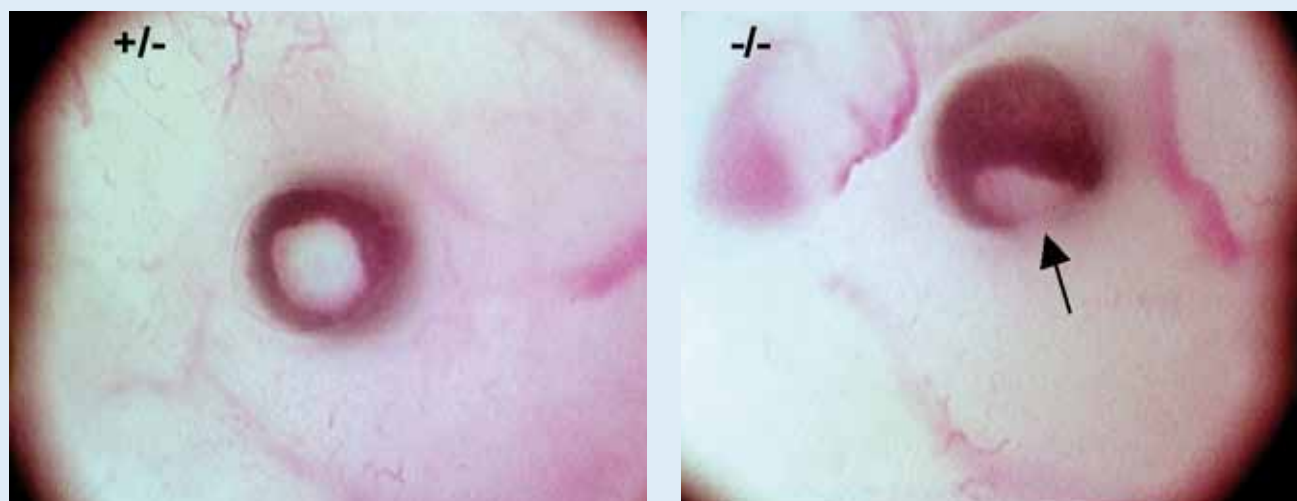
**Figure 3. Mouse wildtype compared with *Pax2¹ᴺᵉᵘ* at day 14.5.** Gross photomicrographs of eyes from embryonic day 14.5 *Pax2¹ᴺᵉᵘ* mouse embryo and its heterozygous littermate. Note ventral absence of retinal tissue with an open optic fissure. Embryo is oriented anterior (nose) to the right.

vesicle [51]. As invagination of the optic vesicle proceeds to form the optic cup, *Pax2* expression becomes restricted to the ventral optic cup and the optic stalk. During the process of optic fissure closure, *Pax2* is expressed at the closing edges of the optic fissure and becomes downregulated as the closure progresses [43]. Later in prenatal development, *Pax2* expression is relegated to the optic stalk and is lost some time in early postnatal life, although in *Pax2* heterozygous mice, the optic fissure does not extend into the optic stalk [43]. Chu *et al.* note that in human fetal eyes, *PAX2* expression is limited to astrocyte precursor cells, including those at the retina–optic nerve boundary [50]. These authors hypothesize that it is a dosage sensitive loss of *Pax2* expression at the retina–optic nerve boundary that may explain the congenital optic nerve anomalies in some patients with renal coloboma (papillorenal) syndrome. Although mutations in *PAX2* are not a common cause of optic fissure closure defects in humans, homozygous disruption of *Pax2* in animal models often leads to such defects, suggesting an important role for *Pax2* in the process of fissure closure [44,45]. Because *Pax2* expression is limited to neuroectodermally-derived tissues and is not expressed in the mesenchyme-derived fetal vasculature, it can be posited that the blood vessel patterning abnormalities are secondary to reduced *Pax2* expression in the optic stalk and retinal astrocyte precursors [49]. In fact, a complex set of interactions occurs during development between the developing neural, glial and vascular elements of the eye [49]. Chan *et al.* found that *Pax2*-expressing astrocytic precursor cells precede the advancement of endothelial precursor cells that form the retinal vasculature, suggesting that these cells may act as a kind of scaffold for the developing vasculature.

*Pax2*-heterozygous mice have smaller kidneys by weight and size than wildtype littermates and histology of these small kidneys show fewer nephrons [9,48]. Assays of cell death in heterozygous *Pax2*¹ᴺᵉᵘ mice show increased rates of apoptosis in cells of the ureteric bud and decreased branching, which results in fewer nephrons of the developing kidney. Dziarmaga *et al.* showed that apoptosis in the cells of the ureteric bud is mediated by the proapoptotic gene *Bcl2*. Targeted disruption of *Bcl2* rescued kidney size and reduced nephron number caused by *Pax2* haploinsufficieny [47]. These findings demonstrate that renal hypoplasia and reduced nephron number result directly from increased cell death in the developing kidney and are not mediated by vascular abnormalities.
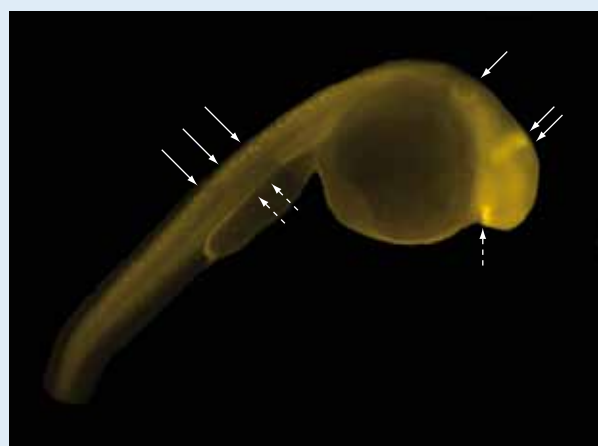


**Figure 4. Zebrafish 24 h postfertilization pax2 expression.** Immunostaining performed with anti-Pax2 (Convance, polyclonal, rabbit anti-Pax2-GST fusion protein) in whole mount wildtype zebrafish embryo at 24 h postfertilization (hpf). Pax2 expression is demonstrated in the developing nephric structures (double dotted arrows) throughout the developing spinal column (triple solid arrows), otic vesicle (single solid arrow), midbrain/hindbrain (double solid arrows), and in the ventral optic cup at the site of the optic fissure (single dotted arrow).
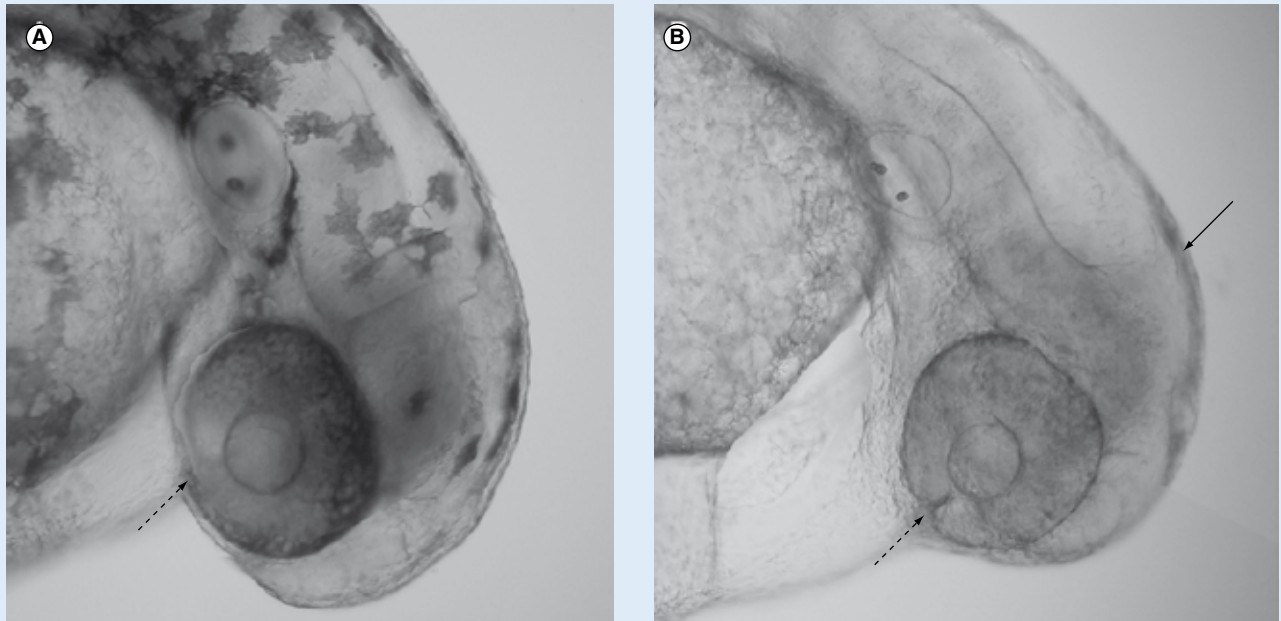
**Figure 5. Zebrafish pax2atu29a mutant, a homozygous null allele, next to a wildtype clutchmate at 33 h postfertilization.**
Although this wholemount photomicrograph was obtained at a time prior to closure of the optic fissure (normally 72 h postfertilization), the optic fissure in the pax2atu29a is still more visible than in the unaffected clutchmate [55]. Note that in the mutant zebrafish **(B)**, there is loss of the midbrain/hindbrain boundary (open arrow in **B**) that is so clearly seen in the wildtype fish (dotetd arrow in **A**). The optic fissure is visible in the mutant (dotted arrow in **A**), and although, not yet closed in this 33 h postfertilization fish, it is easier to visualize (dotted arrow in **B**).

In zebrafish, expression patterns of *pax2* are very similar to those observed in developing mice with *pax2* expression occuring in developing nephric structures, otic structures, midbrain/hindbrain, cells within the spinal column and in the optic stalk and cup of the eye [39]. In zebrafish, *pax2* expression becomes visible at 10 h postfertilization (hpf) in the brain and forms a stripe at the midbrain/hindbrain junction by 14 hpf. At 14 hpf, *pax2* expression is detected in the cells of the prospective optic stalk, auditory vesicle, pronephros and single cells of the hindbrain. At later points during development, single cells in the neural tube, both dorsally and ventrally, express *pax2* [39]. Primarily described as being expressed in the optic stalk and the most proximal parts of the optic cup, *pax2* expression occurs along the edges of the optic fissure. Demonstrated in the whole mount figure (performed at 24 hpf), *pax2* expression also occurs in cells surrounding the optic fissure and stalk (single closed arrow in FIGURE 4).

It is important to mention that the zebrafish genome underwent evolutionary duplication [52]. Therefore ,the zebrafish has two copies of *pax2,* called *pax2a* and *pax2b* [53]. Zebrafish *pax2a* is expressed earlier in development than pax2b [53]. In zebrafish with homozygous *pax2a* mutations of the isthmus organizer of the midbrain/hindbrain is absent, optic fissure closure defects and otic defects occur and nephric development is limited. These anomalies are very similar to those observed in mice with homozygous *Pax2* mutations [44,45]. The fact that phenotypic anomalies observed in zebrafish with

homozygous *pax2a* null mutations are the same as in *Pax2* mutant mice confirms that mutations in *pax2a* are sufficient to recapitulate phenotypic anomalies caused by mutations in *Pax2* null mice [54,55].

An allelic series of zebrafish *pax2a* mutants have been described. Zebrafish *pax2a* mutants were identified by large-scale genetic screening, isolating phenotypes with specific phenotypic anomalies [54,56]. This series of phenotypes were identified by the loss of the midbrain/hindbrain boundary, known as the isthmus, and are called the no isthmus or *noi* phenotype. Subsequent work showed that the *noi* phenotypes harbored point mutations in *pax2a*, the zebrafish orthologue of human *PAX2* and mouse *Pax2*. Both hypomorphic (reduced function) and null alleles have been described [55]. *Noi* phenotypes include loss of the midbrain/hindbrain boundary, abnormalities of nephric structures, and *noi* homozygous mutants die with severe edema and heart failure a few days post-fertilization. Zebrafish *pax2a* homozygous mutants show delayed closure of the optic fissure and colobomatous eye defects **(FIGURE 5)** [57].

### *PAX2* as part of a gene-network of that controls optic fissure closure

Upstream control of *Pax2*/*pax2a* expression in both mouse and zebrafish is under control of sonic hedgehog (shh) protein. Shh/shh is expressed during the early development of cells of the precordal plate. In zebrafish, absence of shh expression leads to loss of pax2.1 expression [58,59], with concomitant ectopic expression of pax6 in the domain typically occupied by pax2.1. In humans with mutations in

*SHH*, optic fissure closure defects have been demonstrated by the presence of uveoretinal colobomas [63].

In mice, loss of Pax2 expression results in an extension of Pax6 expression, with concomitant loss of Pax2 expression in the optic stalk. Conversely, loss of Pax6 expression results in increased Pax2 expression domain and loss of the retinal structures [51]. This suggests that differences in expression of either transcription factor could result in various optic nerve abnormalities [60].

Approximately half of the patients with a clinical phenotype of renal coloboma (papillorenal) syndrome do not have mutations in *PAX2*. This leads to the hypothesis that genes besides *PAX2* cause renal coloboma (papillorenal) syndrome. Few downstream effectors of PAX2 function have been identified. Genes confirmed to be influenced by *PAX2* that are expressed in the developing optic stalk and ventral optic cup would be ideal candidates for investigating this hypothesis.

## Expert commentary

Renal coloboma (papillorenal) syndrome is an autosomal dominant disorder characterized primarily by optic nerve dysplasia. Many affected patients have kidney or urinary tract abnormalities and may suffer from kidney failure. Mutations in *PAX2* are associated with the syndrome, although other genes are also likely to be responsible. Further studies are needed to help understand the basis for optic nerve defects, to determine how *PAX2* functions during eye development and to identify the genetic basis of renal coloboma (papillorenal) syndrome in patients who do not have mutations in *PAX2*. Studies in mice and zebrafish with mutations in *Pax2/pax2* have been useful in extending our insight into the developmental defects that result in renal coloboma (papillorenal) syndrome.

## Five-year view

Although our group has identified a number of point mutations in *PAX2* in patients with renal coloboma syndrome, nearly all studies have not used technology to identify large deletions or chromosomal rearrangements at the *PAX2* locus. As researchers begin to routinely use deletion identification techniques, such as high resolution comparative genomic hybridization and multiplex ligation probe amplification or real-time PCR to identify dosage abnormalities, it is highly probable that large deletions of the *PAX2* locus will emerge as an important cause of renal coloboma (papillorenal) syndrome.

It is anticipated that other genes beside *PAX2* will be discovered to cause renal coloboma (papillorenal) syndrome. As genes that are part of the gene-network controlled by *PAX2* are discovered, it is anticipated that some of these genes will contribute to the phenotype of renal coloboma (papillorenal) syndrome.

Animal models will prove critical to the identification of genes responsible for renal coloboma (papillorenal) syndrome, as well as other ocular conditions that result from abnormal optic fissure development and closure. One model that will, undoubtedly, contribute is zebrafish. Owing to their high clutch size (hundreds of eggs), rapid development of optically clear embryos (optic fissure closure is complete within 72 hpf), ease of pharmacologic intervention by treatment of the water surrounding the fish and facile genetic manipulation through the use of knockdown technologies, such as morpholinos [61,62,64], zebrafish are an ideal model to assay the effects of genetic manipulations or exogenous pharmacologic treatments on optic fissure closure and development [65].

## Key issues

- Renal coloboma (papillorenal) syndrome is an autosomal dominant condition that can be caused by mutations in *PAX2*.
- Dysplastic abnormalities of the optic disc are the most common eye abnormality reported in renal coloboma (papillorenal) syndrome.
- Kidney abnormalities include small dysplastic kidneys (renal hypodysplasia) and vesicoureteral reflux.
- Kidney failure can occur at any age, from the prenatal period to the seventh decade.
- Patients with a history of renal hypodysplasia should have careful eye examination for conditions associated with renal coloboma syndrome.
- Patients with optic nerve dysplasia or eye abnormalities found in renal coloboma (papillorenal) syndrome should be evaluated for kidney disease.
- Both mouse and zebrafish with homozygous mutations in *Pax2/pax2* exhibit failed closure of the optic fissure with complete coloboma. Heterozygous mouse models have optic nerve findings similar to those in human patients.
- Pax2 is expressed in the astrocytic cells of the optic stalk and have a role in vascular routing in the developing optic nerve. This may, in part, explain the observed retinal vascular anomalies in individuals with renal coloboma (papillorenal) syndrome.

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