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# PAX2 mutations in renal–coloboma syndrome: mutational hotspot and germline mosaicism

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The renal–coloboma syndrome (RCS, MIM 120330) is an autosomal dominant disorder caused by *PAX2* gene mutations. We screened the entire coding sequence of the *PAX2* gene for mutations in nine patients with RCS. We found five heterozygous *PAX2* gene mutations: a dinucleotide insertion (2G) at position 619 in one sporadic RCS case, a single nucleotide insertion (619 + G) in three unrelated cases, and a single nucleotide deletion in a familial case. In this familial case, three affected sibs showed a striking ocular phenotypic variability. Each of the sibs carried a 619insG mutation, whilst unaffected parents did not, suggesting the presence of germline mosaicism. Interestingly, the 619insG mutation has been previously reported in several patients and is also responsible for the *Pax2*<sup>1Neu</sup> mouse mutant, an animal model of human RCS. This study confirms the critical role of the *PAX2* gene in human renal and ocular development. In addition, it emphasises the high variability of ocular defects associated with *PAX2* mutations ranging from subtle optic disc anomalies to microphthalmia. Finally, the presence of *PAX2* germline mosaicism highlights the difficulties associated with genetic counselling for *PAX2* mutations. *European Journal of Human Genetics* (2000) 8, 820–826.

**Keywords:** *PAX2*; renal–coloboma syndrome; germline mosaicism; mutational hotspot

## Introduction

In humans, *PAX2* gene mutations are responsible for Waardenburg syndrome type I and III (*PAX3*<sup>1</sup>), aniridia and Peter's anomaly (*PAX6*<sup>2–4</sup>), thyroid dysgenesis (*PAX8*<sup>5</sup>), oligodontia (*PAX9*<sup>6</sup>) and renal–coloboma syndrome (RCS, *PAX2*<sup>7</sup>). RCS is a rare autosomal dominant disorder (MIM 120330) associating optic nerve coloboma and retinal epithelium thinning, renal hypoplasia, vesico-ureteral reflux and mild sensorineural hearing loss. In 1995, Sanyanusin *et al* first reported a *PAX2* mutation in patients with renal and ocular defects.<sup>7</sup> Since then, eight different *PAX2* mutations and a chromosome 10/13 *de novo* translocation have been identified in 13 unrelated patients.<sup>7–14</sup> It has been shown that *PAX2* gene

mutations are associated with both inter and intrafamilial phenotypic variability; all patients share renal and ocular abnormalities, while some present with hearing loss, mental retardation or seizures.<sup>9</sup> Several *Pax2* mutant mice are known: *Pax2*<sup>1Neu</sup> resulting from a *Pax2* frameshift mutation,<sup>15</sup> *Krd* carrying a large chromosomal deletion encompassing the *Pax2* gene (kidney and retinal defect,<sup>16</sup>) and the *Pax2* knock-out mouse.<sup>17,18</sup> All the mouse mutants have kidney and optic nerve or retinal defects.

Here we report the identification of *PAX2* mutations in five out of nine unrelated RCS patients, and two are previously undescribed mutations. In one familial case, unaffected parents did not carry the mutation suggesting the presence of germline mosaicism involving the paternal allele. The finding that the G619 insertion has occurred three times independently in our series as well as in seven other cases of RCS, and that we have also observed a novel 619insGG

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suggests the existence of a poly G mutational hot-spot of the *PAX2* gene.

## Materials and methods

### Patients and families

A total of nine patients with renal abnormalities and coloboma were studied for *PAX2* mutations. Table 1 summarises the clinical features of the patients.

### Detection of *PAX2* mutations and large deletion

Genomic DNA was extracted from peripheral blood samples using standard protocols. PCR was carried out using intronic primers for the 12 exons of the *PAX2* gene.<sup>19</sup> PCR products were labelled by incorporation of <sup>33</sup>PdCTP. Direct DNA sequencing of exon 2 to 5 was performed for all patients. Single-strand conformation polymorphism (SSCP) was used for the other exons to detect nucleotide variation according to standard conditions (MDE gel (Bioproducts, Rockland, ME, USA), electrophoresis overnight at room temperature and at 4W, autoradiography 48 h). Direct DNA sequencing was performed when an abnormal SSCP pattern was detected using the Dye Deoxy Terminator Cycle Sequencing kit (PE Biosystems, Warrington, UK). In addition, an intragenic (AC)<sub>n</sub> highly polymorphic dinucleotide repeat<sup>7</sup> was studied in all patients to rule out large deletions. Karyotype and FISH analysis was performed in the patient uninformative for the CA repeat.

## Results

We screened the entire coding sequence of the *PAX2* gene for mutations in RCS patients and report 3 different *PAX2* mutations (Figure 1) in five unrelated RCS patients with variable ocular findings. Table 1 summarises the phenotype of all the patients and the mutations identified.

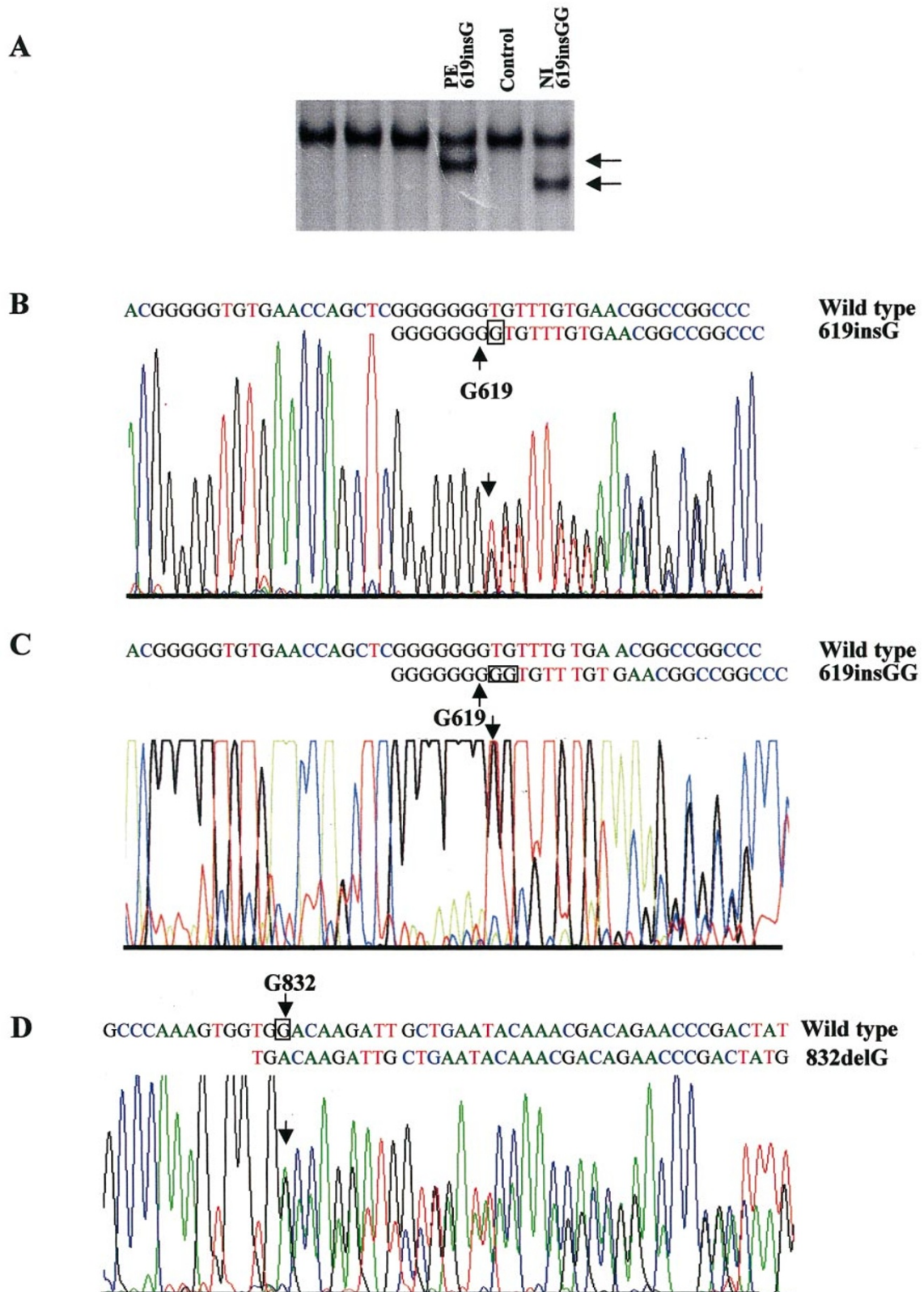
A previously not described *PAX2* gene mutation (a dinucleotide insertion GG at position 619) was identified in an isolated RCS case showing bilateral optic nerve coloboma and bilateral renal hypoplasia (patient NI). This frameshift mutation predicts a premature termination of the protein 3 codons downstream from the mutation (Figure 1A and 1C). The mother did not carry the mutation but the healthy father was not available for DNA study.

A single nucleotide insertion (G) at position 619 was found in an isolated case with bilateral renal hypoplasia associated with bilateral microphthalmia and retinal degeneration (patient RA, Figure 3A). Irides were normal. Parents were not available for DNA study. As with the previous mutation, the 619insG frameshift mutation predicts a premature termination of the protein 27 codons downstream from the insertion. The same mutation was identified in a sporadic case with bilateral renal hypoplasia associated with bilateral retinal coloboma (patient PE, Figure 1A and 1B). Parents were studied and did not carry the mutation suggesting the presence of a *de novo* mutation.

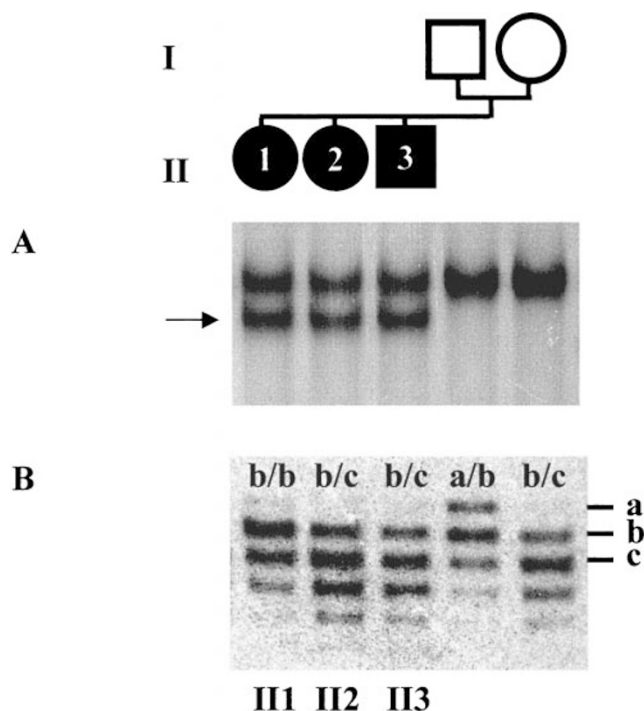
A family with three affected sibs were studied (patients AR, Figure 2). Patient II1 had bilateral renal hypoplasia and a 'morning-glory syndrome' (equivalent to an optic coloboma), whilst II2 and II3 presented with isolated unilateral cystic renal hypoplasia with no ocular manifestation. Careful ophthalmological examination showed an optic disc anomaly in both of them: bilateral papillary dysplasia in II2 (Figure 3B) and bilateral optic nerve coloboma in II3 (Figures 3C and 3D). A 619insG was identified in all three children of this family. Both unaffected parents did not carry the mutation, however, suggesting a germline mosaicism (Figure 2). The study of a *PAX2* intragenic DNA microsatellite marker showed that the mutation was of paternal origin. False paternity was excluded by the study of polymorphic markers.

**Table 1** Clinical features and *PAX2* mutations observed in RCS patients

Case	Clinical features	Nature of mutation	Familial (F) Isolated (I) /Inheritance
NI	Bilateral renal hypoplasia and bilateral optic nerve coloboma	619insGG	I/?
RA	Bilateral renal hypoplasia and bilateral microphthalmia with right optic nerve dysplasia	619insG	I/?
PE	Bilateral renal hypoplasia and bilateral retinal coloboma	619insG	I/ <i>de novo</i>
AR	II1 – Bilateral renal hypoplasia, 'morning glory syndrome' II2 – Unilateral renal hypoplasia and bilateral papillary dysplasia II3 – Unilateral renal hypoplasia, bilateral papillary coloboma	619insG	F/germline mosaicism
MA	1. Unilateral renal agenesis, bilateral optic nerve coloboma and severe myopia 2. Renal insufficiency, slight reduction in kidney size and bilateral optic nerve coloboma	832delG	F/inherited
BA	Bilateral renal hypoplasia in one sib, bilateral renal hypoplasia, vesico-ureteral reflux and unilateral microphthalmia with iris coloboma in one sib	–	F
PH	Bilateral renal hypoplasia and coloboma	–	I
VE	Single pelvic kidney, bilateral retinal coloboma and microphthalmia	–	I
TH	Renal hypoplasia, left chorioretinal and right optic nerve coloboma (Figure 3G)	–	I



**Figure 1** SSCP analysis and sequencing of exon 2 and 3 of the *PAX2* gene. Abnormal exon 2 SSCP bands are indicated by arrows in A; B, C, D show the sequences of the three mutations identified, in exon 2 (B,C) and 3 (D) respectively.



**Figure 2** SSCP (A) and *PAX2* intragenic polymorphic marker analysis (B) in the family AR. The abnormal SSCP band indicated by an arrow is seen only in the three affected children. In B, the three affected children share a common paternal allele (b), whilst the maternal allele is either b (patient II1) or c (patients II2 and II3) thus suggesting a paternal germline mosaicism.

Finally, we identified a single G deletion at position 832 in exon 3 of the *PAX2* gene (Patient MA, Figure 1D). This mutation was identified in a family where a child presented bilateral optic nerve coloboma, severe myopia and unilateral renal agenesis, whilst his father had bilateral optic nerve coloboma with association to a chorioretinal coloboma (Figures 3E and 3F). Renal explorations in the father showed renal insufficiency and slight reduction in kidney size. The 832delG frameshift mutation predicts a premature termination of the protein 61 codons downstream from the deletion and has not been reported previously.

None of these mutations was observed in a panel of 100 control chromosomes.

Finally, the *PAX2* intragenic microsatellite marker study showed the presence of two alleles in all patients except one. In this patient, a karyotype and FISH analysis indicated that both copies of the *PAX2* gene were present. Thus, in all patients tested, no evidence of a large *PAX2* gene deletion could be found.

### Discussion

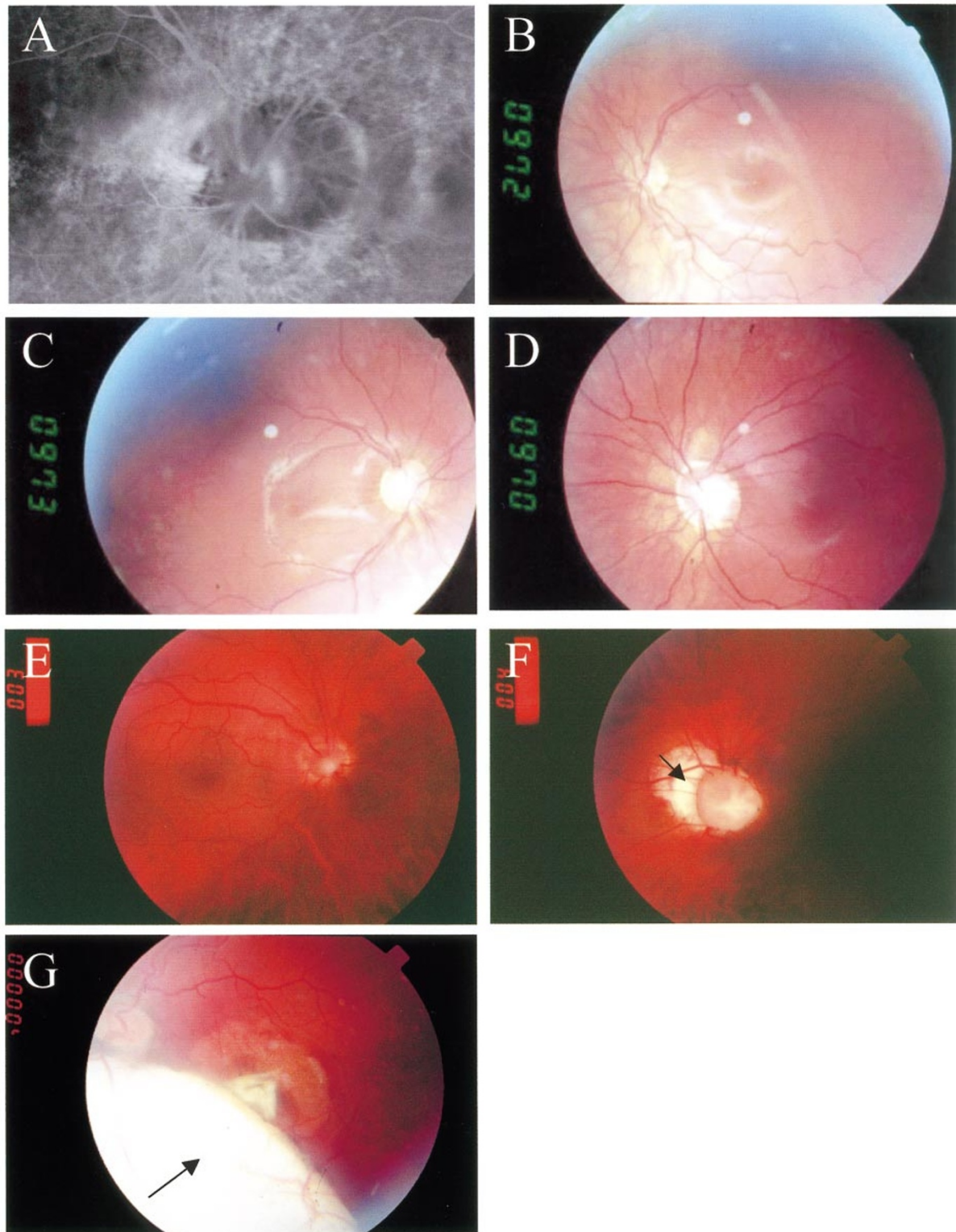
The *PAX* genes comprise a family of developmental genes encoding nuclear transcription factors.<sup>20</sup> They are charac-

terised by a conserved amino acid motif with DNA binding activity, the paired domain. Nine members of this family have been isolated in human and mouse, and classified into four groups sharing structural motifs (ie octapeptide and homeodomain) and sequence similarities. Disruption of at least five *PAX* genes has been shown to result in developmental abnormalities in humans.<sup>1-7</sup> So far, eight different *PAX2* mutations have been reported in 13 patients with RCS.<sup>7-13</sup> In all previously reported cases, patients presented with RCS or renal hypoplasia with mild ocular manifestations described as optic disc dysplasia or papillo-renal syndrome. Here, we report on five additional cases of *PAX2* mutations in patients with RCS. One novel mutation resulted from a 2 guanine nucleotides insertion within a stretch of 7 guanines in exon 2 of the *PAX2* gene. In three other patients, a single G insertion at the same position has been identified. This latter mutation has already been reported in several patients. Finally, we identified a novel guanine deletion at position 832 in a familial RCS. We failed, however, to find either a *PAX2* mutation or a large deletion in 4/9 RCS patients examined. Although the promoter region as well as the non-coding sequences of the *PAX2* gene have not yet been studied, these results suggest that the RCS might be genetically heterogeneous.

Interestingly, in one familial case, a paternal germline mosaicism was observed. Furthermore, in this family, the clinical spectrum was quite variable with ocular anomalies being detected only after careful ophthalmological examination in two out of three sibs. This observation confirms findings of previous studies showing that all patients with *PAX2* mutations present with an abnormal eye fundus, even a very subtle one.

Along with the mutations reported here and unpublished data (Salomon *et al*, 2000 personal communication), 11 different *PAX2* mutations have been hitherto identified in 20 unrelated patients. They are summarised in Figure 4. In addition, a 10/13 translocation within either intron 3 or 4 of the *PAX2* gene has been reported.<sup>14</sup> With the exception of two mutations in exons 5 and 7, all *PAX2* gene mutations reported so far are located in the paired domain (exons 2 and 3). Interestingly, 17/20 mutations are frameshift mutations predicting a truncated and non-functional *PAX2* protein lacking the transactivation domain, the homeodomain or the octapeptide. Therefore, haplo-insufficiency is the most likely mechanism of dominance of *PAX2* mutations in humans. Finally, 12/20 *PAX2* gene mutations are either a deletion or an insertion of one or two Gs at position 619 in a stretch of 7 Gs which could be regarded as a mutational hot spot resulting from polymerase slippage. Interestingly, the *PAX2*<sup>1Neu</sup> mutant mouse has been shown to carry the same G insertion at the very same position. Therefore this mouse mutant represents a natural mouse model for *PAX2* mutations in humans.<sup>15</sup>

In conclusion, our study confirms the critical role of the *PAX2* gene in human renal and ocular development, and



**Figure 3** Ophthalmological examination of the patients. **A:** fluorescein angiography centered on the optic nerve of the right eye of patient RA showing papillary dysplasia. Family AR: fundus of the left eye of patient II2 (**B**) showing papillary dysplasia with abnormal vessels division; right (**C**) and left (**D**) fundus of patient II3 showing bilateral optic nerve coloboma. Patient MA: right (**E**) and left (**F**) fundus showing bilateral optic nerve coloboma, more severe in the left eye with the association to a chorioretinal coloboma (arrow). **G:** Left eye fundus of patient TH showing a large nasal inferior chorioretinal coloboma (arrow).

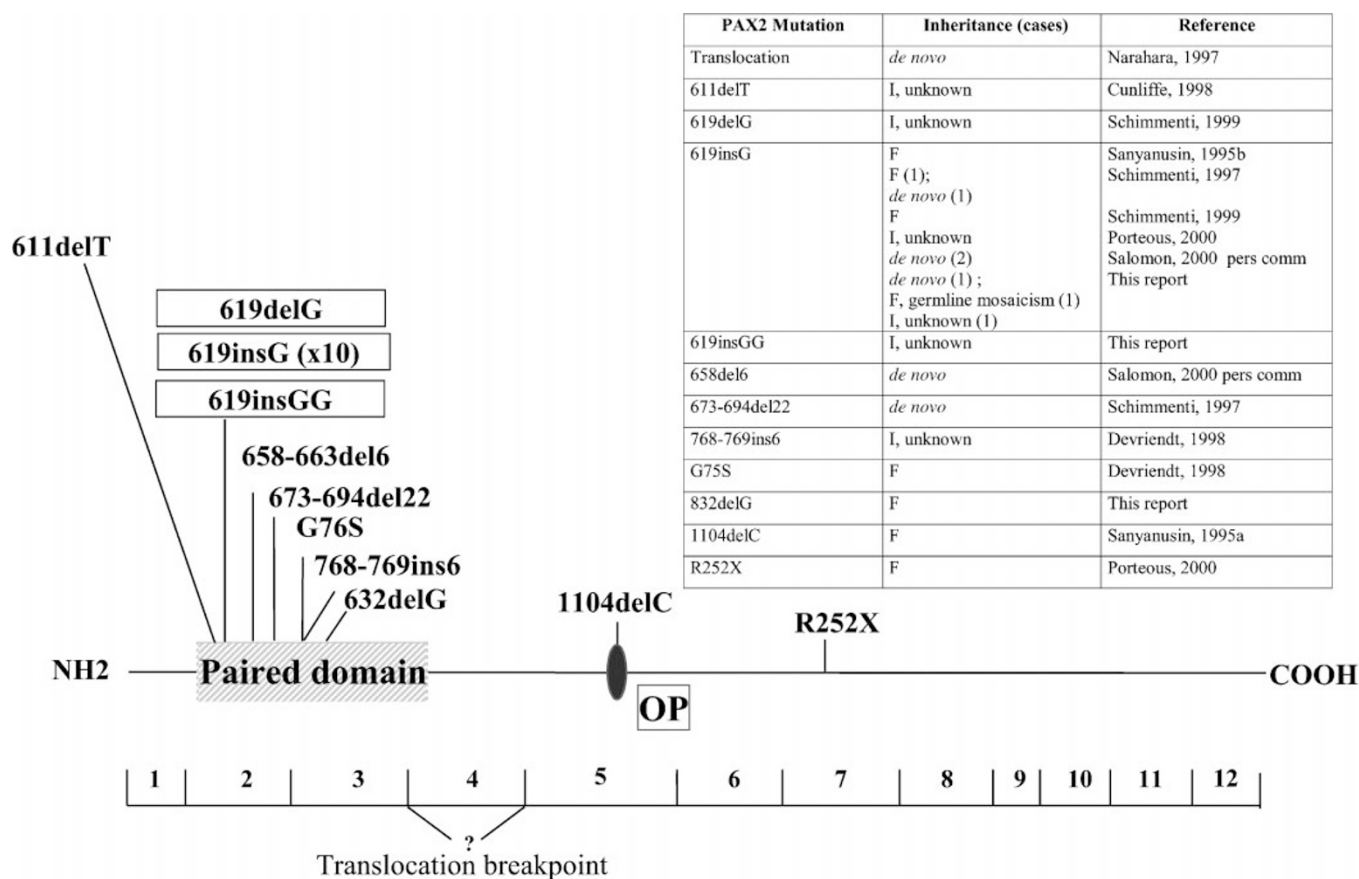


Figure 4 Human PAX2 mutations, F: familial; I: isolated.

further demonstrates the wide variation in the clinical spectrum of patients who have PAX2 mutations. PAX2 mutations are responsible for causing renal hypoplasia associated with various ocular manifestations ranging from subtle optic disc anomalies to microphthalmia. In addition, we describe a novel insertion in a mutational polyG hot spot. This type of mutation represent 60% of PAX2 mutations reported so far (12/20). Finally, the presence of PAX2 germline mosaicism in one family makes genetic counselling difficult in RCS.

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