CASE REPORT

Three New *PAX2* Gene Mutations in Patients with Papillorenal Syndrome

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ABSTRACT

Papillorenal syndrome (PAPRS; Mendelian Inheritance in Man [MIM] 120330) is an autosomal dominant disease characterised by the presence of congenital renal and optic nerve abnormalities associated with mutations of the *PAX2* gene. In this article, the authors present four patients with PAPRS who are carriers of three new *PAX2* mutations, as well as another patient with a possible non-pathogenic variant of the *PAX2* gene. All patients were given a full neurophthalmological examination, and all patients underwent a genetic test for *PAX2*. Patients 1 and 2 presented with the classic signs of PAPRS: renal disease associated with a congenitally abnormal optic disc, whereas patients 3 and 4 only presented with a congenital optic nerve abnormality and no renal involvement. In patients 1 and 2, the optic nerves were affected by the presence of a central excavation within the optic disc, absence of the central retinal artery, as well as multiple cilioretinal arteries radiating from the periphery of the optic disc. Bilateral optic nerve pits were seen in patient 3, and lastly, in patient 4 there was the presence of superficial gliotic tissue on the left optic disc. All patients presented with a missense mutation in the *PAX2* gene, where in patient 4 possibly being only a non-pathogenic variant of the gene. In conclusion, the authors present two patients with classic clinical signs of PAPRS, having two new *PAX2* mutations, which until now have not been described in the current literature; another patient with a new *PAX2* mutation showing only ocular manifestations of the disease, and lastly, a patient who is a carrier of a variant of the *PAX2* gene has a congenitally abnormal optic disc, which is probably not related to PAPRS.

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Introduction

Papillorenal syndrome (PAPRS; Mendelian Inheritance in Man [MIM] 120330) is an autosomal dominant disease characterised by the presence of congenital renal and optic nerve abnormalities associated with mutations of the *PAX2* gene.^{1,2} PAPRS was first described in 1977,^{3,4} when it was termed renal coloboma syndrome, having later been changed to papillorenal syndrome (PAPRS), used in current literature. The latter designation is probably the most accurate, as it cannot be assumed that the optic nerve abnormalities are colobomatus in all cases.⁵ Until now, PAPRS has only been associated with mutations of the *PAX2* gene. However, it is estimated that approximately 50% of individuals with clinical findings characteristic of PAPRS do not present with an identified mutation of the *PAX2* gene.⁵ For this reason, it is important to note that the diagnosis should be based fundamentally on

clinical suspicion when a patient presents as a typical (renal abnormalities and optic disc dysplasia).

Ophthalmologists, as well as nephrologists, should include this syndrome in the differential diagnosis of any patient presenting with isolated abnormalities of the optic nerve or kidneys. Such alterations could easily go unnoticed unless the ophthalmologist and nephrologist respectively consult one another in order to arrive at a diagnosis of PAPRS.

In this article, we will present three patients with PAPRS, who are carriers of three new *PAX2* mutations, as well as another patient with a possible non-pathogenic variant of the *PAX2* gene.

Clinical cases

Patient 1

Patient 1 is female, aged 13 years, who has had poor vision in the left eye (OS) since childhood, as well as

an alteration in renal function under periodic review by a nephrologist for hypoplastic polycystic kidney disease. There was no family history of renal or ocular abnormalities, nor was there any consanguinity between the parents.

On clinical examination, the visual acuities (VA) were 20/30 for the right eye (OD) and 4/200 for the left eye (OS). Colour vision testing by Ishihara was normal for OD; however, she was unable to distinguish any of the plates for OS. Pupils were equal and reactive to light and accommodation, with the presence of a relative afferent pupillary defect (RAPD) OS. Horizontal pendular nystagmus was present along with a sensory exotropia in primary gaze, OD being the fixating eye. Fundus examination revealed bilateral optic nerve abnormalities with a central excavation where neither the central retinal artery nor vein could be seen. However, there were numerous vessels radiating from periphery of the optic disc. The difference between both optic discs was that the central excavation was more prominent OD, and also the fact that fibroglial tissue was present OS (Figure 1). Refraction was OD +2.00/−2.00×160 and OS −2.50/−3.00×20. The anisometropia can explain the amblyopia OS.

Genetic studies were performed using the following method: the 12 coding exons (exons 1–10 of transcript variant 1 and exons 9 and 11 of transcript variant 2) and the exon-intron boundaries of the *PAX2* gene on chromosome 10q24.31 (OMIM

167409) were amplified by polymerase chain reaction (PCR) and sequenced directly. The resulting sequence data were compared with the reference sequences and NM_000278, respectively. The genetic study revealed that the patient carried the heterozygous *PAX2* mutation c.213-2A>G. Sequencing analysis showed an A to G transition at position c.213-2 of the *PAX2* gene in heterozygous state (c.213-2A>G). The nucleotide exchange was located in intron 2 and affected the acceptor splice of exon 3.

To the best of our knowledge, this variant has not been previously reported in the literature. A computer-based comparison of the modified acceptor splice site of exon 3 of the *PAX2* gene with the wild-type sequence was performed with different bioinformatic tools. All programs predicted a loss of the constitutive acceptor splice site of exon 3. Given the position of the transition of this evolutionary highly conserved nucleotide and the prediction by different bioinformatic algorithms, it can be assumed that the mutation is pathogenic.

Patient 2

Patient 2 is male, aged 26, followed periodically by a nephrologist for renal failure secondary to polycystic kidney disease. The patient had a successful renal transplant 2 years ago but complained of bilateral sudden and painless loss of vision while on haemodialysis several months

Figure 1. Fundus image of patient 1 showing the abnormal appearance of both optic discs, with a central excavation. The central retinal arteries and veins cannot be appreciated. However, a number of vessels radiating from the periphery of the optic disc can be seen. The difference between both optic discs lies in the central excavation being more prominent OD, as well as the presence of central fibroglial tissue OS.

prior to having the transplant. Because of this, he was seen by several ophthalmologists but without a clear diagnosis. The patient was finally referred to the neuro-ophthalmology department for further investigation under the suspicion of having bilateral optic neuropathy. There was a family history of renal failure (maternal).

On clinical examination, VA was 20/100 OD and count fingers (CF) OS. He was unable to distinguish any of the plates with either eye during Ishihara colour vision testing. Pupils were equal and reactive to light and accommodation, with a relative afferent pupillary defect (RAPD) OS. Fundus examination revealed bilateral optic nerve abnormalities with central excavations, more prominent OS. It should be noted that there were also the presence of multiple vessels in the periphery of the optic discs, which were better appreciated with fluorescein angiography (Figure 2).

Visual fields revealed a dense central scotoma and superior altitudinal defect in both eyes. Cerebral magnetic resonance imaging (MRI)

showed the presence of isolated lacunar infarcts as well as thinning of both optic nerves.

Genetic studies were performed using the same method for patient 1, having confirmed the patient as a carrier of the *PAX2* mutation c.389C>G (p.Pro130Arg), consisting of a nucleotide exchange from C to G at position c.389 in exon 3 of the *PAX2* gene (c.389C>G), leading to a substitution of the evolutionary highly conserved amino acid proline by arginine at position 130 of the protein (p.Pro130Arg). All eight bioinformatic tools predicted a pathogenic effect for the missense alteration. To the best of our knowledge, this variant has not been described in the literature to date nor has it been included in any databases thus far.

Patient 3

Patient 3 is male, aged 15, was referred to the neuro-ophthalmology department for evaluation after routine eye examination revealed a congenital abnormality of one of his optic nerves. He did not show any altered renal function nor any other medical history of interest.

Figure 2. (A) Fundus image of patient 2 showing the abnormal appearance of both optic nerves, with bilateral central excavations, more prominent OS. (B) Fluorescein angiography showing the presence of multiple vessels in the periphery of the optic disc.

On clinical examination, VA was 20/20 OD and 20/30 OS. Ishihara colour vision testing was normal for both eyes. Pupils were equal and reactive to light and accommodation, without any relative afferent pupillary defect (RAPD). Fundus examination showed the presence of a pit within the optic nerve OD and the presence of gliosis on the surface of the optic nerve OS. The right macula had a normal appearance, whereas the left macula showed the presence of a small amount of subretinal fluid (Figure 3). Posterior optical coherence

tomography (OCT) was performed, which confirmed the presence of intraretinal fluid, compatible with optic pit maculopathy, for which it was assumed that there was also an optic nerve pit underlying the superficial gliosis of the optic nerve OS (Figure 4). Given the acceptable VA, the retinal department decided on conservative follow-up. The MRI results for this patient were normal.

Genetic studies were performed using the same method for patient 1, where he was found to carry

Figure 3. Fundus image of patient 3 showing the presence of an optic nerve pit OD and superficial gliotic tissue on the optic nerve OS.

Figure 4. Macular OCT of patient 3 showing (A) the presence of intraretinal fluid and schisis cavities, compatible with optic pit maculopathy OS (for which it is assumed that there is an optic disc pit underlying the superficial optic nerve gliosis OS); (B) normal macula OD.

the heterozygous missense variant c.988G>A (p.Gly330Ser) in exon 8 of the *PAX2* gene, which was adjacent to the donor splice site of exon 8 and may have impaired splicing. The result was confirmed by sequencing of an independent PCR product. To the best of our knowledge, the variant has not been described in literature to date. No annotation in genetic databases or minimum angle of resolution (MAR) has been documented. All the bioinformatic analysis programs used predicted the alteration to be pathogenic. In addition to causing an amino acid exchange (p.Gly330Ser), c.988G>A also altered the last nucleotide position of exon 8. It is conceivable that the heterozygous missense variant c.988G>A (p.Gly330Ser) in *PAX2* was causative for the phenotype of the patient.

Patient 4

Patient 4 is female, aged 20, referred to the neuro-ophthalmology department for evaluation of an abnormal optic nerve OS, which was discovered incidentally on routine eye examination. The patient did not report any visual disturbance. There was no family history of either ophthalmic or renal disease nor any consanguinity between the parents.

On clinical examination, VA was 20/20 OD and 20/30 OS. Ishihara colour vision testing and intraocular pressure (IOP) measurements were normal for both eyes. Pupils were equal and reactive to light and accommodation, with a relative afferent pupillary defect (RAPD) OS.

On fundus examination, the right eye appeared normal, whereas the presence of an abnormal optic nerve with superficial gliotic tissue was seen OS (Figure 5).

Goldmann visual field testing showed a normal field OS and an inferior altitudinal defect OS. Cranial MRI was otherwise normal despite a decrease in the caliber of the left optic nerve (OS).

Genetic studies were performed using the same method for patient 1, which confirmed the patient to be a carrier of the heterozygous *PAX2* variant c.478G>A (p.Ala160Thr), having consisted in a transition of G to A at position c.478 in exon 4 in heterozygous state, which resulted in a non-conservative substitution of the evolutionary conserved amino acid alanine by threonine at position 160 of the protein sequence. The result was confirmed by sequencing of an independent PCR product. This variation has already been annotated as rare polymorphism (rs201383632; frequency 0.1% in the population). Three bioinformatic programs predicted a benign character, whereas two other programs predicted a pathogenic effect for this alteration. In summary, c.478G>A (p.Ala160Thr) most likely represented a variant of uncertain significance that is non-informative as causative of the patient's clinical features.

Discussion

In this paper we have presented three patients with papillorenal syndrome (PAPRS), who were carriers of three new *PAX2* mutations. Likewise, we have

Figure 5. Fundus image of patient 4 showing a normal optic nerve OD, and an abnormal optic nerve with superficial gliotic tissue OS.

presented another patient with a possible non-pathogenic variant of the *PAX2* gene.

Patients 1 and 2 presented with the classic clinical presentation of PAPRS: renal disease associated with a congenitally abnormal optic disc. However, patients 3 and 4 only had one abnormal optic nerve, without any renal involvement. Clinically, PAPRS includes fundamental alterations in renal function as well as involvement of the optic nerve. Around 92% of patients with the presence of mutations for this syndrome are shown to have renal involvement.² The most frequent involvement (65%) is renal hypodysplasia. This abnormality is characterised by the presence of a small kidney with reduced number of nephrons and a disorganised renal tissue.^{1,2} Other possible renal abnormalities that can be seen in this syndrome include renal hypoplasia, multicystic dysplastic kidney, oligomeganephronia (presence of reduced numbers of nephrons and hypertrophy of the glomeruli and tubules), as well as vesicoureteral reflux.^{1,2}

All these abnormalities can lead to renal insufficiency, which can be seen at any age (as well as from birth), but with presentation at an average age of 19.5 years.^{1,2}

In 1,2), void one-sentence par *PAX2* mutations have been described in patients with isolated renal dysplasia without any ocular involvement, probably due to an allelic heterogeneity of the *PAX2* gene.¹

In this case, patients 1 and 2 presented with polycystic renal hypoplasia and polycystic kidney, respectively, after renal transplant. Patients 3 and 4 did not present with any renal involvement.

Around 77% of patients with *PAX2* mutations present with optic nerve involvement.² The most frequent alteration is the presence of optic nerve dysplasia.² A vacant optic nerve with a central excavation, absence of the central retinal artery, and presence of multiple cilioretinal arteries in radial formation has often been described in scientific literature.⁶ In these cases, fluorescein and Doppler angiography confirm the absence of the central retinal artery and the presence of perfusion caused by the multiple cilioretinal arteries.⁶ Patients 1 and 2 presented with this type of optic nerve abnormality and in particular presented with an abnormal optic nerve showing a prominent central excavation, without the appreciation of the central retinal artery or

vein. Nevertheless, numerous vessels were seen radiating from the periphery of the optic disc. This was better appreciated by the fluorescein angiography performed on patient 2.

What stands out in both these patients is the asymmetric effect on visual acuity, despite the congenital anomalous appearance of both optic nerves. In particular, with patient 1, the eye with the weaker VA (OS) had the largest excavation, whereas with patient 2 there was no excavation in the eye with the weaker VA (OS), but there was a greater degree of peripapillary chorioretinal atrophy.

What is of great value to note is the history of sudden and painless bilateral loss of vision appreciated by patient 2 in the months following renal transplant. This was suggestive of a possible ischaemic optic neuropathy that could have affected the visual acuity of the patient, which could explain the dense central scotoma and relative superior altitudinal defects in both eyes.

In any case, despite being more frequent, the so called vacant optic nerve isn't the only ocular manifestation of PAPRS. Other less frequent optic nerve variants have been described, such as optic disc pitting, optic nerve hypoplasia, and the presence of redundant fibroglial tissue.¹ Likewise, in other publications, the presence of other ocular alterations has been described, such as retinal coloboma and retrobulbar optic nerve cysts.

This is the case with patient 3 who presented with a right optic nerve pit and the presence of gliosis on the surface of the optic nerve OS. There was also a presence of intraretinal fluid, compatible with left optic pit maculopathy, for which it was assumed that there was an optic nerve pit underlying the superficial gliosis of the optic nerve OS. In any case, the VA was normal for both eyes.

In the case of patient 4 who presented with superficial fibroglial tissue OS and a normal looking optic nerve OD, Goldmann visual field testing OS showed a superior altitudinal defect. This patient-glial tissue OS and a normal looking optic nerve OD, Goldrve defect OS was discovered incidentally after routine ophthalmological examination.

It should be noted that there was no abnormal renal function in either of the two patients. In other words, the clinical expression of the *PAX2* mutations only affected the optic nerves. This raises the possibility of *PAX2* mutations being

associated with isolated ocular abnormalities, in the same way that *PAX2* mutations have been previously associated with isolated renal abnormalities, as described in the literature.

Other less frequent abnormalities seen in patients with *PAX2* mutations include neurosensory auditory loss (present in 7% of those affected),¹ central nervous system malformations, mental delay, and ligamentous laxity.⁷

As for the mechanism for which optic nerve abnormalities are produced, laboratory studies on rats and zebrafish having *PAX2/PAX2A* mutations have shown a failure in the closure of the optic fissure, giving rise to a chorioretinal coloboma. However, *PAX2* haploinsufficiency produces a failure in the closure of the optic nerve resulting in optic nerve dysplasia. Likewise, vascular abnormalities accompanying this syndrome can occur secondary to the abnormality in fissure closure. For some authors, these studies prove in one form or another that PAPRS falls within the spectrum of colobomatous malformations of the eye.^{8,9}

PAX2 genes fall within the paired boxfall within the *ry. <eant* that regulate transcription. Nine types of these genes have been described, which differ from each other based on the presence or absence of four domains: pair domain, octapeptide domain, homeodomain, and transactivation domain.² Specifically, *PAX2* genes have 12 exons, with the majority of mutations occurring on exons 2–4 (paired domain). Yet, there are also families with mutations described in other domains.² In our patients, the mutations were localised on intron 2 (patient 1), exon 3 (patient 2), exon 8 (patient 3), and exon 4 (patient 4).

Until 2011, the literature had identified 53 *PAX2* gene mutations in 86 different families.² The most frequently occurring mutation described in this syndrome is c.76dup, also known as c..619insG because of the insertion of an extra residual guanine in a sequence of seven residual guanines.¹

Although haploinsufficiency is the most plausible mechanism for *PAX2* mutations, the possible existence of other mechanisms should not be excluded. For example, many *PAX2* mutations are nonsense or frameshift mutations, ultimately producing abnormal transcripts. In these cases, the norm is that these abnormal transcripts are degraded, but one cannot

exclude the fact that they remain functionally abnormal yet possibly causing additional effects.² In this sense, the slight missense mutations described produce functionally altered mutant proteins with a decrease in stability and a reduction in *PAX2* transactivation.^{8,10,11,12} Additionally, one cannot also exclude the fact that these mutated proteins can cause a truly negative effect on other proteins within the transcription complex, contributing to the pathogenesis of the disease.^{8,10}

The majority of *PAX2* mutations lead to a loss in total expression of one of the alleles or by the expression of a truncated protein. The mechanisms that produce these mutations are varied and include shift in the reading time, disruption of a conserved splice site, genomic deletion or disruption, or introduction of a premature stop codon.²

Missense mutations are less frequent and produce a full-length *PAX2* protein yet with an abnormal function.² All our patients had missense mutations. In patient 1, the nucleotide exchange was located in intron 2 and affected the acceptor splice site of exon 3. In patient 2, the missense mutation was located in exon 3, which leads to a substitution of the evolutionary highly conserved amino acid proline by arginine at position 130 of the protein (p.Pro130Arg). Patient 3 carried the heterozygous missense variant c.988G>A (p. Gly330Ser) in exon 8 of the *PAX2* gene.

In a recent publication, Bower et al.² described several missense variations of benign character. One of those is c.478G>A, which consists of a substitution of amino acid threonine by alanine at position 160 of the protein (p.Alal60Thr). This variation was identified in a foetus with renal agenesis and contralateral multicystic renal dysplasia. Nevertheless, the variation was also present in the healthy mother, for which it was considered to be of benign character.

This is the same variant that was present in patient 4. As previously described, our patient presented with a congenitally abnormal optic nerve OS, with superficial fibroglial tissue, accompanied by an altitudinal defect on visual field testing, despite having almost normal VA (20/30 OS). Given the *PAX2* gene variant is probably apathogenic (three bioinformatic programs predicted a benign character, whereas

two other programs predicted a pathogenic effect for this alteration), the most likely diagnosis of that patient is that of a congenitally abnormal optic nerve without any relation to PAPRS. In any case, the absence of genetic analysis of the patientograms predicted a benign character, whereas two other et al.) does not allow us to be sure that is it not a mild form of clinical expression of PAPRS.

In conclusion, we have presented two patients with a classic clinical presentation of PAPRS having two new *PAX2* mutations not previously described in the scientific literature, another patient with a new *PAX2* mutation with exclusively ocular manifestations, and a final patient with a congenitally abnormal optic disc, found to be a carrier of a *PAX2* variant but without any probable relation to PAPRS. We have therefore amplified the spectrum of *PAX2* mutations currently described in the literature and likewise have noted the possibility of the existence of other patients having *PAX2* mutations with isolated ocular abnormalities.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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