First evidence of SOX2 mutations in Peters' anomaly: Lessons from molecular screening of 95 patients

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First evidence of *SOX2* mutations in Peters' anomaly: lessons from molecular screening of 95 patients

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ABSTRACT

Peters' anomaly (PA) is a rare anterior segment dysgenesis characterized by central corneal opacity and irido-lenticulo-corneal adhesions. Several genes are involved in syndromic or isolated PA (*B3GLCT*, *PAX6*, *PITX3*, *FOXE3, CYP1B1*). Some Copy Number Variations (CNVs) have also been occasionally reported. Despite this genetic heterogeneity, most of patients remain without genetic diagnosis.

We retrieved a cohort of 95 individuals with PA and performed genotyping using a combination of Comparative genomic hybridization, whole genome, exome and targeted sequencing of 119 genes associated with ocular development anomalies.

Causative genetic defects involving 12 genes and CNVs were identified for 1/3 of patients. Unsurprisingly, *B3GLCT* and *PAX6* were the most frequently implicated genes, respectively in syndromic and isolated PA. Unexpectedly, the third gene involved in our cohort was *SOX2*, the major gene of micro-anophthalmia. Four unrelated patients with PA (isolated or with microphthalmia) were carrying pathogenic variants in this gene that was never associated with PA before.

Here we described the largest cohort of PA patients ever reported. The genetic bases of PA are still to be explored as genetic diagnosis was unavailable for 2/3 of patients. Nevertheless, we showed here for the first time the involvement of *SOX2* in PA, offering new evidence for its role in corneal transparency and anterior segment development.

KEYWORDS

Anterior segment dysgenesis, Peters' anomaly, microphthalmia, *SOX2, B3GLCT, PAX6, PITX3, CYP1B1, FOXE3, CNV*

1 INTRODUCTION

Peters' anomaly (PA) is a rare anterior segment dysgenesis (ASD). Its incidence has only been estimated once, around 1.5 per 100,000 live births.[1] Our group recently estimated this incidence of around 2.5/100,000 live births in the Midi-Pyrénées region of France (unpublished data), which is consistent with the previous estimation. The main anatomical lesion observed in PA is a defect in the posterior layers of the cornea —Descemet's membrane in particular— which leads to central corneal opacity of varying extent.[2,3] This opacity is frequently associated with iridocorneal (PA type I) or lenticulocorneal (PA type II) adhesions.[2] The pathophysiology of PA is yet not clearly elucidated. It might result from imperfect separation of the lens vesicle and the surface ectoderm during ocular development (OD).[4] It could also be the consequence of a defect in neural crest cells (NCC) migration, which is crucial for the development of the anterior segment (AS) of the eye.[5]

Careful clinical examination (slit lamp) and imaging techniques such as ultrasound biomicroscopy (UBM) or Optical Coherence Tomography (OCT) are needed to diagnose PA and rule out other causes of corneal opacities, including other ASDs (primary congenital glaucoma and Axenfeld-Rieger anomaly[6]) as well as corneal dystrophies[7], sclerocornea, corneal dermoids and metabolic, infectious or traumatic causes, which have pathophysiologies different from that of PA and can also lead to congenital corneal opacity.[8] PA often results in severe visual impairment due to obstruction of the visual axis, especially as the condition is frequently bilateral.[8,9] Moreover, it is often associated with other ocular anomalies: cataract, glaucoma, microphthalmia and contralateral anophthalmia.[3] Treatments vary and may include penetrating keratoplasty, with some patients who continue to have poor visual prognoses.[9]

PA can be isolated or, more frequently, associated with extraocular features. Peters-plus syndrome (PPS) is the most common syndromic form of PA, caused by biallelic pathogenic variants in *B3GLCT*, which encodes an O-glucosyltransferase.[11] PPS is characterized by developmental delay (DD), short limbs with broad distal extremities, cleft lip or palate, and PA or another ASD. In addition to *B3GLCT*, more than ten other genes that cause isolated or syndromic PA have been reported to date, including the transcription factors *PAX6*,[12] *PITX2*,[6] *PITX3*,[13] *FOXE3*,[14] *FOXC1*[6] and *MAF*,[15] the extracellular matrix–associated gene *COL4A1*[16] and one of the enzymes of the cytochrome P450 superfamily *CYP1B1*.[17] Copy number variations (CNVs), such as the 22q11.2 microdeletion, have also been reported in individuals with syndromic PA.[18] Despite the number of genes known to play a role in PA, previous studies have only reached genetic diagnoses for 1/4 of patients.[19,20]

Thus, this study first sought to determine the contribution of each OD gene in an exceptional cohort of 95 unrelated patients affected by syndromic and non-syndromic PA. Accordingly, we performed array comparative genomic hybridization (aCGH), whole genome, exome or targeted sequencing (TS) in the largest genotyped PA cohort ever reported.

2 METHODS

We retrieved clinical and genetic data from all the patients who were referred to our laboratory (genetic lab of Toulouse University Hospital) for a genetic diagnosis of PA during the fifteen last years. This study was designed in compliance with the tenets of the Helsinki Declaration and patient enrolment had approval by our local Ethics Committee (RNIPH#2021-44) and the CNIL-MR-004 registry (#2206723v0). All the patients or their legal representatives gave informed consent for genetic analysis. From 2006 to 2021, a total of 95 probands with a PA diagnosis were referred by specialists after ophthalmic examination showing the presence of central corneal opacities and iridolenticulocorneal adhesions. During these fifteen years, the genetic diagnosis strategy developed in our lab has followed the different advances made in sequencing technologies. Thus, the genetic diagnosis of PA was initially based on the exploration of the 7 main PA genes (*B3GLCT, PAX6, FOXE3, PITX3, CYP1B, PITX2, FOXC1*) by combining direct sequencing, Multiplex Ligation-dependent Probe Amplification (MLPA) and aCGH (43 patients), then by TS using a 24 ocular genes panel (35 patients) and finally by using a customized 119 genes panel (66 patients), whose method was detailed in previous works,[21,22] allowing both the detection of SNVs and CNVs. This 119 genes panel includes multiple genes associated with isolated or syndromic ASD (including PA), aniridia, coloboma, micro-anophthalmia and congenital cataract (Supp.Data.1). VCF files were first filtered to detect variants with an allelic balance >30%, a depth >30X and a number of occurrences in the run <4 patients (around 50 patients by run). Private annotations of previously detected variants were conserved in our database. These annotations were performed both with informatics tools for variants >5% of allelic frequency in gnomAD (stand-alone criterion of the ACMG recommendations)[23] and manually by a trained geneticist specialized in genetic ophthalmology for other variants. If no variant was found after this first analyse, data were explored removing the filters cited above.

Of note, all patients with negative results were at least screened with the 119 genes panel, except 4 patients who immediately benefited from whole-exome (WES) (P25 and P29, Agilent SureSelectV6, 10X coverage >95%)

and whole-genome (WGS) (P28 and P34, 30X coverage >80%) sequencing. Analyses performed on each patient are detailed in Supp.Data.2.

Variants classified as 'pathogenic', 'likely pathogenic', or 'of uncertain significance' (VUS) according to the ACMG guidelines[23] were reported in this work and confirmed by Sanger sequencing (VUS are detailed in supplementary data 3 and were not confirmed by Sanger sequencing). In the same way, CNVs detected through the 24 or 119 genes panels (P2 and P59) were confirmed by using a customized 180k aCGH (Agilent, California, U.S.A.), enriched in OD genes.

3 RESULTS

A total of 95 unrelated individuals were referred in our lab with a clinical diagnosis of unilateral (24 patients with normal contralateral eye) or bilateral PA (71 patients with bilateral ocular defects). In addition to PA, some individuals exhibited other ocular anomalies: 17 with micro-anophthalmia and 5 with coloboma. Nearly half of patients (39/95, 41%) presented with extraocular features (Supp.Data.2): 17 with neurodevelopmental disorders (DD, intellectual disability or autism spectrum disorders), 11 with growth retardation (GR), 10 with central nervous system defects, 7 with urogenital abnormalities and 6 with cardiac malformations. Of note, four patients had a disease-causing variant that partly or fully explains the extraocular features but poorly explains the ocular phenotype (P69, P73, P80, P85; Supp.Data.2). A quarter of the cohort (22 patients) had a familial history of ocular phenotype (microphthalmia, ASD, cataract and high myopia).

Using the aforementioned genetic screening strategies, we identified a causative variant in 34 patients of the cohort (36%, Figure1, Table1): 30 had a single nucleotide variant (SNV) or CNV involving one of the 119 OD genes (Supp.Data.3A) and 4 had a pathogenic CNV elsewhere in the genome. Genetic defects were identified two times more frequently in patients with a family history of ocular defects (13/22=59%) than those with a sporadic PA (21/73=29%). Genetic diagnoses were also more common in patients with PA that was bilateral (28/71=39%) versus unilateral (6/24=25%) and syndromic (19/39=49%) versus isolated (15/56=27%). In patients with isolated PA, the probability of detecting a causative variant was also higher when the ocular anomaly was bilateral (13/46=28%) versus unilateral (2/12=17%). In the same way, patients with syndromic PA were more likely to have a genetic diagnosis when the ocular defect was bilateral (15/26=58%) rather than unilateral (4/13=31%).

3.1 *B3GLCT* (NM_194318.4), the first cause of syndromic PA

Among the syndromic PA individuals, 18% (7/39) had biallelic pathogenic variants in *B3GLCT*, that was the leading gene responsible for PA (7/95=7%, namely 7/34=**21% of positive cases**). Although some were neonates with incomplete clinical data, all had syndromic PA with documented features concordant with PPS.[11] Interestingly, patient **P93** exhibited sagittal craniosynostosis, which was not previously considered as a distinctive PPS sign. Patient **P58** had a homozygous intragenic deletion (exons 1 and 2, confirmed by MLPA), also found at homozygous state in his symptomatic brother. **P87**, a 22-year-old female with a classic PPS phenotype, was carrying the c.1184+3A>G variant that results in a skipping of the exon 13 observed on transcript analysis (data not shown), *in trans* with the recurrent c.660+1G>A variant[11]. The five other patients (**P3**, **P15**, **P56**, **P71**, **P93**) have biallelic pathogenic *B3GLCT* variants, already documented in the literature, including the c.660+1G>A variant.[11]

3.2 *PAX6* (NM_000280.4), the first cause of isolated PA

Deleterious *PAX6* variants were the most frequent defects found in patients with isolated PA and the second gene associated with PA in our cohort (5/95=5%, namely 5/34=**15% of positive cases**). Defects in the *PAX6* gene are mainly associated with aniridia, but they have also been linked to other ocular anomalies. In particular, *PAX6* was the first gene involved in PA.[12] Four out the five patients had a missense variant, including two novel variants (**P7**: c.38G>C p.(Gly13Ala) and **P74**: c.786G>C p.(Arg262Ser)) and two variants previously described in patients with PA and aniridia (**P33**: c.52G>C p.(Gly18Arg) and **P46**: c.622C>T p.(Arg208Trp)).[24,25] A germline mosaicism was highly suspected in the P33 family because of a recurrence during prenatal diagnosis. Surprisingly, one patient with isolated bilateral PA (**P2**) had a whole gene deletion. Although no clear genotype–phenotype correlation exists, the majority of null *PAX6* variants are rather known to cause aniridia, whereas missense variants cause less typical ocular phenotypes.[24] With the exception of the patient P2, our data support this correlation as 4 out of the 5 patients had a missense variant within the functional domains of the protein and did not exhibit aniridia.

3.3 *SOX2* (NM_003106.4), first involvement in PA

It is noteworthy that four individuals (4/95=4%; 4/34=**12% of positive cases**) were found to harbour heterozygous deleterious variants in *SOX2*, a gene that plays a major role in OD as evidenced by its mutations

responsible for severe eye defects such as micro-anophthalmia.[26] **P45** had unilateral complex microphthalmia with PA (Figure2A), normal left eye and DD. Molecular analysis revealed a novel nonsense variant in *SOX2*, c.22G>T p.(Glu8*). **P91** displayed isolated left anophthalmia and right PA without microphthalmia after examination under general anaesthesia. She was only one-month-old at the time of her examination and does not have any other malformation. Genetic analysis revealed a frameshift recurrent *SOX2*variant: c.70_89del p.(Asp24Argfs*65), yet previously associated with ocular growth defects of various severity (from normal size to anophthalmia).[26–28] **P94** had unilateral right complex microphthalmia with PA visible on OCT (Figure2C) and left optic nerve hypoplasia with a normal AS. He also had DD with cerebral atrophy on MRI, microcephaly and craniofacial dysmorphism (epicanthal folds, wide mouth). Genetic analyses revealed a nonsense *SOX2* variant, c.103A>T p.(Lys35*), found at mosaic state (around 25% in blood) in his mother who has hypogonadotropic hypogonadism without ocular defect. This variant was previously reported in a patient with hypogonadotropic hypogonadism.[29] **P95** had right anophthalmia and left PA without microphthalmia, moderate intellectual disability, partial corpus callosum atrophy, microcephaly (-3.3 SD), GR (-2.25 SD), congenital bilateral pes cavus, hypogonadotropic hypogonadism with bilateral cryptorchidism, testicular atrophy and micropenis. Direct sequencing of *SOX2* revealed the *de novo* reciprocal duplication of the recurrent deletion, c.70_89dup p.(Gly31Thrfs*22), never described before.

3.4 PITX3, CYP1B1 and FOXE3, recurrent causes of isolated PA

3.4.1 PITX3 (NM_005029.4)

We identified pathogenic variants in *PITX3* in three patients (**P14**, **P41**, **P50**) with isolated autosomal dominant PA (3/95=3%; 3/34=**9% of positive cases**). In the **P50** family, the *PITX3* variant explains the familial ASD but poorly explains extra-ocular features displayed only by the proband (DD, GR) and no other cause was found to date. These data (Table1) confirm the intra and inter familial variability described in ocular phenotypes associated with *PITX3* disease-causing variants.[13]

3.4.2 CYP1B1 (NM_000104.4)

We identified six causative *CYP1B1* variants in three individuals (**P5**, **P36**, **P38**) with isolated sporadic PA (3/95=3%; 3/34=**9% of positive cases**) and glaucoma (no data available about glaucoma for P38). All six variants have previously been reported as pathogenic in patients with various types of ASD, including primary congenital

glaucoma.[2,30–32] For two individuals, complete inheritance patterns were not determined due to lack of DNA samples.

3.4.3 FOXE3 (NM_012186.3)

Three individuals (**P64**, **P70**, **P90**) had variants in the *FOXE3* gene (3/95=3%; 3/34=**9% of positive cases**), resulting in the severe ocular phenotype of complex microphthalmia and PA. **P64** was one of two siblings, previously reported with a homozygous missense variant (c.232G>A p.Ala78Thr).[14] Surprisingly, patient **P70** carried a monoallelic c.960A>C stop-loss variant, which is usually associated with milder ocular anomalies of dominant inheritance.[14,33] An undetected modifying factor *in cis* or *in trans* of the stop-loss variant might explain the severity of his phenotype. Although the inheritance pattern was not determined for **P90**, the two pathogenic variants (c.232G>A and c.253dup) were clearly not supported by the same reads (Supp.Data.4).

3.5 *PITX2*, *RARB*, *COL4A1*, *HCCS* and *FOXC1*, rare causes of PA

3.5.1 PITX2 (NM_153426.2)

Patient **P52** had the missense variant c.311T>C p.(Phe104Ser) in the homeobox domain of *PITX2* (1/95=1%; 1/34=**3% of positive cases**). This gene is usually associated with Axenfeld-Rieger syndrome, but occasionally with PA.[6] Hypodontia exhibited by P52 is thus concordant with this syndrome (Table1).

3.5.2 RARB (NM_000965.5)

We identified a heterozygous *RARB* variant in **P65** (1/95=1%; 1/34=**3% of positive cases**) who displayed bilateral complex microphthalmia (with PA and cataract) and spastic tetraparesis. This novel c.1151G>A p.(Gly384Asp) missense variant alters a conserved amino acid close to the p.Arg387Cis/Ser/Leu hotspot previously described in individuals with ASD, DD and spastic paraparesis.[34]

3.5.3 COL4A1 (NM_001845.6)

P27, previously reported,[35] harboured the *COL4A1* c.615+1G>A splicing variant. This novel variant was found in all four affected family members. *COL4A1* is involved in a spectrum of disorders: cerebrovascular disease and various ocular anomalies, including PA.[16] Previous analyses in this family found the c.3947A>G p.(Tyr1316Cys) missense variant in *PTCH1*, inherited from his affected mother, which is now considered as a VUS (Supp.Data3).[35]

3.5.4 HCCS (NM_005333.5)

Targeted *HCCS* analysis in **P6** —a girl previously reported who had syndromic bilateral complex ocular malformation and died at 4 months from a cardiomyopathy— revealed the nonsense variant c.589C>T p.(Arg197*) (1/95=1%; 1/34=**3% of positive cases**).[36] Heterozygous pathogenic *HCCS* variants in females are responsible for microphthalmia and linear skin defects, and are lethal in hemizygous males.[36]

3.5.5 FOXC1 (NM_001453)

Patient **P59** who had isolated bilateral PA (Figure2) was carrying a *FOXC1* duplication detected in a larger 6p25.3 duplication that also encompasses *FOXF2* and *GMD5* (arr[GRCh38]6p25.3(1386760_1822396)x3). The duplication was inherited from his symptomatic mother (bilateral PA and glaucoma). Both *FOXC1* duplications and deletions have been associated with ASD.[6] This emphasizes the importance of *FOXC1* gene dosage during development of the AS.

3.6 CNVs not involving ocular genes, a relatively frequent cause of PA

Four patients had pathogenic CNVs that did not encompass OD genes. Of note, the presence of pathogenic variants in ocular genes that might otherwise explain their ocular phenotypes was excluded by the 119 genes panel. Yet the literature and databases do indicate ocular anomalies for similar CNVs. This highlights the need for careful, systematic ocular examination of individuals with CNVs and, more generally, DD. Moreover, CNVs should be systematically screened in patients with syndromic PA as they accounted for 10% (4/39) of the diagnoses.

3.6.1 22q11.21

Two patients (**P4**, **P83**) had 22q11.2 deletion syndrome (arr[GRCh38]22q11.2(22:19022279_21098156)x1). Although PA has rarely been described in patients with 22q11.2 deletion, it might be a frequent cause of PA. Reis *et al.*[18] pointed out the role of *TBX1* in both, the retinoic acid pathway (critical to OD) and the *PITX2* pathway.

3.6.2 8q21

Chromosomal microarray analysis revealed a 8q21.11q21.2 deletion (10-Mb) in **P72** (arr[GRCh38]8q21.11q21.2(75652023_85480932)x1). PA has already been described in two individuals with

overlapping 8q21.11 microdeletions[37] that span seven OMIM-morbid genes, mostly associated with autosomal recessive disorders. Although two candidate genes have been singled out by Happ *et al.* to explain ocular features in 8q21.11 patients (*ZFHX4* and *PEX2*[37]), additional studies are needed to conclude.

3.6.3 13q31-q33

We identified a *de novo* 13q31.2-q33.3 deletion (27.7-Mb) in **P86** (arr[GRCh38]13q31.1q33.2(82528272_106198042)x1). This deletion encompasses 14 OMIM-morbid genes, including *ZIC2*, previously shown to be important in zebrafish OD.[38] Interestingly, two patients in the Decipher database (decipher.sanger.ac.uk; patients 395338 and 400829) with ASD (aniridia and Axenfeld-Rieger syndrome, respectively) had large deletions partly spanning the 13q31 region, though only patient 395338 exhibited *ZIC2* deletion. Furthermore, not all individuals with 13q deletions have ocular malformations,[39] and *ZIC2* deletion alone is apparently not sufficient for the development of these anomalies.

4 DISCUSSION

In this study, we show that PA is a genetically heterogenous ocular defect. The discovery of new genes, and expansion of the clinical spectra of those already known, are essential to refine our knowledge of AS development, which is driven by multiple interacting factors and tissue components. First, the transcription factor PAX6 plays a key role in OD and guides NCC migration with other transcription factors such as PITX2 and FOXC1. The involvement of transcription factors such as PITX3 and FOXE3, that are crucial for lens vesicle formation and differentiation, shows the importance of lens in AS formation. Retinoic acid pathway also plays a role in AS development with the involvement of *RARB* in PA.[40] In addition, genes encoding extracellular matrix proteins (*COL4A1*) or even enzymes (*B3GLCT*, *CYP1B1*) have been associated with PA. Investigation of all these intricate genes and pathways could teach us more about the PA physiopathology that still remain elusive.

With this cohort of 95 individuals with PA, we bring here the largest overview of clinical and genetic data of PA ever reported. Excepted few CNVs in regions of unknown significance, PA is mainly the consequence of intragenic defects in a dozen of genes, allowing the diagnosis in only 1/3 of patients. Among patients with a diagnosis, some genes are rather associated with syndromic PA (*B3GLCT*, *SOX2, PITX2*, *COL4A1*, *RARB*, *HCCS*) whereas others are rather associated with isolated PA (*PAX6*, *FOXE3*, *FOXC1*, *CYP1B1*, *PITX3*). Moreover, besides inter and intrafamilial variability as illustrated by the **P14** family (*PITX3*), each of these genes accounts for a small number of diagnoses, making difficult to draw any genotype-phenotype correlation.

What our work also shows is that most of the genes associated with PA were known before the advent of next-generation sequencing.[2,19] Its development has however allowed the simultaneous exploration of multiple genes tied to ocular malformations and thereby widened the phenotypic spectrum of the previously known disease-causing genes. These latter include *SOX2*, not previously linked to PA, but which we demonstrated here to be associated with PA condition. *SOX2* defects are mostly associated with severe bilateral ocular anomalies (microphthalmia/anophthalmia) and extraocular features (GR, DD, various organ malformations).[26] Although AS phenotypes (microcornea, posterior embryotoxon, iris hypoplasia, irregularly constricted pupil, sclerocornea, cataract and aphakia) have occasionally been described in complex microphthalmia secondary to *SOX2* variants,[28,41,42] no report in the literature has ever linked PA to a *SOX2* pathogenic variant in a patient. In two patients (**P45** and **P94**), PA seems to be part of a global OD defect as microphthalmia is observed on the same eye. However, the two other patients (**P91** and **P95**) displayed isolated PA on one eye and anophthalmia on the other eye, which is particularly intriguing and suggesting a primary defect of AS development. Of note, all the variants identified in *SOX2* and responsible for PA were heterozygous truncating variants (nonsense or frameshift) positioned early in the gene (before the HMG domain). However, some patients with such truncating variants were reported in the literature with various phenotypes, ranging from clinical anophthalmia to normal eye.[26,43] Furthermore, patient **P91** displays the recurrent frameshift deletion c.70_89del that has been described in patients with various ocular phenotypes.[28,41,42] Therefore, additional genetic variants or environmental effects might be considered to explain *SOX2* phenotypic variability.

SOX2 plays a critical role in eye development, participating in differentiation during the development of the AS.[44,45] It closely interacts with *PAX6* during early lens development, and *SOX2* expression level has been detected in the developing lens vesicle of human embryos.[27,44] This transcription factor also plays a crucial role in NCC formation that is essential in AS development.[46] The alteration of each of these SOX2 functions can therefore possibly impact AS formation and lead to PA. Moreover, *SOX2* appears to be an essential regulator of limbal epithelial stem cells that participates in corneal epithelium renewal and thus corneal transparency.[47] This role of *SOX2* suggests the therapeutic potential of *SOX2* activation in endothelial corneal diseases.[47] *SOX2* activation might also be investigated as a means of treating corneal opacity in PA, resulting from alteration of the posterior layers of the cornea. Thus, this work brings new evidence on the complex role of *SOX2* in OD, as it is involved in ocular growth, choroid fissure closure[27,44] and, as we have now observed, in AS formation.

Through TS using a 119 ocular genes panel, we were able to bring the diagnostic yield to one-third of our PA cohort. Although our approach used mainly TS, we obtained similar results to those of Weh *et al*., who identified a causative variant in 22% (6/27) individuals with PA, using WES filtered on 699 ocular genes.[20] This highlights the low frequency of recently described genes to establish new diagnoses (i.e. *BMP4* for which we did not find any disease-causing variant in our cohort) and the need to look for other mechanisms. In two recent studies that employed filtered exome data or a mixed WGS/WES strategy in their ASD cohorts, the rates of genetic diagnoses among patients with PA were 64% (7/11) and 75% (6/8).[17,48] These higher rates are difficult to interpret given the small number of patients in those two studies. Yet we noticed that all mutated genes identified in their cohorts, with the exception of *TP63*,[48] were covered by our TS strategy. Thus, two-thirds of individuals with PA still lacked genetic diagnosis. Consequently, other genetic causes should be considered. These possibly include variants in the few PA genes that were not explored by our TS strategy such as *TP63*, that has been described only in one patient with PA.[48] Of note, this shows that, despite occasional description of causative genes (i.e *BMP4*, *TP63*), the major genes of PA have probably already been found and other molecular mechanisms should be explored such as variants in deep intronic region or in regulatory elements, usually not covered by standard diagnostic procedures, as previously shown for *PAX6*.[22,49] Interpretation of these variants may be difficult and require complementary techniques, such as RNA studies, to conclude about their role in the patient's phenotype. Furthermore, the significant number of CNVs observed in our cohort suggests that structural variants may also play an important role in PA. Structural variants that alter 3D genome architecture, such as by disrupting topologically associating domains, have also been connected to human diseases.[50] Thus, much remains to be explored in the genetics of PA and the use of new technologies like whole genome and RNA sequencing could help to reveal hidden diagnoses.

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DECLARATIONS

Conflict of interest: None declared.

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Data availability: Features of cohort members are detailed in Supp.Data2. Novel variants described in this study have been submitted to the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/; submission accession numbers: SCV001449117 and SCV001449128).

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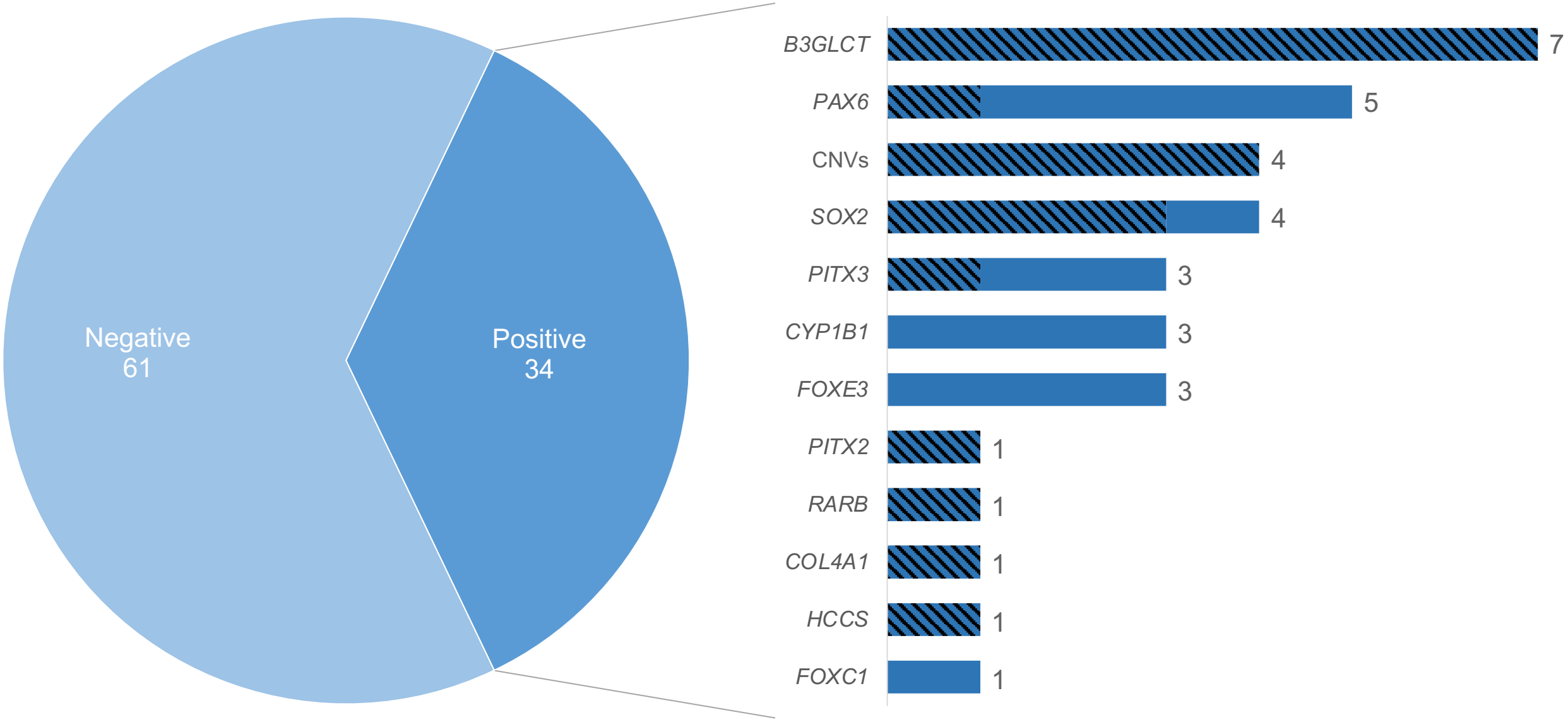
FIGURES LEGENDS

Table1: Genotypic, phenotypic and familial data of the 34 positive patients described in this work.

Figure1: Breakdown of genetic diagnoses in the 95 PA patients cohort. For the 34 patients with positive results, the bar chart indicates the identified genetic causes and the numbers of individuals concerned. Hatched bars indicate (for each gene) the number of patients with extra-ocular features among those with a genetic diagnosis. CNV = copy number variation.

Figure2: A. On top, photograph of **P45** (*SOX2*: p.(Glu8*)) showing right corneal opacity and on bottom ultrasound imaging of her right eye revealing iridocorneal adhesions. **B.** Patient **P59** (*FOXC1* duplication): ultrasound exam shows upper quadrant of right eye. Note corneal thinning and disappearance of posterior hyperechoic line corresponding to Descemet's membrane. **C.** Patient **P94** (*SOX2*: p.(Lys35*)). Optical Coherence Tomography shows iridocorneal adhesion.


Negative
61
Positive
34
B3GLCT
7
PAX6
5
CNVs
4
SOX2
4
PITX3
3
CYP1B1
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FOXE3
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PITX2
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RARB
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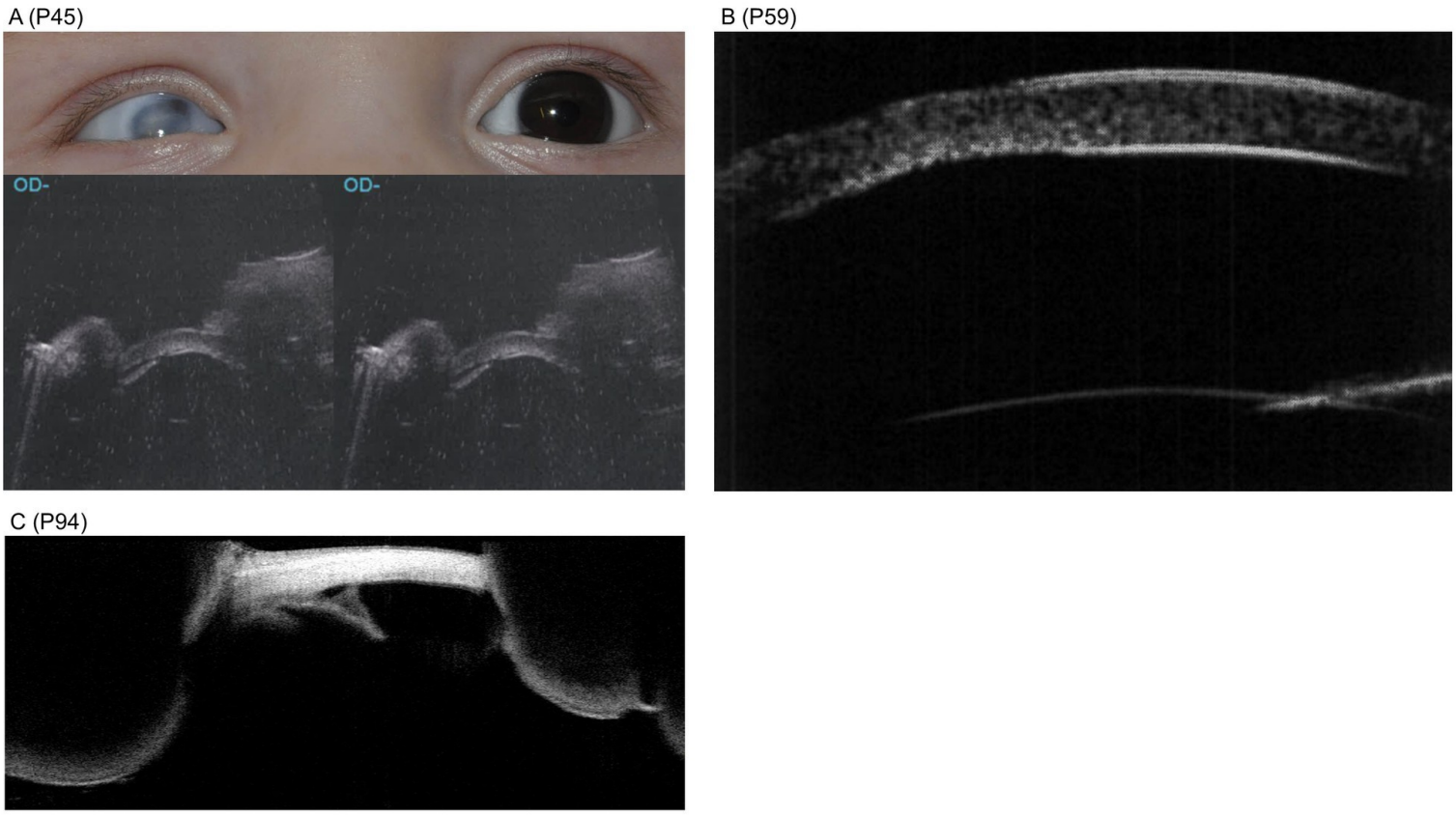
COL4A1
1
HCCS
1
FOXC1
1



CGE_14123_figure 2.jpg

Table 1: Genotypic, phenotypic and familial data of the 34 positive patients described in this work.

The gene content of each CNV is detailed in brackets in the genotype column with the gene thought to cause the eye defect in bold.

Caption: ACC: abnormal corpus callosum, Bilat: bilateral, CFD: craniofacial dysmorphism, DD: developmental delay, GR: growth retardation, het: heterozygous status, homo: homozygous status, ID: intellectual disability, L: left micro: microphthalmia, NP: not performed, R: right, WMH: white matter hyperintensities.

Gene Transcript	**Patient**	**Sex**	**Age years**	**Genotype**	**Ocular phenotype**	**Extraocular features**	**Family history and segregation**
B3GLCT NM_194318.4	P3	M	10	c.[660+1G>A];[1178G>A] p.[?];[(Gly393Glu)]	Bilat PA	GR, DD, atrial septal defect, pulmonary stenosis	**Mother** (asymptomatic): c. [1178G>A];[=] **Father** (asymptomatic): c.[660+1G>A];[=]
	P15	F	0.1	c.[459+1G>A];[459+1G>A] p.[?];[?]	Bilat PA	Ventriculomegaly	**Mother** (asymptomatic): c.[459+1G>A];[=] **Father** (asymptomatic): c.[459+1G>A];[=]
	P56	F	1.6	c.[660+1G>A];[660+1G>A] p.[?];[?]	Bilat PA (corneal graft)	DD, GR, blepharophimosis, ptosis	**Mother** (asymptomatic): c.[660+1G>A];[=] **Father** (asymptomatic): c.[660+1G>A];[=]
	P58	F	0.1	rsa[GRCh38] 13q12.3(B3GLCT)x0 (***B3GLCT*** exons 1 & 2)	Bilat PA	Prominent forehead, GR	**Mother** (asymptomatic): het deletion **Father** (asymptomatic): het deletion **Brother** (ASD, optic disc coloboma, pelvic kidney, intrauterine GR, no DD): homo deletion
	P71	F	0.3	c.[660+1G>A];[660+1G>A] p.[?];[?]	Bilat PA	bilat nephromegaly, CFD	**Mother** (asymptomatic): c.[660+1G>A];[=] **Father** (asymptomatic): c.[660+1G>A];[=]
	P87	F	22	c.[660+1G>A];[1184+3A>G] p.[?];[?]	Bilat PA	GR, short and large hands and feet, atrial septal defect, pes cavus , anus anteposition, DD	**Mother** (asymptomatic): c.[660+1G>A];[=] **Father** (asymptomatic): c.[1184+3A>G];[=]
	P93	M	0.2	c.[660+1G>A];[660+1G>A] p.[?];[?]	Bilat PA	bilat labiopalatal cleft, ACC, short long bones, short hands and feet, sagittal craniosynostosis, CFD	**Mother** (asymptomatic): c.[660+1G>A];[=] **Father** (asymptomatic): c.[660+1G>A];[=]
PAX6 NM_001258462.1	P2	M	0.4	arr[GRCh38]11p13(31141793_32226400)x1 (***PAX6***)	Bilat PA		**Father** (juvenile glaucoma, embryotoxon): no deletion **Mother** (embryotoxon): no deletion ***De novo***
	P7	F	0.1	c.[38G>C];[=] p.[(Gly13Ala)];[=]	Bilat PA	autism spectrum disorder, hypoplasia of depressor anguli oris, fifth fingers clinodactyly	**Mother** (isolated bilat PA): c.[38G>C];[=] Maternal grandparents (asymptomatic):c.38=
	P33	M	0.5	c.[52G>C];[=] p.[(Gly18Arg)];[=]	Bilat micro with ASD (PA and cataract)		**Both parents** (asymptomatic): c.52= **Germline mosaicism**: recurrence in prenatal diagnosis
	P46	M	41	c.[622C>T];[=] p.[(Arg208Trp)];[=]	Bilat PA		**Father** and **8 paternal relatives**: PA or ASD. Familial segregation NP
	P74	F	7	c.[786G>C];[=] p.[(Arg262Ser)];[=]	R: PA		**Mother** (bilat cataract, foveal hypoplasia): c.[828G>C];[=]
SOX2 NM_003106.4	P45	F	1.1	c.[22G>T];[=] p.[(Glu8*)];[=]	R: micro (AL: 16mm at 1 month), PA	ID	Parental segregation NP
	P91	F	0.1	c.[70_89del];[=] p.[(Asp24Argfs*65)];[=]	L: anophthalmia R: PA		*De novo*
	P94	M	2	c.[103A>T];[=] p.[(Lys35*)];[=]	R: PA, micro (enucleation) L: optic nerve hypoplasia	DD, cerebral atrophy (MRI), microcephaly, CFD (epicanthal folds, wide mouth)	**Mother** (hypogonadotropic hypogonadism, no ocular defect) c.103=/A>T (25% mosaicism)
	P95	M	21	c.[70_89dup];[=] p.[(Gly31Thrfs*32)];[=]	R: anophthalmia L: PA	ID, GR, microcephaly, ACC, bilateral cryptorchidism, micropenis, hypogonadotropic hypogonadism	*De novo*
PITX3 NM_005029.4	P14	F	37	c.[38G>A];[=] p.[(Ser13Asn)];[=]	Bilat PA		**Mother** (late-onset cataract): c.[38G>A];[=] **Son** (bilat PA (R: corneal graft), optic disc pallor and hypoplasia): c.[38G>A];[=]

Gene Transcript	**Patient**	**Sex**	**Age years**	**Genotype**	**Ocular phenotype**	**Extraocular features**	**Family history and segregation**
	P41	M	43	c.[640_656dup];[=] p.[(Gly220Profs*95)];[=]	Bilat PA		**2 siblings** (congenital cataract): c.[640_656dup];[=] **Father** (embryotoxon, congenital cataract): not tested, **1 Nephew** (bilat PA): not tested
	P50	M	1.6	c.[640_656dup];[=] p.[(Gly220Profs*95)];[=]	Bilat AP	GR, DD, rhizomelia, short hands, exaggerated cupid's bow	**Mother** (juvenile cataract): c.[640_656dup];[=] multiple **mother's relatives** (not tested): juvenile or congenital cataract
CYP1B1 NM_000104.4	P5	F	28	c.317C>A(;)1200_1209dup p.(Ala106Asp)(;)(Thr404Serfs*30)	Bilat PA with congenital glaucoma		Parental segregation NP
	P36	F	1.3	c.[868dup];[171G>A] p.[(Arg290Profs*37)];[(Thr57*)]	Bilat: PA, glaucoma, R: cataract		**Mother** (asymptomatic): c.[868dup];[=] **Father** (asymptomatic): c.[171G>A]; [=]
	P38	F	41	c.1064_1076del(;)1310C>T p.(Arg355Hisfs*69)(;)(Pro437Leu)	Bilat PA		Parental segregation NP
FOXE3 NM_012186.2	P64	F	4	c.[232G>A];[232G>A] p.[(Ala78Thr)];[(Ala78Thr)]	L: PA and micro		**Both parents** (asymptomatic): c.[232G>A];[=] **Sister** (fœtus, R micro and ASD): c.[232G>A];[232G>A]
	P70	F	3	c.[960A>C];[=] p.[(*320Cys*200)];[=]	Bilat complex micro with PA		**Mother**: bilat micro, Maternal aunt: unilateral micro, **Maternal grandmother**: ocular anomaly Familial segregation NP
	P90	F	0.4	c.[232G>A];[253dup] p.[(Ala78Thr)];[(Ala85Glyfs*200)]	Bilat PA and micro		Parental segregation NP (reads analysis shows the two variants in *trans*) **Mother**: unilateral high myopia
PITX2 NM_153426.2	P52	M	4	c.[311T>C];[=] p.[(Phe104Ser)];[=]	L: aniridia R: micro, PA	Autism spectrum disorder, Hypodontia	Parental segregation NP
RARB NM_000965.5	P65	F	10	c.[1151G>A];[=] p.[(Gly384Asp)];[=]	Bilat micro (AL: L 20mm, R21mm) and PA with cataract	spastic tetraparesis, dystonia	Related parents Parental segregation NP
COL4A1 NM_001845.6	P27	F	2	c.[615+1G>A];[=] p.[?];[=]	Bilat PA		**Mother** (central corneal opacity, embryotoxon, periventricular WMH): c.[615+1G>A];[=] **Brother** (focal epilepsy, intracerebral calcifications, DD): c.[615+1G>A];[=] **Sister** (congenital glaucoma): c.[615+1G>A];[=]
HCCS NM_005333.5	P6	F	0.4	c.[589C>T];[=] p.[(Arg197*)];[=]	L: micro with hypoplastic optic nerve R: PA	death at 4 months (noncompaction cardiomyopathy), GR, ACC, hypoplasia of the cerebral white matter, absent septum pellucidum	*De novo*
FOXC1 NM_001453.3	P59	M	1.3	arr[GRCh38]6p25.3(1386760_1822396)x3 (***FOXC1****, FOXF2, GMDS*)	Bilat PA (predominant on the right eye)		**Mother** (bilat PA and glaucoma): *FOXC1* duplication
CNVs	P4	M	0.6	arr[GRCh38]22q11.2(22:19022279_21098156)x1 (***TBX1****, LZTR1, SLC25A1, COMT*)	Bilat PA (corneal graft)	Immune thrombocytopenia and neutropenia, psoriasis	*De novo*
	P83	F	40	arr[GRCh38]22q11.2(22:19022279_21098156)x1 (***TBX1****, LZTR1, SLC25A1, COMT*)	Bilat PA	Di George syndrome	Parental segregation NP
	P72	F	1	arr[GRCh38]8q21.11q21.2(75652023_85480932)x1 (***PEX2***, ***ZFHX4****, IL7, MRPS28, PMP2, IMPA1, CA2*)	R: PA	ACC, feeding difficulties, CFD (malar hypoplasia, thin eyebrows)	*De novo*
	P86	M	0.4	arr[GRCh38]13q31.1q33.2(82528272_106198042)x1 (*CLDN10, DAOA, DNAKC3, ERCC5, FRF14, GPC6, MIR17HG, NALCN, PCCA, SLC10A2, SLITRK1, SLITRK6, TGDS,* ***ZIC2****)*	L PA	DD, bilat profound deafness, microcephaly, CFD, micropenis, rhizomelia, delayed talus ossification	*De novo*