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Longitudinal genotype-phenotype analysis in 86 PAX6-related aniridia patients

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Abstract

Aniridia is most commonly caused by haploinsufficiency of the PAX6 gene, characterised by variable iris and foveal hypoplasia, nystagmus, cataracts, glaucoma and aniridia related keratopathy (ARK). Genotype-phenotype correlations have previously been described, however detailed longitudinal studies of aniridia are less commonly reported. We identified eighty-six patients from sixty-two unrelated families with molecularly confirmed heterozygous PAX6 variants from a United Kingdom (UK)-based single-centre ocular genetics service. They were categorised into mutation groups and retrospective review of baseline to most recent clinical characteristics (ocular and systemic) were recorded. One hundred and seventy-two eyes were evaluated, with a mean follow up period of 16.3 ± 12.7 years. Nystagmus was recorded in 87.2%, and foveal hypoplasia in 75%. Cataracts were diagnosed in 70.3%, glaucoma in 20.6% and ARK in 68.6% of eyes. Prevalence, age of diagnosis and surgical intervention varied amongst mutation groups. Overall, the missense mutation sub-group had the mildest phenotype, and surgically naïve eyes maintained better visual acuity. Systemic evaluation identified type 2 diabetes in 12.8%, which is twice the UK prevalence. This is the largest longitudinal study of aniridia in the United Kingdom, providing insights into prognostic indicators for patients and guiding clinical management of both ocular and systemic features.
**Introduction**

Aniridia (OMIM 106210) is a rare congenital pan-ocular condition with an incidence of 1:40,000-100,000 that demonstrates no predilection for race or gender (1, 2). It is inherited autosomally dominantly with high penetrance, although there is significant intra- and inter-family phenotypic variability, and a third of cases are sporadic (2). Typical aniridia is characterised by variable iris hypoplasia, which can present with complete absence of the iris (or only a small iris stump seen on gonioscopy), partial iris defects, and in some instances a full iris with only abnormal surface architecture or transillumination defects (3, 4). Patients typically have impaired visual acuity (0.70 – 1.00 logMAR) and nystagmus from birth (1, 2), attributable to foveal hypoplasia (3, 5). The development of cataracts, glaucoma, and aniridia-related keratopathy (ARK) is common, and further contributes to a progressive deterioration in vision (1, 2, 6-8).

Aniridia is caused by heterozygous mutations involving the paired box 6 gene (*PAX6*) which is considered the master regulator of the eye (9, 10). *PAX6* (OMIM 607108) encodes a transcription factor which is highly expressed throughout the developing eye, and postnatally in the cornea, conjunctiva, iris, ciliary body, lens and retina (11, 12). Typical aniridia phenotypes are usually associated with loss-of-function heterozygous *PAX6* mutations which result in haploinsufficiency (13-17), with nearly 70% (i.e., nonsense, frameshift from insertion-deletion [indels] and most intronic/splicing variants) leading to the introduction of a premature termination codon (PTC). Other variants causing *PAX6* haploinsufficiency include whole gene deletions or mutations in the *PAX6* 3' regulatory region (18, 19). Mutations leading to the C-terminal extension (CTE) of *PAX6* protein are less common, with some reports linking these variants to more severe aniridia phenotypes, comparable to PTCs (3, 20, 21). In contrast, missense *PAX6* variants are usually linked to milder aniridia cases or, more frequently, to non-aniridia related phenotypes like microphthalmia and ocular coloboma (OMIM 120430 and 120200) or foveal hypoplasia 1 (OMIM 136520) (18, 22-24).
Outside of the eye, **PAX6** expression plays a vital role in the embryological development of the brain (25, 26) and pancreas (27), and postnatally it influences the expression and secretion of various pancreatic derived hormones, including insulin (27). Systemically, isolated **PAX6** mutations have been associated with reports of type 1 and 2 diabetes (25, 26, 28), obesity (28), brain anatomical and neurodevelopmental anomalies, neurobehavioral, and autism spectrum disorder (29-35). Furthermore, aniridia is also observed in syndromes with systemic involvement which includes WAGR syndrome (Wilms tumour, aniridia, genitourinary anomalies and mental retardation, OMIM 194072), and the closely related WAGRO syndrome (Wilms tumour, aniridia, genitourinary anomalies, mental retardation, and obesity, OMIM 612469) (36). Both conditions involve deletions of the **PAX6** gene, as well as **WT1** (WAGR), or **WT1** and **BDNF** (WAGRO) (18).

The spectrum of **PAX6** mutations and clinical phenotypes of aniridia patients have been well described (3, 7, 8, 37-42), and most studies have reported no clear genotype-phenotype correlations (8). More recent studies report cross-sectional data, but do not map disease progression. Hence, the purpose of our study is to examine the longitudinal natural history of aniridia, with a focus on genotype-phenotype correlations in a large cohort of molecularly confirmed **PAX6** patients.
Results

Molecular and clinical characteristics of patients

One hundred and seventy-two eyes from 86 individuals (52 females, 34 males) were evaluated in this study. There were 62 families in total, 60 patients from 36 families with familial aniridia, 18 patients with sporadic aniridia with de novo PAX6 pathogenic variants, and 8 individuals in which the family history was not documented. Ethnicity was not documented in 41.9% of individuals, however, 40.7% were white British (n=35), 8.1 % white European (n=7), 3.5% Black (n=3), and 5.8% Asian (n=5). The age at baseline visit ranged from 1 month to 66 years (mean 15.7 ± 16.0 years). The age at final visit ranged from 1 year to 71 years (mean 31.7 ± 18.1 years, excluding 4 patients with a single visit). Detailed genotype and phenotype data for all patients are provided in supplemental Table 1, and a summary of patient demographics and clinical characteristics are presented in Table 1.

Spectrum of heterozygous PAX6 variants

Detailed genetic results for all 86 patients included in this study are presented in supplementary Table 1. Patients were divided into 6 groups according to the type of PAX6 variants identified; nonsense (14/86, 16.3%), frameshift (21/86, 24.4%), intronic/splice site (15/86, 17.4%), C-terminal extension (CTE) (14/86, 16.3%) and missense (19/86, 22.1%) groups. PAX6 whole gene deletions were identified in 2 patients (2.4%) and a larger deletion encompassing PAX6 3' regulatory region in ELP4 gene was detected in 1 patient (1.2%); these patients were grouped into the gene deletion category.

There were 48 different variants identified in this cohort, with only one novel frameshift variant, c.345_351dupTAACATA p.(Pro118*), which occurred sporadically in a patient (Patient 23-i) presenting with bilateral complete iris hypoplasia, foveal hypoplasia and cataracts.
Supplementary Table 1. The majority of variants were located in exons 5 (9 in total; 3 frameshift and 6 missense), 6 (9 in total; 1 nonsense, 3 frameshift and 5 missense), 9 (2 nonsense) and 13 (4 CTE) (Figure 1A). The majority of intronic variants are also located in intron 6 (Figure 1B). Variants causing the introduction of a PTC are distributed across the gene, while all CTE are located in exon 13. As supported by other cohort studies, missense variants are exclusively located in exons 5 and 6, which code for the paired domain of the PAX6 protein. The exception is patient 50-I which was identified with a change in the starting codon in exon 4, c.2T>G, p.(Met1Arg) (43). This variant is predicted to cause failure of translation or initiation from cryptic sites, but due to the amino acid change (Met to Arg) it was classified into the missense group.

Patient 21-I has the variant c.174C>T in exon 6, which is predicted in silico to be synonymous (p.(Gly58=)). However, functional characterisation of this variant showed that it leads to exon 6 shortening by introducing a new donor site and consequent altering the reading frame, resulting in the formation of a PTC (p.(Arg59Valfs*12)) and likely transcript degradation by NMD (44, 45); hence, it was classified as a frameshift. Patient 47-I was identified with 2 independent PAX6 variants: intronic variant c.917-1G>C and missense variant c.1112C>A, p.(Thr371Asn) (20). However, the missense change is predicted benign by in silico tools (not shown), classifying this patient into the intronic variant group.

**Longitudinal changes in visual acuity**

Longitudinal changes in best corrected visual acuity (BCVA) were assessed across decades to map the natural history and the cohort was divided into subgroups based on the type of mutation and history of surgical intervention (for the following ocular indications; cataract, glaucoma and ARK) (Figure 2). A total of 165 eyes from 83 patients were included in the BCVA analysis. Ninety-five eyes from 50 patients did not have a history of surgical intervention (i.e.,
surgically naïve eyes), whereas the remaining 70 eyes from 38 patients underwent at least one ocular surgical procedure. In the surgically naïve eyes, mean ± SD BCVA in the 1st decade of life was 0.85 ± 0.14 LogMAR, with a progressive decline in visual acuity observed over the following decades. Compared against the mean of all mutations, patients with intronic and CTE variants trended towards a worse visual outcome with increasing age, whilst patients with gene deletions, frameshift and missense mutations maintained stable visual acuity over time (Figure 2A). For the first 5 decades, surgically naïve eyes maintained a mean BCVA LogMAR of 1.0, compared to those having undergone surgical intervention who had a mean BCVA of more than 1.5 LogMAR (Figure 2B). Although the BCVA is shown to worsen in the 6th decade of the surgically naïve group, this was compromised of patients with CTE variants only, and therefore not an accurate comparison. Amongst eyes which had undergone surgical intervention, only the missense group maintained a better visual acuity than the overall mean. All other mutation sub-groups demonstrated a trend towards a gradual decline in visual acuity from the third decade onwards (Figure 2C), except for the gene deletion group which had the most progressive visual decline from the first decade (Figure 2C). The gene deletion group is small (4 eyes from 2 patients) and therefore the visual acuity trends observed for gene deletions is only reflective of observed natural history of our cohort and larger number of patients with gene deletions will be required for future studies.

Nystagmus and degree of foveal hypoplasia varies with mutation sub-group

Nystagmus was recorded in 75/86 patients (87.2%) and foveal hypoplasia was documented in 129/172 eyes (75%); in the remaining patients where nystagmus or fovea hypoplasia was not recorded, the cases were excluded from further analysis. Spectral domain optical coherence tomography (SD-OCT) macula scans were available for 29 eyes from 17 patients to classify foveal hypoplasia into mild (grades 1 and 2) and severe (grades 3 and 4). The grade of foveal hypoplasia was symmetrical between both eyes of each patient where both OCT images were available. There was a significant difference in severity between mutation groups
(p<0.001) with intronic (z-score=2.2) and frameshift (z-score=2.0) sub-groups displaying a more severe foveal hypoplasia, while the missense (z-score=-4.0) sub-group presented milder grades (Table 2).

Of 29 eyes from 17 patients used for FH grading analysis, only 4 eyes from 2 individuals were from the same family, of which all demonstrated the same grade of foveal hypoplasia. Due to the small number, further analysis could not be performed on intrafamilial variability. Spearman’s correlation analysis was performed on foveal hypoplasia grade and first recorded visual acuity, this demonstrated a significant positive correlation between higher foveal hypoplasia grade and worsening visual acuity (rho=0.46, p=0.012).

**Spectrum of iris hypoplasia varies with PAX6 specific mutation sub-groups**

Iris abnormalities were reported in 154/172 eyes (89.5%), the majority displayed ocular symmetry, however asymmetric iris hypoplasia was observed in 3 patients, from the frameshift (19-I), CTE (30-I) and missense (62-I) mutation groups. Fifty-two eyes had grade 6 complete iris hypoplasia (30.2%), with higher prevalence in nonsense (42.9%), intronic (46.7%), and gene deletion (33.3%) sub-groups, and at a lower prevalence in the missense (18.4%) and CTE (17.9%) groups. Conversely, partial iris hypoplasia (grades 1-5) was seen in 65 eyes, and was more prevalent in the missense (50%) and CTE (53.6%) sub-groups, with lower prevalence of nonsense (7.1%), intronic (33.3%), and gene deletion (16.7%) patients (Table 1). Eighteen eyes (10.5%) had normal iris architecture with no structural abnormalities documented, these were observed in the missense (26.3%), CTE (14.3%) and frameshift (9.5%) mutation sub-groups. Thirty-six eyes had documented iris abnormalities, but the degree was not recorded (20.9%). For severity analysis, grades 1-4 were considered mild, and grades 5-6 considered severe. Overall, the missense mutation group had significantly milder (z-score =-3.5) grades of iris hypoplasia compared with nonsense (z-score=4.0),
frameshift (z-score=2.7), intronic (z-score=2.7) and gene deletion (z-score=2.0) groups, which demonstrated significantly higher grades of iris hypoplasia (Table 2).

Within this cohort, iris hypoplasia grade was fully documented for 10 families which consisted of 2 affected members. In four families there was no intrafamilial variability relating to iris hypoplasia (18, 25, 45, and 59). Seven families (19, 27, 31, 34, 55, 58 and 60) demonstrated mild intrafamilial variability (no iris abnormality to mild iris hypoplasia, or severe grades of aniridia only), and one family (42) showed more significant differences from mild grade 1 to severe grade 6 iris hypoplasia.

**Natural history of cataracts in PAX6 patients**

Cataracts were diagnosed in 121/172 eyes (70.3%) from 62 individuals (59 bilateral and 3 unilateral, 23 males and 39 females). The mean age of cataract diagnosis was 17.4 ± 12.9 years. No statistically significant difference was detected in the age of onset between different mutation sub-groups (p = 0.053) (Figure 3A) and gender had no role in the prevalence (p = 0.12). However, the prevalence varied significantly (p = 0.047) with missense and frameshift mutations exhibiting a lower prevalence (57.9%, z-score=-1.9 and 59.5%, z-score=-1.8, respectively) compared to other groups (Table 2). Cataract surgery was performed in 65 eyes (53.7%, 29 bilateral and 7 unilateral, 3 males and 43 females) at a mean age of 33.2 ± 14.7 years. Patients’ age at the time of cataract operation was statistically different between groups (p = 0.029); the mean age in the missense sub-group was significantly younger (20.8 years) compared to individuals in the frameshift (43.8 years, p = 0.001) and intronic (36.8 years, p = 0.014) mutations (Figure 3B). Comparing mean visual acuity of operated and unoperated eyes, there is no trend towards improvement in visual acuity in the operated group (Figure 3C).

**Natural history of glaucoma in PAX6 patients**
In total there were 37/172 eyes with recorded glaucoma, however, two eyes (from 2 different patients) had secondary glaucoma following surgical intervention so were excluded from further statistical analysis; (i) patient 53-I was a Caucasian female who had undergone cataract surgery at 8 and 10 weeks of age for right and left cataracts respectively, and subsequently developed secondary glaucoma requiring cyclodiode laser to the left eye at 15 months of age, and uses topical glaucoma treatment in both eyes (although the right eye maintained a healthy cup: disc ratio of 0.2) and (ii) patient 46-III, of unknown ethnicity, developed glaucoma 16 months following penetrating keratoplasty in their right eye aged 23, without developing glaucoma in their surgically naïve left eye. Thirty-five eyes (20.6%) from 19 individuals (16 bilateral and 3 unilateral, 7 males and 12 females) remained, information on whether the glaucoma was primary or secondary to previous ocular surgery was only available in 20 eyes from 12 patients. Sixteen eyes developed glaucoma prior to any surgical intervention, supporting a diagnosis of primary glaucoma. In 2 eyes it was not clear if prior surgery would have influenced the development of glaucoma; (i) patient 13-I had glaucoma in the right eye diagnosed age 32 and underwent cataract surgery aged 23, however the left eye of the same individual was diagnosed with glaucoma aged 31 and was surgically naïve until aged 36, hence it is likely that both eyes were predisposed to primary glaucoma, and (ii) patient 19-III underwent left eye cataract surgery aged 20 and developed glaucoma in the operated eye aged 49, although they did not have signs of glaucoma in the unoperated eye at their last visit (aged 49) and there is no family history of glaucoma, further long-term monitoring of this patient may provide more conclusive evidence.

Overall, the mean age of glaucoma diagnosis was 25.0 ± 17.3 years. Gender had no effect on the prevalence of glaucoma (Spearman’s rho = 0.05, p = 0.54). No significant difference in the mean age at glaucoma diagnosis was observed between mutation groups (p = 0.22) (Table 2 and Figure 4A). The prevalence of glaucoma was significantly different between the individual mutation groups (p < 0.001) with gene deletions having a higher prevalence (100%, z-
score=4.9), compared to the frameshift mutations with the lowest prevalence (11.4%, z-score=1.6) (Table 2). Glaucoma surgery (including cyclodiode laser, tube surgery, and trabeculectomy) were performed in 18 eyes (51.4%, 6 bilateral and 6 unilateral; 5 males and 7 females). The mean age of glaucoma surgery was 30.7 ± 19.5 years. Although patients’ age at the time of glaucoma operation varied between mutation groups, the differences did not reach statistical significance (p = 0.085), likely due to the small number of eyes in the subgroups (Table 2 and Figure 4B). Patients with no surgical intervention for glaucoma maintained a consistently better visual acuity compared with patients who underwent glaucoma surgery (Figure 4C).

**Natural history of Aniridia-related keratopathy (ARK) in PAX6 variants**

Clinical features of ARK were recorded in 118 eyes (68.6%, 116 bilateral and 2 unilateral; 51 males and 67 females), of which 23 eyes had corneal surgery (19.5%, 11 unilateral and 6 bilateral; 8 males and 14 females). The mean age of first ARK surgery was 40.2 ± 13.8 years with no significant difference between mutation sub-groups (p = 0.68) (Figure 5A). The prevalence of ARK was significantly different between the groups (p = 0.005), with a higher prevalence in the CTE (z-score=2.1) and nonsense (z-score=2.1) mutations, and a lower prevalence in those with missense variants (z-score=-3.2) (Table 2). Gender had no influence on prevalence (p = 0.21). Visual acuity is consistently better in ARK eyes with no surgery from the 1st decade through to the 6th decade (Figure 5B).

**Associated ocular and systemic features in patients with PAX6 variants**

Other observed ocular findings amongst the cohort included ptosis (27 eyes, 15.7%), optic nerve hypoplasia (15 eyes, 8.7%), optic nerve coloboma (1 eye, 0.6%), severe microphthalmia (1 eye, 0.6%), ectopia lentis (11 eyes, 6.4%), retinal detachment (9 eyes, 5.2%), and evisceration was performed in 2 painful blind eyes with no perception of light from a single
patient (patient 1-I) (1.2%) (Figure 6A). Ptosis was observed in 14 patients (13 bilateral, 1 unilateral) and was first documented prior to any surgery in 15 eyes, after any form of surgical intervention in 8 eyes, and unknown surgical status in 4 eyes. No clear correlation with any of the different mutation groups was detected.

Retinal detachment was recorded in 9 eyes (5.2%) from 6 individuals, representing a deletion of the PAX6 3’ regulatory region, a nonsense variant (2 unrelated patients with p.[Arg240*]), CTE (p.[Asp413Glu*112] and p.[*423Leuext*14]), and an intronic mutation (c.357+5G>A). There were no missense or frameshift variants in this cohort (Figure 6A). Of these, 6 occurred in operated eyes, 2 in surgically naïve eyes, and the surgical status is unknown in one eye.

Several systemic associations were present including obesity (23.3%, n=20), type 2 diabetes (12.8%, n=11), asthma (12.8%, n=11), hypothyroidism (7%, n=6) and learning difficulties (7%, n=6) (Figure 6B), but these were not significantly linked to any mutation group. Other neurological features with lower frequency included autism (2.3%, n=2), central auditory processing disorder which was diagnosed in 1 patient (1.2%) and suspected in another, and structural brain anomalies including parietal lobe cavernoma (1.2%, n=1), absent pineal gland (1.2%, n=1), and an abnormality to the posterior aspect of the corpus callosum (1.2%, n=1).

Common ocular and systemic features are detailed in supplementary Table 1.
Discussion

We present a longitudinal natural history study of aniridia patients with molecularly confirmed PAX6 mutations over a mean 16.3 years follow-up. To the best of our knowledge, this is the largest clinically relevant study on genotype-phenotype correlation with respect to systemic associations, and ocular prognosis including long-term visual acuity, cataracts, glaucoma and ARK in the United Kingdom (for a graphical summary see Figure 7).

The majority of PAX6 variants found in our cohort lead to PAX6 haploinsufficiency, of which one was novel (patient 23-I) and a further 5 were recently reported by the NHS Wessex Regional Genetics Laboratory, where we sent our patients for diagnostic genetic testing(46). Cross et al described the genotypes of their PAX6 cohort and reported the respective phenotypes derived from the genetic request form(46). In contrast, in this study we present detailed phenotypic descriptions and report novel significant genotype-phenotype correlations that emerged over time. Missense mutations had the mildest disease course with increased association of non-aniridia phenotypes, better visual acuity and lower grades of iris and foveal hypoplasia, but a younger age of surgical intervention for cataract and glaucoma surgery. For those fewer missense patients with more severe iris hypoplasia (grade 5 or 6), the mutations either abolished the PAX6 start codon (c.2T>G, p.[Met1Arg]) or could in reality affect splicing mechanisms with consequent transcript degradation through nonsense-mediated decay (NMD), hence mimicking loss of function variants and leading to classical severe aniridia phenotypes (44, 47). The previously published variant c.372C>A, p.(Asn124Lys) resulted in severe disruption of the PAX6 DNA-binding activity during early eye formation, hence giving rise to a left microphthalmia and right optic nerve coloboma (23, 48).

A recent analysis of 15 patients with deletions in the PAX6 3' regulatory region showed significantly milder phenotypes compared to all other mutations, with no reported nystagmus,
ARK or foveal hypoplasia (47). We had one patient (patient 3-I) with a 3'UTR gene deletion extending to involve ELP4 and DCDC1; they presented with partial iris hypoplasia, cataracts, glaucoma and a retinal detachment in a surgically naïve eye, but no reported ARK.

Previous studies have suggested that patients with PTC and CTE variants have a severe phenotype (3, 49), our data concurred with levels of visual acuity. However, they all demonstrated varying degrees of cataract, glaucoma and ARK prevalence, and the CTE subgroup trended towards milder grades of both foveal and iris hypoplasia compared to frameshift and nonsense groups. Despite frameshift and nonsense mutations being loss-of-function alleles with the worst visual acuities among operated eyes, each sub-group had different preponderances, for example, nonsense variants resulted in higher prevalence of cataract, glaucoma and ARK, whereas frameshift variants resulted in significantly more severe grade of foveal hypoplasia. These differences may be due to non-coding regulatory elements or other genetic modifiers, which are potentially tissue-specific, but further studies with increased number of patients would add evidence towards these phenotypic correlations. Identifying natural history patterns between variant subtypes will help guide clinical trial outcomes measures for mutation-specific therapeutics like nonsense suppression therapy which targets nonsense in-frame PTCs.

Clinical features such as foveal hypoplasia was observed in three-quarters of patients, and nystagmus in 87.2%, corresponding with reported prevalence rates of 78%-93% and 58%-95%, respectively (3, 7, 39, 47, 50). Previously, the most severe grade of foveal hypoplasia (grade 3 or 4) was associated with PTCs, and milder grades (grade 1 and 2) associated with gene deletions (51). We found significantly more severe grades of foveal hypoplasia in the frameshift and intronic mutation groups; no foveal imaging was available for patients in the gene deletion group to assess. Grade of foveal hypoplasia is a predictor of visual acuity (52),
a positive correlation between higher foveal hypoplasia grade and worsening visual acuity was
demonstrated in our study. Hence, foveal hypoplasia grading may be a useful tool in predicting
visual potential in young infants/children and play a role in guiding visual prognosis.

Both operated and unoperated eyes demonstrated a progressive decline in vision with
increasing age, but the non-surgical group maintained a better mean visual acuity for the first
five decades. Cataracts were the most common ocular co-morbidity, with a mean age of
surgery being 33.2 ± 14.7 years, in keeping with the reported mean of 20-30 years in the
current literature (7, 38, 53, 54). The missense sub-group were operated on earlier (mean age
of surgery was 20.8 ± 11.9), this is likely due to a better baseline visual acuity and a greater
subjective reduction in vision due to the lens opacities. PAX6 has been identified in cases of
congenital cataract(55), and a case series on cataract surgery in patients with congenital
aniridia demonstrated some improvement in visual acuity following cataract surgery over a
follow up period of up to 18 months (53). Our study explored the visual acuity changes over
an average 16.3 years in patients with all forms of cataract and found no significant difference
in visual acuity amongst eyes which had undergone cataract surgery (53.7%) compared to
eyes with unoperated cataract (46.3%). This may be due to additional progressive
glaucomatous changes and ARK, which can also impede on visual acuity despite cataract
surgery (38).

The detection rate of glaucoma (20.6%) and the mean age at diagnosis (25.0 ± 17.3 years)
was within published timeframes, childhood or early adulthood (7, 28, 56), with prevalence
rates of between 15-66.7% (7, 8, 38, 39, 47, 50, 56). Gramer et al reported high variability in
the age of glaucoma onset in aniridia with 70% diagnosed between 20-69 years of age (56).
We found variability in the mean age of glaucoma diagnosis amongst the mutation groups
ranging from 19.3 to 50.7 years of age, but no significant difference in the mean age at
diagnosis. Glaucoma surgery (including laser procedures) was performed in 51.4%, this is in-line with other studies, which have reported between 36-71.4% requiring surgical intervention (7, 28, 38). One study analysing genotype-phenotype correlations in families with aniridia from Australasia and Southeast Asia identified 11 patients with glaucoma, of which 4 underwent surgical intervention (glaucoma filtration surgery or glaucoma drainage device) at a mean age of 13 ± 5.6 years (7). This departs from our mean age of first surgical intervention at 30.7 ± 19.5 years but may be impacted by the differences in the study population. The majority of eyes in our study had primary glaucoma, with only 2 cases having clear secondary glaucoma following surgical intervention. A prospective cases series on congenital aniridia with cataracts reported no incidence of secondary glaucoma following cataract surgery after a mean follow up postoperatively of 10.2 ± 6.4 months, with a mean patient age at surgery of 25.4 ± 14.77 years (53), but a recent study has reported an overall higher prevalence of secondary glaucoma amongst patients with ARK requiring advanced corneal surgery, compared with patients with ARK treated medically (57).

Published cohort studies have reported the age of ARK onset as between 19-33 years of age using differing grading criteria (7, 57). It has also been suggested that a minimal degree of keratopathy is present in all aniridia patients (6). It is likely that early ARK features are either underreported or go undetected unless specifically investigated and published standard grading criteria are not always documented in patient clinical notes. Hence, a limitation of this study is that the age of ARK onset was not accurately discernible, and it was not possible to apply an ARK grading in this investigation. Although our prevalence rate was 68.6% (118/172 eyes), in line with that previously reported (48-80%) (7, 38, 47, 57), the true rates may be higher. A prospective study would facilitate the acquisition of more detailed information on the development and progression of ARK and its relationship to various surgical interventions. A recent study from Lagali et al described an association between PAX6 mutation types and severity of ARK, with whole gene deletions followed by PTC and CTE variants associated with
the most severe grades of ARK. Milder disease forms were in turn associated with missense changes and the mildest forms of ARK in patients with non-PAX6 variants (6, 58). Accordingly, we found a significantly higher prevalence of ARK in CTE and nonsense mutation sub-groups, but a significantly lower prevalence with missense mutations, and the absence of ARK in patient 3-I with a 3’UTR gene deletion extending to involve ELP4 and DCDC1. The two patients with whole gene deletion showed presence of ARK with history of corneal surgery, which is consistent with severe ARK diagnosis. In severely affected ARK eyes requiring advanced corneal surgery, a higher rate of glaucoma has been reported in literature (57) which may contribute to worse visual outcomes in the operated group in our study. Conversely, for conservatively managed glaucoma patients, we observed better visual acuity and reduced need for surgery (seen in the missense sub-group).

Amongst the systemic associations observed in our cohort, obesity and type 2 diabetes were the most common. Obesity has been reported in both syndromic and non-syndromic aniridia (7, 28, 36, 59), 23.3% of our patients were affected, but this is below the estimated UK’s general population prevalence of 27.8% (60). Type 2 diabetes, however was observed in 12.8% of our cohort, which is higher than a previously reported 7% prevalence in a survey of aniridia patients (28), and is twice the prevalence of diabetes in the UK general population (4.5% - 6%) (61, 62). Cases of glucose intolerance, type 1 and 2 diabetes have been reported amongst aniridia patients (25, 59, 63, 64) but type 1 diabetes was not observed in our cohort.

The development of type 2 diabetes is multifactorial, with contributions from genetics, epigenetics, lifestyle factors and obesity (65, 66). One study reported 5 unrelated aniridia patients, 4 of which had PAX6 mutations, all with either glucose intolerance or diabetes (63), but the causal relationship for diabetes remains unclear as the majority of patients with aniridia do not develop this. Mice with heterozygous Pax6 variants were shown to have decreased
insulin levels (67). Our results suggest that whilst obesity may have an impact on the
development of diabetes in some aniridia patients, the presence of PAX6 mutations is a likely
factor. We therefore recommend a low threshold for diabetic assessment to enable clinicians
to effectively monitor and modify risk factors to delay the onset or severity in aniridia.

The retrospective data acquired from the medical records and images of patients can pose
some limitations especially relating to accuracy/detail of documentation. The number of clinical
visits can also be irregular. None-the-less, this study has delineated strong genotype-
phenotype correlations, which can inform disease prognosis and clinical management
decisions. However, phenotypic variability exists within mutation sub-groups, suggesting the
presence of potential genetic modifiers that require further investigation and/or increased
numbers of patients. Prospective studies would help to address limitations, especially in
identifying corneal changes in ARK, and to increase data on smaller represented mutation
groups such as gene deletions. In the recent phase 2 ataluren trial for nonsense-mediated
aniridia (NCT02647359), change from baseline in maximum reading speed with both eyes
was considered the primary outcome measure, secondary outcomes included BCVA in both
eyes, critical print size, reading acuity, severity of corneal keratopathy and iris area measured
at 48 weeks. This study can guide the choice of outcome metrics that may be employed for
future trials, for example, visual acuity in unoperated eyes remains stable over long periods of
follow-up hence not the optimum best clinical trial endpoint, especially for short trials. It also
provides insight into the optimum window where intervention and monitoring and early
detection may prevent ocular co-morbidities and slow disease progression. For future studies
more in-depth analysis of prospective corneal changes may provide measurable parameters
for patients undergoing treatment for ARK.

In conclusion we present a natural history evaluation of 86 patients from the United Kingdom
with molecularly confirmed PAX6 mutations, and describe the phenotypical differences in
prevalence, severity, and onset of ocular co-morbidities. We provide a valuable tool for
clinicians and scientists to aid prognostication, monitoring and measuring treatment outcomes in individuals with aniridia.
Materials and methods

Subjects

Moorfields Eye Hospital NHS Foundation Trust (MEH) receives secondary and tertiary referrals for patients with congenital aniridia from throughout the United Kingdom. Patients who have had genetic testing and received a molecular diagnosis are recorded within a genetics module of the hospital electronic patient record (OpenEyes Electronic Medical Record, Apperta Foundation, Sunderland, Tyne And Wear, UK). In this study, we interrogated the genetics database retrospectively to identify all families with molecularly confirmed PAX6 heterozygous mutations on 1st March 2020. Eighty-six patients from 62 families with clinically diagnosed isolated aniridia and PAX6 heterozygous variants were included in this study. Patients diagnosed with non-aniridia related phenotypes and WAGR/WAGRO syndrome were excluded. We established a database to collect phenotyping data from a retrospective review of clinical records and ocular imaging data gathered as part of standard patient care from the first (baseline) clinical visit to their most recent attendance (mean 16.3 ± 12.7 years, range 0.5 – 61.3 years). The following clinical parameters were recorded: visual acuity (LogMAR), iris and foveal morphology using established standard grading scales (see below), ocular and systemic co-morbidities (including cataracts, glaucoma, ARK and diabetes) and surgical interventions.

Molecular screening

All patients gave informed written consent for genetic testing. Direct Sanger sequencing of the PAX6 gene (Genbank accession number NM_000280.4/ ENST00000643871.1 was used for variant nomenclature and exon numbering) was performed at the National Health Service (NHS) Wessex Regional Genetics Laboratory (Salisbury, UK), and the Rare & Inherited Disease Genomic Laboratory at Great Ormond Street Hospital (London, UK). Variant analysis
including pathogenicity prediction and novelty was performed using Alamut® Visual v.2.15 (Interactive Biosoftware, France) and the publicly available Leiden Open Variation Database (LOVD) PAX6 Mutation Database (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6).

The Wessex Regional Genetics Laboratory recently published all novel PAX6 variants including patients from our cohort, hence only one novel variant remained which has been submitted to ClinVar.

**Visual acuity**

Best corrected visual acuity (BCVA) was recorded in LogMAR (and converted to this if given in Snellen for statistical analysis). Visual acuity of 1/60 or counting fingers, hand movements, light perception and no perception of light was recorded as 1.98, 2.28, 2.6 and 3.0 respectively (68, 69). Patients with their first baseline visit during infancy often had fixing and following visual acuity. For data analysis, fixing and following was excluded, but their first recorded Snellen or LogMAR visual acuity was taken as the first visual acuity recording.

The age at presentation and follow up periods varied amongst patients as reflected by their age and clinical need. Therefore, not all patients had a documented visual acuity for each decade. But where this data was available, 12-month timepoints were collected and a mean visual acuity for the decade was calculated.

**Iris morphology**

The iris morphology was graded by a single grader (VK), and grading as described by Grønskov et al., (70) was adapted for this study; no iris abnormality (grade 0), stromal hypoplasia, iris abnormalities and centrally located pupil (grade 1), stromal hypoplasia, iris abnormalities and eccentric pupil (grade 2), circumpupillary aplasia with iris abnormalities (grade 3), atypical sector coloboma with less than half circumference absent (grade 4),
subtotal iris hypoplasia with more than half circumference absent (grade 5), and complete iris hypoplasia (grade 6). For statistical analysis, grades 1-4 were considered as mild iris hypoplasia, while grades 5 and 6 were classified as severe iris hypoplasia.

**Foveal hypoplasia**

Foveal hypoplasia and nystagmus were identified through clinical notes. Where imaging was available, both the Heidelberg Eye Explorer software (Heyex, Heidelberg Engineering, Heidelberg, Germany), and Topcon IMAGEnet 6 software (Topcon, Corp., Japan) were used to assess the degree of foveal hypoplasia in OCT macula scans. Single line scans were excluded on the basis that the imaging may not have captured the fovea. Volumetric OCT scans of the macula were assessed and the grade of foveal hypoplasia assigned by a single grader (author VK) using the grading system described by Thomas et al., (52). The normal foveal structures observed on OCT includes the extrusion of the plexiform layers, foveal pit, outer segment lengthening and outer nuclear layer widening (52); Grade 1 foveal hypoplasia was assigned when extrusion of the plexiform layers is absent and all other foveal features are present, grade 2 hypoplasia includes features of grade 1 and the absence of the foveal pit, grade 3 includes features of grade 2 and the absence of outer segment lengthening, and in grade 4 all foveal features are absent (52). For statistical analysis, grades 1 and 2 were considered as mild foveal hypoplasia, while grades 3 and 4 were regarded as severe.

**Ocular co-morbidity and surgical intervention**

For individuals who developed glaucoma, the date of onset was taken as the commencement of intraocular pressure (IOP) lowering drugs, those with ocular hypertension or developed secondary glaucoma were excluded. For age of onset, patients with cataracts or glaucoma on their first baseline visit were excluded, unless at or below 1 year of age, as the date of onset could not be accurately ascertained. All procedures requiring patient consent were recorded
as a form of surgical intervention including all types of cataract extraction; glaucoma laser
treatment, tube surgery, and trabeculectomy; and ARK-related superficial keratectomy,
corneal grafts, limbal stem cell transplants and keratoprosthesis. Aniridic fibrosis syndrome is
a rare complication seen in aniridia and was not identified in our cohort.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2013 (Microsoft, Redmond,
Washington, USA) and SPSS v. 26 (IBM, Armonk, New York, USA). Continuous data were
presented as population mean ± standard deviation (SD), while categorical data was
presented in frequency and/or percentage. Kruskal-Wallis test (one-way analysis of variance
on ranks) was used to compare continuous variables across mutation groups. When a
statistically significant difference was detected, Dunn’s post hoc test was used to assess
pairwise comparisons. Contingency tables along with Chi-square test or likelihood ratio were
used to compare categorical variables (two-tailed testing). Adjusted standardized residuals (z-
score) were used a post hoc test. Cells with a z-score absolute value larger than 1.96 were
considered statistically significant. Spearman’s signed-rank correlation coefficient (rho) was
used to investigate correlation between foveal hypoplasia and visual acuity. P value less than
0.05 was considered statistically significant.

Study approval

This study was approved by Moorfields Eye Hospital and the National Research Ethics
Committee (REC12/LO/0141) and was conducted in adherence to the tenets of the
Declaration of Helsinki; informed written consent was obtained from all participants.

Author Contributions
MM supervised the research. VK acquired data. VK, AH, DLC analysed data, VK, AH, DLC and MM wrote the manuscript.

**Acknowledgements**

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13. Vincent MC, Pujo AL, Olivier D, Calvas P. Screening for PAX6 gene mutations is consistent with haploinsufficiency as the main mechanism leading to various ocular defects. European journal of human genetics : EJHG. Feb 2003;11(2):163-9. doi:10.1038/sj.ejhg.5200940


**Figure 1.** Distribution of the 48 different *PAX6* variants identified in this cohort. (A) *PAX6* coding sequence (CDS) is shown with numbered exons and colours representing the respective protein domains: paired domain (PD, blue, exons 5-7), homeodomain (HD, green, exons 8-10) and proline-serine-threonine-rich domain (dark grey, exons 10-13). Linker region is represented in light grey (exons 7-8). Striped boxes represent non-coding exons 1-4. Variants are represented by white squares (nonsense), white triangles (frameshift), black circles (missense) and black diamonds (C-terminal extension). Novel variant is highlighted in red. (B) Schematic of *PAX6* including intronic regions (white boxes) was used to show
distribution of intronic/splice site variants, represented as white circles. Both schematics represent \textit{PAX6} transcript \textsc{NM}\_000280.4 encoding protein isoform \textsc{NP}\_000271.1. (C) Schematic representation of deletions in 11p13 encompassing either whole \textit{PAX6} gene or the regulatory regions 3' of \textit{PAX6} in \textit{ELP4} gene, \textit{PAX6} is highlighted in black and neighbour genes are represented by grey boxes. Coloured bars represent approximate coordinates of deletions identified in 3 patients in this study (Patients 1-i, 2-i, and 3-i). The exact chromosomal coordinates were not obtained from the genetic screening service. Adapted from (9).

\textbf{Figure 2.} Best corrected visual acuity (BCVA) in patients with \textit{PAX6} mutations. Line charts demonstrate the longitudinal change in BCVA in this cohort in different mutation groups with surgically naïve eyes (A), all eyes with or without surgical intervention (B) and comparing
mutation groups with a history of surgical intervention (C). Data represent mean ± standard deviation. LogMAR: logarithm of the minimum angle of resolution, CTE: C-terminal extension.

Figure 3. PAX6 patients with cataract. Box-and-whiskers plots represent the differences in age at diagnosis (A) and age at surgery (B) between mutation groups. The blue box represents the 25th to 75th percentiles. The black line within the box represents the median. Whiskers extend to the minimum and maximum values. All data points were superimposed on the graph. Kruskal-Wallis and Dunn’s post hoc tests were used to compare between mutation groups. (C): Line graph demonstrates the change in logarithm of the minimum angle of resolution (LogMAR) visual acuity in patients with cataract. Data present mean ± standard deviation. Black dashed arrow and blue arrow represent the mean age at cataract diagnosis and surgery, respectively. CTE: C-terminal extension, ns: not significant, *p < 0.05, ***p < 0.001
**Figure 4. PAX6 patients with glaucoma.** Box-and-whiskers plots represent differences in age at diagnosis (A) and age at surgery (B) between mutation groups. The blue box represents the 25th to 75th percentiles. The black line within the box represents the median. Whiskers extend to the minimum and maximum values. All data points were superimposed on the graph. Kruskal-Wallis test was used to compare between mutation groups. (C): Line graph demonstrates change in logarithm of the minimum angle of resolution (LogMAR) visual acuity in glaucomatous patients. Data present mean ± standard deviation. Black dashed arrow and blue arrow represent the mean age at glaucoma diagnosis and surgery, respectively. CTE: C-terminal extension, ns: not significant.
Figure 5. PAX6 patients with aniridia-related keratopathy (ARK). (A): Box-and-whiskers plot represents the difference in the age at corneal surgery between mutation groups. The blue box represents the 25th to 75th percentiles. The black line within the box represents the median. Whiskers extends to the minimum and maximum values. All data points were superimposed on the graph. No statistically-significant difference was observed between groups (Kruskal-Wallis test). (B): Line graph demonstrates the change in logarithm of the minimum angle of resolution (LogMAR) visual acuity in patients with ARK. Data present mean ± standard deviation. Blue arrow represents the mean age at surgery. CTE: C-terminal extension, ns: not significant.
Figure 6. Complex ocular features and systemic features detected in this cohort. (A) Distribution of the number of eyes with atypical aniridia or complex ocular phenotypes within the different mutation groups: microphthalmia (n=1), ptosis (n=27), optic nerve (ON) hypoplasia (including one ON coloboma) (n=14), ectopia lentis (n=11) and retinal detachment (n=9). (B) Distribution of systemic features among patients in the different groups: obesity (n=20), type 2 diabetes (T2D) (n=11), hypothyroidism (n=6), hypertension (n=9), hypercholesterolemia (n=4) and asthma (n=11).
Figure 7. Reference guide for age of cataract and glaucoma development relating to PAX6 variants. The degree of complete and partial iris hypoplasia, versus full iris structure amongst variant sub-groups are listed, but it shows no correlation with the average age at cataract and glaucoma onset based on mutation-types which included nonsense (n=14), intronic (n=15), gene deletion (n=3), CTE (n=14), frameshift (n=21), and missense (19). Prevalence of the cataracts and glaucoma in percentages are shown within the arrow. Unknown data is excluded from reference guide.
Table 1. Patient demographics and characteristics

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>Gene Deletion</th>
<th>Intronic</th>
<th>CTE</th>
<th>Missense</th>
<th>Frameshift</th>
<th>Nonsense</th>
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<tbody>
<tr>
<td>No. of Participants</td>
<td>86</td>
<td>3 (3%)</td>
<td>15 (17%)</td>
<td>14 (16%)</td>
<td>19 (22%)</td>
<td>21 (24%)</td>
<td>14 (16%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35 (41%)</td>
<td>0</td>
<td>7 (47%)</td>
<td>5 (36%)</td>
<td>4 (21%)</td>
<td>11 (52%)</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>Female</td>
<td>51 (59%)</td>
<td>3 (100%)</td>
<td>8 (53%)</td>
<td>9 (64%)</td>
<td>15 (79%)</td>
<td>10 (48%)</td>
<td>6 (43%)</td>
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<tr>
<td>Mean age at last visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.7 ± 18.1</td>
<td>40 ± 15.1</td>
<td>35.7 ± 18.7</td>
<td>36.9 ± 16.4</td>
<td>24.4 ± 15.8</td>
<td>31.3 ± 20.1</td>
<td>31.5 ± 19.4</td>
</tr>
</tbody>
</table>

| No iris abnormality      |              |               |          |     |          |            |          |
| (Grade 0)                |              |               |          |     |          |            |          |
| No. of eyes              | 18/172       | 0/6           | 0/30     | 4/28 | 10/38    | 4/42       | 0/28     |
|                          | (10.5%)      | (0%)          | (0%)     | (14.3%) | (26.3%) | (9.5%)     | (0%)     |

| Iris hypoplasia and      |              |               |          |     |          |            |          |
| other iris               |              |               |          |     |          |            |          |
| abnormalities (Grade 1-5)|              |               |          |     |          |            |          |
| No. of eyes              | 65/172       | 1/6           | 10/30    | 15/28 | 19/38    | 18/42      | 2/28     |
|                          | (37.8%)      | (16.7%)       | (33.3%)  | (53.6%) | (50%)    | (42.9%)    | (7.1%)   |

| Complete iris            |              |               |          |     |          |            |          |
| hypoplasia               |              |               |          |     |          |            |          |
| (Grade 6)                |              |               |          |     |          |            |          |
| No. of patients          | 52/172       | 2/6           | 14/30    | 5/28 | 7/38     | 12/42      | 12/28    |
|                          | (30.2%)      | (33.3%)       | (46.7%)  | (17.9%) | (18.4%)  | (28.6%)    | (42.9%)  |

<p>| Nystagmus                |              |               |          |     |          |            |          |
| No. of patients          | 75/86        | 2/3           | 13/15    | 12/14 | 17/19    | 18/21      | 13/14    |
|                          | (87.2%)      | (66.6%)       | (86.7%)  | (85.7%) | (89.5%)  | (85.7%)    | (92.9%)  |</p>
<table>
<thead>
<tr>
<th></th>
<th>Number of eyes</th>
<th>Gene deletion</th>
<th>Intronic</th>
<th>CTE</th>
<th>Missense</th>
<th>Frameshift</th>
<th>Nonsense</th>
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<tr>
<td><strong>Iris hypoplasia severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (grade 1-4)</td>
<td>68 (50.0%)</td>
<td>0 (50.0%)</td>
<td>6 (25.0%)</td>
<td>13</td>
<td>27 (75.0%)</td>
<td>22 (64.7%)</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Severe (grade 5-6)</td>
<td>68 (50.0%)</td>
<td>4 (100.0%)</td>
<td>18 (75.0%)</td>
<td>11</td>
<td>9 (25.0%)</td>
<td>12 (35.3%)</td>
<td>14 (100.0%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Z score</td>
<td>2.0</td>
<td>2.7</td>
<td>-0.4</td>
<td>-3.5</td>
<td>2.7</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Foveal hypoplasia severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild (grade 1-2)</td>
<td>13 (44.8%)</td>
<td>-</td>
<td>0</td>
<td>2</td>
<td>9 (100.0%)</td>
<td>2 (20.0%)</td>
<td>0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Severe (grade 3-4)</td>
<td>16 (55.2%)</td>
<td>-</td>
<td>5 (100.0%)</td>
<td>1</td>
<td>0</td>
<td>8 (80.0%)</td>
<td>2 (100.0%)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Z score</td>
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<td>2.2</td>
<td>-0.8</td>
<td>-4.0</td>
<td>2.0</td>
<td>1.3</td>
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<tr>
<td><strong>Cataract</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Prevalence</td>
<td>121 (70.3%)</td>
<td>6 (100.0%)</td>
<td>24 (80.0%)</td>
<td>21</td>
<td>22 (57.9%)</td>
<td>25 (59.5%)</td>
<td>23 (82.1%)</td>
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<tr>
<td>Z score</td>
<td>1.6</td>
<td>1.3</td>
<td>0.6</td>
<td>-1.9</td>
<td>-1.8</td>
<td>1.5</td>
<td></td>
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</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td>17.4 ± 12.9</td>
<td>2.9 ± 2.4</td>
<td>18.7 ± 16.2</td>
<td>19.5</td>
<td>17.2 ± 9.9</td>
<td>22.9 ± 11.0</td>
<td>11.8 ± 11.8</td>
<td>0.053</td>
</tr>
<tr>
<td><strong>Operations</strong></td>
<td>65 (53.7%)</td>
<td>4 (66.7%)</td>
<td>11 (45.8%)</td>
<td>9</td>
<td>14 (63.6%)</td>
<td>12 (44.4%)</td>
<td>15 (71.4%)</td>
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<tr>
<td><strong>Age at operation</strong></td>
<td>33.2 ± 14.7</td>
<td>30.0 ± 12.8</td>
<td>36.8 ± 13.2</td>
<td>33.0</td>
<td>20.8 ± 11.9</td>
<td>43.8 ± 15.1</td>
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<td><strong>Glaucoma</strong></td>
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### Prevalence

<table>
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<th>35 (20.6%)</th>
<th>6 (100%)</th>
<th>7 (24.1%)</th>
<th>4 (14.3%)</th>
<th>5 (13.5%)</th>
<th>5 (11.4%)</th>
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<td>Z score</td>
<td>4.9</td>
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<td>-1.2</td>
<td>-1.6</td>
<td>1.1</td>
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<td></td>
</tr>
</tbody>
</table>

### Age at diagnosis

| Age at diagnosis | 25.0 ± 17.3 | 17.8 ± 12.6 | 20.0 ± 0.0 | 19.3 ± 13.0 | 28.5 ± 26.0 | 50.7 ± 2.3 | 19.8 ± 13.6 | 0.22 |

### Operations

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<th>Operations</th>
<th>18 (51.4%)</th>
<th>4 (66.7%)</th>
<th>2 (28.6%)</th>
<th>0</th>
<th>3 (60.0%)</th>
<th>4 (80.0%)</th>
<th>5 (62.5%)</th>
<th></th>
</tr>
</thead>
</table>

### Age at operation

| Age at operation | 30.7 ± 19.5 | 32.8 ± 17.5 | 30.5 ± 7.8 | 13.0 ± 10.4 | 50.8 ± 7.5 | 23.6 ± 74.0 | 0.085 |

### Aniridia-related keratopathy

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>118 (68.6%)</th>
<th>4 (66.7%)</th>
<th>22 (73.3%)</th>
<th>24 (85.7%)</th>
<th>18 (47.4%)</th>
<th>26 (61.9%)</th>
<th>24 (85.7%)</th>
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<tbody>
<tr>
<td>Z score</td>
<td>-0.1</td>
<td>0.6</td>
<td>2.1</td>
<td>-3.2</td>
<td>-1.1</td>
<td>2.1</td>
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### Operations

<table>
<thead>
<tr>
<th>Operations</th>
<th>23 (29.5%)</th>
<th>4 (100.0%)</th>
<th>4 (18.2%)</th>
<th>3 (12.5%)</th>
<th>1 (5.6%)</th>
<th>5 (19.2%)</th>
<th>6 (25.0%)</th>
<th></th>
</tr>
</thead>
</table>

### Age at operation

| Age at operation | 40.2 ± 13.8 | 39.7 ± 17.0 | 39.3 ± 18.9 | 42.7 ± 9.7 | 34.0 | 48.4 ± 15.7 | 33.0 ± 9.3 | 0.68 |

Data are presented as mean ± standard deviation or frequency and percentage. *P* values are based on Kruskal-Wallis test or Pearson’s Chi-square test. CTE: C-terminal extension mutation.

Table includes known grades for foveal and iris hypoplasia. Unknown grades were excluded from prevalence and severity.