HHS Public Access

Author manuscript

Exp Eye Res. Author manuscript; available in PMC 2025 January 01.

Published in final edited form as:

Exp Eye Res. 2024 January ; 238: 109746. doi:10.1016/j.exer.2023.109746.

Reduction of lens size in *PAX6*-related aniridia

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Abstract

Heterozygous mutation of *PAX6* in humans leads to congenital aniridia (OMIM 106210) which is typified by congenital iris and foveal defects, and later onset glaucoma, aniridic keratopathy, and cataract. Mice heterozygous for *Pax6* mutations phenocopy many aspects of aniridia including the iris defects, keratopathy and cataract, although *Pax6* mutant mice have small lenses, a phenotype which is not typically reported in human aniridia, perhaps due to difficulties in measuring lens diameter during typical ophthalmic examinations as the lens periphery is shielded by the iris. In order to overcome this, records of patients diagnosed with congenital aniridia between April 2015 and May 2021 at the Necker-Enfants Malades Hospital, and genetically confirmed with a disease-causing *PAX6* variant, were retrospectively reviewed for those with normal axial length whose iris defects allowed visualization of the lens margins and corneal diameter to allow calculation of a lens/corneal diameter ratio. This, value was compared with values obtained from a cohort of patients with Sjödell grade IV oculocutaneous albinism type 1 (OCA1; OMIM 203100) which allowed visualization of the lens periphery via iris transillumination. This analysis revealed that patients with congenital aniridia had a significantly lower lens/corneal ratio when compared to those with albinism, suggesting that humans haploinsufficient for *PAX6*, like mice, rats, frogs, and zebrafish, exhibit reductions in lens size.

The *PAX6* gene encodes a transcription factor that is essential for the primary induction of eyes from the head ectoderm and the later development of nearly every ocular structure (Cvekl and Callaerts, 2016; Cvekl and Tamm, 2004). Heterozygous variants in *PAX6* cause congenital aniridia, a panocular disease usually diagnosed by the presence of congenital

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Conflict of Interest: none

defects of the iris and fovea leading to photophobia, nystagmus and low vision (Daruich et al., 2023; Daruich et al., 2022; Landsend et al., 2021). Later in life, additional sight threatening conditions develop in these patients including glaucoma (Bajwa et al., 2019), corneal keratopathy (Latta et al., 2021), and cataract (D'Oria et al., 2021; Voskresenskaya et al., 2021) suggesting that two copies of the *PAX6* gene are required for the normal homeostasis of the trabecular meshwork, cornea and lens.

Much of what is known about the function of *Pax6* in the developing eye has been elucidated using either genetically modified animals or those harboring spontaneous mutations in the *Pax6* gene, with both humans (Glaser et al., 1994; Hodgson and Saunders, 1980) and animals homozygous for germline Pax6 mutations completely lacking eyes due to a failure of lens placode development (Cvekl and Callaerts, 2016; Cvekl and Tamm, 2004; Matsuo, 1993; Nakayama et al., 2015). Mice heterozygous for germ line mutations in *Pax6* phenocopy many aspects of aniridia including congenital iris hypoplasia (Davis-Silberman et al., 2005; Ramaesh et al., 2009; Singh et al., 2002; Wang et al., 2017b), the development of juvenile/adult onset keratopathy (Davis et al., 2003; Hickmott et al., 2018; Ramaesh et al., 2003), glaucoma (Cole et al., 2023), and cataract. (Wang et al., 2017a; Duncan et al., 2000). However, *Pax6* heterozygous mice do not phenocopy the foveal hypoplasia seen in human aniridia as mice do not have a foveated retina and these animals are typically microphthalmic (the Small eye (Sey) phenotype) while aniridia patients seldom meet the diagnostic criteria for microphthalmia (Abdolkarimi et al., 2022; Voskresenskaya et al., 2021). Currently it is believed that Sey microphthalmia is likely driven by a congenital reduction in lens size arising at the lens placode stage (van Raamsdonk and Tilghman, 2000) which results in reduced globe diameter since mouse lenses fill a majority of the ocular volume (Abdolkarimi et al., 2022; Voskresenskaya et al., 2021).

However, it is not currently established whether humans heterozygous for *PAX6* disease-causing variants have smaller than normal lenses. Measurements of lens thickness, which is often a clinical surrogate for lens size in humans due to difficulties in measuring lens diameter due to the iris masking the lens rim, suggest that aniridia patients are born with normally sized lenses (Voskresenskaya et al., 2021). However, lens diameter measurements (which are possible in aniridic eyes when the iris fails to develop) in a Russian aniridia cohort have yielded measurements smaller than reported in human cadaver lenses of similar age (Voskresenskaya et al., 2021). Despite this, it was still possible that *in vivo* measurements made in those with aniridia were misleading due to optical artifacts since the study was unable to measure lens diameter using a similar method in a control group (Voskresenskaya et al., 2021).

To overcome these limitations, clinical records of the 356 patients diagnosed with congenital aniridia between April 2015 and May 2021 by the Ophthalmology Department of the Necker-Enfants Malades University Hospital were retrospectively evaluated to identify those with genetically verified *PAX6* disease-causing variants, phakic eyes, transparent corneas, no microcornea/microphthalmia, iris defects that allow visualization of the horizontal lens periphery, and the availability of high quality anterior segment photographs (taken in either awake or anesthetized patients) that clearly show both the border between the sclera and cornea and the lens periphery which left 22 patient records available for study (Table

1). Similarly, a cohort of 8 patients with oculocutaneous albinism (Sjödell grade IV) was identified and used as a control group as this condition allows visualization of the lens through the translucent iris in anterior segment photographs (Sjodell et al., 1996) and is not typically associated with either lens or corneal defects (Summers, 2009) (Table 1).

In all 30 subjects studied, any apparent lens defects were determined, horizontal corneal and lens diameter was measured, then a ratio between lens and corneal diameter (lens diameter/corneal diameter X 100) obtained in order to control for the variable photographic conditions used for the clinical photographs found in the electronic medical records (Figure 1, Table 1). This analysis revealed that lens diameter as a percentage of corneal diameter (raw numbers shown in the 4th column of table 1) was significantly lower (and more variable) in aniridia patients compared to albinism patients (Mean= 80.4, SD 7.6 vs 86.9, SD 1.2, respectively, p=0.0023, Mann Whitney test) indicating that the diameter of the aniridic lens was in fact reduced compared to those with albinism in this cohort of patients.

It should be acknowledged that this conclusion is weakened because the “control group” suffers from albinism which is a vision compromising eye disease although corneal and lens defects are not typical features of albinism, likely because neither structure contains melanocytes. Notably though, the reduction in lens diameter in aniridia observed here, in concert with the prior observation that lens thickness is unchanged in young aniridia patients (Voskresenskaya et al., 2021), is in good accordance with prior clinical observations that the aniridic lens is misshapen (D’Oria et al., 2021; Mehta et al., 2004). It could also explain the observation that aniridic eyes have “stretched” zonules as these would be expected to result from the formation of a smaller than normal diameter lens in a normal sized eye, while this would also be expected to result in the often reported torn zonules and lens dislocations in these patients (D’Oria et al., 2021; Eden et al., 2008; Gramer et al., 2012; Jusufovic et al., 2014).

Importantly, the reduction in lens size seen in aniridia correlates well with the lens phenotype of frogs (Nakayama et al., 2015), zebrafish (Takamiya et al., 2020), rats (Fujiwara et al., 1994), and mice harboring heterozygous *Pax6* mutations (Abdolkarimi et al., 2022), particularly the mouse Pax6$^{tm1/Pgr}$ allele (on the NMRI genetic background) which exhibits reductions in lens size and abnormalities in lens shape while retaining lens transparency through weaning (Voskresenskaya et al., 2021). Further studies will leverage this observation by using mice heterozygous for the Pax6$^{tm1/Pgr}$ allele to elucidate the pathogenic mechanisms underlying the postnatal lens abnormalities typically associated with congenital aniridia.

Financial Support:

National Eye Institute EY028597 (MKD)

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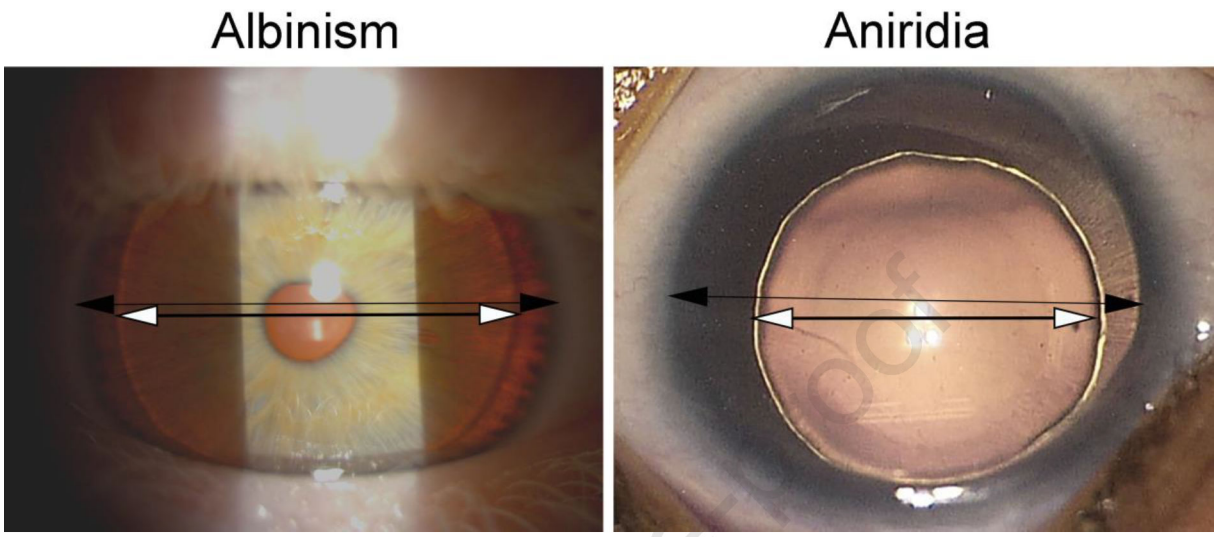

Figure 1. Example anterior segment photographs used in this study

This study was designed in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the APHP Centre. All patient data were obtained from the BAMARA database of CRMR OPHTARA (Centre de Référence de Maladies Rares en Ophtalmologie, accredited by French Health Ministry and Europe ERN.Eye)) with informed consent. Albinism photographs are slit lamp images optimized for iris retroillumination taken on a Topcon Slit Lamp. Aniridia images are anterior segment photographs taken on a Zeiss Callisto Eye Microscope. Horizontal corneal width (line with black arrowheads) and horizontal lens width (line with white arrowheads) were measured and the ratio used to determine relative lens size in each patient.

Table 1:

Horizontal lens diameter measured in relationship to horizontal cornea diameter (measured white to white) in aniridia and albinism patients. Individuals who met the aniridia inclusion criteria had iris defects which allowed the lens periphery to be viewed while also having corneal and overall ocular dimensions within the range of normal for the patient's age (*ie* patient did not meet the clinical criteria for microcornea or microphthalmia). Individuals who met the albinism inclusion criteria had Sjodell grade IV iris pigmentation defects, which allowed visualization of the horizontal lens margins.

Patient number	Pathology	If Aniridia: 1=complete, 2= partial; 3=other	Lens diameter as percentage of corneal diameter	Lens anomalies present	PAX6 anomaly: 1= positive for *PAX6* variant, 2= deletion of *PAX6*, 3= positive for *TYR* variant
1-AN	Aniridia	1	66		1
2-AN	Aniridia	1	80		1
3-AN	Aniridia	2	77		1
4-AN	Aniridia	3 Coloboma	76	Coloboma	1
5-AN	Aniridia	1	92		1
6-AN	Aniridia	2	82		1
7-AN	Aniridia	2	79		1
8-AN	Aniridia	1	80		1
9-AN	Aniridia	1	84		1
10-AN	Aniridia	1	92		2
11-AN	Aniridia	2	85		2
12-AN	Aniridia	1	88		2
13-AN	Aniridia	3 Coloboma	58	Coloboma	1
14-AN	Aniridia	1	77	Lenticonus	1
15-AN	Aniridia	1	80		1
16-AN	Aniridia	1	85	Coloboma	1
17-AN	Aniridia	1	86		1
18-AN	Aniridia	1	82		1
19-AN	Aniridia	1	83		1
20-AN	Aniridia	1	76		1
21-AN	Aniridia	2	79		1
22-AN	Aniridia	2	82		1
1-ALB	Albinism	NA	84.5		3
2-ALB	Albinism	NA	87		3
3-ALB	Albinism	NA	88		3
4-ALB	Albinism	NA	88		3
5-ALB	Albinism	NA	86		3
6-ALB	Albinism	NA	87		3
7-ALB	Albinism	NA	88		3
8-ALB	Albinism	NA	87		3