



Clinicopathologic correlation of aniridia: Optical coherence tomography angiography and histopathologic observations

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ARTICLE INFO

Keywords:

Aniridia
Histopathology
OCT angiography
Foveal hypoplasia
PAX6

ABSTRACT

Purpose: To describe optical coherence tomography angiography (OCTA) findings in a patient with aniridia and correlate with representative histopathology.

Observations: OCTA images of the macula of a pediatric aniridic patient, who has nystagmus and impaired vision bilaterally, demonstrate a complete absence of the foveal avascular zone (FAZ) in both the superficial and deep vascular complexes (SVC and DVC). In addition, larger superficial blood vessels were found to be abnormally diving from the SVC into the DVC. Similarly, immunofluorescence with confocal microscopy imaging of a retinal histopathology specimen from a 2 month old aniridic patient demonstrated larger vessels diving in the same manner.

Conclusions and importance: This study highlights the clinical, imaging and histopathologic findings of aniridia. Supine OCTA imaging, performed during examination under anesthesia, allowed for visualization of retinal microvasculature in eyes with nystagmus. The histopathology images helped validate OCTA findings that, with further investigation, may lead to new information about the development of abnormal retinal microvasculature.

1. Introduction

Aniridia is a rare genetic disorder that causes panocular abnormalities. Early hallmark findings include partial or total absence of the iris highly suggestive of aniridia, and early nystagmus that is usually evident by 6 weeks of age.¹ Patients often have decreased visual acuity and can develop comorbidities including cataracts, glaucoma, and progressive keratopathy secondary to limbal stem cell deficiency.

Aniridia results from a heterozygous mutation of paired box gene 6 (PAX6), which is an important gene for eye development in utero.² About two thirds² to seventy percent¹ of cases are in individuals with a known family history of aniridia. Gene deletions causing aniridia may involve not only PAX6 but also the Wilms tumor suppressor gene 1 (WT1), which is in close approximation to PAX6 on chromosome 11.² This type of deletion is associated with WAGR syndrome, where individuals have a Wilms tumor, aniridia, genitourinary abnormalities and intellectual disability. Manifestation of these four traits varies greatly in WAGR syndrome, but aniridia is usually present to some degree.³

There is also evidence demonstrating that aniridia is associated with

abnormal retinal vasculature (asymmetry, reduced number and less arching patterns of superficial blood vessels) compared to normal eyes, as evidenced by fundus photos.⁴ This raises the question of whether the mutations that cause aniridia affect the retinal vasculature in other ways.

Optical coherence tomography angiography (OCTA) can be used to elaborate these changes with additional information about smaller vessels and the position of vessels within the retinal structure, making it a valuable tool to study retinal microvasculature abnormalities that may be present in aniridia. Portable^{5,6} OCTA systems used during examination under anesthesia have proven to be reliable in capturing images in children with retinal disease, especially infants and those with nystagmus, making portable OCTA devices particularly helpful for aniridia cases. Here we present a unique OCTA observation in an aniridic patient and a clinico-histopathologic correlation.

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<https://doi.org/10.1016/j.ajoc.2020.100919>

Received 8 June 2020; Received in revised form 13 August 2020; Accepted 31 August 2020

Available online 11 September 2020

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2. Case report

2.1. Methods

2.1.1. Optical coherence tomography angiography

An investigational, portable OCTA system (Spectralis HRA + OCT with Flex module, Heidelberg Engineering, Heidelberg, Germany),⁶ which has a movable stand and adjustable arm, was used to capture images of a subject with aniridia who was undergoing examination under anesthesia and operative procedures. A standard tabletop device (Spectralis HRA + OCT, Heidelberg Engineering, Heidelberg, Germany) was used to capture images from a healthy age matched control subject. Volumetric scans of the macula were acquired as 512 A scans/B scan and 512 B scans/volume with $10^\circ \times 10^\circ$ scan size and approximately 6 μm resolution.

After scan acquisition, *en face* OCTA images of the superficial vascular complex (SVC) and deep vascular complex (DVC) were created from the volumetric scans using automatic segmentation of the retinal layers by the Spectralis software. A grader (AT) reviewed the automatic segmentation to check for any necessary segmentation correction, although no correction was needed. The SVC is defined as the retinal slab from the inner limiting membrane to 17 μm above the lower boundary of the inner plexiform layer. The DVC is defined as the slab from 17 μm above the lower boundary of the inner plexiform layer to the lower boundary of the outer plexiform layer. In addition, the Spectralis software's projection artifact removal was used for the DVC images.

2.1.2. Histopathology

Immunofluorescence analysis was performed on paraffin embedded sections from a different patient with aniridia who had expired. Eyes were donated post mortem and sent to Florida Lions Eye Bank for evaluation. The sections were initially deparaffinized using serial alcohol dilutions. Antigen retrieval, permeabilization, and blocking was performed. The samples were incubated overnight with a rabbit polyclonal CD31 antibody (1:50; Abcam, Cambridge, MA) for 12 hours. Samples were washed three times in Phosphate buffered saline (PBS) and incubated for 2 hours with goat anti-rabbit AlexaFluor 594. Samples were washed three times with PBS and counterstained with 1X PureBlu DAPI (BioRad, Hercules, CA). Images were obtained using a laser scanning confocal microscope (Leica SP8, Leica microsystems, Buffalo Grove, IL).

3. Clinicopathologic correlation

3.1. Clinical history

A 7 year old patient with aniridia and a PAX6 gene mutation underwent examination under anesthesia and OCT and OCTA imaging in the operating room with the portable Heidelberg Flex module system. There was a known family history of PAX6 mutation at the time of birth. At 2 months of age, the patient was noted to have nystagmus and bilateral thin rims of iris consistent with aniridia. By 4 months of age, the patient required medical intervention for glaucomatous features. The patient has been regularly followed and has had clear corneas, bilateral subclinical cataracts and advanced glaucoma necessitating medical and surgical intervention. At the latest visit, visual acuity was 20/150 with correction in both eyes.

Histopathologic analysis was performed on a specimen from a 2 month old male with a history of WAGR syndrome (Wilms tumor, Aniridia, Genitourinary abnormalities and Intellectual disability) who developed respiratory difficulties and expired.

3.2. OCTA and histopathologic correlation

The 7 year old aniridic patient displayed foveal hypoplasia in both eyes, as evidenced by a complete lack of foveal pit morphology and outer

retinal maturation, in contrast to the fovea from a healthy age matched control (Fig. 1). In the right eye of the 7 year old aniridic patient, the foveal avascular zone (FAZ) was completely absent in both the SVC and DVC layers, in comparison to the clear avascular regions seen in a healthy age matched control (Fig. 2A–D). In addition, large superficial vessels were observed to abnormally violate the SVC/DVC boundary. These vessels originate from the SVC and are observed to dive into the DVC (Fig. 2C and D). OCTA signal superimposed onto the OCT structure, representative of retinal blood flow, shows the exact course of the vessels as they dive from the SVC to the DVC (Fig. 2E and F).

Retinal histopathologic sections from a representative aniridic decedent demonstrate prominent retinal blood vessels extending into the inner nuclear layer (within the DVC boundaries), similar to the “diving” vessels present on OCTA. (Fig. 3).

4. Discussion

Here we present a retinal OCTA imaging and histopathologic correlation of aniridia. The absent FAZ and foveal pit are in line with evidence demonstrating that the size of the FAZ correlates with the size of the foveal pit, although the mechanistic relationship is unclear.⁷ The absent FAZ of both the SVC and DVC are also similar findings seen in other etiologies of foveal hypoplasia.^{8–10} In a small sample of foveal hypoplasia subjects, Pakzad-Vaezi et al.⁹ observed that the FAZ's in the DVC of eyes with albinism were less developed than both normal eyes and eyes with idiopathic foveal hypoplasia. They also noted that the eyes with albinism tended to have worse visual acuity compared to eyes with idiopathic foveal hypoplasia and raised the question as to whether this was due in part to a less developed FAZ. This hypothesis would depend on whether the FAZ in the DVC affects development and specialization of the outer retina (cone packing and elongation of inner and outer segments), which is most important for visual acuity.^{11,12}

Outer retinal specialization can occur independent of foveal pit formation.^{13,14} There is currently no evidence supporting a direct impact of the FAZ on outer retinal specialization in the fovea, therefore, further investigation into the effects of the FAZ is needed.

The diving vessels in the OCTA images from our aniridic subject parallel similar findings observed by Hsu et al.¹⁵ who observed vessels in

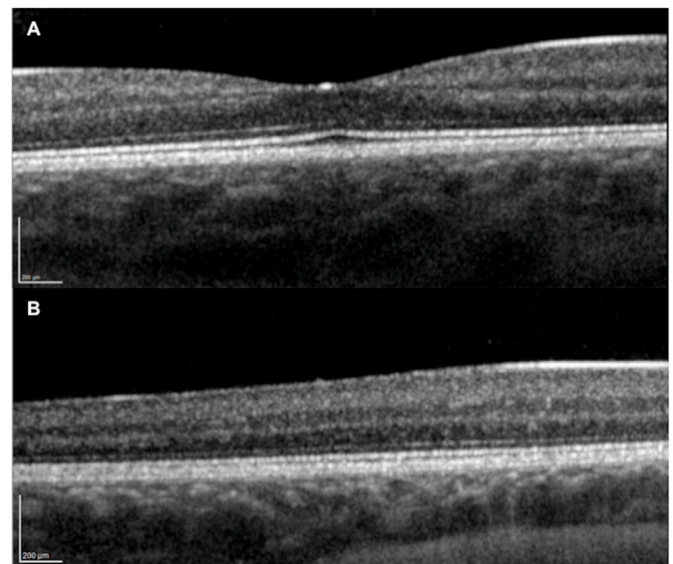


Fig. 1. Optical Coherence Tomography of Aniridia. Spectral domain optical coherence tomography demonstrating: A) the macula of a healthy age matched control with a normal foveal pit and expected maturation of the fovea (inner retinal layers that have migrated out of the pit, a widened outer nuclear layer and elongated inner and outer segments); B) foveal hypoplasia secondary to aniridia, with lack of foveal pit morphology or outer retinal maturation.

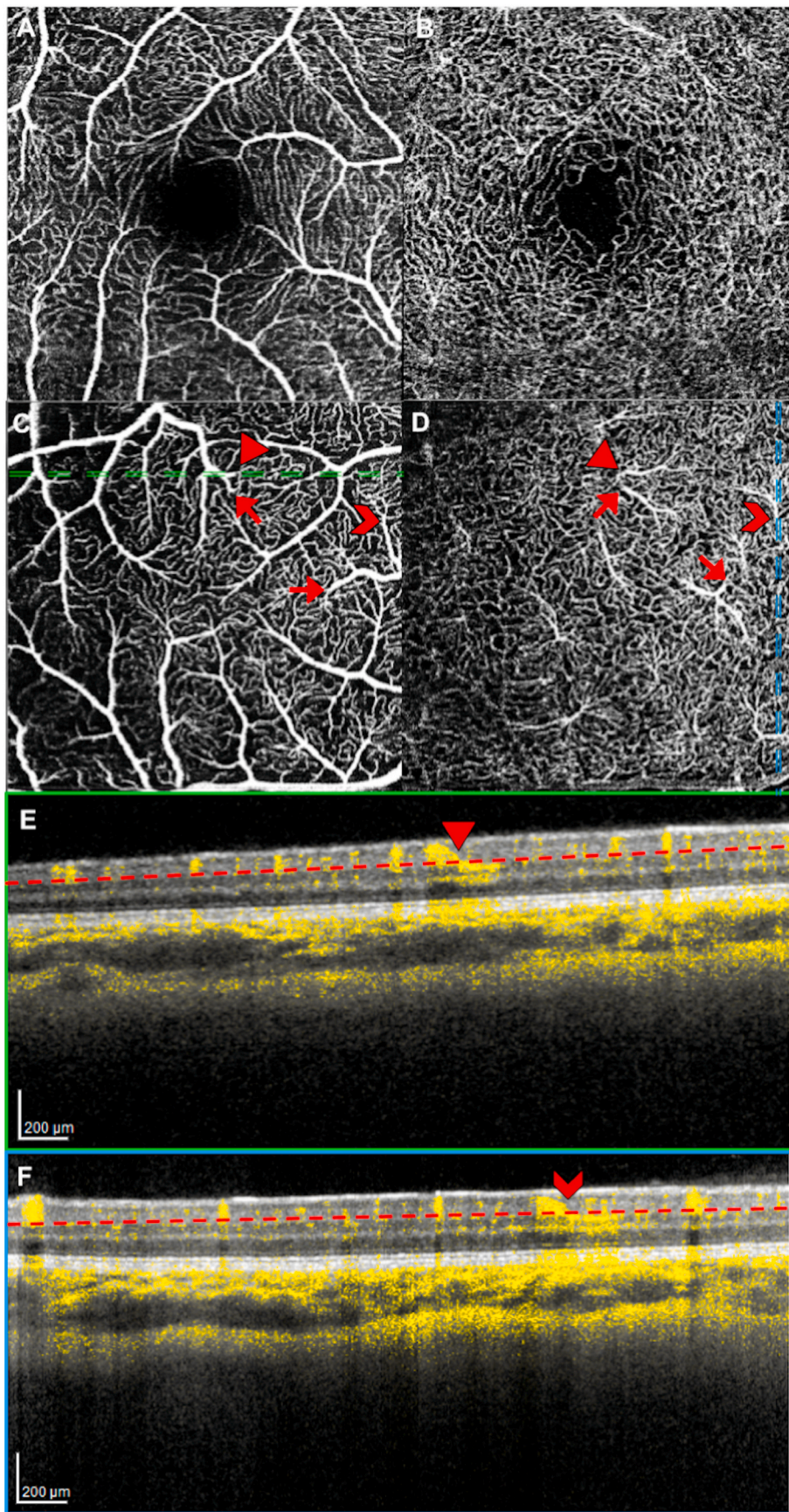


Fig. 2. OCT Angiography of Aniridia. Optical coherence tomography angiography demonstrating: age matched control images of the superficial vessel complex (SVC) (A) and deep vessel complex (DVC) (B), with an appropriately developed foveal avascular zone; Lack of foveal avascular zone in both the SVC (C) and DVC (D) in an eye with aniridia, with red arrows pointing to blood vessels as they course through the SVC and after they dive into the DVC. Corresponding flow data overlaying optical coherence tomography (OCT) structural images (E, F) visualize the path of large diving blood vessels (red arrows) from the superficial vessel complex to the deep vessel complex (red dotted line marks the SVC/DVC boundary within the inner plexiform layer). The OCT B scan in (E) corresponds with the green dotted line in (C) and the OCT B scan in (F) corresponds with the blue dotted line in (D). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

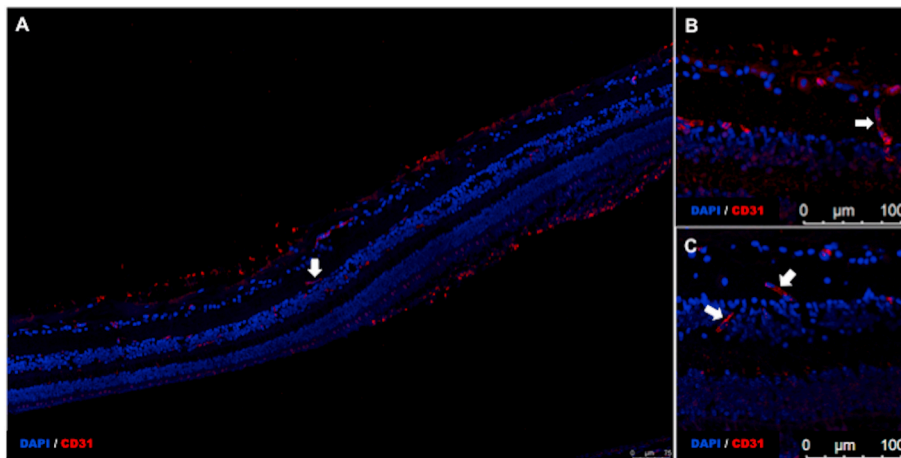


Fig. 3. Histopathology of Aniridia. Immunofluorescence on paraffin embedded sections from an eye with aniridia at low (A) and high magnification (B,C). CD31 (red), an endothelial cell marker and DAPI (blue), a counterstain for cell nuclei, demonstrate prominent retinal vessels extending into the inner nuclear layer (within the deep vessel complex boundaries) of the retina (arrows), similar to the “diving” vessels present on OCTA. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the SVC of infants with retinopathy of prematurity that crossed into the DVC. In contrast to those cases, the subject in this study has different pathology and is of significantly older age in the context of retinal vascular development. Thus, it is difficult to draw conclusions based on these two imaging studies. However, seeing diving vessels in multiple pathologies demonstrates that this observation may be an abnormal retinal microvascular development that should be further investigated.

The histopathology observation in the aniridic decedent represents, to our knowledge, the only *ex vivo* microscopy analysis of a retinal specimen from an aniridic subject. A Pubmed search, completed on May 5, 2020, of the terms *histopathology* and *aniridia* found no other similar reports. Although the histopathology finding helps validate the OCTA observation, histopathology from additional diseased and normal subjects need to be investigated.

Our limited knowledge of retinal microvasculature development comes from histopathology studies. Hughes et al.¹⁶ demonstrate in human retina specimens that extensions of the inner microvascular network form the outer microvascular network through angiogenesis. The inner network continues to remodel through vessel retraction.¹⁶ One postulation might be that the diving vessels in this study are remnant extensions from the inner microvascular network that failed to retract during early development.

Supine OCTA imaging under anesthesia has allowed for the study of retinal microvasculature in patients with nystagmus. The OCTA observations in this report bring to light questions about abnormal retinal microvasculature developments and whether they serve an important role in retinal diseases like aniridia. The histopathology images help to validate the OCTA findings, however, the findings from this case report need to be studied in additional eyes.

5. Conclusions

This study highlights the clinical, imaging and histopathologic findings of the foveal retina in aniridia. Supine OCTA imaging under anesthesia allowed for study of retinal microvasculature in eyes with nystagmus. The histopathology images support the OCTA findings that, with further studies, may lead to new information about the development of abnormal retinal microvasculature.

Patient consent

Patient’s guardian provided consent for OCTA imaging and use of the images.

IRB approval by the Duke University IRB was obtained for this study.

Funding

This study was supported by grants from the VitreoRetinal Surgery Foundation (AT) and Heidelberg Engineering (AT, LV). Research equipment was provided by Heidelberg Engineering. Neither group had any role in design, analysis, writing or decision to submit this report.

Financial disclosures

Lejla Vajzovic: Orbit Biomedical: research and equipment grant, consulting; Alcon: consulting; Alimera Sciences: consulting; Allergan: consulting; Aerie: consulting; Bausch & Lomb: consulting; DORC: consulting; Guidepoint: consulting; Novartis: research grant; Roche/Genentech: research grant, consulting; Second Sight: research grant, consulting; Evolve Medical Education: consulting. Anthony Therattil: Orbit Biomedical: research grant.

Sharon Freedman: Qlaris: consulting.

The following authors have no financial disclosures: AN, STH, MPK, SRD.

Authorship

All authors attest that they meet the current ICMJE criteria for authorship.

Acknowledgments

None.

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