Published in final edited form as:

Exp Eye Res. 2019 January ; 178: 91–98. doi:10.1016/j.exer.2018.09.017.

Detection of microvascular retinal changes in type I diabetic mice with optical coherence tomography angiography

Hironori Uehara¹, Timilai Lesuma¹, Parker Stocking², Nathan Jensen¹, Sangeetha Ravi Kumar¹, Mingyang Aaron Zhang¹, Susie Choi¹, Xiaohui Zhang¹, Bonnie Archer¹, Lara Carroll¹, and Balamurali K. Ambati^{1,*}

¹Dept. of Ophthalmology, Moran Eye Center, University of Utah, 65 Mario Capecchi Dr., Salt Lake City, UT 84132

²Rocky Vista University College of Osteopathic Medicine, 255 E. Center St. Ivins, UT 84738

Abstract

Optical coherence tomography (OCT) angiography is a dye-free and non-invasive angiography which allows visualization of retinal and choroid vascular flow, enabling observation of highly permeable and three dimensional vasculature. Although OCT angiography is providing new insights in human retinal and choroidal diseases, a few studies have been reported in experimental mice. In this study, to determine the potential of OCT angiography in experimental mice, we sought to examine whether OCT angiography can detect vascular change in type I diabetic mice. To conduct age dependent analysis, 2 and 6 month old male type 1 diabetic *Ins2^{Akita/+}* and age matched C57BL/6J mice were used. OCT angiography was performed by Heidelberg Spectralis OCT Angiography Module with 30 degree lens + mouse adapter lens. We acquired the OCT angiography image from the peripheral nasal position. For analysis of OCT angiography images, OCT angiography positive area were used for vascular density. We analyzed vascular density from the retinal surface (inner limiting membrane) to 120µm depth with 4µm steps in order to correlate vascular density vs depth (N=4 per group). Vascular density of both mouse strains demonstrated three different peaks. By comparing with the OCT image, the first peak (superficial), second peak (intermediate) and third peak (deep) were located in nerve fiber layer/ganglion cell layer, inner plexiform layer/inner nuclear layer and outer plexiform layer/outer nuclear layer, respectively. We calculated vascular density of these peaks separately. In C57BL/6J mice, the vascular density in all three layers do not show significant difference between 2- and 6-month-old. On the other hand, 6-month-old *Ins2^{Akita/+}* mice showed a significant decrease of the vascular density in all three layers compared to 2-month-old *Ins2^{Akita/+}* mice. Also, the vascular density of 6-month-old *Ins2^{Akita/+}* mice in the deep layer showed a significant decrease compared to 2- and 6-month-old C57BL/6J mice. Thus, OCT angiography successfully detects retinal vascular difference between type I diabetic mice and control mice, and age-dependent vasculature change in type I diabetic mice. The diabetic mice demonstrated reduced vascular density due to reduced density of flowing deep

*Corresponding author: bambati@gmail.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

vessels. Importantly, we observed this difference without retinal blood leakage, hemorrhage or neovascularization. Our analysis (vascular density vs retinal depth) suggests that OCT angiography is useful for *in vivo* detection of retinal vasculature alteration in experimental mice.

Keywords

Optical coherence tomography angiography; murine diabetic retinopathy

1. Introduction

Optical coherence tomography (OCT) has become indispensable to the clinical practice of ophthalmology for various diseases such as age-related macular degeneration, glaucoma and diabetic retinopathy (Adhi and Duker, 2013; Branchini et al., 2013; Kniggendorf et al., 2016; Manjunath et al., 2011; Thomas and Duguid, 2004; Van Melkebeke et al., 2018; Wang et al., 2016). Precise structural observation of retina and choroid by OCT leads to accurate diagnosis of disease states. Historically, OCT was not able to assess flow or vascular status. To observe retinal/choroid vasculature, dye-based angiographies (fluorescein angiography (FA) and indocyanine green angiography) are primarily used. However, the dye-based angiographies are invasive and may cause some complications (Obana et al., 1994; Yannuzzi et al., 1986). Also, two-dimensional imaging of dye-based angiographies can limit diagnosis of vascular conditions.

Recently, OCT angiography has emerged as a non-invasive and dye-free angiography for observing retinal/choroid vasculature with flow (Fingler et al., 2009; Jia et al., 2012; Liu et al., 2012; Mariampillai et al., 2008; Wang et al., 2007). In humans, the three dimensional angiogram can enable detailed retinal/choroid vasculature observation in various eye diseases. In age-related macular degeneration, choroid neovascularization and choriocapillaris loss, (some of which are difficult to observe by dye-based angiography due to high permeability), have been observed (Jia et al., 2015; Ma et al., 2017; Malamos et al., 2017) with OCT angiography. In glaucoma, (Jia et al., 2014; Yarmohammadi et al., 2016) and diabetic retinopathy, (Nesper et al., 2017; Sambhav et al., 2017) loss of perfused vessels and flow have been observed. Thus, OCT angiography can be considered as an ophthalmic diagnostic tool that complements conventional dye-based angiography.

Few OCT angiography studies on experimental mice have been reported (Alnawaiseh et al., 2017; Alnawaiseh et al., 2016; Giannakaki-Zimmermann et al., 2016). In this study, we examine whether OCT angiography can detect retinal vasculature alteration in non-diabetic vs. diabetic mice. An important distinction of this study is the three-layer visualization utilized in imaging via OCT angiography and its importance to providing the distinction between diabetic and non-diabetic mice.

2. Material and Methods

2.1 Mice

All animal experiments were conducted in accordance with the ARVO Declaration on Use of Animals in Research, and performed after approval by the University of Utah IACUC.

Male C57BL/6J and male *Ins2^{Akita/+}* were purchased from Jackson Labs. Male mice were chosen since male *Ins2^{Akita/+}* mice develop a more severe diabetic phenotype than females (Barber et al., 2005).

2.2 Murine OCT angiography

Mice were anesthetized by 80mg/kg ketamine (Zetamine, VetOne®, Boise, Idaho) and 10mg/kg xylazine (AnaSed® LA, VetOne®). Pupils were dilated with 1% tropicamide (Alcon Laboratories, Inc., Fort Worth, Texas). To prevent corneal drying, we used a rigid contact lens (Cantor & Nissel Ltd, Brackly, Northamptonshire, United Kingdom) (Figure 1A) with base optic zone radius (BOZR 1.70), diameter (DIAM 3.20) and power (PWR 0.00) which fits the mouse cornea (Figure 1B). During imaging and post imaging, the mouse were kept on water circulating heat pad (T/pump, Stryker, Kalamazoo, Michigan) to maintain body temperature. For OCT angiography, we used Heidelberg HRA+OCT with 30 degree lens and blue mouse adaptor lens (Heidelberg Engineering, Heidelberg, Germany). The software version 6.7 to 6.9 were used. The scan size is $10^\circ \times 10^\circ$. A-scan and B-scan are 768 and 512, respectively. We focused on retinal vasculature in the nasal position around 30° (one image) from optic nerve.

2.3 OCT angiography image analysis

Each OCT angiography image from inner limiting membrane (0 μ m) to a depth of 120 μ m was analyzed at 4 μ m steps by Image J. Briefly, each image was binarized using the average intensity of the avascular image as a threshold. A single threshold was used for all analysis. The positive pixel number/total pixel ratio is designated as OCTA positive density. For the comparison, we used ANOVA and subsequent Student's t-test, with $p < 0.05$ being considered as significant. Error bar is standard deviation.

3. Results

3.1 Optimal conditions to acquire OCT angiography in mice

The fundus image quality directly affects OCT angiography image acquisition. Although various factors affect image quality, anesthesia-induced cataract is the most common. The focus to the retina is critical. We generally used 15–25D power of the Heidelberg Spectralis lens in this study to visualize retinal blood vessel in the infrared image as shown in Figure 1C. Also, to get the appropriate focus, we used a 30 degree lens with blue mouse adaptor lens (Figure 1D). In most cases, a sharp infrared image reduces image duplication risk. Finally, Figure 1E shows a mouse positioned for OCT angiography.

3.2 Data analysis of OCT angiography in mice

Upon OCT angiography in mouse, we found two challenges to proper data analysis. First, depending on the focus, we found the image to show duplicate vessels. Figure 2A–C shows an example of OCT angiography image duplication. The vessel in the blue circle at 16.37D shows a single vessel (Figure 2A). However, with increasing diopter (21.92 and 26.66D), the vessel in the blue circle starts to be duplicated in the image (Figure 2B–C). On the other hand, the vessel in the red circle at 16.37D shows duplication but with increasing diopters the image shows a single vessel (Figure 2A–C). To avoid the image duplication, we

determined the appropriate focus empirically. In most cases, a sharp infrared image reduces the image duplication risk but we were not able to avoid image duplication completely. Therefore, we removed duplicated images from our analysis manually. Another issue is that of mirror OCT angiography images at the avascular photoreceptor space (Figure 2D–E). The mirror images were always observed at the outer segment of photoreceptor (around 200µm depth from inner limiting membrane). Therefore, in this study we did not analyze OCT angiography images in the outer segment layer.

Figure 3 shows a representative OCT image and OCT angiography analysis. We measured vascular density from the inner limiting membrane (retinal surface) to 120µm depth in 4µm steps. As shown in Figure 3, mouse retinal vessels can be isolated into three layers in both non-diabetic C57BL/6J mice (Figure 3A) and diabetic *Ins2^{Akita/+}* mice (Figure 3B). This spatial separation of retinal vasculature by OCT angiography is superior aspect compared to fluorescein angiography (supplemental figure). By comparison with OCT image, the superficial (first peak), intermediate (second peak) and deep (third peak) are located in nerve fiber layer/ganglion cell layer, inner plexiform layer/inner nuclear layer and outer plexiform layer/outer nuclear layer, respectively.

3.3 Vascular density of young and aged C57BL/6J mouse

To determine whether OCT angiography can detect alterations of retinal vasculature over time, we examined non-diabetic C57BL/6J mouse at 2 months old (young) and 6 months old (aged). Figure 4A shows the representative OCT angiography image of superficial, intermediate and deep retina at 2 months old in C57BL/6J mouse. Figure 4B shows vascular density from the inner limiting membrane to 120µm depth. Figure 5A–B shows the representative OCT angiography image and vascular density in 6-month-old C57BL/6J mice. There were no significant differences between 2 and 6 months in vascular density of C57BL/6J mice.

3.4 Vascular density of young and aged *Ins2^{Akita/+}* mouse

As in control C57BL/6J mice, we examined retinal vasculature in young and aged diabetic *Ins2^{Akita/+}* mouse. 2-month-old *Ins2^{Akita/+}* mouse showed the similar phenotype to C57BL/6J (Figure 6A–B). On the other hand, 6-month-old *Ins2^{Akita/+}* mouse demonstrated a very different phenotype (Figure 7A–B), with reduced deep vascular density. These results indicate that OCT angiography can detect attenuation of flowing vasculature in aged diabetic *Ins2^{Akita/+}* mouse.

3.5 Deep vasculature changes in aged *Ins2^{Akita/+}* mouse

Finally, OCT angiography positive densities in each group were compared (Figure 8). We separately calculated the vascular volume density in each layer. In the superficial layer, C57BL/6J showed $16.6 \pm 5.6\%$ (2-month-old) and $14.3 \pm 5.6\%$ (6-month-old) (not statistically significant), while *Ins2^{Akita/+}* showed $23.1 \pm 1.8\%$ (2-month-old) and $14.3 \pm 3.5\%$ (6-month-old) ($p < 0.01$). In the intermediate layer, C57BL/6J showed $17.8 \pm 5.1\%$ (2-month-old) and $17.0 \pm 3.8\%$ (6-month-old) (not statistically significant), and *Ins2^{Akita/+}* showed $22.9 \pm 0.9\%$ (2-months-old) and $13.9 \pm 5.4\%$ (6 months-old) ($p < 0.05$). However, ANOVA for the four groups in the superficial and intermediate layer was not significant. In the deep layer,

C57BL/6J showed $18.6 \pm 5.7\%$ (2-month-old) and $19.0 \pm 1.8\%$ (6-month-old) (not statistically significant), and *Ins2^{Akita/+}* showed $17.3 \pm 3.5\%$ (2-month-old) and $7.9 \pm 3.2\%$ (6-month-old) ($p < 0.01$). Furthermore, the deep vascular density of 6-month-old *Ins2^{Akita/+}* was lower than that of both C57BL/6J groups (ANOVA, $p < 0.01$; Student's t-test to other groups, $p < 0.05$). In an analysis of total retinal vascular density (0–120 μm), C57BL/6J showed $17.8 \pm 4.1\%$ (2-month-old) and $17.2 \pm 2.0\%$ (6-month-old), and *Ins2^{Akita/+}* showed $20.6 \pm 1.6\%$ (2-month-old) and $10.7 \pm 3.2\%$ (6-month-old). 6-month-old *Ins2^{Akita/+}* demonstrated a significant decrease of total vascular density (ANOVA, $p < 0.01$; Student's t-test to other groups, $p < 0.05$).

3.6 Inner plexiform layer decrease in aged *Ins2^{Akita/+}* mouse

OCT angiography spatial analysis yields an interesting observation. The table 1 below shows the distance from retinal surface to the peak of each vasculature layer.

Since this analysis is in 4 μm steps, some standard deviations showed 0. Intermediate and deep layer depth from the surface in 6 months old *Ins2^{Akita/+}* mouse decrease significantly compared to the other groups (both layers ANOVA $p < 0.001$). Also, we measured thickness of the corresponding layers from OCT images (Table 2).

Importantly, NFL/GCL/IPL separation in mouse retinal OCT is difficult since each boundary is often obscure. From these observations, the intermediate peak shift in aged *Ins2^{Akita/+}* mouse suggests inner plexiform layer loss which is observed in human diabetic retinopathy (van Dijk et al., 2009). Thus, OCT angiography can be a useful method for detecting retinal vasculature and structure alteration in the mouse.

4. Discussion

The prevalence of diabetes mellitus continues to surge, with an estimated increase from 415 million people, 8.8% of the globe's population in 2015, to 642 million people, roughly 10.4% of the population in 2040 (Ogurtsova et al., 2017). The pathogenesis of diabetes can lead to a variety of ocular complications, the most common and serious of these being diabetic retinopathy, which is identified in approximately one third of all diabetics. In the United States, 40% of people with type 2 diabetes, and 86% of those with type 1 diabetes have diabetic retinopathy (Cheung et al., 2010). Early diagnosis of diabetic retinopathy can provide opportunities for earlier intervention that would hopefully prevent vision loss, structural or functional retinal impairment, and a chance to treat or reverse the systemic condition.

OCT angiography is not only a non-invasive method to visualize retinal vasculature but also enables us to analyze vascular density spatially. In this study, utilizing this benefit, we successfully detected age dependent alteration of retinal vasculature in the type 1 diabetic *Ins2^{Akita/+}* mouse. Generally, *Ins2^{Akita/+}* mice have elevated blood glucose level from 4.5 weeks of age (Barber et al., 2005). The superficial and intermediate retinal vasculature trend towards a relatively higher density in *Ins2^{Akita/+}* than control mice (Figure 8). This may indicate retinal blood flow changes as early as 2 months of age in *Ins2^{Akita/+}*. At 6 months of age, *Ins2^{Akita/+}* mice show a significant decrease of deep retinal vasculature compared to the other groups (Figure 8). In a previous study, we found that 6-month-old *Ins2^{Akita/+}* mice

developed decreased retinal vascular density, pericyte loss and retinal blood leakage (Cahoon et al., 2015). OCT angiography provides precise vascular density in each layer and is enough to detect diabetic retinopathy symptoms in mice.

Previously, McLenachan S. et al. reported that the deep vascular layer increased in aged *Ins2^{akita/+}* mice by FA (McLenachan et al., 2015), which is different from our observations in our current study. We speculate this difference is due to the methodological difference between FA and OCT angiography. At first, OCT angiography has a superior spatial retinal vasculature observation than FA. In FA, the depth information of retinal vasculature is not available, in which the observers know where to scan subjectively by their experience and skill. In OCT angiography, the precise depth information allows us to separate each vascular layer objectively, as shown in Figures 4–8. Second, the object of OCT angiography is different from FA. Since fluorescein of FA is a small molecule (376 Da), it can perfuse into even occluded vessels (Gong et al., 2016). On the other hand, OCT angiography detects flow of blood, especially moving erythrocytes. Therefore, OCT angiography does not visualize blood vessel without flow. We believe these methodological differences between the current work and McLenachan's work (2015) result in different conclusions.

The reduced deep vasculature layers and the inner plexiform layer loss in aged diabetic *Ins2^{akita/+}* mouse are similar phenotypes to human diabetic retinopathy (Nesper et al., 2017; Sambhav et al., 2017; van Dijk et al., 2009). There was a controversy whether *Ins2^{akita/+}* mouse has symptoms related to human diabetic retinopathy or not. Barber et al and Cahoon et al showed higher vascular permeability in *Ins2^{akita/+}* mice (Barber et al., 2005; Cahoon et al., 2015), while absence of diabetic retinopathy symptom in *Ins2^{akita/+}* mice by histology and FA was reported (McLenachan et al., 2013). Our *in vivo* finding with OCT angiography, which indicates decrease of blood flow in deep vascular layer, strongly suggest that the aged *Ins2^{akita/+}* mouse has an early stage of non-proliferative diabetic retinopathy.

Through this study, we identified several technical difficulties of OCT angiography utilization in mice. First, cataract, which is induced by anesthesia and drying of the corneal surface, reduces quality of OCT angiography or even precludes OCT angiography. Body temperature control and contact lens reduce the risk of cataract development. Therefore, during OCT angiography we monitored infrared image quality to maintain OCT angiography quality. Second, we found OCT angiography image duplication occurs sometimes. Although we speculated the focus may affect image duplication, unfortunately we were not able to eliminate this problem. Since image duplication would cause overestimation of retinal vascular density, we removed duplicated images from our analysis manually. However, in order to keep quality and reproducibility of OCT angiography analysis in large data sets, automatic image control will be necessary. Third, the mirror image of retinal vasculature in the avascular photoreceptor layer was observed. In this study, since *Ins2^{akita/+}* mice did not show neovascularization in avascular areas, therefore this artifact does not affect our observation. However, OCT angiography application to subretinal neovascularization needs careful consideration of this effect. Finally, mouse motion, especially heart beats, may affect OCT angiography image acquisition. Although Heidelberg image tracking system enable image acquisition with heart beats, fixing the position of the mouse head could avoid this problem.

In conclusion, OCT angiography successfully detects age-dependent retinal vasculature alterations in type 1 diabetic *Ins2^{Akita}/+* mouse. Our results demonstrate that *in vivo* monitoring of retinal vasculature spatially by OCT angiography is useful for detection of retinal vasculopathy. Although there are several difficulties of the application of OCT angiography to experimental mice, OCT angiography can provide precise observation of *in vivo* retinal vasculature. Future studies could include evaluation of other mouse strains, e.g., Akimba mice or JR5558 mice, which also express vascular anomalies with high permeability, or murine models of diabetes or macular degeneration after treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the National Institutes of Health/NEI (R01EY026029) and unrestricted grant from Research to Prevent Blindness, New York, NY. To the Department of Ophthalmology & Visual Sciences, University of Utah.

References

- Adhi M, Duker JS, 2013 Optical coherence tomography – current and future applications. *Current opinion in ophthalmology* 24, 213–221. [PubMed: 23429598]
- Alnawaiseh M, Brand C, Bormann E, Wistuba J, Eter N, Heiduschka P, 2017 Quantitative analysis of retinal perfusion in mice using optical coherence tomography angiography. *Experimental eye research* 164, 151–156. [PubMed: 28889963]
- Alnawaiseh M, Rosentreter A, Hillmann A, Alex AF, Niekamper D, Heiduschka P, Pap T, Eter N, 2016 OCT angiography in the mouse: A novel evaluation method for vascular pathologies of the mouse retina. *Experimental eye research* 145, 417–423. [PubMed: 26946073]
- Barber AJ, Antonetti DA, Kern TS, Reiter CE, Soans RS, Krady JK, Levison SW, Gardner TW, Bronson SK, 2005 The *Ins2Akita* mouse as a model of early retinal complications in diabetes. *Investigative ophthalmology & visual science* 46, 2210–2218. [PubMed: 15914643]
- Branchini L, Regatieri C, Adhi M, Flores-Moreno I, Manjunath V, Fujimoto JG, Duker JS, 2013 Effect of Intravitreal Anti-Vascular Endothelial Growth Factor Therapy on Choroidal Thickness in Neovascular Age-Related Macular Degeneration Using Spectral-Domain Optical Coherence Tomography. *JAMA ophthalmology* 131, 693–694. [PubMed: 23494008]
- Cahoon JM, Rai RR, Carroll LS, Uehara H, Zhang X, O'Neil CL, Medina RJ, Das SK, Muddana SK, Olson PR, Nielson S, Walker K, Flood MM, Messenger WB, Archer BJ, Barabas P, Krizaj D, Gibson CC, Li DY, Koh GY, Gao G, Stitt AW, Ambati BK, 2015 Intravitreal AAV2.COMP-Ang1 Prevents Neurovascular Degeneration in a Murine Model of Diabetic Retinopathy. *Diabetes* 64, 4247–4259. [PubMed: 26340930]
- Cheung N, Mitchell P, Wong TY, 2010 Diabetic retinopathy. *Lancet (London, England)* 376, 124–136.
- Fingler J, Zawadzki RJ, Werner JS, Schwartz D, Fraser SE, 2009 Volumetric microvascular imaging of human retina using optical coherence tomography with a novel motion contrast technique. *Optics express* 17, 22190–22200. [PubMed: 19997465]
- Giannakaki-Zimmermann H, Kokona D, Wolf S, Ebner A, Zinkernagel MS, 2016 Optical Coherence Tomography Angiography in Mice: Comparison with Confocal Scanning Laser Microscopy and Fluorescein Angiography. *Translational Vision Science & Technology* 5, 11.
- Gong H, Song Q, Wang L, 2016 Manifestations of central retinal artery occlusion revealed by fundus fluorescein angiography are associated with the degree of visual loss. *Exp Ther Med* 11, 2420–2424. [PubMed: 27313672]

- Jia Y, Bailey ST, Hwang TS, McClintic SM, Gao SS, Pennesi ME, Flaxel CJ, Lauer AK, Wilson DJ, Hornegger J, Fujimoto JG, Huang D, 2015 Quantitative optical coherence tomography angiography of vascular abnormalities in the living human eye. *Proceedings of the National Academy of Sciences of the United States of America* 112, E2395–2402. [PubMed: 25897021]
- Jia Y, Tan O, Tokayer J, Potsaid B, Wang Y, Liu JJ, Kraus MF, Subhash H, Fujimoto JG, Hornegger J, Huang D, 2012 Split-spectrum amplitude-decorrelation angiography with optical coherence tomography. *Optics express* 20, 4710–4725. [PubMed: 22418228]
- Jia Y, Wei E, Wang X, Zhang X, Morrison JC, Parikh M, Lombardi LH, Gattley DM, Armour RL, Edmunds B, Kraus MF, Fujimoto JG, Huang D, 2014 Optical coherence tomography angiography of optic disc perfusion in glaucoma. *Ophthalmology* 121, 1322–1332. [PubMed: 24629312]
- Kniggendorf VF, Novais EA, Kniggendorf SL, Xavier C, Cole ED, Regatieri CV, 2016 Effect of intravitreal anti-VEGF on choroidal thickness in patients with diabetic macular edema using spectral domain OCT. *Arquivos brasileiros de oftalmologia* 79, 155–158. [PubMed: 27463625]
- Liu G, Lin AJ, Tromberg BJ, Chen Z, 2012 A comparison of Doppler optical coherence tomography methods. *Biomedical Optics Express* 3, 2669–2680. [PubMed: 23082305]
- Ma J, Desai R, Nesper P, Gill M, Fawzi A, Skondra D, 2017 Optical Coherence Tomographic Angiography Imaging in Age-Related Macular Degeneration. *Ophthalmology and Eye Diseases* 9, 1179172116686075. [PubMed: 28579843]
- Malamos P, Tsolkas G, Kanakis M, Mylonas G, Karatzenis D, Oikonomopoulos N, Lakoumentas J, Georgalas I, 2017 OCT-Angiography for monitoring and managing neovascular age-related macular degeneration. *Current eye research* 42, 1689–1697. [PubMed: 28937833]
- Manjunath V, Goren J, Fujimoto JG, Duker JS, 2011 Analysis of choroidal thickness in age-related macular degeneration using spectral-domain optical coherence tomography. *American journal of ophthalmology* 152, 663–668. [PubMed: 21708378]
- Mariampillai A, Standish BA, Moriyama EH, Khurana M, Munce NR, Leung MK, Jiang J, Cable A, Wilson BC, Vitkin IA, Yang VX, 2008 Speckle variance detection of microvasculature using swept-source optical coherence tomography. *Optics letters* 33, 1530–1532. [PubMed: 18594688]
- McLenachan S, Chen X, McMenamin PG, Rakoczy EP, 2013 Absence of clinical correlates of diabetic retinopathy in the Ins2Akita retina. *Clinical & experimental ophthalmology* 41, 582–592. [PubMed: 23433122]
- McLenachan S, Magno AL, Ramos D, Catita J, McMenamin PG, Chen FK, Rakoczy EP, Ruberte J, 2015 Angiography reveals novel features of the retinal vasculature in healthy and diabetic mice. *Experimental eye research* 138, 6–21. [PubMed: 26122048]
- Nesper PL, Roberts PK, Onishi AC, Chai H, Liu L, Jampol LM, Fawzi AA, 2017 Quantifying Microvascular Abnormalities With Increasing Severity of Diabetic Retinopathy Using Optical Coherence Tomography Angiography. *Investigative ophthalmology & visual science* 58, Bio307–bio315. [PubMed: 29059262]
- Obana A, Miki T, Hayashi K, Takeda M, Kawamura A, Mutoh T, Harino S, Fukushima I, Komatsu H, Takaku Y, et al., 1994 Survey of complications of indocyanine green angiography in Japan. *American journal of ophthalmology* 118, 749–753. [PubMed: 7977601]
- Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, Cavan D, Shaw JE, Makaroff LE, 2017 IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice* 128, 40–50. [PubMed: 28437734]
- Sambhav K, Abu-Amero KK, Chalam KV, 2017 Deep capillary macular perfusion indices obtained with OCT angiography correlate with degree of nonproliferative diabetic retinopathy. *European journal of ophthalmology*, 0.
- Thomas D, Duguid G, 2004 Optical coherence tomography--a review of the principles and contemporary uses in retinal investigation. *Eye (London, England)* 18, 561–570.
- van Dijk HW, Kok PHB, Garvin M, Sonka M, DeVries JH, Michels RPI, van Velthoven MEJ, Schlingemann RO, Verbraak FD, Abramoff MD, 2009 Selective Loss of Inner Retinal Layer Thickness in Type 1 Diabetic Patients with Minimal Diabetic Retinopathy. *Investigative ophthalmology & visual science* 50, 3404–3409. [PubMed: 19151397]
- Van Melkebeke L, Barbosa-Breda J, Huygens M, Stalmans I, 2018 Optical Coherence Tomography Angiography in Glaucoma: A Review. *Ophthalmic research*, 1–13.

- Wang RK, Jacques SL, Ma Z, Hurst S, Hanson SR, Gruber A, 2007 Three dimensional optical angiography. *Optics express* 15, 4083–4097. [PubMed: 19532651]
- Wang YT, Tadarati M, Wolfson Y, Bressler SB, Bressler NM, 2016 Comparison of Prevalence of Diabetic Macular Edema Based on Monocular Fundus Photography vs Optical Coherence Tomography. *JAMA Ophthalmol* 134, 222–228. [PubMed: 26719967]
- Yannuzzi LA, Rohrer KT, Tindel LJ, Sobel RS, Costanza MA, Shields W, Zang E, 1986 Fluorescein angiography complication survey. *Ophthalmology* 93, 611–617. [PubMed: 3523356]
- Yarmohammadi A, Zangwill LM, Diniz-Filho A, Suh MH, Manalastas PI, Fatehee N, Yousefi S, Belghith A, Saunders LJ, Medeiros FA, Huang D, Weinreb RN, 2016 Optical Coherence Tomography Angiography Vessel Density in Healthy, Glaucoma Suspect, and Glaucoma Eyes. *Investigative ophthalmology & visual science* 57, Oct451–459. [PubMed: 27409505]

Highlights

- OCT angiography enables three dimensional retinal vasculature analysis in mice
- Aged diabetic mice show diminished deep retinal vascular layer
- Aged diabetic mice demonstrate a similar phenotype to human diabetic retinopathy

Figure 1. Condition of OCT angiography in mouse.

(A) Contact lens for mouse from Cantor&Nissel. (B) Mouse eye with/without contact lens.

(C) Infrared image with Cantor&Nissel contact lens. (D) Lens of Heidelberg Spectralis used for mouse study. (E) Mouse position for OCT angiography imaging.

Figure 2. Duplication and mirror image in OCT angiography.

(A-C) Different diopter OCT angiography images. The vessels in the blue and red circles show duplication depending on the dioptric power. (D) OCT angiography around inner nuclear layer. (E) OCT angiography of the same position around outer segment of photoreceptor. Although the outer segment of photoreceptor are avascular, a mirror image is observed.

(A) C57BL/6J

OCT with OCT angiography

OCTA analysis

(B) *Ins2^{Akita/+}*

OCT with OCT angiography

OCTA analysis

Figure 3. Spatial analysis of OCT angiography.

Representative OCT image with OCT angiography signal and OCT angiography analysis.

(A) 6-month-old C57BL/6J, (B) 6-month-old diabetic *Ins2^{Akita/+}*. OCT angiography signals are overlaid on OCT images as yellow. From OCT angiography image, vascular density from the retinal surface (inner limiting membrane) to 120μm depth with 4mm steps is measured. Generally, three different peaks (superficial, intermediate and deep) are observed.

Figure 4. 2-month-old C57BL/6J OCT angiography image and its analysis.

(A) Representative OCTA images of 2-month-old C57BL/6J in superficial, intermediate and deep vascular layers. In the merged image, red, green and blue correspond to superficial, intermediate deep vascular layers respectively. (B) Vascular density from inner limiting membrane (0 μm) to 120 μm . N=4. Error bar is standard deviation.

Figure 5. 6-month-old C57BL/6J OCT angiography image and its analysis.

(A) Representative OCTA images of 6-month-old C57BL/6J in superficial, intermediate, and deep vascular layers. In the merged image, red, green and blue correspond to superficial, intermediate, and deep vascular layers respectively. (B) Vascular density from inner limiting membrane (0μm) to 120μm. N=4. Error bar is standard deviation.

Figure 6. 2-month-old *Ins2^{Akita/+}* OCT angiography image and its analysis.

(A) Representative OCT angiography images of 2-month-old *Ins2^{Akita/+}* in superficial, intermediate, and deep vascular layers. In the merged image, red, green and blue correspond to superficial, intermediate, and deep vascular layers respectively. (B) Vascular density from inner limiting membrane (0 μm) to 120 μm . N=4. Error bar is standard deviation.

Figure 7. 6-month-old *Ins2^{Akita/+}* OCT angiography image and its analysis demonstrates vascular attenuation.

(A) Representative OCT angiography images of 6-month-old *Ins2^{Akita/+}* in superficial, intermediate, and deep vascular layers. In the merged image, red, green and blue correspond to superficial, intermediate, and deep vascular layers respectively. (B) Vascular density from inner limiting membrane (0 μm) to 120 μm . N=4. Error bar is standard deviation.

Figure 8. Deep vascular change in aged *Ins2^{Akita/+}*.

Three different layers (superficial, intermediate and deep) are analyzed separately as shown in the schematic diagram (top graph). C57BL/6J mice do not show the alteration of vasculature density. On the other hand, aged diabetic *Ins2^{Akita/+}* mouse (6-month-old) shows decreased vascular density over time compared with other groups. p-values were calculated by ANOVA and two-tailed Student's t-test.

Table 1.
The distance from retinal surface to the peak of each vasculature layer.

	Superficial Peak	Intermediate Peak	Deep Peak
C57BL/6J, 2-month-old	10±0µm	54±3.3µm	86±3.3µm
C57BL/6J, 6-month-old	10±0µm	54±0µm	86±0µm
<i>Ins2^{Akita/+}</i> , 2-month-old	10±0µm	52±2.3µm	83±2.0µm
<i>Ins2^{Akita/+}</i> , 6-month-old	10±0µm	45±2.0µm [*]	76±4.0µm [*]

^{*} indicates p<0.01 compared to the other groups by two-tailed Student's t-test

Table 2.
Thickness of Nerve fiber layer (NFL)/Ganglion cell layer (GCL)/Inner plexiform layer (IPL), Inner nuclear layer (INL) and Outer plexiform layer (OPL) from OCT images.

	NFL/GCL/IPL	INL	OPL
C57BL/6J, 2-month-old	64.9±1.8μm	16.8±2.9μm	17.8±0.96μm
C57BL/6J, 6-month-old	65.3±1.2μm	16.0±3.2μm	18.1±1.2μm
<i>Ins2^{Akita/+}</i> , 2-month-old	66.8±2.4μm	16.3±1.9μm	17.3±2.2μm
<i>Ins2^{Akita/+}</i> , 6-month-old	53.6±3.6μm [*]	16.7±3.8μm	17.7±1.0μm

^{*} indicates p<0.01 compared to the other groups by two-tailed Student's t-test.