Characterization of Fatty Acid Binding Protein 7 (FABP7) in the Murine Retina

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PURPOSE. To characterize the mouse retina lacking fatty acid binding protein (FABP7−/−).

METHODS. Immunohistochemistry (IHC) was performed in 8-week-old mice to localize FABP7 in the retina. Retinal thickness was measured using image-guided spectral-domain optical coherence tomography images. Electoretinography was carried out to assess retinal function. Fundus photography and fundus fluorescein angiography were performed on FABP7−/− and littermate wild-type (WT) mice, and retinal vascular changes were calculated using Singapore I Vessel Assessment (SIVA) analysis. Blood glucose levels were measured in the 8-week-old WT and FABP7−/− mice. In addition, retina was processed for trypsin digestion and retinal flat mounts for isolectin staining. Transcript levels of FABP7, VEGF, GFAP and Na+/K+ATPase were quantified using real-time PCR, and protein expression was analyzed by IHC and Western blot.

RESULTS. Fatty acid binding protein 7 is expressed in the inner nuclear layer, outer plexiform layer, and photoreceptor inner segments. No significant difference in retinal thickness and ERG responses was observed between FABP7-deficient and WT retinas. FABP7−/− mice have significantly decreased retinal venular caliber retinal arteriolar fractal dimension compared with WT littermates. FABP7−/− mice showed significant increased areas of fluorescein leakage in the retina. FABP7−/− mice exhibited elevated high blood glucose levels compared with WT mice. Trypsin digested FABP7−/− mice retina showed increased acellular strands and endothelial cell drop outs, and reduced microvasculature branching compared with WT retina. FABP7−/− mice retina also have increased GFAP and VEGF expression.

CONCLUSIONS. Fatty acid binding protein 7 is expressed in the retina and might play an important role in maintaining retinal vasculature.

Keywords: retina, cell biology, binding protein, retinal vasculature

Fatty acid binding protein 7 (FABP7), also known as brain lipid-binding protein, belongs to a family of intracellular lipid-binding proteins known to bind and solubilize long-chain polyunsaturated fatty acids (PUFAs), as well as control intracellular lipid dynamics.1 Fatty acid binding protein 7 expression is confined primarily to the brain, except for hepatic kupffer cells.2 In the embryonic brain, FABP7 localizes primarily in the ventricular germinal cells, and is highly expressed in radial glia and immature astocytes. Its expression, however, is remarkably attenuated in the astrocytes of adult brain, consistent with its role in glial cell differentiation and proliferation.3 Fatty acid binding protein 7 knockout mice (FABP7−/−) are viable, and showed neither macroscopic nor microscopic abnormalities in both embryonic and mature brains.4 The most distinct phenotype in FABP7 knockout mice is the enhancement of fear memory and anxiety, presumably through the modulation on neuronal N-methyl-D-aspartate receptor activity, mediated by a 4% decrease in the total brain content of docosahexaenoic acid.
(DHA, representing n-3 FAs), and a corresponding increase of arachidonic acid (representing n-6 FAs).\textsuperscript{4}

In humans, FABP7 has been associated with psychiatric disorders, such as schizophrenia.\textsuperscript{5,6} Upregulation of FABP7 is associated with poor prognosis in glioblastoma.\textsuperscript{7–9} Conferring its stem cell features, and promotes metastasis and invasion through cell proliferation and migration.\textsuperscript{10} Fatty acid binding protein 7 is a well-established marker for neuro-progenitor stem cells.\textsuperscript{12,13} In the adult brain, FABP7 regulates the proliferative response of astrocytes to injury.\textsuperscript{14} More recently, FABP7 also has been shown, in astrocytes, to modulate receptor-mediated signal transduction in response to external stimuli via regulation of lipid raft function. In particular, Caveolin-1 expression is reduced in the absence of FABP7, with corresponding reduction of ligand-dependent accumulation of Toll-like receptor 4 and glial cell-line–derived neurotrophic factor receptor alpha 1.\textsuperscript{1,15}

Although FABP7 was previously thought to be expressed exclusively in the brain, recent reports suggest a role in retinal diseases. Transcriptome analysis of retina obtained from type I diabetic rats showed upregulation of FABP7 mRNA that is not normalized by insulin treatment.\textsuperscript{16} In animal models of neurodegeneration, retinal astrocytes and Müller glial cells express FABP7 after kainate-induced injury, as part of their gliotic, de-differentiation, and regenerative efforts.\textsuperscript{17} Last, FABP7 has been shown in vitro to bind preferentially to DHA\textsuperscript{18,19} This is of particular interest to the retina because DHA constitutes a major structural component of photoreceptor phosopholipid membranes. Reduced plasma levels of DHA have been observed in various retinal diseases, such as in patients with retinitis pigmentosa.\textsuperscript{20–22} In addition, an inverse relationship between diets high in DHA and progression of AMD has been demonstrated in patients.\textsuperscript{23} However, the role of FABP7 in the retina is yet to be studied. In this article, we characterize the expression, localization, and function of FABP7 in the mouse retina.

**Materials and Methods**

**Animals**

Wild-type (WT) C57BL/6 mice were purchased from the National University of Singapore (Singapore). FABP7\textsuperscript{−/−} mice were obtained from Professor Owada’s laboratory, Institute of Laboratory Animals, Yamaguchi University School of Medicine, Ube, Japan.\textsuperscript{4} In our facility, FABP7\textsuperscript{−/−} mice were further crossedbreed with C57BL/6 WT mice for at least 10 generations to produce WT and FABP7\textsuperscript{−/−} mice used in the present study. Animals were housed on a 12-hour light/12-hour dark cycle with food and water provided ad libitum. Eight-week-old mice were used in the study. Handling and care of all animals were performed according to the guidelines approved by the SingHealth Institutional Animal Care and Use Committee, Singapore, and is conducted in accordance with the ARVO recommendations for animal experimentation.

**Blood Glucose Measurements**

Animals were fasted for 4 hours before glucose measurements. Animals were kept on warmed pads for 5 to 10 minutes for better visualization of the veins and the collection site was wiped with 70% ethanol before blood collection. Blood samples were obtained by pricking the lateral tail veins using sterile 27-G needles. Blood was milked gently from the tail, allowing one droplet of blood to be placed on glucose test strips. Random blood glucose was read using a hand-held glucometer (Accuchek Performa; Roche Diagnostics, Basel, Switzerland). Direct pressure was applied on the tail to achieve hemostasis.

**Fundus Photography and Fundus Fluorescein Angiography (FFA)**

Digital color fundus photographs were taken using a MICRON IV comprehensive system for rodent retinal imaging (Phoenix Research Labs, Pleasonton, CA, USA) after pupil dilation with topical administration of 1% tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA) and 2.5% phenylephrine (Bausch and Lomb Pharmaceuticals, Inc., Tampa, FL, USA) ophthalmic solutions. Mice were anesthetized with a combination of ketamine (20 mg/kg body weight) and xylazine (2 mg/kg body weight).\textsuperscript{24} For FFA, mice were injected intraperitoneally with 10% sodium fluorescein dye at a dose of 0.01 mL per 5 to 6 g body weight and fundus images were obtained using MICRON IV. Quantitation of vascular leakage was analyzed using the ImageJ version 1.48 software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA). Mean intensity values of the capillaries and vascular leakage were acquired and measured by excluding the value of large vessels (with gray value more than 100).

**Insulin Measurements**

Wild-type and FABP7\textsuperscript{−/−} mice were starved for 6 hours and blood was collected from the tail vein into EDTA-coated tubes. Plasma was isolated and insulin levels were determined using an Ultrasensitive Insulin ELISA kit (Alpco, Windham, NH, USA).

**Optical Coherence Tomography (OCT)**

Image-guided spectral-domain OCT images of 2-μm resolution were taken using inSight software (Phoenix Research Labs, Plesanton, CA). During acquisition of images, mouse cornea was always kept moist with application of ophthalmic gel. The relative intensity of the ellipsoid zone (EZ) was estimated using ImageJ version 1.48; National Institutes of Health). At each region of interest, measurement of the EZ and the inner nuclear layer (INL) intensity was performed using a selection of 20 pixels laterally and 6 pixels vertically. The relative intensities of the EZ was determined as EZ:INL intensity ratio.

**Singapore I Vessel Assessment (SIVA) Analysis**

Digital colored fundus photography images were used for SIVA analysis, a semiautomated computer-assisted program, used to measure specific retinal vascular parameters (tortuosity, branching angle, fractal dimension, and caliber) of the mice retinal vasculature for FABP7\textsuperscript{−/−} and littermate WT mice as described earlier.\textsuperscript{25,26} Retinal vascular tortuosity reflects the straightness of the vessels (smaller tortuosity value indicates straighter retinal vessels). Retinal vascular tortuosity was computed as the integral of the curvature square along the path of the vessel, normalized by the total path length; this measure is dimensionless, as it represents a ratio measure. The estimates were summarized as retinal arteriolar and venular tortuosity separately, representing the average tortuosity of arterioles and venules, respectively. Retinal vascular fractal dimension was calculated from a skeletonized line tracing using the box-counting method and represents a “global” measure that summarizes the whole branching pattern of the retinal vascular tree. Larger values of this dimensionless entity indicate a more complex branching pattern. Retinal vascular caliber was calculated based on the revised Knudston–Parr-Hubbard formula, as described earlier. Retinal arteriolar and venular calibers were summarized using the six largest
arterioles and venules (in terms of vessel diameter) measured from photographs, as central retinal artery equivalent and central retinal vein equivalent, respectively. Briefly, trained graders were masked to experimental layout to measure the parameters listed above using a set of standardized protocol. The standard measured area was defined within the region between 0.5 and 2.0 disc diameters away from the disc margin.

Electroretinography (ERG)

Eight-week-old animals were dark-adapted overnight (for at least 12 hours) and all the procedures were carried out under dim red light. Anesthesia and pupil dilation were induced as described above. Animals were lightly secured to a stage with fastener strips across the upper and lower back to ensure a stable, reproducible position for ERG recordings. Body temperature was maintained at 37 to 38°C with a pumped-water heating pad (TP500 T/Pump; Gaymar Industries, Orchard Park, NY, USA) fixed to the top of the stage. Electroretinograms were recorded (Epsilon; Diagnosys LLC, Lowell, MA, USA) with corneal monopolar electrodes. A gold-cup electrode was placed in the mouth to serve as the reference electrode, and a silver–silver chloride electrode (Grass-Telefactor, West Warwick, RI, USA) was placed in the tail to serve as the ground electrode. Recordings were performed at a wide range of stimulus intensity (−3.0 to 1.0 log cd.s/m²) in dark-adapted (scotopic) condition. The response at each flash intensity was an average of at least five trials. Signals were band-pass filtered from 1 to 100 Hz and were acquired at 1 kHz. Each animal underwent an ERG recording session for approximately 30 minutes.

Hematoxylin and Eosin Staining

The mice were euthanized with an intravenous injection of pentobarbital (100 mg/kg). Dissected eye cups were incubated in Perfix (4% paraformaldehyde [PFA], 20% isopropanol, 2% trichloroacetic acid, and 2% zinc chloride) for 24 hours, dehydrated in graded alcohol series, and embedded in paraffin; 5-μm tissue sections were cut using a microtome (Leica Microsystems, Heidelberg, Germany) and placed on polylysine-coated glass slides. The slides were heated at 60°C for 30 minutes on a hot plate, and stained with hematoxylin for 40 seconds and eosin for 25 seconds (Surgipath Europe, Ltd., Peterborough, Cambridgeshire, UK). The tissue sections were washed in tap water, mounted with paramount and imaged under bright field using a Zeiss AX10 fluorescence microscope equipped with an Axio Vision 4.7.1 Imaging System (Carl Zeiss Microscopy GmbH, Jena, Germany).

Retinal Flat Mounts

Retina whole mounts were prepared as previously described27 with minor modifications. Wild-type and FABP7−/− eyes were enucleated and fixed in 4% PFA in PBS for 15 minutes at room temperature. The eyes were then transferred to cold 1x PBS on ice for 5 to 10 minutes. The neural retina and choroid/RPE were dissected separately and placed in cold (−70°C) ethanol. Retinas were then rinsed in PBS and blocked in 1% Triton-X/ PBS for 30 minutes. The whole mounts were then incubated with Isolectin GS-B4 from Griffonia simplicifolia, Alexa Fluor 594 Conjugate (I21413 1:100; Molecular Probes, Eugene, OR, USA) overnight at 4°C. Stained whole mounts were flat-mounted with Prolong Gold (Invitrogen, Carlsbad, CA, USA) and left overnight. All the imaging was performed with a laser-scanning confocal fluorescence microscope.

Trypsin Digest

Trypsin digests of the mice retina were prepared as previously described,28 with minor modifications. Wild-type and FABP7−/− eyes were enucleated and fixed in 10% neutral buffered formalin for 5 hours at room temperature. Retinas were dissected out and washed with filtered water five times in 30-minute intervals with gentle shaking. Trypsin digest was performed by overnight incubation in 1x TrypLE (Invitrogen) at 37°C. The retinas were washed with filtered water and incubated in water for 2 hours to allow the retinal tissue to detach from the retina vasculature network. The retinal vasculatures were prepared by peeling off the internal limiting membrane and removal of adherent retinal tissue by repeated washes in filtered water using mild agitation from a 1-ml pipette. Retinal vasculatures were unfolded and mounted on a glass slide and allowed to air dry completely. Retinal vasculature flat mounts were stained using a periodic acid-Schiff staining kit (ab150680; Abcam, Cambridge, MA, USA) and mounted with Permount (Fisher Scientific, Fair Lawn, NJ, USA). The slides were visualized under bright field with a Nikon imaging system (Nikon Corporation, Tokyo, Japan).

Real-Time PCR

Retinas were dissected after enucleation of eyes of WT and FABP7−/− mice. Isolated tissue was placed immediately in 100-μl RLT buffer consisting of 1% β-mercaptoethanol. Total RNA was extracted using an RNeasy Mini Kit according to manufacturer’s instructions (Qiagen, Valencia, CA, USA). RNA samples were reverse-transcribed using Superscript III with random primers (Life Technologies, Carlsbad, CA, USA). Real-time PCR was performed on a LightCycler 480 II System (Roche, Indianapolis, IN, USA) using SYBR green and genespecific primers to quantify FABP5, FABP7, FABP12, glial fibrillary acidic protein (GFAP), Na+K+-ATPase, and VEGF transcripts. The real-time PCR primers used in this study are listed in the Table. The PCR cycle involves preincubation at 95°C for 5 minutes, and 45 amplification cycles at 95°C for 10 seconds, 60°C for 15 seconds, and 72°C for 30 seconds. Melting curve analysis was carried out to verify amplification reaction. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene.
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**Immunohistochemistry**

Enucleated eyes taken from 8-week-old mice were placed into 4% PFA in 1x PBS for fixation before washing them in 1x PBS. Eyes were subsequently placed in a gradient of sucrose prepared in 1x PBS before embedding in Tissue Freezing Medium (Leica Biosystems, Heidelberg, Germany) and frozen at −80°C. Frozen eye blocks were placed in −20°C for 30 minutes to thaw before sectioning. Tissue sections of 8-μm thickness were cut using a cryostat (Microm, Heidelberg, Germany) and frozen at −80°C. Sections were dried for 30 minutes to −80°C. Sections were then incubated with either a single primary antibody for single immunofluorescence staining or two primary antibodies for double immunofluorescence staining at 4°C overnight. Slides were washed three times with 1x PBS before incubation with a respective secondary antibody for single immunofluorescence staining or two different respective secondary antibodies for double immunofluorescence staining. Slides were washed three times with 1x PBS before being mounted with Histo mount (Fisher Scientific, Waltham, MA, USA) and UltraCruz (Santa Cruz Biotechnology, Dallas, TX, USA) mounting medium. All sections were imaged with a Nikon Ti-E confocal microscope (Eclipse Ti-E with Confocal Micros C2; Nikon Corporation), at 2048 × 2048-pixel resolution.

**Western Blots**

Retinas were isolated from WT and FABP7−/− mice and immediately snap frozen in dry ice and subsequently stored at −80°C. Tissues were lysed and homogenized using motor pestle in 100 μl radioimmunoprecipitation assay buffer containing 1% protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA). Samples were centrifuged at 12,879g for 10 minutes at 4°C. Supernatants were collected and protein quantification was assessed using Pierce BCA Protein Assay Kit (Thermo Scientific). Protein was loaded onto 4% to 12% Bis-Tris gel (Life Technologies, Carlsbad, CA, USA) and run at 160 V for 30 minutes and transferred onto polyvinylidene difluoride (PVDF) membrane at 20 V for 1 hour. The blots were blocked in 5% nonfat dry milk containing 0.1% Tween-20 in 1x Tris-sodium/potassium ATPase alpha-5 (1:200, mouse monoclonal, MAB5356 EMD; Millipore, Billerica, MA, USA), opsin short wavelength (SW) (1:400, goat polyclonal, SCI-456; Santa Cruz Biotechnology, Dallas, TX, USA), opsin medium wavelength/long wavelength (MW/LW) (1:100, goat polyclonal, SC2217; Santa Cruz Biotechnology), sodium/potassium ATPase alpha-5 (1:200, mouse monoclonal, MA3-915; Thermo Fisher Scientific, Waltham, MA, USA), GFAP (1:250, polyclonal rabbit, 20354; Dako, Carpinteria, CA, USA), VEGF-A (1:100, rabbit polyclonal, ab96154; Abcam), isolectin fluorescein conjugated (1:100, FL-1201; Vector Laboratories, Burlingame, CA, USA), and Desmin (1:50, mouse monoclonal, M0760; Dako) were performed using optimized dilutions of primary antibodies. Tissue sections on slides were fixed with 4% PFA for 10 minutes, followed by two washes in 1x PBS and blocking in 1% BSA for 30 minutes. Slides were then incubated with either a single primary antibody for single immunofluorescence staining or two primary antibodies for double immunofluorescence staining at 4°C overnight. Slides were washed three times with 1x PBS before incubation with a respective secondary antibody for single immunofluorescence staining or two different respective secondary antibodies for double immunofluorescence staining. Slides were washed three times with 1x PBS before being mounted with Histo mount (Fisher Scientific, Waltham, MA, USA) and UltraCruz (Santa Cruz Biotechnology, Dallas, TX, USA) mounting medium. All sections were imaged with a Nikon Ti-E confocal microscope (Eclipse Ti-E with Confocal Micros C2; Nikon Corporation), at 2048 × 2048-pixel resolution.

**Statistics**

Data were analyzed using GraphPad Prism 6.0 (Graphpad Software, Inc., La Jolla, CA, USA). Student’s t-test was used to compare data between two groups; P < 0.05 was considered to be significant.

**FABP7−/− Mice Display Subtle Changes in Retinal Thickness and Function**

To further understand the function of FABP7 in the retina, we looked at mice lacking FABP7 protein (FABP7−/−).24 In view of the localization of FABP7 to inner nuclear, outer plexiform, and photoreceptor ISs, we were interested to know if there were any structural or functional abnormalities in retina lacking FABP7. Optical coherence tomography analysis of FABP7−/− mice showed normal retinal thickness of 230.6 ± 7.02 μm, which is similar to that of the WT mice 232.18 ± 6.28 μm (Fig. 2A). We also looked into the relative intensity of the ellipsoid zone between the two groups but found no significant differences (data not shown). In hematoxylin-eosin (H&E) staining, FABP7−/− mice showed minor architecture disorganization in the photoreceptor IS/outer segment (OS) compared with littermate WT mice (Fig. 2B). Moreover, normal ERG waveforms were observed in FABP7−/− mice, with no significant differences in average a-wave amplitude and b-wave amplitude (10 cd.s/m²) compared with WT mice (Fig. 2C). Next, we asked if loss of FABP7 leads to compensatory changes in FABP5 and FABP12. Analysis of mRNA expression revealed a decreasing, but not significant, trend in FABP5 levels (Supplementary Fig. S1C) but a significant increase in FABP12 (P < 0.01; Supplementary Fig. S1C).

**FABP7−/− Mice Develop Defects in Retinal Venular Caliber and Retinal Arteriolar Fractal Dimensions**

Much to our surprise, despite the primary localization of FABP7 to the ONL, OPL, and photoreceptors, FABP7−/− mice showed...
abnormalities in the retinal vasculature. Fundus photographs of FABP7−/− mice were analyzed with SIVA, a computer-aided integrated platform for retinal vasculature analysis. FABP7−/− mice have significantly decreased retinal venular caliber (Fig. 3N; 26.61 ± 1.15 μm; P = 0.018) and decreased retinal arteriolar fractal dimension (Fig. 3O; 0.98 ± 0.04 μm; P = 0.016) compared with WT mice (28.30 ± 2.24 μm and 1.03 ± 0.06 μm), respectively. However, this decrease was not associated with significant differences in retinal arteriolar or venular tortuosity (Figs. 3Q, 3R). With increasing age, retinal arteriolar caliber narrowing is associated with the absence of FABP7 in older mice of 12 weeks old (data not shown). Fundus fluorescein angiography depicted subtle differences in retinal vasculature, such as reduced venular caliber in FABP7−/− mice (Fig. 3I) compared with WT (Fig. 3C), consistent with SIVA analysis. In addition, there was increased vessel permeability in FABP7−/− mice as shown by fluorescein dye leakage on FFA (Fig. 3J). Quantitation of vascular leakage using Image J (Supplementary Fig. S3) confirmed that FABP7−/− mice have increased vascular leakage (mean intensity of 10.91, n = 10, P < 0.01) compared with WT (mean intensity of 9.24, n = 10).

**FABP7−/− Mice Exhibit a Vascular Phenotype**

We next asked the question of whether FABP7−/− animals display an inflammatory and vascular phenotype. We found that
FABP7−/− mice exhibit significantly higher blood glucose levels (Fig. 4A) compared with their WT littermates (8.18 ± 0.29, n = 19 for WT and 10.36 ± 0.29, n = 22 for FABP7−/−; P < 0.001) but there were no significant differences in the plasma insulin levels (Supplementary Fig. S4). Trypsin digestion showed increased acellular strands and less microvasculature branching in FABP7−/− mice retina compared with FABP7 WT retina (Fig. 4B). Similarly, FABP7−/− mouse retina showed reduction in
FIGURE 3. *FABP7*−/− mice exhibit leaky vessels with defective retinal venular caliber and retinal arteriolar fractal dimensions. (A–L) Retinal fundus photography and FFA images of WT (A–F) compared with *FABP7*−/− mice (G–L). *FABP7*−/− mice have an increase in areas of fluorescein leakage (white arrow in [J]), indicative of increased vascular permeability. (M–R) Singapore I vessel assessment analysis was performed on retinal vasculature from fundus photographs in both WT and *FABP7*−/− mice. As shown in the box plot graphs, *FABP7*−/− mice had significantly decreased retinal venular caliber (26.61 ± 1.15 μm, *P* = 0.018) compared with WT mice (28.30 ± 2.24 μm). In addition, *FABP7*−/− mice showed significantly decreased retinal arteriolar fractal dimension (0.98 ± 0.04 μm, *P* = 0.016) compared with WT mice (1.03 ± 0.06 μm). There are, however, no differences in retinal arteriolar caliber, retinal venular fractal dimension, and retinal venular/arteriolar curvature tortuosity between WT and *FABP7*−/− mice.
microvasculature density and branching, as compared with WT retina, as observed in the isolectin-stained retinal flat mounts (Fig. 4C), consistent with the histopathologic findings following trypsin digestion. \( FABP7^{-/-} \) retina depicts increased activity of Müller cells, as shown by increased expression of GFAP in the inner retina (Figs. 5A, 5B). This was associated with increased VEGF expression, particularly in the nerve fiber layer/ganglion cell layer, as well as the photoreceptor IS/OS layers (Figs. 5C, 5D). Western blot analysis showed a corresponding increase in both GFAP and VEGF expression in \( FABP7^{-/-} \) total retinal lysate (Fig. 5E), associated with a corresponding increase in the VEGF mRNA by real-time PCR (Fig. 5F).

**DISCUSSION**

We present in this study for the first time, expression, localization, and functional role of FABP7 in the retina. Fatty acid binding protein 7 is expressed primarily in the INL, outer retina, where it colocalizes with \( \text{Na}^+\text{K}^+\text{ATPase} \) in the photoreceptor IS, as well as the OPL, where photoreceptors synapse with the horizontal cells. The photoreceptor ISs and OSs are unique due to their high phospholipid content, and, because FABP7 has been shown to bind to DHA in vitro,\(^\text{19}\) it is not surprising that FABP7 is localized to the photoreceptor ISs. Photoreceptor membrane phospholipids are heavily acylated with PUFAs, with DHA being the most abundant PUFA present in the retina.\(^\text{31}\) Docosahexaenoic acid in the RPE cells is either taken up systemically from the choriocapillaries, or from phagocytosis of photoreceptor tips. Docosahexaenoic acid is recycled within the retina, as it is actively taken up by the IS in a paracrine fashion through the inter-photoreceptor matrix, before being returned to photoreceptor OSs for biogenesis of disc membranes.\(^\text{52}\) The regulation and role of FABP7 in this process remains largely unknown.
Fatty acid binding protein 7 expression in the retina was first reported in a transcriptome analysis of retina obtained from type I diabetic rats, suggesting a role in diabetic retinopathy.16 Despite the significant expression of FABP7 in the retina, FABP7−/− mice exhibit only very subtle changes in retinal structure and function in the present study. Optical coherence tomography analysis showed similar retinal thickness in FABP7−/− mice compared with littermate WT mice. FABP7−/− mice displayed normal ERG responses, with no significant differences in a-wave and b-wave amplitude.

**Figure 5.** FABP7−/− depict inflammatory and vascular phenotype. (A–D) Immunohistochemistry of WT versus FABP7−/− mice retina, showing increased expression of both GFAP and VEGF in FABP7−/− mice retina. Glial fibrillary acidic protein expression is increased mostly in the inner retina, whereas VEGF expression is increased through the retinal layers, particularly in the photoreceptor layers. Scale bar: 50 μm. (E) Western blot of total retinal lysate shows a corresponding increase in VEGF (by 1.4-fold) and GFAP protein levels (by >2.0-fold) (n = 6). (F) Quantitative PCR of total retinal lysate shows a corresponding increase in VEGF mRNA (n = 6).
compared with WT mice. Retinas enriched in DHA and n-3 very long chain fatty acids have been shown to have supranormal ERG responses, with high amplitudes for both scotopic and photopic a-waves and b-waves.33 Normal a-wave and b-wave amplitudes in our FABP7/C0 mice suggest that FABP7 is not crucial for photoreceptor function under physiological conditions. Rather FABP7 may play a role in regulating photoreceptor function in response to “stress” conditions.

Our study suggests that loss of FABP7 results in elevated serum random glucose levels, but the mechanism underlying this is unclear. Fatty acid binding protein 7 expression is confined primarily to the brain, except for hepatic Kupffer cells and pancreatic islets of Langerhans.2 Fatty acid binding protein 7 may have a yet-defined role in glucose metabolism through insulin secretion.

Definitive studies linking the FABP family of proteins to glucose regulation are lacking. However, adipocyte fatty acid binding protein (FABP4) and liver fatty acid binding protein (FABP1) have both been implicated in the development of insulin resistance.1 In particular, human FABP1 expression correlates positively with obesity and insulin resistance, with a positive Spearman correlation with fasting blood glucose in nondiabetic subjects.34 Increased FABP1 expression is associated with streptozocin-induced diabetes in rats, and type 1 diabetes in mice.35-37

Interestingly, despite absence of FABP7 colocalization with desmin-stained pericytes and isolectin-stained endothelial cells, FABP7-deficient retina exhibits a vascular phenotype. FABP7/C0 mice have significantly decreased retinal venular caliber and retinal arteriolar fractal dimension, as seen in SIVA analysis. However, this was not associated with significant difference in retinal arteriolar or venular tortuosity. In humans, wider retinal venular caliber has been shown to be present in prediabetic retinopathy state as well as in metabolic syndrome.38 Often reflecting microvascular changes that occur early in the development of diabetes.39 Greater retinal fractal dimension, representing increased geometric complexity of the retinal vasculature, is also independently associated with early diabetic retinopathy signs in type 1 diabetes.40 Generalized retinal arteriolar narrowing, on the other hand, is an early characteristic sign of hypertensive retinopathy.41,42 It is unclear which pathophysiological process underpins these changes in retinal vasculature in FABP7/C0 mice, perhaps it is a result of changes in blood flow associated with retinal hypoxia,43 or alternatively, it may reflect inflammatory processes due to impaired metabolism, as supported by epidemiologic findings of changes in retinal venules with elevated systemic inflammatory markers.44,45 In light of elevated serum glucose levels in FABP7/C0 mice, the decreased retinal venular caliber and retinal arteriolar fractal dimension observed in FABP7/C0 mice are likely early changes, which precede the later changes of increased retinal venular caliber and increased retinal arteriolar fractal dimension commonly observed in chronic diabetes or hypertension. Indeed, with increasing age, 12-week-old FABP7/C0 mice have been shown to have narrower retinal arteriolar caliber compared with littermate WT mice (data not shown). Moreover, trypsin digestion of the retinas from FABP7/C0 mice exhibited increased acellular strands and less microvasculature branching. This phenomenon of pericyte dropout, and subsequent endothelial cell loss, is pathogenic of changes in early diabetic retinopathy.46

It remained to be seen whether the observed changes in the retinal vasculature of FABP7/C0 mice are due to mild hyperglycemia or retina-specific effect of FABP7. In established models of streptozocin-induced diabetic C57B6 mice, hallmark of early diabetic retinopathy, such as acellular capillaries, apoptosis of vascular cells, and pericyte ghost in the retina, were typically observed only at 6 months after induction of diabetes, at significantly higher levels of serum glucose >14 mM.47 In contrast, the FABP7/C0 mice developed retinal vascular changes at an accelerated rate, at 2 months, despite being only mildly hyperglycemic (10.35 ± 0.2931 mM). This seems to suggest that the observed retinal vasculature phenotype in the FABP7/C0 mice may be a retinaspecific effect, but this remains to be clarified with further experiments. Correspondingly, these changes in the retinal vasculature are associated with activation of Müller glial cells, as characterized by upregulation of GFAP in the FABP7/C0 retina. This is a feature common to early events observed in diabetic rats,48 reflecting an increased inflammatory response in the FABP7/C0 retina. In addition, by IHC staining, VEGF expression was increased in all layers of the retina (ganglion cell layer, inner plexiform, INL/ONL), particularly in the photoreceptor and RPE layers. This was shown quantitatively by quantitative PCR (qPCR) and Western blot analysis. It is possible that this increase in VEGF expression is driving the vasculature changes observed in the FABP7/C0 retina.

Accumulating evidence suggests that photoreceptor cells play a major role in oxidative stress and local inflammation early in the diabetic retina, through the production of reactive oxygen species;49 however, the exact mechanism by which this occurs is presently unknown. Fatty acid binding protein 7 also has been recently identified to be upregulated, in a hypoxia-inducible factor-1α (HIF-1α)-dependent manner, after chronic treatment with anti-VEGF (anti-VGF) in a mouse xenograft model of human U87 glioblastoma cells in severe combined immunodeficiency (SCID) mice. Fatty acid binding protein 7 increases fatty acid uptake into tumor cells for protection against reactive oxygen species toxicity.50 One can speculate that FABP7 plays a similar role in the retina to mediate oxidative stress under “stressed” conditions, such as in diabetes or in response to chronic anti-VEGF treatment; however, further work is required to elucidate this mechanism and its relation to DHA content. We conclude that FABP7 is present in the retina and plays an important role in the maintenance of retinal vasculature.

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