Altered Anterior Segment Biometric Parameters in Mice Deficient in SPARC

Henrietta Ho,1 Hla M. Htoon,2 Gary Hin-Fai Yam,2–4 Li Zhen Toh,2 Nyein Chan Lwin,2 Stephanie Chu,2 Ying Shi Lee,2 Tina T. Wong,1–5 and Li-Fong Seet2–4

1Singapore National Eye Centre, Singapore
2Singapore Eye Research Institute, Singapore
3Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
4Duke-NUS Medical School Singapore, Singapore
5School of Materials Science and Engineering, Nanyang Technological University, Singapore

Correspondence: Li-Fong Seet, Singapore Eye Research Institute, 20 College Road, The Academy Discovery Tower, #06-98, Singapore 169856; seet.li.fong@seri.com.sg, Tina T. Wong, Glaucoma Service, Singapore National Eye Centre, 11 Third Hospital Avenue, Singapore 168751; tina.wong.t.l@snec.com.sg.
Submitted: July 5, 2016
Accepted: November 19, 2016
Citation: Ho H, Htoon HM, Yam GH-F, et al. Altered anterior segment biometric parameters in mice deficient in SPARC. Invest Ophthalmol Vis Sci. 2017;58:386–393. DOI:10.1167/iovs.16-20261

PURPOSE. Secreted protein acidic and rich in cysteine (SPARC) and Hevin are structurally related matricellular proteins involved in extracellular matrix assembly. In this study, we compared the anterior chamber biometric parameters and iris collagen properties in SPARC-, Hevin- and SPARC-/Hevin-null with wild-type (WT) mice.

METHODS. The right eyes of 53 WT, 35 SPARC-, 56 Hevin-, and 63 SPARC-/Hevin-null mice were imaged using the RTVue-100 Fourier-domain optical coherence tomography system. The parameters measured were anterior chamber depth (ACD), trabecular-iris space area (TISA), angle opening distance (AOD), and pupil diameter. Biometric data were analyzed using analysis of covariance and adjusted for age, sex, and pupil diameter. Expression of Col1a1, Col8a1, and Col8a2 transcripts in the irises was measured by quantitative polymerase chain reaction. Collagen fibril thickness was evaluated by transmission electron microscopy.

RESULTS. Mice that were SPARC- and SPARC-/Hevin-null had 1.28- and 1.25-fold deeper ACD, 1.45- and 1.53-fold larger TISA, as well as 1.42- and 1.51-fold wider AOD than WT, respectively. These measurements were not significantly different between SPARC- and SPARC-/Hevin-null mice. The SPARC-null iris expressed lower Col1a1, but higher Col8a1 and Col8a2 transcripts compared with WT. Collagen fibrils in the SPARC- and SPARC-/Hevin-null irises were 1.5- and 1.7-fold thinner than WT, respectively. The Hevin-null iris did not differ from WT in these collagen properties.

CONCLUSIONS. SPARC-null mice have deeper anterior chamber as well as wider drainage angles compared with WT. Therefore, SPARC plays a key role in influencing the spatial organization of the anterior segment, potentially via modulation of collagen properties, while Hevin is not likely to be involved.

Keywords: SPARC, Hevin, OCT; anterior chamber, collagen
Since the first descriptions of SPARC, several new members containing the signature ECM calcium-binding (EC) domain immediately preceded by a follistatin-like module, have been described. Among the members, Hevin (also known as SPARC-like 1, SC-1, Mast9, and ECM2) exhibits the highest similarity to SPARC, with the amino acid identity between mouse SPARC and Hevin at 70%. Like SPARC, Hevin possesses the conserved collagen-binding motif that supports binding to fibrillar and network collagen. Hence, similar to SPARC, Hevin is potentially involved in the development and remodeling of a variety of tissues including the central nervous system, heart, lung, kidney, muscles, and eye via the regulation of collagen fibrillogenesis and modulation of the ECM structure. Deletion of Hevin in the mouse resulted in a similar phenotype as the SPARC-null mouse including the development of cataracts, enhanced solid tumor growth, and altered wound healing. Moreover, the copper-binding and EC domains of SPARC and Hevin are similar, suggesting potential compensatory functions for the two proteins.

Collagen is a critical determinant of tissue architecture, chamber geometry, and biomechanical function of the heart. Dysfunctional collagen maturation, as caused by SPARC deficiency, has been shown to aggravate cardiac rupture and increased incidence of cardiac failure after myocardial infarction. We therefore hypothesize that SPARC and/or Hevin may also play key roles in determining anterior chamber characteristics that may have implications for functional properties of the eye. Indeed, SPARC-null mice have been shown to feature lower intraocular pressures. However, it remains unknown whether SPARC or Hevin deficiency alters the anterior segment structure of the eye. In this study, we examined the effect of deficiency in SPARC, Hevin, or both SPARC and Hevin, on anterior chamber characteristics by anterior segment optical coherence tomography (AS-OCT).

The major anterior segment biometric parameters measured include anterior chamber depth (ACD), trabecular-iris space area (TISA), and angle opening distance (AOD). By comparing these biometric measurements in the knockout mice with wild-type (WT) mice, we demonstrate that SPARC, but not Hevin, is involved in determining key anterior segment structures such as ACD and AOD. Furthermore, we reveal that the altered biometric parameters in the SPARC-deficient mice are associated with reduced type I collagen transcript expression as well as decreased collagen fibril thickness in the iris.

**Materials and Methods**

**Animal Husbandry**

All animal experiments were approved by the Institutional Animal Care and Use committee and treated in accordance with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. Both SPARC-null and Hevin-null strains backcrossed onto a 129SVE background were kind gifts from E. Helene Sage of the Benaroya Research Institute at Virginia Mason (Seattle, WA, USA). We mated SPARC- and Hevin-null mice to generate SPARC/Hevin-null double-null mice. Wild-type mice were obtained from the inbreeding of heterozygous mice. All mice were bred and maintained at the SingHealth Experimental Medicine Centre (Singapore General Hospital, Singapore). Genotypes were determined by performing polymerase chain reaction on purified tail DNA. We imaged mice aged at least 8 weeks when adulthood was reached, and when the iridocorneal angle and its structures have completed morphogenesis.30

**Anterior Segment-Optical Coherence Tomography (AS-OCT) Imaging**

Mice were anesthetized by intraperitoneal injection of a 0.1 mL ketamine/xylazine mixture containing 2 mg/mL xylazine hydrochloride (Troy Laboratories, Smith-field, Australia) and 20 mg/mL ketamine hydrochloride (Ketamine, Parnell Laboratories, Alexandria, Australia) before being imaged using the Fourier-domain OCT system (Optovue, Inc., Fremont, CA, USA). The system used in this study was fitted with an additional corneal lens adapter (cornea anterior module-low magnification [CAM-L]) on the device to provide a scan length of 2 to 6 mm and an image size of 12 × 8 mm. Mice were positioned on an adjustable platform placed before the chinrest of the device. Scans were centered on the pupil and taken along the horizontal axis (nasal-temporal angles at 0–180°). A series of images were obtained, from which three clear scans were selected, and an average value for each parameter (see below) was obtained. All images were taken and measured using OCT system software (RTVue version 6.1.0.4; Optovue, Inc.). Images of the right eye were analyzed for all the genotypes.

The following anterior chamber parameters were measured manually (Fig. 1):

1. Anterior chamber depth (ACD) was defined as the distance from the posterior cornea surface to the anterior surface of the lens.
2. Angle width parameters. Angle opening distance (AOD) 500 is defined as the perpendicular distance between the trabecular meshwork and the iris at 500 μm anterior to the scleral spur. However, as the angle structures are poorly defined in mice due to size, the point where the iris and the inner sclera wall met was taken to represent a rough estimate of the scleral spur. Therefore, AOD 500 is defined as the distance between the posterior cornea and anterior iris surface, along a line perpendicular to the cornea at 500 μm from the point of iridociliary contact. Traditionally, trabecular-iris space area 500 (TISA 500) is bounded anteriorly by the AOD 500 line, posteriorly by a line drawn from the scleral spur perpendicular to the plane of the inner scleral wall to the opposing iris, superiorly by the inner corneoscleral wall, and inferiorly by the iris surface. As the landmarks for the posterior limit of TISA 500 are too small to be clearly identified in mice eyes, the posterior perpendic-
ular line is replaced by a single point at the angle of iridoscleral contact that yields a triangular area (Fig. 1).

3. Pupil diameter (PD) was measured as a line drawn between the most central points of the nasal and temporal iris pigment epithelium.

**Real-Time Quantitative Polymerase Chain Reaction (qPCR)**

Iris specimens were lysed by sonication and total RNA was recovered with Trizol (Invitrogen Corp., Carlsbad, CA, USA) and the RNeasy extraction kit (Qiagen, Valencia, CA, USA) as described previously. Total RNA was reverse-transcribed into cDNA using random hexamer primers (Invitrogen Corp.) with reverse transcriptase (Superscript III; Invitrogen Corp.). All qPCR reactions, comprising the master mix (Power SYBR Green PCR Master Mix; Applied Biosystems, Inc., Carlsbad, CA, USA), were performed in triplicates in volumes of 10 μL in 384-well microtiter plates and run using the Roche LightCycler 480 System (Roche Diagnostics Corp., Indianapolis, IN, USA). All mRNA levels were measured as CT threshold levels. Rpl13a was determined to be the most suitable housekeeping gene of irises by the 2\(^{-}\text{ΔΔCT}\) method. Primers used for qPCR were: Rpl13a-forward, 5′-GAGGTCGGGTGGAAGTACCA-3′, and Rpl13a-reverse, 5′-TGACACCTGGCCTTTTCCTTT-3′; Gapdh-forward, 5′-GAGGTCGGGTGGAAGTACCA-3′, and Gapdh-reverse, 5′-TGCATCTTGGCCTTTTCCTT-3′; Rna18s1-forward, 5′-AGTACCCACACCTACCCCAA-3′, and Rna18s1-reverse, 5′-GAGGTCGGGTGGAAGTACCA-3′; Actb-forward, 5′-GAGGTCGGGTGGAAGTACCA-3′, and Actb-reverse, 5′-GAGGTCGGGTGGAAGTACCA-3′; Gapdh-forward, 5′-ATGCCGCTCTTCTGATCTAC-3′, and Gapdh-reverse, 5′-GAGGTCGGGTGGAAGTACCA-3′; and Col8a1-forward, 5′-GAGGTCGGGTGGAAGTACCA-3′, and Col8a1-reverse, 5′-GAGGTCGGGTGGAAGTACCA-3′; Col8a2-forward, 5′-GAGGTCGGGTGGAAGTACCA-3′, and Col8a2-reverse, 5′-GAGGTCGGGTGGAAGTACCA-3′.

The average ΔCT value, calculated from the WT irises for each gene, was used for calculating the fold change in gene expression in the SPARC- and Hevin-null irises by the 2\(^{-}\text{ΔΔCT}\) method. Primers used for qPCR were: Rpl13a-forward, 5′-GAGGTCGGGTGGAAGTACCA-3′, and Rpl13a-reverse, 5′-TGACACCTGGCCTTTTCCTTT-3′; Col1a1-forward, 5′-CITCACCCTACAGCCACCTTGTG-3′, and Col1a1-reverse, 5′-CTTGTTGTTTTTGATTGAGTAC-3′; Col1a2-forward, 5′-AGTACCCACACCTACCCCAA-3′, and Col1a2-reverse, 5′-TGACACCTGGCCTTTTCCTTT-3′; Col8a2-forward, 5′-GAGGTCGGGTGGAAGTACCA-3′, and Col8a2-reverse, 5′-TGACACCTGGCCTTTTCCTTT-3′.

**Transmission Electron Microscopy (TEM)**

Iris tissues were fixed sequentially with 3% glutaraldehyde (Electron Microscopy Sciences), ultrathin sections were examined using the JEM-2100 transmission electron microscope (JEOL USA, Inc., Peabody, MA, USA). The iris sections were imaged in at least five random fields. Fibril thickness was measured using Image J software (http://image.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA).

**Table 1. Characteristics of WT and Knockout Mice Imaged by AS-OCT**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WT, n = 53</th>
<th>SPARC-Null, n = 35</th>
<th>Hevin-Null, n = 56</th>
<th>SPARC-/Hevin-Null, n = 63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, wk</td>
<td>19.2 ± 7.6</td>
<td>11.9 ± 3.8</td>
<td>15.4 ± 7.0</td>
<td>14.0 ± 5.3</td>
</tr>
<tr>
<td>Sex, %male</td>
<td>64.2</td>
<td>54.3</td>
<td>26.8</td>
<td>46.0</td>
</tr>
<tr>
<td>PD, μm</td>
<td>728.5 ± 322.5</td>
<td>1478.6 ± 352.2</td>
<td>814.7 ± 306.3</td>
<td>1145.7 ± 460.7</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.

**Statistical Analysis**

Comparison of ACD, TISA, and AOD between groups was performed using analysis of covariance (ANCOVA) with post hoc Bonferroni adjustment. We used mice of different ages and sexes to sample biometric differences associated primarily with genotype and which are representative of each genotypic population as a whole. Differential PD (Table 1) can affect the assessment of anterior chamber biometrics, including TISA and AOD. Hence, to determine the biometric differences between genotypes, corrected for variations caused by PD, age, and sex, the latter variables were adjusted statistically in the ANCOVA analysis. The significance of differences in collagen transcript expression and fibril thickness between the genotypes was determined by 1-way ANOVA with Bonferroni post hoc adjustment. All the statistical analyses were performed using statistical software (SPSS, version 19; IBM SPSS Statistics for Windows, Armonk, NY, USA). P < 0.05 was considered to be statistically significant.

**Results**

A total of 53 WT, 35 SPARC-, 56 Hevin- and 63 SPARC-/Hevin-null mice were measured by AS-OCT. The average ages of the mice imaged were 19.2 ± 7.6, 11.9 ± 3.8, 15.4 ± 7.0 and 14.0 ± 5.3 weeks for WT, SPARC-, Hevin-, and SPARC-/Hevin-null mice, respectively (Table 1). Since differential PD (Table 1) can affect the assessment of anterior chamber biometrics, including TISA and AOD, all biometric analyses described henceforth were corrected for PD, as well as age and sex.

**Anterior Chamber Depth**

The mean ACDs of WT, SPARC-, Hevin-, and SPARC-/Hevin-null mice were 368.65 ± 11.42, 472.64 ± 14.70, 389.49 ± 11.14, and 460.81 ± 9.75 μm, respectively (Fig. 2A). The ACDs of SPARC- and SPARC-/Hevin-null mice were significantly different from WT (Fig. 2A), with the ACDs of both these genotypes trending toward being significantly deeper than WT by 1.28- and 1.25-fold respectively (Table 2). There was no significant difference found for ACDs between WT and Hevin-null mice (Fig. 2A).

**Trabecular-Iris Space Area**

Trabecular-iris space area was measured using a surrogate triangular area due to poor definition of the scleral spur by OCT. Mean TISA 500 for WT, SPARC-, Hevin-, and SPARC-/Hevin-null mice were 0.047 ± 0.0030, 0.068 ± 0.0039, 0.051 ± 0.0030, and 0.072 ± 0.0026 mm\(^2\), respectively (Fig. 2B). In comparison with WT, the TISA 500 in SPARC- and SPARC-/Hevin-null mice were statistically larger by about 1.45- to 1.53-fold respectively (Table 2). TISA 500 was not significantly different between WT and Hevin-null mice (Fig. 2B).

**Angle Opening Distance**

The mean AODs of WT, SPARC-, Hevin-, and SPARC-/Hevin-null mice were 184.77 ± 12.55, 262.94 ± 15.89, 209.06 ± 12.04 and 278.62 ± 10.54 μm, respectively (Fig. 2C). SPARC- and SPARC-/Hevin-null eyes had significantly greater AODs compared with WT by 1.42- to 1.51-fold, respectively (Table 2). As before, no significant difference in AOD was found between WT and Hevin-null mice (Fig. 2C).
The biometric parameters of ACD, TISA, and AOD, which were significantly different between WT and both SPARC- and SPARC-/Hevin-null mice (Fig. 3), were not significantly different between SPARC- and SPARC-/Hevin-null mice. These suggest that the phenotype of greater ACD, TISA and AOD in the SPARC-/Hevin-null mouse is likely to be contributed by SPARC deficiency alone.

**Collagen Transcript Expression and Fibril Thickness**

We previously showed that increased iris expression of COL1A1 transcript was associated with differential anterior chamber biometrics such as a shallower ACD in primary angle closure compared to primary open angle eyes.1 Moreover, mutation in the Col8a2 gene resulted in mice with an abnormally large anterior chamber.37 Thus collagen expression may potentially be involved in determining anterior chamber characteristics. To evaluate the potential association of these collagen genes with anterior segment biometric measurements, the iris tissues from WT and knockout mice were analyzed for mRNA expression of Col1a1, Col8a1, and Col8a2, by quantitative PCR (Fig. 4A). Data from the WT irises were used as baseline values for comparisons of fold changes in gene expression in the knockout irises. While Hevin-null irises did not show significant difference from WT for the collagen transcripts analyzed, the SPARC- and SPARC/Hevin-null irises expressed significantly lower levels of Col1a1 (Fig. 4A). On the other hand, only SPARC-null irises demonstrated higher levels of both Col8a1 and Col8a2 mRNAs compared with WT (Fig. 4A). Additionally, we determined that collagen fibrils in SPARC- and SPARC-/Hevin-null irises were 1.5- and 1.7-fold thinner than those in WT, respectively (Fig. 4B). In contrast, collagen fibrils in the Hevin iris did not appear significantly different in thickness relative to WT. Since Col1a1 mRNA expression and collagen fibril thickness were not significantly different between SPARC- and SPARC-/Hevin-null irises, it is likely that alteration in collagen properties in the SPARC-/Hevin-null mice is contributed by SPARC deficiency alone. Taken together, our data indicate an association between altered collagen characteristics, including reduced Col1a1 transcript expression and thinner collagen fibrils, and the distinct anterior chamber characteristics in the SPARC knockout mice, supporting the involvement of type I collagen in determining anterior chamber geometry.

**DISCUSSION**

We show for the first time in this study that deficiency in SPARC resulted in a deeper anterior chamber and wider anterior chamber angle in the mouse. This suggests that SPARC is strongly influential in determining anterior chamber biometry, while Hevin, although highly related in structure to SPARC, is not likely to be involved. By analyzing SPARC/Hevin-null mice in tandem, we confirmed that SPARC deficiency was sufficient to confer alterations to anterior chamber biometrics, whereas Hevin loss was not necessary and did not exacerbate the biometric alterations. We further reveal that decreased type I collagen expression and reduced collagen fibril thickness in the iris is associated with the biometric changes observed in the SPARC and SPARC/Hevin knockout mice.

While studies have shown that matricellular proteins including SPARC are likely to be important in the pathogenesis of glaucoma by modulating fibrosis and the deposition of extracellular matrix that affects the conventional aqueous outflow facility,6 8 9 the potential influence of these proteins in determining ocular structure has not been demonstrated. Previous attempts at determining the potential role of SPARC in dictating anterior segment ocular structures did not identify alterations in the SPARC-null mouse.27 Similarly, when type I collagen, a physiologic target of SPARC activity, was mutated so as to be resistant to hydrolysis by matrix metalloproteinase, anterior chamber structure alterations were not detected.40 The latter two studies relied on either light microscopy or histologic analyses to detect anterior segment changes. However, these methods are inherently inaccurate due to
dependence on the integrity of the tissue sections being analyzed. On the other hand, noncontact and noninvasive AS-OCT, which provides for objective and quantitative detection of spatial ocular differences, has been expedient in revealing differences in anterior chamber organization in live animals. Multiple AS-OCT studies have previously reported a negative correlation between pupil size and AOD/TISA. Instead of standardizing pupil size by adjusting light intensity, we had conducted all the AS-OCT measurements under ambient room lighting and statistically corrected for variations in pupil diameter. However, it is noted that the pupil diameters of SPARC- and SPARC-/Hevin-null eyes appeared significantly larger than WT (P < 0.001) while no such difference was detected between WT and Hevin-null eyes under similar lighting conditions. The relatively larger pupil diameters in the SPARC- and SPARC-/Hevin-null eyes may suggest a deficiency in the capacity of these irises to respond to light. This raises the interesting possibility that dynamic properties of the SPARC- and SPARC-/Hevin-null irises may deviate from WT.

Since SPARC modulates collagen assembly and deposition, SPARC deficiency is expected to result in global alteration in collagen deposition in the SPARC-null mouse, including the anterior segment of the eye. Indeed, electron microscopy has demonstrated that collagen fibrils in the conjunctiva were abnormal in SPARC-deficient mice. We confirmed in this study that the level of type I collagen transcript as well as collagen fibril thickness in the SPARC-null iris was significant lower than in the WT mouse, contrasting with the apparently normal collagen characteristics in the Hevin-null iris. Given that collagen plays an important role in regulating the flexibility and pliability of tissues such as bones, a deficiency in SPARC expression, resulting in a combined defect of reduced deposition and abnormal assembly of thinner collagen fibrils, may potentially lead to ocular tissues being more elastic. We therefore speculate that the spatial organization of the anterior segment is particularly sensitive to collagen content and assembly, both of which are regulated by SPARC.

Our study also established that the larger ACD observed in the SPARC-null mice was not associated with deficient type VIII collagen expression, contrary to the previous observation that mice with a defective Col8a2 gene sported abnormally large anterior chamber. It is arguable that this Col8a2 gene mutation, for which the effect on type VIII collagen fibrillogenesis is yet unclear, may affect anterior chamber characteristics differently compared with alterations of its expression level alone. Deletion of SPARC was in fact, associated with increased type VIII collagen transcription, suggesting that SPARC is involved in restricting type VIII collagen expression. Interestingly, the simultaneous lack of Hevin abolished this SPARC-null effect, implying that type VIII collagen expression is potentially dependent on the balance between SPARC and Hevin expression. Since the anterior

Figure 3. Anterior segment OCT images of WT, SPARC-, Hevin-, and SPARC-/Hevin-null eyes. Examples of SPARC- and SPARC-/Hevin-null anterior chambers that appeared dramatically different from WT and Hevin-null eyes are shown. Values indicated are the means of the parameters measured for WT (n = 53), SPARC- (n = 55), Hevin- (n = 56), and SPARC-/Hevin-null (n = 63) mice. Scale bar: 250 μm.
chamber characteristics in the SPARC-/Hevin-null eyes are similar to the SPARC-null counterpart, reflecting the similar Col1a1 but not type VIII collagen transcript levels, the overriding impression is that type I collagen is specifically involved in specifying anterior segment organization.

We did not detect gross anterior segment structural anomalies in the Hevin-null mice as compared with WT. The structural homology between SPARC and Hevin has previously prompted speculation that these two proteins may share similar functions, and possibly compensate for each other. Indeed, the loss of both SPARC and Hevin was necessary to suppress angiogenesis in the foreign body response, suggesting compensatory effects for these two proteins. However, the foreign body response itself differed between SPARC- and
Hevin-null mice. While SPARC-null mice displayed reduced fibrotic collagen encapsulation at the implant site, Hevin-null mice showed enhanced biomaterial-induced inflammation.42 There are other major differences between the two members that may suggest distinct functions for the two family members. While Hevin is found predominantly in neural tissues, SPARC is highly expressed in connective tissue and bone. In addition, unlike SPARC, Hevin did not affect the total quantity of collagen deposited.22 In the eye, SPARC expression has been demonstrated in the cornea, retina, lens,43,44 the trabecular meshwork, ciliary epithelium,45 and in aqueous and trabecular meshwork cells, nor was it induced by TGF-β.46 In contrast, Hevin expression was not detected in cultured human trabecular meshwork cells, nor was it induced by TGF-β.47,48 Intraocular pressure alterations, while reduced in SPARC-null mice,27 was not detected in Hevin-null mice.49 All these data suggest that SPARC and Hevin have differential functions in the eye, including their involvement in regulating anterior chamber characteristics.

In conclusion, we demonstrate that SPARC-null mice have deeper anterior chambers as well as wider drainage angles compared with WT, in association with lower Col1a1 transcript expression and reduced collagen fibril thickness in the iris. In contrast, Hevin deficiency appeared to have little impact on anterior chamber biometric parameters or collagen characteristics. SPARC is therefore specifically involved in affecting anatomical features of the anterior chamber of the eye, possibly via modulation of collagen properties.

Acknowledgments

Supported by a SingHealth Foundation Research Grant (SHF/FG583P/2014) to LFS and the Translational and Clinical Research (TCR) Programme (NMRC/TRC/002-SEBI/2008) from the Singapore National Research Foundation, administered by the Singapore Ministry of Health’s National Medical Research Council to TTW. Animal studies were partially funded by the SERI core grant (NMRC/CG/015/2013).

Disclosure: H. Ho, None; H.M. Htoon, None; G.H.-F. Yam, None; L.Z. Toh, None; N.C. Lwin, None; S. Chu, None; Y.S. Lee, None; T.T. Wong, None; L.-F. Seet, None

References