SCLERAL PITS IN CHOROIDEREMIA

Implications for Retinal Gene Therapy

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Purpose: We report a novel finding on spectral domain optical coherence tomography in patients with choroideremia, which we describe as scleral pits (SCPs).

Methods: Cross-sectional observational case series of 36 patients with choroideremia, who underwent ophthalmic examination and multimodal imaging, including optical coherence tomography of the macula. Optical coherence tomography images were reviewed for SCP, which were defined as discrete tracts of hyporeflectivity that traverse the sclera with or without the involvement of Bruch membrane, retinal pigment epithelium, and retina. Unpaired two-tailed *t*-test with Welch correction was used for statistical analysis.

Results: Of the 36 patients, 19 had SCP in at least one eye. Scleral pits were confined to areas of advanced chorioretinal degeneration and never involved the foveola. Type 1 SCP affected only the sclera, whereas Type 2 SCP also involved the Bruch membrane and the retinal pigment epithelium. Type 3 SCP additionally had a full-thickness retinal defect. Patients with SCP were significantly older (51 ± 2 vs. 33 ± 4 years; $P < 0.05$) and had lower best-corrected visual acuity (20/160 vs. 20/30 or 0.9 ± 0.2 vs. 0.2 ± 0.07 logarithm of the minimum angle of resolution; $P < 0.05$) than patients without SCP. Patients with SCP had a greater myopic refractive error compared with patients without SCP (−2.6 ± 0.5 vs. −0.3 ± 0.5D; $P < 0.05$), but there was no significant correlation between the number of SCPs with refraction. Short posterior ciliary arteries were observed to enter the eye through one Type 3 SCP.

Conclusion: Scleral pits are, to the best of our knowledge, a novel optical coherence tomography finding in advanced choroideremia that likely represents the abnormal juxtaposition of penetrating short posterior ciliary arteries with the retina.

RETINA 0:1–6, 2017

Choroideremia is an X-linked (Xq21) recessive disease caused by mutations in the *CHM* gene, which encodes the Rab escort protein-1 (REP1).[1–4] It is a relatively rare disease with a prevalence of 1:50,000.[5] Early clinical manifestations include a loss of peripheral and night vision, which progress to tunnel vision and eventual loss of central vision in later stages of the disease.[6] During childhood, the fundus characteristics may manifest as peripapillary atrophy and widespread pigment clumping at the level of the retinal pigment epithelium (RPE), which then evolve later into lobular atrophic areas involving the retina, RPE, and the choriocapillaris.[7] These areas can coalesce, resulting in a pale fundus from the revelation of underlying scleral tissue covered by preserved large choroidal vessels.[8–10] Although the disease primarily manifests in men, carrier women may also be affected, but usually they demonstrate patchy and less severe degeneration because of random lyonization of the X-chromosome.[11,12] The order of cell death in choroideremia is unclear. Most reports show that RPE is primarily affected with secondary degeneration of the photoreceptors and choriocapillaris; however, other studies alternatively suggest that photoreceptors degenerate first or that both RPE and photoreceptors degenerate independently with secondary damage to choroid.[13–17]

Multimodal imaging using optical coherence tomography (OCT) and other imaging techniques in choroideremia are limited because of rarity of the disease. Previous reports have demonstrated changes such as choroidal neovascularization, cystoid macular

edema, epiretinal membranes, outer retinal tubulations, intralaminar bridges, macular hole formation, and macular holes complicated by retinal detachment.[15,18–23] The OCT study by Jacobson et al[15] showed that retinal remodeling in choroideremia starts with retinal thickening, followed by photoreceptor degeneration, RPE depigmentation, and then retinal thinning with neuronal cell death and loss of laminar architecture.[14] A recent study using en face OCT and OCT angiography showed that at different stages, RPE and choriocapillaris loss exceeded photoreceptor loss.[24] In this study, we report a novel OCT finding in choroideremia that is correlated with disease course and may play a role in presurgical considerations for gene- and cell-based therapies.

Methods

The study was performed in accordance with the tenets of the Declarations of Helsinki, and the study protocol was approved by each institution's research ethical committee. Informed consent was obtained from all subjects. Clinically confirmed cases of choroideremia were reviewed from our pool of patients seen at Casey Eye Institute (Portland, OR) and Department of Ophthalmology, Rigshospitalet, Glostrup (Copenhagen, Denmark). Inclusion criteria were male subjects aged 18 years or older, having a clinical phenotype with or without genetic diagnosis of choroideremia, and having a disease clinically visible within the macular region. The exclusion criteria were history of amblyopia in either eye, concurrent retinal disease of other etiology, or presence of motion or media-opacity artifact in the OCT image.

All patients had detailed clinical examination including best-corrected visual acuity, intraocular pressure, and refraction. Snellen chart or Early Treatment of Diabetic Retinopathy Study chart was used for visual acuity testing, which were converted to logarithm of the minimum angle of resolution visual acuities for statistical purposes. Manifest refraction was documented for all eyes except in case of pseudophakia or poor visual acuity. After dilation, patients underwent imaging with spectral domain OCT, infrared reflectance, fluorescein angiography, and indocyanine green angiography using the SPECTRALIS (wavelength: 870 nm; Heidelberg Engineering Co, Heidelberg, Germany), 30° or 50° color fundus photograph using the FF450 (Carl Zeiss Meditec, Dublin, CA), and widefield fundus autofluorescence (FAF) using the Optos 200Tx confocal scanning laser ophthalmoscope (Optos PLC, Marlborough, MA). The OCT was obtained in high-resolution mode with 97 b scans centered on the fovea. Because our cohort consisted of patients with a wide age range at two different clinical centers, it was not possible to perform an identical scanning protocol on all patients.

Scleral pits (SCPs) were defined as discrete tracts of low reflectivity that traversed the sclera with or

Table 1. Patients With Choroideremia and SCP

Patients	Age (years)	*CHM* Mutation
P1	36	Arg253Stop
P2	51	Hemizygous A>G nucleotide substitution at the position of intron 6
P3	59	c.1044_1047delCAGC
P4	66	c.1584?1587delTGTT
P5	54	ND
P6	48	ND
P7	35	p.Tyr323Stop:c.969T>A
P8	65	c.1010_1015del TCATGCinsCA
P9	52	c.525_526delAG
P10	49	ND
P11	47	c.1609+1delG
P12	56	c.907C>T
P13	61	c.1179insC
P14	67	c.1153insC
P15	44	del exon 1-15
P16	56	c.555-556 del AG
P17	49	c.2T>A
P18	26	del exon 13
P19	56	c.1680-1681del TT

ND, no mutation detected in *CHM* or genetic testing not available.

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Supported by National Institute of Health: 1K08 EY0231186-01 (M.E.P.); 1K08EY026650-01 (P.Y.); and P30 EY010572 (Casey Eye Institute), Research to Prevent Blindness: unrestricted departmental funding to Casey Eye Institute, Choroideremia Research Foundation, Danish Association of the Blind (S.K.C.), and Foundation Fighting Blindness: C-CL-0711-0534-OHSU01 (Casey Eye Institute); CD-NMT-0714-0648 (P.Y.); and CD-NMT-0914-0659 (M.E.P.).

This study was an investigator-initiated study conducted using data from patients who were part of a multicenter choroideremia natural history study sponsored by NightstaRx, Ltd (London, United Kingdom). A. Girach is an employee of NightstaRx, otherwise none of the other authors declare any competing interests or received any payment from NightstaRx for the development of this study or for manuscript preparation. R.G. Weleber serves on the scientific advisory board for Applied Genetic Technologies Corp (AGTC); and serves on the scientific advisory board for the Foundation Fighting Blindness (FFB) (these relationships have been reviewed and managed by Oregon Health and Science University); and received travel expenses from FFB and AGTC and other support from Sanofi, all outside the submitted work.

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without the involvement of Bruch membrane, RPE, and retina. The number of SCP was counted for each eye within the area of the OCT volume scan, which consists of a 20° × 20° area or 20° × 15° area centered on the fovea. To fully visualize the extent of certain SCP, enhanced-depth imaging OCT was additionally performed in five subjects. The SCP identified on OCT was further characterized on infrared reflectance and FAF. All data collected from the right and left eyes from each patient were highly correlated (data not shown), and thus, the averaged values of both eyes were used to represent each patient. Unpaired two-tailed *t*-tests with Welch correction were used for statistical analysis.

Results

Of 36 patients with choroideremia included in the study, 19 had SCP in at least one eye (53%). Of those, 13 had SCP in both eyes. Genetic testing demonstrating a mutation in *CHM* was available for 26 patients (16 with SCP vs. 10 without SCP; Table 1). Regardless of genetic confirmation, all patients had clinical findings and a family history consistent with choroideremia. Two eyes were excluded: one because of presence of a choroidal neovascular membrane and the other because of a technical error. Scleral pits were localized to areas of advanced chorioretinal degeneration but never involved the foveola. Three types of SCP were observed (Figure 1). Type 1 had intact Bruch membrane, residual RPE, and retina. Type 2 had a break in Bruch membrane and RPE with or without focal retinal excavation, whereas Type 3 had focal full-thickness retinal loss. Type 1 SCP was difficult to detect on fundus examination or with infrared reflectance or FAF imaging, and was only visible on OCT. Type 2 and 3 SCPs were seen on fundus examination as focal round depressions that were hyporeflective on infrared reflectance and hypoautofluorescent on FAF, which was indicative of the RPE defect. Enhanced-depth imaging OCT performed in five subjects showed that SCP extended deeply into the outer sclera. Fundus angiography was performed for one patient (No. P10) with Type 3 SCP, wherein the

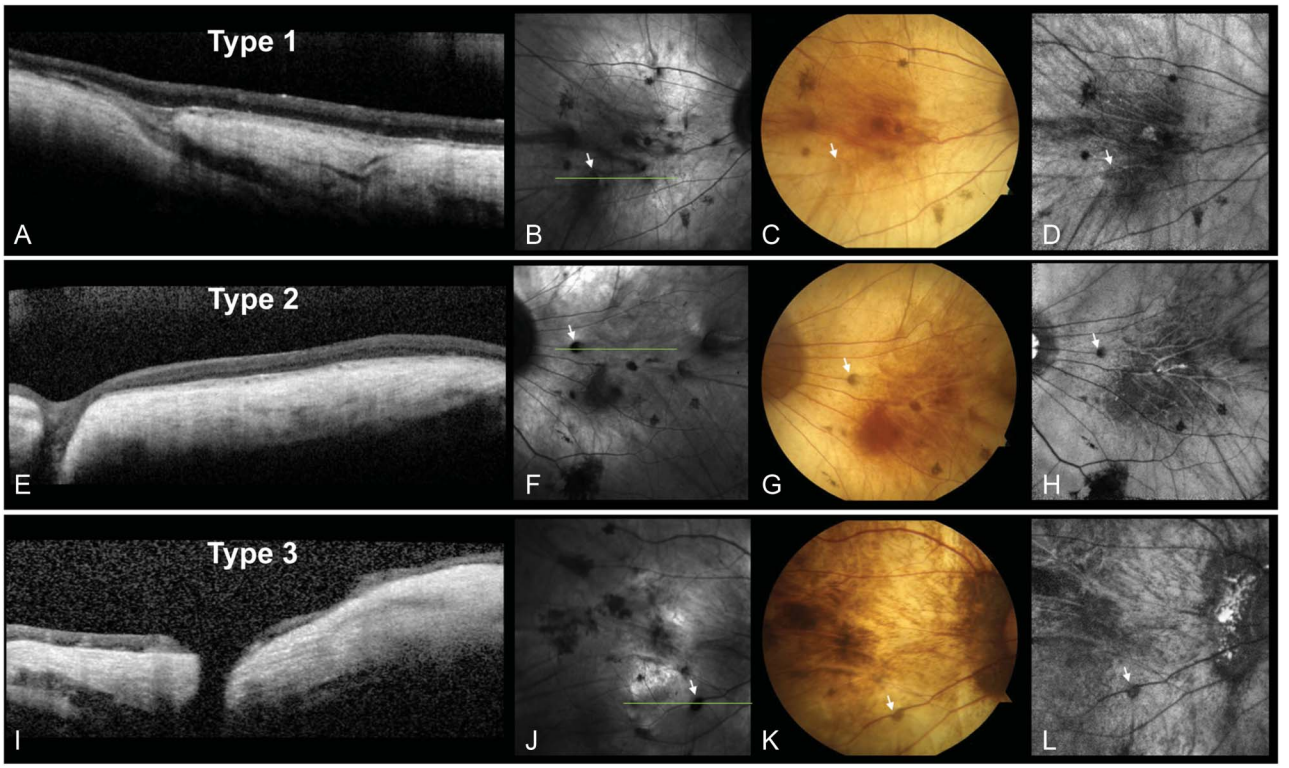

Fig. 1. Three types of SCPs. Upper row (**A**–**D**): Type 1 SCP, patient no. P3, right eye. **A.** Optical coherence tomography reveals subtle focal posterior bowing of the retina, RPE, and choroid above an area of scleral cavitation and oblique tract. This Type 1 SCP is visible as a hyporeflective spot on infrared (**B**, arrow) and a subtle gray lesion on color fundus photograph (**C**, arrow) but is not visible on FAF (**D**, arrow). Middle row (**E**–**H**): Type 2 SCP, patient no. P3, left eye. **E.** Optical coherence tomography shows prominent posterior bowing of the retina with no discernible retinal laminations, RPE, or choroidal layers, culminating in a sclera cavitation and tract at a right angle to the retinal surface. Type 2 SCP is easily visible as a prominent hyporeflective spot on infrared (**F**, arrow), a dark spot on color (**G**, arrow), and hypoautofluorescent spot on FAF (**H**, arrow). Lower row (**I**–**L**): Type 3, patient no. P10, right eye. **I.** Optical coherence tomography reveals a full-thickness focal defect of the retina, RPE, and choroid with no visible membrane separating the equally hyporeflective space of the vitreous and scleral tracts. Type 3 SCP is similar in appearance to Type 2 SCP on infrared, color, and FAF (**J**–**L**, arrows).

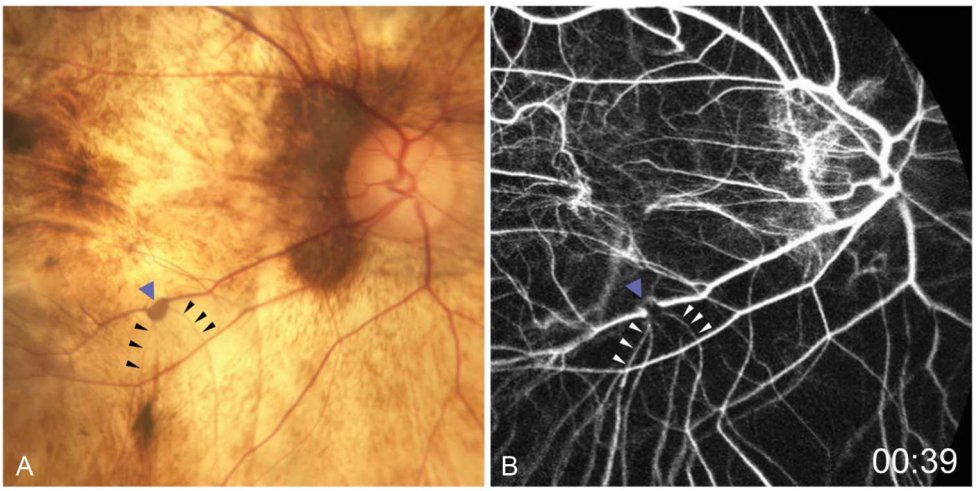

Fig. 2. Angiogram of a Type 3 SCP. Magnified color photograph of a Type 3 SCP (**A**, blue arrowhead) from Figure 1K (patient no. P10, right eye) showing apparent interruption of a segment of a large retinal vein. Fluorescein angiogram reveals that the retinal vein is patent with decreased flow in semicircinate path (**B**, blue arrowhead). Short posterior ciliary arteries appear to enter the globe through this pit (**A**, black arrowheads; and **B**, white arrowheads).

cavitation seemed to cause an interruption in a retinal vessel. Fluorescein angiography revealed that the retinal vessel filled normally but had a circuitous route following a path around the edge of the SCP lesion (Figure 2), whereas indocyanine green angiography showed no discrete cyanescence that correlated with the SCP.

Patients with SCP were significantly older (51 ± 2 vs. 33 ± 4 years; $P < 0.05$) and had poorer acuity (20/160 vs. 20/30 or 0.9 ± 0.2 vs. 0.2 ± 0.06 logarithm of the minimum angle of resolution; $P < 0.05$) compared with patients without SCP (Figure 3). Patients with SCP had a greater myopic refractive error compared with patients without SCP (−2.6 ± 0.5 vs. −0.3 ± 0.5D; $P < 0.05$). However, there was no significant correlation between the number of SCPs with age, best-corrected visual acuity, or refraction. There was no significant difference in intraocular pressure between the group with SCP and the group without SCP. This study was not powered to determine correlations based on types of SCP because of the low numbers of subjects and presence of more than one type in some eyes.

Discussion

We have characterized SCPs as a novel OCT finding in patients with choroideremia, which are discrete fovea-sparing lesions that involve the macula, and appear as dramatic structural defects of the sclera, and to varying degrees, Bruch membrane, RPE, and retina. The spectrum of SCP in choroideremia seems phenotypically different from those typically found in pathologic myopia that is characterized as a posterior thinning and outpouching of the scleral on OCT.[25,26] However, some SCP in choroideremia resemble the peripapillary lesions in pathologic myopia as described by Ohno-Matsui et al.[27] In addition, Ohno-Matsui et al also showed that short posterior ciliary arteries penetrate the eye at the sites of SCP, which is similar to our findings in choroideremia. Although the patients with choroideremia in our cohort were not high myopes, the patients with SCP were significantly more myopic than those without SCP, suggesting that moderate levels of myopia may exacerbate choroidal thinning and contribute to risk of developing SCP in choroideremia. Patients with SCP were also significantly older and had significantly poorer visual acuity than those without SCP, which suggests that SCP is a phenomenon that develops late in the disease course of choroideremia. Previous reports showed that choroideremia becomes more asymmetric in older patients,[28] which may explain the unilaterality of SCP in about a third of our patients.

Previous histological reports of choroideremia have demonstrated degeneration in the retina, RPE,

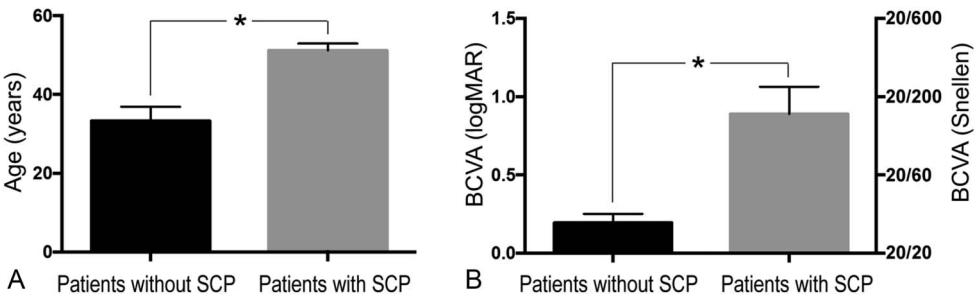

Fig. 3. Patients with SCPs were significantly older (**A**), and had worse best-corrected visual acuity (**B**) than patients without SCPs. SCP, scleral pit; BCVA, best-corrected visual acuity; asterisk, $P < 0.05$.

and choriocaplliaris;[12,13] however, there are no known histological descriptions that correspond with SCP as described in this study. Without histopathology, it is unknown whether SCP are still lined with vascular endothelial cells, and whether Type 3 SCP are indeed full-thickness perforations of the globe or whether a thin hyporeflective membrane between the two spaces remains. Based on the results of angiography and similarities to peripapillary SCP in high myopia, the etiology of SCP in choroideremia is most likely due to the abnormal juxtaposition of penetrating short posterior ciliary arteries with the retina as a result of severe choroidal atrophy.

It is unknown whether SCP is specific to choroideremia or may also be found in other choroidal dystrophies. In addition, this study was also limited by the retrospective nature and relatively small sample size; however, choroideremia is a rare disease. Studies in larger groups of patients with choroideremia will be necessary to determine whether the different types of SCP identified in this study are correlated with different *CHM* mutations.

Scleral pits are, to the best of our knowledge, a novel OCT finding in choroideremia, especially in patients with reduced visual acuity and advanced disease. It is unknown whether vitrectomy or subretinal injection may disrupt the residual Bruch membrane and RPE layer of an SCP, which would hasten drainage and reduce anatomical sequestration of subretinal-delivered therapeutic agents, especially because it pertains to retinal gene- and cell-based therapies. If so, treatment efficacy may be affected, not only by limiting retention of the therapeutic agent, but also by compromising subretinal space–associated immune tolerance from potential exposure of the vector capsid or stem-cell antigens to choroidal dendritic cells and lymphatics. These potential issues may be especially problematic for Type 3 SCP, even if a thin membrane remains that cannot not be visualized by OCT separating the two compartments. Additional studies will be needed to determine whether areas with numerous SCP should be avoided in the ongoing retinal gene therapy clinical trials for choroideremia.

Key words: choroideremia, scleral pits, CHM, short posterior ciliary arteries.

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