**Choroidal Caverns in Stargardt Disease**

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Received: October 16, 2021
Accepted: January 26, 2022
Published: February 14, 2022

Citation: Mucciolo DP, Giorgio D, Lippera M, et al. Choroidal caverns in Stargardt disease. Invest Ophthalmol Vis Sci. 2022;63(2):25. https://doi.org/10.1167/iovs.63.2.25

Recessive Stargardt disease (STGD1) is the most common form of inherited macular dystrophy. The disease is associated with pathogenic sequence variants of the ABCA4 gene coding for a visual cycle transport protein. Impaired function of this protein leads to the accumulation of lipofuscin and their derivatives within the retinal pigment epithelium (RPE). STGD1 is characterized by a progressive loss of central visual function caused by the degeneration of photoreceptors and RPE cells in the macula. Recently, Querques et al. described angular hyporeflective cavities called "choroidal caverns" in patients affected by geographic atrophy (GA), assuming that these findings could arise from nonperfused ghost vessels and persistence of stromal pillars where the vessels were originally situated. Dolz-Marco et al. correlated a histologic survey of donor eyes with multimodal imaging from a clinical case series to demonstrate that choroidal caverns were lipid globules, suggesting that choroidal caverns might represent a common normal physiological lipid depot for photoreceptor metabolism. Further works have expanded the prevalence of choroidal caverns in other disorders.

Up to now, these characteristic choroidal features have rarely been investigated in patients affected by STGD1. To the authors' knowledge, only two previous studies have reported on the multimodal imaging of choroidal caverns in patients affected by STGD1; however, no study has specifically focused on the qualitative and quantitative investigation of choroidal caverns and on their relationship with clinical outcomes in STGD1.

In the present work, we have described the choroidal caverns in a large cohort of patients affected by STGD1 evaluating the prevalence of choroidal caverns, their localization and relation with choriotelial structures.

**Materials and Methods**

We retrospectively reviewed the database of patients affected by STGD1 at the Reference Center for Hereditary Retinal Degenerations at the Eye Clinic in Florence in the period from 2012 to 2017. The criteria for STGD1 diagnosis have been described in previous articles. All the STGD1 patients included in the study had undergone molecular genetic testing showing at least two causative ABCA4 mutations. Clinical phenotype was evaluated according to the Fishman classification.

As part of standard clinical assessment, all patients underwent a complete ophthalmologic examination: best corrected visual acuity (BCVA), biomicroscopy of the anterior segment, measurement of intraocular pressure, and fundus examination. Color fundus photographs (FF450 Retinograph; Carl Zeiss Meditec, Jena, Germany; or Daytona, [Image 491x30 to 543x48].

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positive principles of the Helsinki Declaration. The study was approved by the local Institutional Review Board (Careggi Hospital, Italy). The mean age of the patients with choroidal caverns was 47.4 ± 11.5 years (range, 23–75 years), whereas the mean age of onset of the disease was 24.7 ± 11.8 years (12–57 years). The mean BCVA was significantly lower in eyes with choroidal caverns compared to those without choroidal caverns (1.01 ± 0.29 vs. 0.81 ± 0.44; p = 0.03).

All eyes with choroidal caverns presented phenotype III and IV according to the Fishman classification. More precisely, of the eyes with choroidal caverns (23 eyes), 19 eyes (19/23; 82.6%) of 17 patients presented stage III and four eyes (4/23; 17.3%) of four patients presented stage IV (Table 2C). A subgroup analysis to compare the eyes with choroidal caverns and the eyes without choroidal caverns in advanced STGD1 (stage III + stage IV, 102/172 eyes of 51 patients) did not reveal significant differences in the BCVA, SFCT, and GA extension (Table 3).

Of the eyes of patients with stage III and IV (102/172 eyes, 51 patients; 59.3%), 48 eyes of 24 patients, 28 eyes of 14 patients, and 26 eyes of 13 patients underwent DRI OCT Topcon, Cirrus HD-OCT and Spectralis HRA-OCT, respectively. Using DRI OCT Topcon we detected choroidal caverns in seven eyes (7/48, 14.5%), whereas using Cirrus HD-OCT we discovered choroidal caverns in four eyes (4/28, 14.2%). Finally, using Spectralis HRA-OCT we discovered choroidal caverns in 12 eyes (12/26 [46.1%]). In our series all the patients were examined using only one OCT device. OCTA was available for eight eyes (8/172 eyes; 4.6%) of six patients with choroidal caverns (patients P21, P23, P29, P36, P70, P81) and did not reveal any flow signal inside these lesions (Fig.).

**OCT and Clinical Findings Correlations**

The demographics and main clinical features of the study population are summarized in Tables 2A to 2C. The mean age of the patients with choroidal caverns was 47.4 ± 11.5 years (range, 23–75 years), whereas the mean age of onset of the disease was 24.7 ± 11.8 years (12–57 years). The mean BCVA was significantly lower in eyes with choroidal caverns compared to those without choroidal caverns (1.01 ± 0.29 vs. 0.81 ± 0.44; p = 0.03).

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**OCT Findings**

Using structural OCT, choroidal caverns were detected in 23 eyes (23/172 eyes [13.3%]) of 21 patients (bilaterally only in two patients: patients P21 and P36). The total number of choroidal caverns detected was 63. Choroidal caverns appeared as hyperreflective cavities of variable size located in the choroidal tissue. They presented a mild hyperreflective rim and a nonhomogeneous hyperreflective tail (Fig.).

The mean greatest diameter of the choroidal caverns was 67.14 ± 34.5 μm (range, 21–197 μm). In our study, 49 choroidal caverns were located in the Sattler layer and 14 in the Haller layer. No choroidal caverns were detected in the choriocapillaris. Two choroidal caverns were located in the subfoveal area whereas 61 were extrafoveal. Five choroidal caverns were visible at the edge of the macular atrophy, and 58 were located inside the area of atrophy. The mean SFCT was significantly reduced in eyes with choroidal caverns in comparison with those observed in eyes without choroidal caverns (245.1 ± 82.4 μm vs. 285.9 ± 94.3; p = 0.05).

**RESULTS**

Eighty-six patients (172 eyes) from 78 independent families characterized by biallelic mutations in the ABCA4 gene were included in the study (Table 1). Thirty-four patients were male (34/86 [39.5%]), and 52 were female. The mean age of the patients was 40.4 ± 15.6 years (range, 14–85 years) with a mean age of onset of the disease of 24.4 ± 14.0 years (range, 6–58 years). The mean BCVA (LogMar) was 0.76 ± 0.38 (range, 1.6–0) with a mean spherical equivalent of −1 ± 1.93 (range, −5.50 to 2.5). In all patients the clinical picture according to the Fishman classification was the same in both eyes: 40 eyes (40/172 [23.2%]) of 20 patients were classified as phenotype I, 30 eyes (30/172 [17.4%]) of 15 patients as phenotype II, 80 eyes (80/172; 46.5%) of 40 patients as phenotype III, and 22 eyes (22/172 [12.7%]) of 11 patients as phenotype IV.

**DISCUSSION**

In this study we have reported on choroidal caverns, a characteristic finding observed in the choroid of patients affected by STGD1. Up to now, these unusual findings have been reported in patients affected by different diseases, such as atrophic age-related macular degeneration (AMD), pachychoroidal diseases, and Best disease and has rarely been...
Table 1. Genetic Features of ABCA4 Related STGD Patients

| Patient ID | ABCA4 Mutations                                      | Presence of Choroidal Caverns (Y/N) |
|------------|------------------------------------------------------|-------------------------------------|
| P1         | c.2791G>A (p.Val931Met)                               |                                | N                     |
| P2         | c.4532+1G>A (p.? )                                   |                                | Y                     |
| P3         | c.5461+10T>C p.(?)                                   |                                | Y                     |
| P4         | c.5882G>A (p.Gly1961Glu)                              |                                | Y                     |
| P5         | c.4793C>A (p.Ala1598Asp)                              |                                | Y                     |
| P6         | c.5882G>A (p.Gly1961Glu)                              |                                | Y                     |
| P7         | c.461C>T (p.Thr1545=)                                 |                                | Y                     |
| P8         | c.5018+2T>C p.(?)                                    |                                | Y                     |
| P9         | c.2345G>A (p.Trp782*)                                 |                                | Y                     |
| P10        | c.4676+1G>A (p.? )                                   |                                | Y                     |
| P11        | c.5714+5G>A (p.? )                                   |                                | Y                     |
| P12        | c.5929G>A (p.Gly1977Ser)                              |                                | Y                     |
| P13        | c.5961_5964del (p.Asp1988Profs*3)                     |                                | Y                     |
| P14        | c.4793C>A (p.Ala1598Asp)                              |                                | Y                     |
| P15        | c.3564G>A (p.Glu1122Lys)                              |                                | Y                     |
| P16        | c.364C>T (p.Arg212Cys)                                |                                | Y                     |
| P17        | c.5917del (p.Val1973*)                                |                                | Y                     |
| P18        | c.5917del (p.Val1973*)                                |                                | Y                     |
| P19        | c.5018+2T>C p.(?)                                    |                                | Y                     |
| P20        | c.5882G>A (p.Gly1961Glu)                              |                                | Y                     |
| P21        | c.5961_5964del (p.Asp1988Profs*3)                     |                                | Y                     |
| P22        | c.4793C>A (p.Ala1598Asp)                              |                                | Y                     |
| P23        | c.3564G>A (p.Glu1122Lys)                              |                                | Y                     |
| P24        | c.3610G>A (p.Asp1204Asn)                              |                                | Y                     |
| P25        | c.5917del (p.Val1973*)                                |                                | Y                     |
| P26        | c.2791G>A (p.Val931Met)                               |                                | Y                     |
| P27        | c.3292C>T (p.Arg1098Cys)                              |                                | Y                     |
| P28        | c.4139C>T (p.Pro1380Leu)                              |                                | Y                     |
| P29        | c.4139C>T (p.Pro1380Leu)                              |                                | Y                     |
| P30        | c.1846G>A (p.Glu616Lys)                               |                                | Y                     |
| P31        | c.3564G>A (p.Glu1122Lys)                              |                                | Y                     |
| P32        | c.634C>T (p.Arg212Cys)                                |                                | Y                     |
| P33        | c.5087G>A (p.Ser1696Asn)                              |                                | Y                     |
| P34        | c.610G>A (p.Asp1204Asn)                               |                                | Y                     |
| P35        | c.2888G>T (p.Gly963Val)                               |                                | Y                     |
| P36        | c.2300T>A (p.Val767Asp)                               |                                | Y                     |
| P37        | c.4775G>A (p.Gly1961Glu)                              |                                | Y                     |
| P38        | c.3056G>A (p.Thr1019Met)                              |                                | Y                     |
| P39        | c.4982G>T (p.Glu328*)                                 |                                | Y                     |
| P40        | c.2791G>A (p.Val931Met)                               |                                | Y                     |
| P41        | c.5381C>A (p.Ala1794Asp)                              |                                | Y                     |
| P42        | c.1714C>T (p.Arg572*)                                 |                                | Y                     |
| P43        | c.5917del (p.Val1973*)                                |                                | Y                     |
| P44        | c.4775G>A (p.Gly1961Glu)                              |                                | Y                     |
| P45        | c.5917del (p.Val1973*)                                |                                | Y                     |
| P46        | c.1622T>C (p.Leu541Pro)                               |                                | Y                     |
| P47        | c.3261A>C (p.Glu1087Asp)                              |                                | Y                     |
| P48        | c.3212C>T (p.Phe984Leu)                               |                                | Y                     |
| P49        | c.3806T>C (p.Leu1209Pro)                              |                                | Y                     |
| P50        | c.5882G>A (p.Gly1961Glu)                              |                                | Y                     |
| P51        | c.247_250dup p.(Ser84Thrfs*16)                        |                                | Y                     |
| P52        | c.6122T>C (p.Leu2041Pro)                              |                                | Y                     |
| P53        | c.5882G>A (p.Gly1961Glu)                              |                                | Y                     |
| P54        | c.5882G>A (p.Gly1961Glu)                              |                                | Y                     |
| P55        | c.5917del (p.Val1973*)                                |                                | Y                     |
| P56        | c.5018+2T>C p.(?)                                    |                                | Y                     |
| P57        | c.5146+1C>G (p.(Gly1728Ser)                           |                                | Y                     |
| P58        | c.5018+2T>C p.(?)                                    |                                | Y                     |
| P59        | c.4793C>T (p.Arg1553Cys)                              |                                | Y                     |
| P60        | c.6416G>T (p.Arg2149Leu)                              |                                | Y                     |
| P61        | c.6446G>T (p.Arg2149Leu)                              |                                | Y                     |
| P62        | c.610G>A (p.Asp1204Asn)                               |                                | Y                     |
| P63        | c.5917del (p.Val1973*)                                |                                | Y                     |
| P64        | c.286A>G (p.Asn96Asp)                                 |                                | Y                     |
reported in patients affected by STGD1.7,12 The prevalence of choroidal caverns varied in literature: 12.5% and 42% in eyes affected by GA,6,10 and 52% in eyes affected by pachychoroid disease.8 In our study, we have reported a mean prevalence of 13.3% for choroidal caverns (23/172 eyes of 21 patients) in STGD1. However, we should take into account the Y: yes; N: no.
Dolz-Marco et al. demonstrated a cavern size of 61 μm, whereas eyes affected by pachychoroid disease tended to have a larger maximum size, ranging from 249 to 486 μm. However, in STGD1, the development of choroidal caverns could be directly related to the process of RPE impairment and atrophy: first, we observed choroidal caverns only in eyes affected by stage III and IV STGD1 (more advanced stages); second, choroidal caverns were found in areas of the choroid underneath RPE atrophy, localized in both the Sattler and Haller layer, and no choroidal caverns were discovered in the choriocapillaris (CC) layer; third, no choroidal caverns were discovered outside the area of macular atrophy or in the surrounding retina. We know that the health of the RPE is crucial for choroid architecture development whereas RPE impairment causes progressive choroidal sclerosis. The RPE secretes a variety of growth factors, and an increasing body of evidence indicates that RPE-derived trophic factors are essential for normal choroidal development. Choroidal caverns appear as empty structures characterized by the absence of any pathological blood flow detectable using OCTA; for this reason, the focal obliteration of some choroidal vessels may be a possible explanation for the development of choroidal caverns in STGD1; however, we cannot definitely exclude that choroidal caverns may correspond to tiny vessels characterized by a slow flow which is not detectable using OCTA. For all these reasons we suggest that the RPE impairment observed in the STGD1 patients, more evident in stage III and IV, could be responsible for the development of the choroidal caverns.

We have also investigated the association between choroidal caverns and some clinical features of the disease, such as SFCT, BCVA, and the extension of macular atrophy, the latter was measured only in stage III and IV STGD1 patients.

Up to now, several articles have taken into consideration the association between different OCT parameters and specific features of STGD1. SFCT has been found to be positively associated with a better BCVA and a higher foveal thickness and inversely related to duration of the disease, severe fundus alterations, and electroretinogram (ERG) abnormalities. Vural et al. recently reported a statistically significant correlation between subfoveal SFCT and BCVA, inner and outer retinal thickness and paracentral multifocal electroretinogram (mf-ERG) responses, whereas Arrigo et al. reported that STGD1 eyes with choroidal caverns were associated with lower BCVA, retinal layer thinning, and higher hyperreflective foci numbers.

In our study, we observed that patients with choroidal caverns presented a thinner choroid and worse visual acuity in comparison with patients without choroidal caverns, and these differences are significant. However, this result may be partially due to the more advanced stage of disease (stage III and IV) that characterized all patients with choroidal caverns in comparison with patients without; in fact, from past studies we know that both the BCVA and SFCT have been found to be inversely related to duration of the disease and more severe fundus alterations.

For this reason, we took into consideration only patients with more advanced disease stages (stage III and IV) and excluded patients with stage I and II, and we compared the patients with and without choroidal caverns: we did not detect differences in either the BCVA or the SFCT. Further-
more, no differences were detected in the GA area extension in these two study groups. Therefore the BCVA, SFCT, and GA area extension did not differ between eyes with and without choroidal caverns in the more advanced stages of the disease; we could not identify and clarify the prognostic role of choroidal caverns in STGD1 patients. Future studies are necessary to better highlight the role of choroidal caverns in STGD1.

Finally, in our work we have evaluated possible genotype-phenotype correlation between specific genetic variants of the ABCA4 gene and the presence of choroidal caverns. However, we are unable to show a genotype/phenotype association between specific genetic variants and the presence of choroidal caverns in our cohort.

We are aware of some limitations of our study, which include its retrospective nature, the lack of histological data of the choroidal caverns, and the use of different OCT devices; however, we studied in detail a large number of genetically characterized patients.

In conclusion, we identified choroidal caverns in STGD1 as hyporeflective cavities of variable size characterized by the absence of flow using OCTA; we found similar morphological and prevalence data with the choroidal caverns previously reported in AMD; the choroidal caverns are present only in advanced stages of STGD1, suggesting a degenerative origin caused by the RPE impairment, more evident in stage III and IV of the disease.

Acknowledgments

Disclosure: D.P. Mucciolo, None; D. Giorgio, None; M. Lippera, None; V. Dattilo, None; I. Passerini, None; E. Pelo, None; A. Sodi, None; G. Virgili, None; F. Giansanti, None; V. Murro, None

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