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Correlation of Outer Retinal Degeneration and Choriocapillaris Loss in Stargardt disease using *en face* OCT and OCT Angiography

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Abstract

Purpose: Measure and correlate degeneration of the inner segment/outer segment junction (IS/OS), retinal pigment epithelium (RPE) and choriocapillaris (CC) in Stargardt disease (STGD)

Design: Prospective cross-sectional study

Methods Setting: Casey Eye Institute

Population: 23 patients with STGD

Procedures: OCT angiography (OCTA) scans were centered on the fovea. OCT slab projection and *en face* boundary maps were used to create masks to measure total IS/OS loss or RPE atrophy, as well as regions of isolated IS/OS loss, isolated RPE atrophy, matched IS/OS and RPE degeneration, or intact IS/OS and RPE. CC vascular density (CCVD) was quantified from CC angiogram.

Outcome Measures: Area of loss and CCVD for different region types of degeneration were quantified and correlated.

Results: The total area of IS/OS loss was strongly correlated with total area of RPE atrophy ($r=0.96$, $p<0.0001$) by a ratio of 1.6:1 ($r^2=0.90$). CCVD within regions of matched degeneration

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($85.6 \pm 2.7\%$; $p < 0.0001$), isolated IS/OS loss ($93.6 \pm 1.0\%$; $p = 0.0011$), and isolated RPE atrophy ($94.1 \pm 1.1\%$; $p = 0.0065$) were all significantly lower than normal ($99.0 \pm 0.17\%$). There was a trend for CCVD within intact areas ($97.6 \pm 0.38\%$) to decline as the area diminished ($r = 0.68$).

Conclusions: Photoreceptor and RPE degeneration exhibit a strong relationship wherein IS/OS loss is 1.6 fold greater than RPE atrophy, supporting the theory that photoreceptor degeneration precedes RPE in STGD. Both photoreceptor and RPE degeneration contribute synergistically to CCVD attenuation, but extra-lesional CCVD also tended to be abnormal. The findings and techniques in this study may be of utility in developing endpoints for clinical trials.

Introduction:

ABCA4-related retinopathy, commonly referred to as Stargardt disease (STGD) is an inherited macular dystrophy with a prevalence of 1 in 10,000, and is typically associated with mutations in the *ABCA4* gene, which is inherited in an autosomal recessive manner.¹ While STGD commonly presents as a maculopathy characterized by yellow pisciform fleck deposits and macular atrophy,² there are more severe phenotypes that present with generalized cone or cone-rod dysfunction.^{3–5} Additionally, less severe phenotypes, previously referred to as fundus flavimaculatus, can manifest with pisciform deposits extending to the mid-periphery but without central atrophy.^{6,7} The STGD spectrum has also been categorized based on age of onset, wherein the early-onset group of 10 years or younger exhibited more severe disease and rapid decline in visual acuity,⁸ while the late-onset group of 45 years or older typically had milder disease with preserved fovea and visual acuity for many years.^{5,7,9–11} The severity of STGD has also been classified by the level of retinal dysfunction as measured by the full field electroretinogram (ERG).^{12,13} Rarely, mutations in the *ABCA4* gene have also been implicated in retinitis pigmentosa.^{14,15}

Mutations in the *ABCA4* gene result in a loss of function in the encoded flippase protein that leads to progressive accumulation of all-trans-retinal and N-retinylidenephosphatidylethanolamine, which irreversibly forms di-retinoid-phosphatidylethanolamine (A2PE) in the photoreceptor outer segments and in turn leads to accumulation of di-retinoidpyridinium-ethanolamine (A2E) in the retinal pigment epithelium (RPE).^{16–25} It is thought that the aforementioned bisretinoids and their precursors may be photooxidized to generate cytotoxic compounds, however it remains to be determined which ones directly cause photoreceptor versus RPE cell death.¹⁶ Moreover, the accumulation and photooxidation of bisretinoids is interrelated to the activation of complement system and accumulation of membrane attack complex, which exacerbate cell death in STGD.^{26–29} To further elucidate the pathophysiology of STGD, highly detailed volumetric in-vivo imaging of the photoreceptor and RPE layers in patients with STGD is now possible with recent advancements in optical coherence tomography (OCT) technology.

With the advent of ultra-high speed OCT, detailed *en face* structural data can be extracted from the volumetric OCT data and quantified using semi-automated segmentation software.^{30,31} Moreover, through the detection of motion and flow over time, OCT angiography (OCTA) provides novel information on the retinal and choroidal vasculature, which has the following advantages over conventional dye-based angiography: 1) it is a non-invasive

procedure in that intravenous dye is not required, 2) it allows registration and tracking for comparative follow-up studies, and 3), it produces three-dimensional flow information of retinal vessels and choriocapillaris (CC) that is quantifiable and not confounded by dye extravasation.^{32,33} In this study, we use *en face* OCT and OCTA to characterize the dynamics of structural and vascular degeneration, respectively, of the retina and CC in patients with STGD.

Methods:

This was a prospective cross-sectional study of 23 patients (46 eyes) with a clinical diagnosis of STGD recruited between July 2015 and June 2016 from the Ophthalmic Genetics Clinic at Casey Eye Institute. The study protocol was approved prospectively by Oregon Health & Sciences University (OHSU) IRB, adhered to the tenets of the Declaration of Helsinki, and complied with HIPAA. The IRB approved the collection and analysis of de-identified clinical history, ocular imaging, and genetic test results. Informed consent was obtained from all participants. Inclusion criteria were a diagnosis of autosomal recessive STGD based on the pedigree and clinical phenotype of fleck deposits with or without genetic testing, or at least two pathogenic mutations in the *ABCA4* gene. The exclusion criteria were history of concurrent retinal disease of other etiology, or presence of motion or media-opacity artifact in the OCTA image.

All patients received an ophthalmic examination, fundus autofluorescence (AF) using either the Optos (200Tx confocal scanning laser ophthalmoscope; Optos PLC) or Spectralis (Heidelberg Engineering, Heidelberg, Germany) imaging systems, and OCTA imaging (RTVue-XR Avanti with AngioVue; Optovue Inc, Fremont, CA). The OCTA scans contain both volumetric flow (decorrelation) data and volumetric structural (reflectance) data, which covered $6 \times 6 \text{ mm}^2$ and were centered on the fovea (Fig 1). Our customized reading software COOL-ART (Center for Ophthalmic Optics & Lasers-Angiography Reading Toolkit)³⁴ was used to segment retinal layers and generate *en face* thickness maps. The slabs used to create *en face* OCT images of the inner segment/outer segment junction (IS/OS) and RPE were each 9 microns thick, and the position of the slabs was manually optimized for each eye (Fig 1a–b). Using custom Matlab (Natick, MA) software, a panel of experts (TA, AAA, RCP and SSG) determined the exact position of the OCT slabs to best capture information on degeneration of the IS/OS and the RPE layers. The IS/OS slab was positioned to comprise the hyper-reflective IS/OS band on OCT B-scans. To detect RPE atrophy and avoid Bruch's membrane (BM), the RPE slab encompassed only the apical one-third of the hyper-reflective RPE band on OCT B-scans with manual adjustment by ± 1 to 2 pixels as needed. Even though *en face* OCT and AF imaging of RPE atrophy is correlated to loss of melanin versus lipofuscin, respectively, prior studies have shown that there is good agreement for the detection of RPE atrophy between the two modalities in STGD.³⁵ Therefore, corresponding AF images was used for reference to ensure there were no gross errors with the final slab position."

Once the $6 \times 6 \text{ mm}^2$ *en face* slab projections of the IS/OS or RPE layer were created, three experienced graders (TA, AAA, and MJG) used the freehand selection tool in ImageJ V 2.0 (open source) to outline the areas of IS/OS layer loss and RPE layer (Fig 1c–d). Graders

were instructed to continuously review the B-scans during the grading process to ensure the masking accurately reflected the anatomical loss. Graders were blinded to each other's results. No normals were used for control given that healthy macula have no RPE or IS/OS loss so there is nothing to grade. The outlined boundaries of degeneration were used as maps to create an IS/OS loss mask or RPE atrophy mask, wherein areas of degeneration could be quantified (Fig 1e). Among the three graders, areas of IS/OS loss or RPE atrophy were analyzed for correlation statistics, and subsequently averaged for further statistical analysis.

Representative boundary masks from one grader, TA, were used for additional analysis. Using the “Subtract” and “And” functions from the ImageJ calculator tool, the IS/OS loss maps and RPE atrophy maps for each eye were overlaid to create a composite map (Fig 1f) that defined four types of regions in each eye: 1) common areas of matching IS/OS loss and RPE atrophy, 2) common areas of matching IS/OS and RPE preservation, as well as areas of mismatched degeneration wherein 3) IS/OS was lost in isolation with intact RPE, or 4) RPE was atrophic in isolation with intact IS/OS (Fig 1g). The boundary maps of these four regions were used as masks to quantify the area of each respective region for each eye.

A commercial version of split-spectrum amplitude-decorrelation angiography algorithm (SSADA) was used on the AngioVue system³⁶ to compute flow signal and generate volumetric OCTA scans (304×304×640 pixels).³⁷ Using the COOL-ART³⁴ software, the outer boundary of BM was semi-automatically detected, and CC angiogram (6×6 mm²) was generated by projecting the angiographic signal from 9 to 15 microns below the outer boundary of BM. Shadow graphic projection artifact from large retinal blood vessels was minimized by a previously described technique.³³ Choriocapillaris vascular density (CCVD), which is defined as the percentage area occupied by CC blood vessels, was evaluated for the entire *en face* angiogram in both STGD patients and normal healthy subjects.³⁸ The CCVD was also calculated for the areas defined by the boundary masks as described above for STGD patients (Fig 1h). Separate graders that were blinded to the masking analysis were used to analyze the images for CCVD in STGD patients and normal subjects.

All data from the right and left eye of each patient were highly-correlated (data not shown) and thus the averaged values of both eyes were used to represent each patient. Non-parametric linear regression analysis, non-parametric ANOVA (Kruskal-Wallis), Mann Whitney, and Spearman correlation were used for statistical analysis. Dunn's and Bonferroni correction was used for multiple comparisons. To determine inter-grader agreement, intraclass correlation coefficients (ICC) with 95% confidence intervals (CI) were calculated.

Results:

In our cohort of 23 patients with STGD, the mean age was 37 (range 13–62 years) with a preponderance of females with an approximately 2:1 ratio. Thirteen patients (57%) possessed two *ABCA4* pathogenic mutations, seven (30%) had only 1 identified pathogenic mutation, and three patients declined genetic testing (Table 1). The mean best-corrected visual acuity at the time of imaging was 20/70 (range 20/20–20/200) or 0.55 ±0.31 logMAR (range 0.0 –1.0 logMAR). Correlation analysis did not show that age was significantly

correlated with BCVA, which is not surprising given this is not a longitudinal study, but rather a cross-sectional study of a heterogeneous population of STGD patients with early or late onset disease with or without foveal atrophy (Fig 2, top).

The areas of degeneration calculated using masks from the boundary maps of total IS/OS loss or RPE atrophy among the three graders were highly correlated with an ICC (95% CI) of 0.997–0.999 and 0.985 (0.971–0.993), respectively. Among STGD patients, there was a large spectrum of total IS/OS loss (range 0.090–36 mm²) and RPE atrophy (range 0.037–36 mm²), with a trend towards the average area of degeneration for IS/OS being greater than RPE (16.10 ± 3.04 vs. 10.97 ± 2.39 mm², respectively; $p = 0.20$; Fig 2, middle). Indeed, within the imaged area, seven patients had complete loss (~36 mm²) of IS/OS, of which three also had complete loss of RPE. In other words, no patient had complete loss of RPE without also having complete loss of IS/OS. Excluding the floor effect of data points with complete loss (red circles), the total IS/OS loss and total RPE atrophy was strongly correlated with each other ($r = 0.96$, $p < 0.0001$), and linear regression showed the data was best fit by a linear equation with a slope of 1.6 ($r^2 = 0.90$; Fig 2, bottom). This data describes a relationship in which IS/OS loss is 1.6 times more widespread than RPE atrophy in STGD. Total IS/OS loss or RPE atrophy was only moderately correlated with age ($r = 0.48$ and 0.49 , respectively) or BCVA ($r = 0.61$ and 0.49 , respectively), but was not significant after correcting for multiple comparisons (Table 2, Fig S1).

Composite IS/OS-RPE masks were created from IS/OS loss and RPE atrophy masks with the exception of data with a floor effect of complete IS/OS and RPE degeneration within the 6×6 mm² area of analysis, which were excluded. The composite masks revealed four types of regions, which were color coded as follows: black represents areas of matching degeneration of IS/OS loss and RPE atrophy, white represents matching areas of intact IS/OS and RPE, gray represents areas of mismatched degeneration with intact RPE and isolated IS/OS loss, and red represents areas of mismatched degeneration with intact IS/OS and isolated RPE atrophy (Fig 3, top). There were similar amounts of matched degeneration (47%, 3.87 ± 0.93 mm²), as opposed to mismatched degeneration (53%, 4.41 ± 1.07 mm²; Fig 3, bottom left). Among the areas of mismatched degeneration, there was significantly greater areas of isolated IS/OS loss (86%, 3.79 ± 1.0 mm²) than isolated RPE atrophy (14%, 0.63 ± 0.15 mm², $p = 0.0005$; Fig 3, bottom right), which indicates that photoreceptor IS/OS degeneration is more widespread than RPE atrophy and commonly spreads into areas of intact RPE in STGD. The area of total IS/OS loss or total RPE atrophy was strongly and significantly correlated with matched degeneration ($r = 0.86$ or 0.99), mismatched degeneration ($r = 0.93$ or 0.84), and isolated IS/OS loss ($r = 0.93$ or 0.83), but not isolated RPE atrophy ($r = 0.51$ or -0.03 ; Table 2; Fig S2). Moreover, the area of isolated IS/OS loss was strongly and significantly correlated with matched or mismatched degeneration ($r = 0.84$ or 0.97), but isolated RPE atrophy was not ($r = -0.066$ or -0.075 ; Table 2; Fig 4). These data indicate that as the overall area of degeneration enlarges, the various types of regions identified by composite masking also enlarge proportionally except for isolated RPE atrophy, which was uncommonly observed and tended to be associated with rare IS/OS tubulations.

Analysis of the choriocapillaris by OCTA within the total $6 \times 6 \text{ mm}^2$ area showed that there was a trend towards declining CCVD with increasing age in STGD patients, but the correlation was moderate ($p = -0.50$) and lost significance when corrected for multiple comparisons, whereas normal subjects had relatively stable CCVD over time (Table 2; Fig 5, top). The normal individuals had a mean age of 34 (range 24–49 years; $n=10$). There was also a moderate correlation between declining CCVD with worsening BCVA ($r = -0.47$), but it also lost significance after Bonferroni correction (Table 2; Fig 5, middle). The total CCVD in STGD patients ($92.0 \pm 2.0 \%$) was significantly lower than normal subjects ($99.0 \pm 0.17 \%$; $p = 0.0044$; Fig 5, bottom), and negatively correlated with greater areas of total IS/OS loss ($r = -0.77$), total RPE loss ($r = -0.75$), matched degeneration ($r = -0.76$), and isolated IS/OS loss ($r = -0.69$), but not with isolated RPE atrophy (Fig S3). These results showed that total CCVD attenuation had a much stronger relationship with the severity of photoreceptor and RPE degeneration than with age or BCVA. Moreover, isolated RPE atrophy was uncommon and likely contributed too small an area to have a significant relationship with total CCVD.

Composite IS/OS-RPE masks were also used to analyze the CCVD within the four region types. Regions with areas near zero were excluded from analysis because CCVD could not be meaningfully assessed. The CCVD within regions of matched degeneration ($85.6 \pm 2.7 \%$), isolated IS/OS loss ($93.6 \pm 1.0 \%$), and isolated RPE atrophy ($94.1 \pm 1.1 \%$) were all significantly lower than the CCVD in normal subjects ($99.0 \pm 0.17 \%$; $p < 0.0001$, $=0.0011$, $=0.0065$, respectively; Fig 5, bottom). However, there was no significant difference among the CCVD within regions of isolated IS/OS loss, isolated RPE atrophy, or matched degeneration ($p > 0.99$). These data show that either IS/OS or RPE degeneration may independently contribute to a similar decline in CCVD, which tended to be milder than that associated with combined IS/OS and RPE attenuation.

There was no correlation between the CCVD within regions of isolated IS/OS loss or isolated RPE atrophy and the size of the area of degeneration (Table 2; Fig S4, bottom). In contrast, the extent of CCVD attenuation within areas of matched degeneration was moderately correlated with the increasing size of the respective area, however it was not significant after correcting for multiple comparisons ($r = -0.48$; Table 2; Fig S4, top left). The CCVD within areas of no degeneration (intact IS/OS and RPE) in STGD patients ($97.6 \pm 0.38 \%$) was significantly greater than areas of matched degeneration ($p = 0.0004$), but not significantly different than areas of isolated IS/OS or RPE degeneration (Figure 5 bottom). Even though the CCVD within seemingly intact areas of retina and RPE in STGD was not significantly different than normal subjects (STGD: 97.6 ± 0.38 vs normal: $99.0 \pm 0.17 \%$), it had a range with a lower limit (STGD: 93.8 to 99.6 % vs normal: 97.9–99.6 %) and there was moderate correlation of worsening CCVD within diminishing areas of intact structure that was just beyond the threshold for significance after Bonferroni correction ($r = 0.68$; Table 2; Fig S4, top right). Taken together, these data suggest that both intra-lesional and extra-lesional CCVD tended to worsen as the degenerative lesion expands, which may explain why the total CCVD was much more strongly correlated with the size of the degenerative lesion.

Discussion:

The *en face* OCT and OCTA findings in STGD have been reported previously, however, here we utilize an *en face* slab projection method and boundary maps to create IS/OS loss or RPE atrophy masks that not only allow for the analysis of total IS/OS loss or RPE atrophy, but also IS/OS-RPE composite masks that allow for the analysis of regions with isolated IS/OS loss, isolated RPE atrophy, matched IS/OS and RPE degeneration, or intact IS/OS and RPE. These masks allow for quantification and correlation analysis among different region types of degeneration, as it pertains to area of degeneration, CCVD, age, and BCVA.

With regard to *en face* OCT, Sodi, et al., Greenstein, et al., and Melillo, et al. have published various observations regarding RPE atrophy and IS/OS attenuation using various slab projection techniques.^{35,39,40} Both Sodi, et al., and Greenstein, et al., utilized similar slab methods as our study to produce *en face* projections of the IS/OS layer, whereas Melillo, et al., measured the transverse extents of IS/OS loss on OCT b-scans. All three groups used a sub-RPE slab projection method to create *en face* images of the RPE atrophy by visualizing light penetration into the choroid. The sub-RPE method favors the detection of areas of severe RPE atrophy with obvious light penetration, whereas our RPE slab method measures apical RPE signal which may be more sensitive to a wider spectrum of RPE thinning. Another group, Gomes, et al., performed diameter measurements of foveal IS/OS and RPE atrophy on transverse OCT b-scans.⁴¹ Despite the differing methods and different cohort sizes (Sodi, n=32; Greenstein, n=16; Melillo, n=98; Gomes, n=11), all four groups found that the extent of IS/OS loss was greater than RPE atrophy, which is consistent with our findings. Furthermore, we show here for the first time to our knowledge, a strong correlation wherein IS/OS loss is 1.6 times more widespread than RPE atrophy. Taken together with our observation that isolated RPE does not contribute significantly to overall degeneration, these structural studies support the theory that photoreceptor outer segment degeneration generally occur before RPE atrophy and not vice-versa in STGD.

Choriocapillaris loss in STGD has also been shown in prior OCTA studies.^{42–47} Battaglia Parodi, et al., showed that CCVD was worse in STGD eyes that had chorioretinal atrophy,⁴⁶ and both Pellegrini, et al., and Muller, et al., found that the choriocapillaris flow signal was visibly diminished within areas of RPE atrophy in STGD, but not outside the areas of RPE atrophy.^{42,47} Similar to these prior studies, the CCVD within areas of isolated RPE atrophy in our STGD patients was significantly lower than normal, but we also showed the same thing in areas of isolated IS/OS loss. Moreover, we found that the CCVD in areas of combined RPE and IS/OS attenuation tended to be even more abnormal, supporting the theory that the degeneration of both layers is synergistically associated with choriocapillaris attenuation in STGD. Furthermore, our data showed that the extent of total CCVD attenuation was strongly correlated with the magnitude of RPE degeneration, as well as photoreceptor degeneration.

In addition, de Carlo, et al., have reported that areas of abnormal choriocapillaris appeared to be smaller than the corresponding regions of RPE and photoreceptor loss in STGD.⁴³ In our experience, the boundary between abnormal and normal areas CCVD was difficult to visualize and map reliably so our study did not quantify the size of the CCVD defect.

However, using composite IS/OS-RPE boundary masks, we demonstrated that the total CCVD was much more strongly-correlated than intralesional CCVD to the size of the degenerative lesion, which suggests that extra-lesional CCVD may contribute to the level of total CCVD attenuation in STGD. Our results are in contradiction to the findings of de Carlo and colleagues, but indirectly supported by the microperimetry studies of Strauss, et al., showing extra-lesional areas of decreased cone and rod-dependent sensitivity.⁴⁸ Taken together with the findings of Strauss and colleagues, our results suggest that relative photoreceptor dysfunction with mild choriocapillaris attenuation may precede lesion expansion in STGD.

Prior studies have reported correlations of IS/OS loss, RPE atrophy, or CCVD with age and/or BCVA.^{39,44,45,49} Arepalli, et al., reported that foveal IS/OS atrophy was inversely correlated with visual acuity,⁴⁹ and Melillo, et al., have shown that macular RPE atrophy area increases exponentially with age in STGD.³⁹ Ratra, et al. and Mastropasqua, et al. showed that choriocapillaris vascular index and CCVD were correlated with decreasing visual acuity and parafoveal macular thickness, respectively.^{44,45} Similarly, our data showed a trend towards a moderate correlation between IS/OS loss, RPE atrophy, and CCVD attenuation with age or BCVA, however our statistical significance was limited by multiple comparisons. It is also likely that our cohort was more heterogeneous than other studies due to inherent differences in sampling error, wherein the composition of patients with late onset disease and foveal sparing versus those with early onset disease with foveal atrophy would directly influence these correlations. Stratification of our data set according to these patient types was limited by access to historical records and inaccurate recall in our older patients. Our cohort (n =23) was also smaller than that of Melillo (n =98) and Ratra (n =39), but greater than Arepalli (n =17) and Mastropasqua (n =12).

This study was limited by relatively small sample size, however STGD is a rare disease and future multicenter studies would be required to obtain larger datasets. While seven subjects were excluded from composite mask and correlation analysis due to floor effect, the data from the remaining 16 patients was still sufficient to show statistically significant correlations even with Bonferroni correction that resulted in a much stricter p-value of 0.0013 for determination of significance (table 2). In addition, the determination of slab position and grading of primary masks was a manual process and time-consuming, however we implemented a standardized protocol and the degree of inter-grader agreement was extremely high. Future work will be directed towards developing algorithms to automate these processes and determine its agreement with manual analysis, as well as increasing the number of patients. This study is also limited by its cross-sectional nature, and future longitudinal studies will be helpful in confirming the findings of our study. The conclusions of this study were drawn solely on findings from retinal structure alone, so future structure-function studies will also be necessary assess the dynamics of photoreceptor and RPE dysfunction with degeneration in STGD.

We show quantitative evidence that photoreceptor and RPE degeneration exhibit a strong relationship wherein photoreceptor IS/OS loss is 1.6 fold more widespread than RPE atrophy, supporting the theory that photoreceptor degeneration may precede RPE in STGD. Furthermore, our study also showed that IS/OS loss and RPE atrophy contribute

synergistically to CCVD attenuation, but extra-lesional CCVD also tended to be abnormal, which may be associated with possible early dysfunction of photoreceptor or RPE. These findings along with the techniques used in this study may be of considerable utility in determining novel endpoints for gene and stem cell therapy clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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a. Funding/Support

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b. Financial Disclosures:

Oregon Health & Science University (OHSU), Yali Jia and David Huang have significant financial interests in Optovue, a company that may have a commercial interest in the results of this research and technology. These potential conflicts of interest have been reviewed and managed by OHSU.

Yali Jia: Optovue Inc.: Financial support, patent.

David Huang: Optovue, Inc.: patent royalty, stock ownership, research grant and material support, speaker honorarium and travel support.

Richard Weleber and Mark Pennesi: Commercial Relationship: Scientific Advisory Boards for the Foundation Fighting Blindness. This relationship has been reviewed and managed by OHSU.

Simon Gao: Employee of Genentech since Feb 2017

Miao Zhang: Employee of Topcon since March 2018

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Biography

Dr. Yang is currently an assistant professor in ophthalmic genetics and ocular immunology at the Casey Eye Institute, and is a member of the Oregon Retinal Degeneration Center. The focus of his research is to better understand the pathophysiology of various inherited retinal degenerations, and the role of secondary immune-mediated damage. With the support of a career development award from the Foundation Fighting Blindness and a K08 from the National Eye Institute, he is currently conducting clinical trials and translational bench

research in an effort to bring new medical treatments to the clinic for patients with inherited retinal degenerations.

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Figure 1: Methods for *en face* slab projection, boundary maps, and mask analysis

Sample images from patient 2 showing one b-scan with preserved IS/OS and RPE and another with only isolated RPE atrophy (a). Slab position (blue line) is optimized manually for both IS/OS and RPE layer, utilizing autofluorescence images as reference for the later (b). The *en face* projections of the IS/OS and RPE slab is shown along with the boundary lines (cyan) of IS/OS and RPE atrophy, respectively, from one grader (c, d). The IS/OS loss mask (dark yellow) and RPE atrophy mask (dark cyan) are created from the boundary maps (e), then overlaid to create the IS/OS-RPE composite mask (f). The composite mask identifies four types of regions of degeneration, which are used for the analysis of area (g)

and CCVD (**h**). IS/OS, inner segment/outer segment layer; RPE, retinal pigment epithelial layer; OCTA, OCT angiography; CCVD, choriocapillaris vessel density.

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Figure 2: Age vs. BCVA, and total RPE atrophy vs. total IS/OS loss

(**Top**) Scatter plot of age vs. BCVA, which shows no correlation. (**Middle**) On average, total IS/OS loss tended to be greater than total RPE atrophy, however it was not statistically significant and the data was skewed by floor effect of complete loss of layers (36 mm²) in 6 patients for IS/OS loss and 3 patients for RPE atrophy. (**Bottom**) Scatter plot of total RPE atrophy vs. IS/OS atrophy shows a strong correlation, excluding data points that suffer from floor effect (red circles). For reference, the dotted line represents a 1:1 relationship. BCVA, best-corrected visual acuity; IS/OS, inner segment/outer segment layer; RPE, retinal pigment epithelial layer

Figure 3: Composite mask analysis of area of loss

(**Top**) Samples images of composite analysis, which is color-coded to show the four different patterns of degeneration: areas of no degeneration (white), matched degeneration (black), isolated IS/OS loss (gray), and isolated RPE atrophy (red). Patients 2, 19, and 11 show large areas of matched degeneration and isolated IS/OS loss, while patients 3, 10, and 14 show diffusely intact IS/OS and RPE with central area of mixed types of degeneration. Both patient 2 and 14 show foveal preservation of IS/OS and RPE. Overall, isolated RPE atrophy was not common. (**Bottom left**) On average, the area of matched degeneration was similar to the area of mismatched degeneration (isolated IS/OS loss + isolated RPE atrophy). (**Bottom right**) The area of isolated IS/OS loss was significantly greater than the area of

isolated RPE atrophy. IS/OS, inner segment/outer segment layer; RPE, retinal pigment epithelial layer; Asterisk, $p = 0.0005$.

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Figure 4: Correlation analysis of region types of degeneration

(Left top and bottom) Scatter plots of isolated IS/OS loss vs. matched or mismatched degeneration show a strong correlation. **(Right top and bottom)** Scatter plots of isolated RPE atrophy vs. matched or mismatched degeneration show no correlation. Data points with floor effect (red) were excluded from analysis. IS/OS, inner segment/outer segment layer; RPE, retinal pigment epithelial layer.

Figure 5: Age or BCVA vs. CCVD, and composite mask analysis of CCVD

(**Top and middle**) Scatter plot of age or BCVA vs. CCVD show a moderate correlation of declining CCVD with increasing age or decreasing BCVA (increasing logMAR), however statistical significance was limited by multiple comparisons (see table 2). (**Bottom**) Total CCVD from normal subjects were significantly greater than total CCVD and the CCVD within the various region types of degeneration in Stargardt patients, except for areas of intact IS/OS and RPE. The CCVD within intact areas were significantly greater than the CCVD within areas of matched degeneration in Stargardt patients. Solid line with error bar represents mean and 95% confidence interval. BCVA, best-corrected visual acuity; IS/OS, inner segment/outer segment layer; RPE, retinal pigment epithelial layer; OCTA, OCT angiography; CCVD, choriocapillaris vessel density; Asterisk, $p < 0.05$.

Table 1.**Patient Demographics and Genotype**

	Gender	Age	<i>ABCA4</i> allele 1	<i>ABCA4</i> allele 1
1	F	14	c.5882G>A	c.4469G>A
2	F	62	c.1222 C>T	ND
3	M	35	TD	TD
4	M	35	c.5714+5G>A	c.2894A>G
5	F	19	c.6449G>A	c.5196+1137G>A
6	F	25	c.4537C>G	ND
7	M	21	c.5882G>A	c.4139C>T
8	F	59	c.2041C>T	ND
9	M	55	c.5196+1137G>A	c.4774-27T>C
10	F	19	c.2588G>C	c.1222C>T
11	M	53	c.5461-10T>C	ND
12	F	56	c.2947A>G	ND
13	F	21	c.1622T>C	c.3113C>T
14	F	42	c.4222T >C	c.4918C>T
15	F	13	c.4739T>C	c.4469G>A
16	F	52	TD	TD
17	M	26	c.4457C>T	c.5461-10T>C
18	F	55	TD	TD
19	F	16	c.5714+5G>A	c.4469G>A
20	M	56	c.4234C>T	ND
21	F	49	c.6229C>T	890 bp deletion ^a
22	F	48	c.5899-2delA	ND
23	F	23	c.2588G>C	c.161G>A
Male =7		Mean =37	2 alleles identified n= 13	
Female =16		SD =17.1	1 allele identified n= 7 TD n= 3	

ND, not detected; TD, testing declined;

^a spanning exon 28 and 29

Table 2:

Correlation Statistics

Age vs.	Spearman r	p value	BCVA vs.	Spearman r	p value
BCVA	-0.1127	0.6085	-	-	-
Total IS/OS loss	0.4833	0.0195	Total IS/OS loss	0.6053	0.0022
Total RPE atrophy	0.4853	0.0189	Total RPE atrophy	0.5929	0.0029
Isolated IS/OS loss	0.5312	0.0132	Isolated IS/OS loss	0.2245	0.3280
Isolated RPE atrophy	0.2446	0.2852	Isolated RPE atrophy	-0.3626	0.1062
Matched loss	0.5892	0.0049	Matched loss	0.4945	0.0227
Mismatched loss	0.5260	0.0143	Mismatched loss	0.2375	0.3000
CCVD (6×6 mm2)	-0.5036	0.0143	CCVD (6×6 mm2)	-0.4736	0.0224
Total IS/OS loss vs.			Total RPE atrophy vs.		
Total RPE atrophy*	0.9647	<0.0001	-	-	-
Isolated IS/OS loss*	0.9265	<0.0001	Isolated IS/OS loss*	0.8331	<0.0001
Isolated RPE atrophy	0.5077	0.0466	Isolated RPE atrophy	-0.0301	0.8998
Matched loss*	0.8643	<0.0001	Matched loss*	0.9910	<0.0001
Mismatched loss*	0.9324	<0.0001	Mismatched loss*	0.8406	<0.0001
CCVD (6×6 mm2)*	-0.7684	<0.0001	CCVD (6×6 mm2)*	-0.7459	0.0002
Isolated IS/OS loss vs.			Isolated RPE atrophy vs.		
Isolated RPE atrophy	-0.1677	0.4797	-	-	-
Matched loss*	0.8389	<0.0001	Matched loss	-0.0663	0.7813
Mismatched loss*	0.9744	<0.0001	Mismatched loss	-0.0745	0.7550
CCVD (6×6 mm2)*	-0.6917	0.0007	CCVD (6×6 mm2)	0.2505	0.2868
CCVD (isolated IS/OS loss)	-0.1233	0.6045	CCVD (isolated RPE atrophy)	0.2420	0.4015
Matched loss vs.			Mismatched loss vs.		
Mismatched loss*	0.8313	<0.0001	-	-	-
CCVD (6×6 mm2)*	-0.7620	<0.0001	CCVD (6×6 mm2)	-0.4948	0.0226
CCVD (matched loss)	-0.4834	0.0308			
			Intact IS/OS+RPE vs.		
			CCVD (intact IS/OS+RPE)	0.6842	0.0017

* Bonferroni correction: $p = 0.0013$ for significance

BCVA, best-corrected visual acuity; IS/OS, inner segment/outer segment junction; RPE, retinal pigment epithelium; Matched loss, total area of both IS/OS loss and RPE atrophy; Mismatched loss, total area of either isolated IS/OS or RPE atrophy; CCVD, choriocapillaris vessel density; Asterisk, comparisons that remain significant after Bonferroni correction for multiple comparisons.