


## Original Article

# Age-related scattered hypofluorescent spots on late-phase indocyanine green angiography: the multimodal imaging and relevant factors

Ling Chen MD, Xiongze Zhang MD PHD, Bing Liu MD, Lan Mi MD and Feng Wen MD PHD 

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China

### ABSTRACT

**Importance:** Fundus aging and its imaging features.

**Background:** To characterize the demographic and multimodal-imaging features of age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA).

**Design:** A hospital-based retrospective study.

**Participants:** Eight hundred and seventy-five normal fundi fellow eyes from 875 patients underwent indocyanine green angiography (ICGA), fluorescence angiography (FA), autofluorescence (AF) and spectral-domain optical coherence tomography (OCT).

**Methods:** Demographic information, medical records and multimodal imaging data were reviewed.

**Main Outcome Measures:** Diameter of ASHS-LIA and its grade, subfoveal choroidal thickness (SFCT).

**Results:** ASHS-LIA was identified in 233 patients (26.6%) aged 33 to 87 years (mean:  $65.8 \pm 8.4$  years). Patients with ASHS-LIA were significantly older and had a higher male proportion than those without ASHS-LIA (both  $P < 0.001$ ). The occurrence and grade of ASHS-LIA increased with age (all  $P < 0.001$ ). Age (OR = 1.093) and male gender

(OR = 1.550) were the independent relevant factors of ASHS-LIA ( $P < 0.001$ , and  $P = 0.002$ , respectively). The incidence of ASHS-LIA in polypoidal choroidal vasculopathy (PCV) patients (53.2%) was the highest (all  $P < 0.001$ ). ASHS-LIA mainly located in macular region (diameter: 100–500  $\mu\text{m}$ ), and could be confluent. No corresponding abnormalities were detected via multimodal imaging, including FA, AF and OCT. The mean SFCT had no significant difference between eyes with and without ASHS-LIA ( $P = 0.221$ ).

**Conclusions and Relevance:** ASHS-LIA was observed on late-phase ICGA, mainly located in macular region. No corresponding abnormalities were detected by other multimodal imaging, including FA, AF and OCT. The occurrence and grade of ASHS-LIA increased with age. Moreover, ASHS-LIA might be not correlated with SFCT, but correlated with PCV.

**Key words:** age-related hypofluorescent spots, fundus aging, indocyanine green angiography, multimodal imaging, polypoidal choroidal vasculopathy.

### INTRODUCTION

Age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) were first reported by Shiraki *et al.* in 1999.<sup>1</sup> Their study, with a relatively small sample size

■ **Correspondence:** Dr Feng Wen, State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 South Xianlie Road, Guangzhou 510060, China. Email: wenfeng208@foxmail.com

Received 31 December 2017; accepted 3 April 2018.

**Funding sources:** This investigation was supported by grants from the National Natural Science Foundation of China (grant numbers: 81470647).

© 2018 The Authors Clinical & Experimental Ophthalmology published by John Wiley & Sons Australia, Ltd on behalf of Royal Australian and New Zealand College of Ophthalmologists

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

including eyes with both a normal fundus and dry age-related maculopathy, demonstrated that ASHS-LIA was age-related but not correlated with dry age-related maculopathy. ASHS-LIA was distributed in the posterior pole, especially in the macular region. The hypofluorescent spots were noted on late-phase indocyanine green angiography (ICGA), and it was different from the hypofluorescent lesions common in patients with inflammatory conditions involving the choroid such as multifocal choroiditis and acute posterior multifocal placoid pigment epitheliopathy (APMPPE).<sup>2,3</sup> However, there was no article further describing this finding, and the multimodal imaging features of ASHS-LIA, including autofluorescence (AF) and optical coherence tomography (OCT) were not described before. So far, what ASHS-LIA really represents is still not clear.

ICGA is mainly used in the diagnosis of fundus diseases, especially choroidal diseases. As we know, indocyanine green (ICG) dye is water-soluble and may bind to lipid due to polarity but does not bind to hydrophobic neutral lipids. Research showed that ICG dye was located within retinal and choroidal vessels in the early-phase after injection; however, it was extravasated into the choroidal stroma and accumulated within the retinal pigment epithelium (RPE) over time.<sup>4,5</sup> Therefore, fundus fluorescence characteristics on late-phase ICGA might be very important to indicate the pathogenesis of the fundus disease. In addition, the multimodal imaging including colour fundus photography (FP), fundus fluorescein angiography (FFA), AF and OCT might contribute to analyse what ASHS-LIA really represents.

The purpose of the present study was to characterize the demographic and multimodal imaging features of ASHS-LIA and to explore its possible underlying relevant factors.

## METHODS

This is a retrospective, cross-sectional study. All patients who were referred to the Zhongshan Ophthalmic Center for ICGA, FFA, colour FP, AF and OCT examination between January 2014 and April 2017 were reviewed. Patients who had a previous ophthalmological intervention procedure, such as laser coagulation, vitrectomy, anti-vascular endothelial growth factor injection or photodynamic dynamic therapy in one or both eyes, were excluded from this study. Furthermore, patients with poor-quality images affecting fluorescence judgement, or patients with bilateral lesions were excluded. In addition, patients with inflammatory conditions involving the choroid such as multifocal choroiditis and APMPPE were also excluded. After these criteria were applied, 875 normal fundi fellow eyes

from 875 patients were eligible for inclusion in this study. It must be paid for attention that 'normal fundi fellow eyes' in this study were defined as no definite fundus diseases, such as retinal vascular diseases, choroidal neovascular diseases, RPE-related diseases or hereditary fundus diseases, and so on.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center at Sun Yat-sen University. Potential risks associated with ICGA and FFA examination were fully discussed with the patients, and written informed consent was obtained from all included patients.

Demographic information, medical records and multimodal imaging data were reviewed. The best-corrected visual acuity (BCVA) was measured with Snellen charts; Colour FP was performed with a Zeiss FF450 Plus fundus camera (Carl Zeiss, Inc., Jena, Germany). ICGA, FFA and AF were performed with Heidelberg Retina Angiogram (Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany). OCT was performed with an HRA + OCT Spectralis (Heidelberg Engineering, Heidelberg, Germany). Two ophthalmologists (Ling Chen and Xiongze Zhang) independently assessed and diagnosed the patients, assessed whether there was ASHS-LIA, and characterized its ICGA and multimodal imaging features, if ASHS-LIA exists. In addition, the subfoveal choroidal thickness (SFCT) was detected by enhanced depth imaging OCT (EDI-OCT) as previous method.<sup>6</sup> When the two ophthalmologists disagreed, the final decision was made by consulting a retinal specialist (Feng Wen).

Statistical analyses were performed with SPSS Version 21.0 software (SPSS, Inc., Chicago, IL, USA). Descriptive statistics (means  $\pm$  standard deviations [SDs]) of normally distributed variables and geometric means with 95% confidence intervals (CIs) of non-normally distributed variables were calculated. Comparisons were evaluated by independent sample *T*-test or one-way analysis of variance (ANOVA) (normal data), or  $\chi^2$  test (non-normal data). Multiple logistic regression models were constructed to assess the independent relevant factors for ASHS-LIA. Odds ratios (ORs) and 95% CIs are shown. Significant differences were defined for *P*-values <0.05.

## RESULTS

### Patients

A total of 875 eyes from 875 patients were included in this study. The ages ranged from 6 to 90 years (mean: 54.2  $\pm$  16.4 years), and the male: female ratio was 1.6:1. Among the 875 patients, ASHS-LIA was detected in 233 patients (26.6%). The ages of

these patients ranged from 33 to 87 years (mean:  $65.8 \pm 8.4$  years), and the male:female ratio was 2.6:1.

Table 1 shows the demographic characteristics of patients with and without ASHS-LIA. A total of 233 patients were determined with ASHS-LIA, and the average age was  $65.8 \pm 8.4$  years, which was significantly older than those without ASHS-LIA (mean:  $50.0 \pm 16.5$  years) ( $t = -13.957$ ,  $P < 0.001$ ). The male proportion in patients with ASHS-LIA was 2.6:1, significantly higher than in patients without ASHS-LIA (1.4:1) ( $X^2 = 14.204$ ,  $P < 0.001$ ). Moreover, to explore the relationship between age and ASHS-LIA, patients were divided into five groups according to age ( $\leq 30$ , 31–45, 46–60, 61–75 and  $\geq 76$  years), and the incidence of ASHS-LIA in the different age groups was calculated as 0% (0/100), 1.4% (2/144), 20.4% (55/269), 47.8% (142/297) and 52.3% (34/65), respectively. Patients aged 61–75 years and patients older than 75 years had significantly higher occurrence rates of ASHS-LIA compared with other age groups ( $X^2$  test, all  $P < 0.001$ ); nevertheless, there was no significant difference between the 61–75 year age group and the greater-than-75 age group ( $X^2 = 0.432$ ,  $P = 0.511$ ). In addition, patients aged 46–60 years had significantly higher occurrence rates of ASHS-LIA compared with patients aged 31–45 years and patients younger than 30 years ( $X^2$  test, both  $P < 0.001$ ).

Among the total of 875 included patients, ASHS-LIA was observed in 53.2% (175/329) of PCV patients, 26.5% (22/83) of nAMD patients, 20% (7/35) of dry AMD patients, 6.5% (10/153) of CSC patients, 0% (0/41) of patients with normal fundi and 8.1% (19/234) of patients with other fundus diseases (including pathological myopia, idiopathic choroidal neovascularization, retinal macroaneurysms, central retinal vein occlusion, branch retinal vein occlusion, epiretinal membrane, idiopathic pigmentary epithelial detachment, focalchoroidal atrophy, choroidal hemangioma, choroidal melanoma, choroidal osteoma, coats' disease, optic neuropathy and syphilitic retinitis). Table 2 showed the incidence of ASHS-LIA and demographic characteristics

**Table 1.** Demographic characteristics of patients with and without ASHS-LIA<sup>†</sup>

	With ASHS-LIA	Without ASHS-LIA	<i>P</i> value*
<i>n</i>	233	642	-
Age	$65.8 \pm 8.4$	$50.0 \pm 16.5$	$P < 0.001$
Male (%)	72.1	58.1	$P < 0.001$

<sup>†</sup>Data represent *n*, means  $\pm$  SD or %. ASHS-LIA, age-related scattered hypofluorescent spots on late-phase indocyanine green angiography.

**Table 2.** The incidence of ASHS-LIA and demographic characteristics by different diagnosis<sup>†</sup>

	Age	Male (%)	ASHS-LIA <i>n</i> / <i>T</i> (%)
PCV	$63.5 \pm 8.5$	69.6	175/329 (53.2)
nAMD	$66.9 \pm 10.5$	57.8	22/83 (26.5)
dry AMD	$63.9 \pm 10.9$	51.4	7/35 (20)
CSC	$46.5 \pm 11.7$	75.8	10/153 (6.5)
Normal	$37.4 \pm 16.8$	51.2	0/41 (0)
Other	$43.2 \pm 17.4$	46.6	19/234 (8.1)

<sup>†</sup>Data represent the means  $\pm$  SD, *n*/*T* or %. ASHS-LIA, age-related scattered hypofluorescent spots on late-phase indocyanine green angiography; CSC, central serous chorioretinopathy; *n*, the number of patients with ASHS-LIA; nAMD, neovascular age-related macular degeneration; PCV, polypoidal choroidal vasculopathy; *T*, the total number of patients.

by different diagnosis. Furthermore, the incidence of ASHS-LIA in PCV patients was the highest, significantly higher than that in patients with nAMD, dry AMD, CSC and normal fundi ( $X^2$  test, all  $P < 0.001$ ).

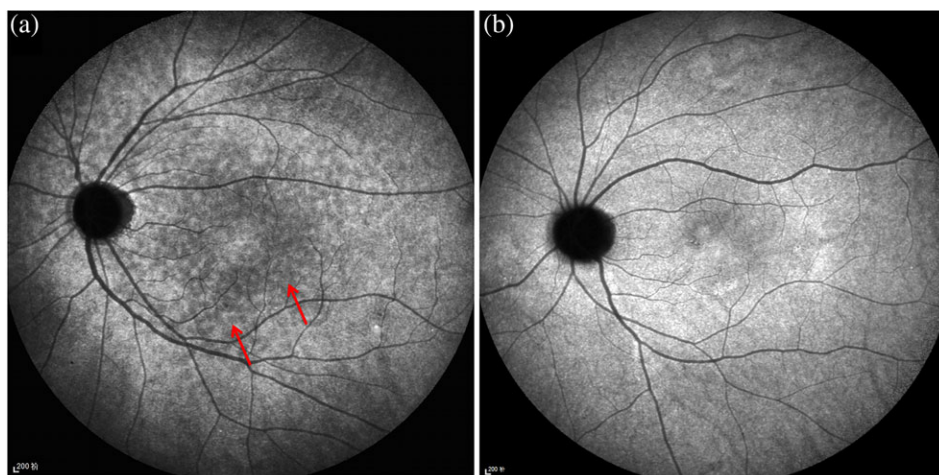
## ICGA characteristics of ASHS-LIA

In our study, ASHS-LIA was observed as early as 15 min after ICG dye injection and was obvious by 30 min post-injection (Fig. 1). ASHS-LIA mainly located in the macular region, even the whole posterior pole. When the whole posterior pole was involved, ASHS-LIA occasionally spread to the nasal near mid-periphery retina, but no spots were observed in the temporal near mid-periphery or the far mid-periphery retina in our study. The diameter of ASHS-LIA ranged from 100 to 500  $\mu$ m, with the majority between 200 and 300  $\mu$ m. Moreover, ASHS-LIA could be confluent, initially appearing in the macular region. According to the region of involvement, we divided ASHS-LIA into three grades (Fig. 2a–c), as follows: grade 1, macular region (68 out of 233), grade 2, macular region and around the optic disc (140 out of 233), and grade 3, throughout the posterior pole (25 out of 233). Confluent ASHS-LIA can occur at all levels (Fig. 2d–f). In addition, our study indicated that the mean age of patients was significantly correlated with the grade of ASHS-LIA (one-way ANOVA, all  $P < 0.001$ ). Nevertheless, there was no significant correlation between the grade of ASHS-LIA and the sex of the patient (Table 3).

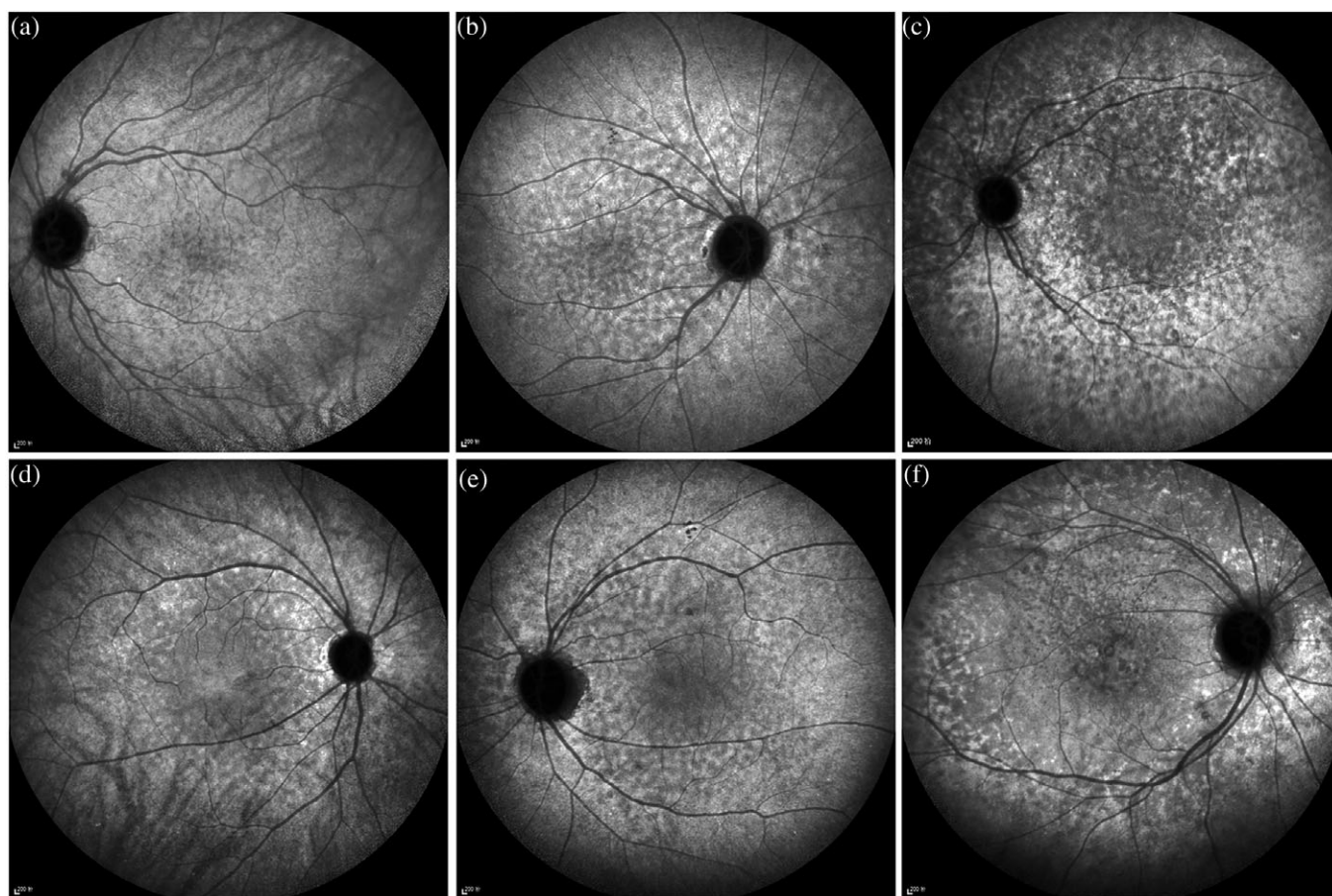
## Multimodal imaging features of ASHS-LIA

Among the 233 patients with ASHS-LIA, all patients underwent dilated colour FP, AF, FFA and OCT. No corresponding changes were observed via colour FP, AF, FFA and OCT in all cases. In brief, colour FP showed an almost normal fundus, and





**Figure 1.** ICGA characteristics of ASHS-LIA. (a) Normal fundi fellow eye from a 59-year-old man whose right eye was diagnosed with polypoidal choroidal vasculopathy. Age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) (red arrow) were distributed in the macular region and around the optic disc (30 min after dye injection). (b) Normal fundi fellow eye from a 42-year-old woman whose right eye was diagnosed with idiopathic choroidal neovascularization. There was no ASHS-LIA in the late-phase ICGA.



**Figure 2.** The grades of ASHS-LIA based on the region of involvement. Different grades of age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) (30 min after dye injection) were shown. (a) ASHS-LIA mainly distributed in the macular region; (b) ASHS-LIA distributed in the macular region and around the optic disc; (c) ASHS-LIA distributed throughout the whole posterior pole; (d) ASHS-LIA distributed in the macular region, with partial confluence; (e) ASHS-LIA distributed in the macular region and around the optic disc, with partial confluence; (f) ASHS-LIA distributed throughout the whole posterior pole, with partial confluence.

there was no abnormal fluorescence corresponding to ASHS-LIA on AF. Apart from faint uneven hypofluorescent spots were observed on very early stage of FFA in some patients, most patients showed uniform background fluorescence from early stage to

late stage after fluorescein injection, with no abnormal fluorescence spots corresponding to ASHS-LIA. Additionally, OCT showed that retinal structures were generally normal and that the RPE band was intact and smooth, with no intraretinal, subretinal or

**Table 3.** Demographic characteristics of patients with different grade of ASHS-LIA<sup>†</sup>

	Grade 1 (n = 68)	Grade 2 (n = 140)	Grade 3 (n = 25)	P values*		
				Grade 1 versus grade 2	Grade 2 versus grade 3	Grade 1 versus grade 3
Age	57.2 ± 6.2	68.1 ± 5.1	76.6 ± 7.6	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
Male (%)	73.5	70.7	76	0.673	0.590	0.809

<sup>†</sup>Data represent *n*, means ± SD or %. ASHS-LIA, age-related scattered hypofluorescent spots on late-phase indocyanine green angiography; grade 1, ASHS-LIA in the macular region; grade 2, ASHS-LIA in the macular region and around the optic disc; grade 3, ASHS-LIA throughout the posterior pole.

sub-RPE deposition corresponding to ASHS-LIA (Figs 3 and 4). In addition, the mean SFCT had no significant difference between eyes with ASHS-LIA (295.1 ± 82.3) and without ASHS-LIA (286.9 ± 88.8) (*t* = 1.225, *P* = 0.221).

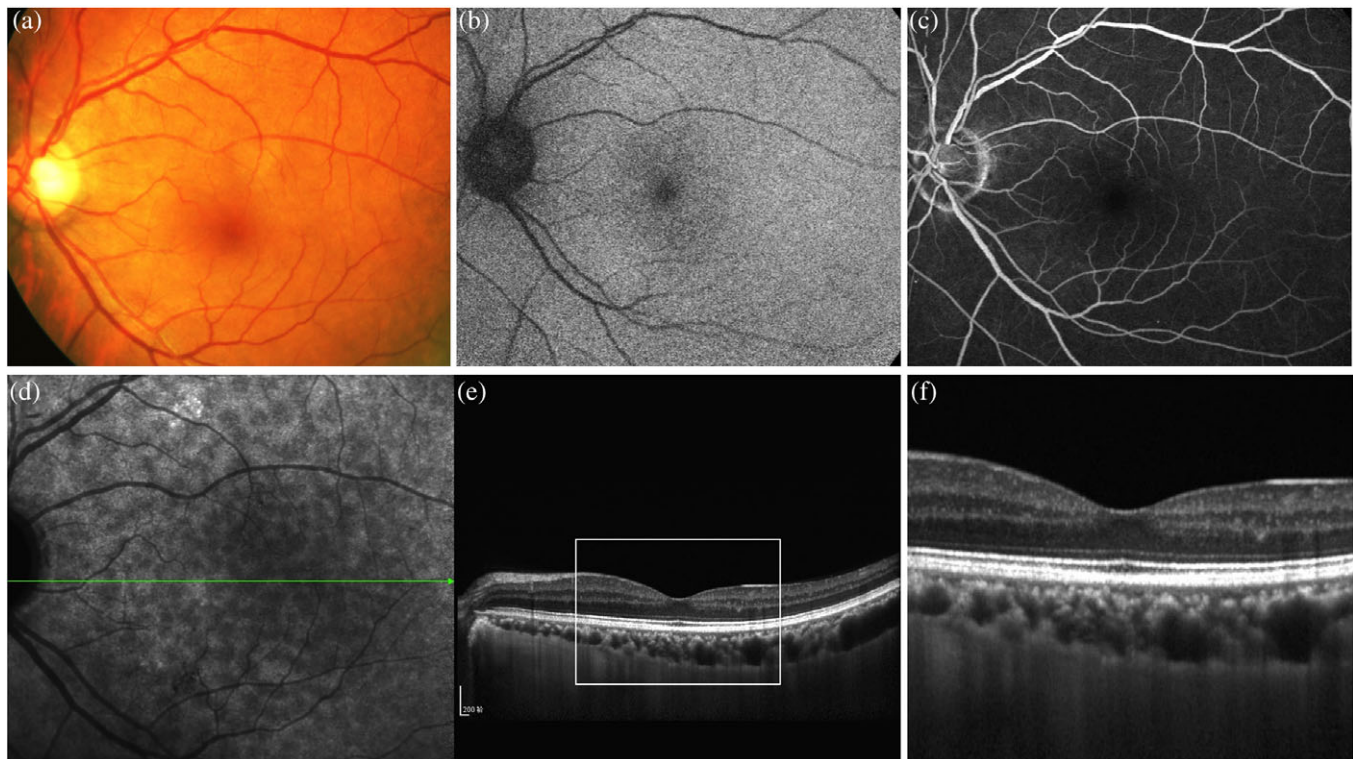
### Independent relevant factors of ASHS-LIA

To determine the independent relevant factors of ASHS-LIA, multiple logistic regression models were constructed. Factors including age (continuous variable) and gender (two categorical variables) were included in the multiple logistic regression analysis.

The odds ratio (OR) for ASHS-LIA was 1.093 per year of age (95% CI = 1.076–1.111) (*P* < 0.001), and the  $\beta$  value was 0.089. In addition, compared to female patients, male patients had an increased occurrence of ASHS-LIA, by approximately 1.799-fold (OR = 1.799; 95% CI = 1.248–2.591) (*P* = 0.002), and the  $\beta$  value was 0.587 (Table 4).

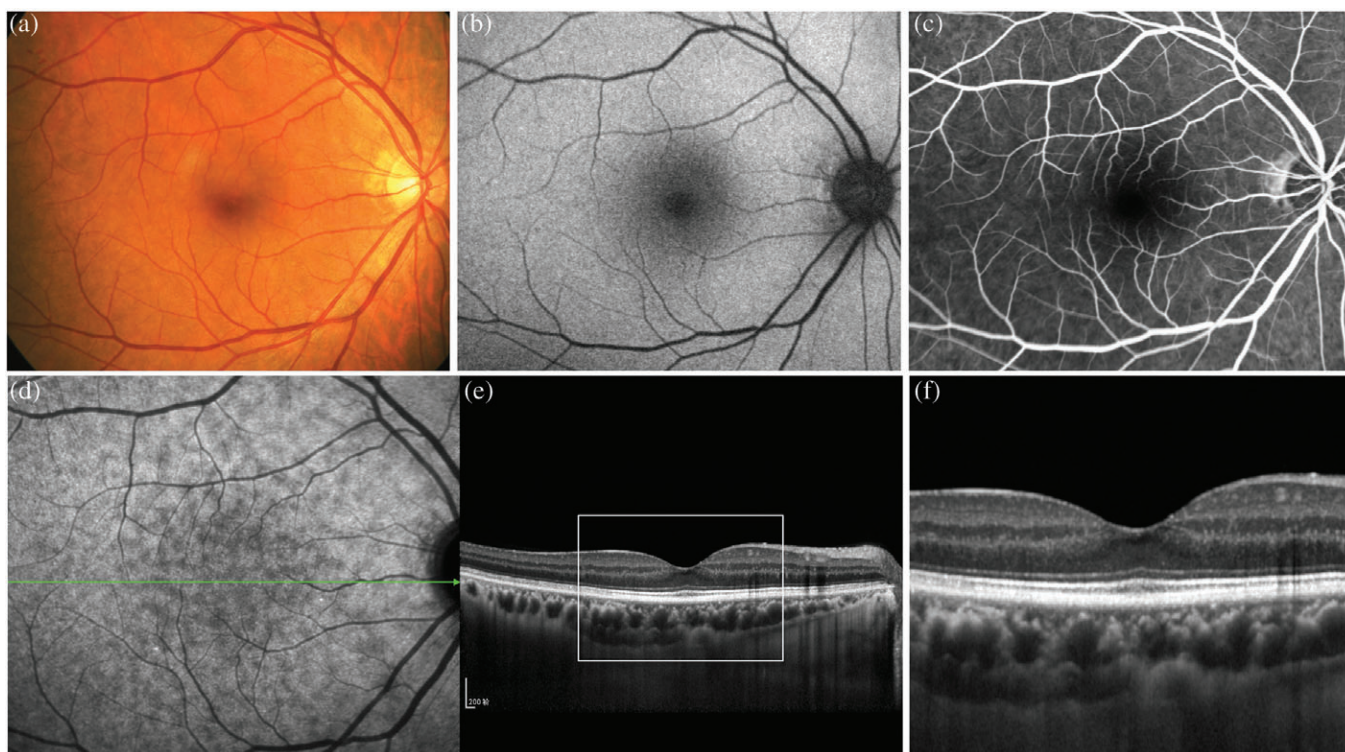
### DISCUSSION

In the present study, we characterized the demographic and multimodal-imaging features of ASHS-LIA and analysed its possible related factors based



**Figure 3.** Multimodal imaging of ASHS-LIA. Normal fundi fellow eye from a 64-year-old man whose right eye was diagnosed with neovascular age-related macular degeneration. No corresponding changes were present in the colour fundus photography (a) blue autofluorescence (b) and fluorescein fundus angiography (c). (d) Age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) were distributed in the macular region and it's around on ICGA (30 min after dye injection). (e) Spectral-domain optical coherence tomography (OCT) showed that the retinal structure was generally normal, and the RPE band was intact and smooth, with no intraretinal, subretinal or sub-RPE deposition corresponding to ASHS-LIA. (f) Magnified OCT image showing the white rectangle region in the image (e).





**Figure 4.** Multimodal imaging of ASHS-LIA. Normal fundi fellow eye from a 54-year-old man whose left eye was diagnosed with polypoidal choroidal vasculopathy. No corresponding changes were present in the colour fundus photography (a) and blue autofluorescence (b). (c) Uneven hypofluorescent spots were vaguely observed on early-stage of fluorescein fundus angiography (30 s after dye injection). (d) Age-related scattered hypofluorescent spots on late-phase indocyanine green angiography (ASHS-LIA) were distributed in the macular region and it's around on ICGA (30 min after dye injection). (e) Spectral-domain optical coherence tomography (OCT) showed that the retinal structure was generally normal, and the RPE band was intact and smooth, with no intraretinal, subretinal or sub-RPE deposition corresponding to ASHS-LIA. (f) Magnified OCT image showing the white rectangle region in the image E.

on clinical data from 875 consecutive patients received ICGA, FFA, AF, colour FP and OCT examination in our ophthalmic centre.

Our study showed that the average age of patients with ASHS-LIA was significantly higher than those without ASHS-LIA. Age stratification analysis also indicated that the occurrence of ASHS-LIA was positively correlated with old age. Multiple logistic regression analysis demonstrated that age was an independent relevant factor of ASHS-LIA. These findings indicated that age was a very important relevant factor for ASHS-LIA, so we speculated that ASHS-LIA might represent the aging of the fundus. Shiraki *et al.* reported that ASHS-LIA was observed in patients ranging in age from 51 to 80 years,<sup>1</sup> but

in our study, patients with ASHS-LIA ranged in age from 33 to 87 years. This might be due to the fact that we included a larger number of patients and therefore a more comprehensive sample. In addition, the proportion of male in patients with ASHS-LIA was significantly higher than the proportion in patients without ASHS-LIA. And multiple logistic regression analysis demonstrated that male gender was an independent relevant factor of ASHS-LIA. Nevertheless, it is still unclear how male gender affects the occurrence of ASHS-LIA. We speculated that it might be associated with a marked male preponderance in common fundus diseases such as age-related macular degeneration (AMD), polypoidal choroidal vasculopathy (PCV) and central serous chorioretinopathy (CSC),<sup>7–9</sup> considering that we included normal fundi fellow eyes of patients with common fundus diseases.

Our study indicated that ASHS-LIA could be observed only by ICGA, and was obvious by 30 min post-injection of dye. It has been demonstrated in rats, primates and humans that ICG dye is located within retinal and choroidal vessels in the early-phase after injection; however, it is extravasated into the choroidal stroma and accumulated

**Table 4.** Independent relevant factors of ASHS-LIA<sup>†</sup>

	$\beta$	OR [95% CI]	P value*
Age	0.089	1.093[1.076–1.111]	P < 0.001
Male	0.587	1.799[1.248–2.591]	0.002

<sup>†</sup>Data represent  $\beta$  values and ORs. ASHS-LIA, age-related scattered hypofluorescent spots on late-phase indocyanine green angiography.

within the RPE over time.<sup>4,5</sup> Therefore, under normal conditions, homogeneous background fluorescence was observed in late-phase ICGA. A lot of previous studies demonstrated that more neutral lipid accumulated in Bruch's membrane (BrM) with aging, increasing its hydrophobicity and decreasing its diffusional capacity.<sup>10–15</sup> As we know, ICG is water-soluble and does not bind to hydrophobic neutral lipids, which may explain the ICGA characteristics and age-association of ASHS-LIA and indicate that ASHS-LIA may represent neutral lipid accumulating in BrM. In addition, ASHS-LIA mainly located in the macular region, and the grade of ASHS-LIA increased with age. That was in accordance with the distribution and progression of neutral lipid accumulating in the retina.<sup>16</sup> Nevertheless, why ASHS-LIA was arranged in such a regular pattern and look similar among subjects, rather than irregularly shaped lesions or streaks, was still unclear. We speculated that it might be partly explained by the hydrophobicity and surface tension of neutral lipids.<sup>17</sup>

Then, if ASHS-LIA does represent neutral lipid accumulating in the retina, why no sub-RPE deposition was observed on OCT? As we know, BrM is the innermost 2–4  $\mu\text{m}$  of the choroid (subjacent to the RPE), consisting of five layers of connective tissue.<sup>18</sup> Lipoproteins begin to accumulate by the fourth decade of life, and gradually form the 'lipid wall' (a thin tightly packed layer of 0.4–2  $\mu\text{m}$ ) between the inner collagenous layer of BrM and RPE basal lamina in many older eyes.<sup>19–21</sup> The minimum resolution of OCT is about 5  $\mu\text{m}$ , therefore, the lipid wall cannot be showed by OCT. To explore the relevant factors of ASHS-LIA, the SFCT was detected, and our results showed that the mean SFCT had no significant difference between eyes with and without ASHS-LIA, which means that there might be no correlation between ASHS-LIA and SFCT changes.

In addition, the experiment *in vitro* demonstrated that cultured human RPE cells take up ICG dye, which might involve active transport.<sup>22</sup> Therefore, RPE cells dysfunction could result in the abnormality of ICG dye taking up, which might result in hypofluorescence on late-phase ICGA, and might be involved in the formation of ASHS-LIA. Additionally, although we evaluated the ASHS-LIA in normal fundi fellow eyes, our results showed that the incidence of ASHS-LIA was the highest in PCV patients. Therefore, we speculated that ASHS-LIA might be related to PCV, but we must consider the selection bias, for more PCV patients were included in this study.

There are several limitations to our study that need to be considered. Firstly, we evaluated the ASHS-LIA in normal fundi fellow eyes of patients

in our study. These eyes may be regarded as clinically 'normal eyes', but they are not from 'normal subjects'. Secondly, this is a clinic-based retrospective study, selection bias may occur. The prevalence of PCV is relatively high in Chinese populations, so more PCV patients were included in this study. Thirdly, although we speculated that ASHS-LIA might represent neutral lipid accumulating in the retina, we have no direct pathological evidence and we cannot explain its regular arrangement pattern. However, considering difficulties in obtaining ICGA images in normal subjects, and the number of patients is large enough and it encompasses a large age range, therefore, our findings are comprehensive, reliable and meaningful.

In conclusion, ASHS-LIA was observed on late-phase ICGA, and mainly located in macular region. No corresponding abnormalities were observed by other multimodal imaging modalities, including colour FP, FA, AF and OCT. The occurrence and grade of ASHS-LIA increased with age. Moreover, ASHS-LIA might be not correlated with SFCT, but correlated with PCV.

## REFERENCES

- Shiraki K, Moriwaki M, Kohno T, Yanagihara N, Miki T. Age-related scattered hypofluorescent spots on late-phase indocyanine green angiograms. *Int Ophthalmol* 1999; **23**: 105–9.
- Jung JJ, Mrejen S, Freund KB, Yannuzzi LA. Idiopathic multifocal choroiditis with peripapillary zonal inflammation: a multimodal imaging analysis. *Retin Cases Brief Rep* 2014; **8**: 141–4.
- Opremcak EM. Acute posterior multifocal placoid pigment epitheliopathy (APMPPE). *Insight* 2013; **38**: 21.
- Chang AA, Morse LS, Handa JT *et al.* Histologic localization of indocyanine green dye in aging primate and human ocular tissues with clinical angiographic correlation. *Ophthalmology* 1998; **105**: 1060–8.
- Matsubara T, Uyama M, Fukushima I, Matsunaga H, Takahashi K. Histological proof of indocyanine green angiography--healthy eyes. *Nippon Ganka Gakkai Zasshi* 1999; **103**: 497–505.
- Koizumi H, Yamagishi T, Yamazaki T, Kawasaki R, Kinoshita S. Subfoveal choroidal thickness in typical age-related macular degeneration and polypoidal choroidal vasculopathy. *Graefes Arch Clin Experiment Ophthalmol* 2011; **249**: 1123–8.
- Wong CW, Yanagi Y, Lee WK *et al.* Age-related macular degeneration and polypoidal choroidal vasculopathy in Asians. *Prog Retin Eye Res* 2016; **53**: 107–39.
- Daruich A, Matet A, Dirani A *et al.* Central serous chorioretinopathy: recent findings and new physiopathology hypothesis. *Prog Retin Eye Res* 2015; **48**: 82–118.
- Cheung CM, Li X, Cheng CY *et al.* Prevalence, racial variations, and risk factors of age-related macular

- degeneration in Singaporean Chinese, Indians, and Malays. *Ophthalmology* 2014; **121**: 1598–603.
10. Feeney-Burns L, Ellersieck MR. Age-related changes in the ultrastructure of Bruch's membrane. *Am J Ophthalmol* 1985; **100**: 686–97.
  11. Bird AC. Bruch's membrane change with age. *Br J Ophthalmol* 1992; **76**: 166–8.
  12. Moore DJ, Hussain AA, Marshall J. Age-related variation in the hydraulic conductivity of Bruch's membrane. *Investig Ophthalmol Vis Sci* 1995; **36**: 1290–7.
  13. Curcio CA, Johnson M, Huang JD, Rudolf M. Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration. *J Lipid Res* 2010; **51**: 451–67.
  14. McCarty WJ, Chimento MF, Curcio CA, Johnson M. Effects of particulates and lipids on the hydraulic conductivity of Matrigel. *J Appl Physiol* 2008; **105**: 621–8.
  15. Huang JD, Curcio CA, Johnson M. Morphometric analysis of lipoprotein-like particle accumulation in aging human macular Bruch's membrane. *Invest Ophthalmol Vis Sci* 2008; **49**: 2721–7.
  16. Johnson M, Dabholkar A, Huang JD, Presley JB, Chimento MF, Curcio CA. Comparison of morphology of human macular and peripheral Bruch's membrane in older eyes. *Curr Eye Res* 2007; **32**: 791–9.
  17. Chorlay A, Thiam AR. An asymmetry in monolayer tension regulates lipid droplet budding direction. *Biophys J* 2018; **114**: 631–40.
  18. Curcio CA, Johnson M, Huang JD, Rudolf M. Aging, age-related macular degeneration, and the response-to-retention of apolipoprotein B-containing lipoproteins. *Prog Retin Eye Res* 2009; **28**: 393–422.
  19. Curcio CA, Johnson M, Rudolf M, Huang JD. The oil spill in ageing Bruch membrane. *Br J Ophthalmol* 2011; **95**: 1638–45.
  20. Ruberti JW, Curcio CA, Millican CL, Menco BP, Huang JD, Johnson M. Quick-freeze/deep-etch visualization of age-related lipid accumulation in Bruch's membrane. *Invest Ophthalmol Vis Sci* 2003; **44**: 1753–9.
  21. Curcio CA, Presley JB, Malek G, Medeiros NE, Avery DV, Kruth HS. Esterified and unesterified cholesterol in drusen and basal deposits of eyes with age-related maculopathy. *Exp Eye Res* 2005; **81**: 731–41.
  22. Chang AA, Zhu M, Billson F. The interaction of indocyanine green with human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 2005; **46**: 1463–7.