Foveal Cone–Photoreceptor Integrity in Aging Macula Disorder

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Purpose. To establish the relation between AMD stage and a quantitative measure for the integrity of foveal cone photoreceptors related to the optical Stiles-Crawford effect.

Methods. Fifty-six AMD eyes and 57 control eyes were included in the final analysis. AMD was graded in accordance with the International Classification System into five mutually exclusive stages. Stages 0 to 1 were labeled no AMD, stages 2 to 3 were labeled early AMD, and stage 4 was labeled late AMD. Fundus reflectometry, together with a model-fit procedure, provided information on directional cone reflectance (Rd), a quantitative measure for the integrity of foveal cone photoreceptors. Optical densities of macular pigment (MPOD) and melanin (MOD) were also obtained. A general linear model analysis was used to compare Rd, MPOD, and MOD among the AMD stages.

Results. Mean Rd was lower in early AMD (0.92%, $P < 0.001$) and late AMD (0.86%, $P < 0.001$) compared with mean Rd in the no-AMD stage (1.76%). Mean MPOD was not different in early AMD (0.53, $P = 0.05$), but it was lower in late AMD (0.19, $P < 0.001$) compared with mean MPOD in the no-AMD stage (0.42). Mean MOD was lower in early (1.09, $P = 0.001$) and late (1.01, $P = 0.004$) AMD compared with mean MOD in the no-AMD stage (1.23).

Conclusions. Foveal cones show signs of misalignment and/or outer segment deterioration in early AMD. Melanin rather than macular pigment may play a protective role against AMD, although loss of these ocular pigments can also be caused by AMD. (*Invest Ophthalmol Vis Sci.* 2008;49:2077–2081) DOI: 10.1167/iovs.07-1181

In 1933, the British researchers Stiles and Crawford[1] reported that light entering the human eye at the center of the pupil was several times more effective in producing the sensation of vision than light entering near the pupil margin. The physiological explanation of this phenomenon, later called the Stiles-Crawford Effect (SCE), is that cone photoreceptors yield a directional sensitivity. Waveguide properties of cone photoreceptor inner segments, guiding the light to the outer segment photopigments, optimize absorption of axial incident light rather than off-axis light. The optical equivalent of this psychophysical effect is called the optical SCE. A small fraction of the incident light is reflected back toward the pupil. In a healthy retina, more light is reflected toward the middle of the pupil, where most cone photoreceptors are aimed. In disease, the optical SCE is a sensitive indicator of cone photoreceptor disturbances.[2]

Aging macula disorder (AMD), as we now prefer to call age-related macular degeneration,[3] is a degenerative disease primarily affecting the macula and an increasingly prevalent cause of irreversible blindness in the industrialized world.[4–9] Early AMD is characterized by drusen and pigmentary abnormalities with relatively few visual symptoms. However, in its late stage, AMD often leads to a disabling central scotoma.

It is known that drusen disturb the orderly alignment of overlying cone photoreceptors.[4,10] The retinal pigment epithelium (RPE) supplies the photoreceptors with nutrients and maintains the integrity of the subretinal space.[11] Thus, RPE changes may also affect the optical quality of the involved cone photoreceptors.[12] Several electrophysiological and psychophysical studies aimed at examining visual function in early AMD have found disturbances in light sensitivity and in adaptation throughout the retina regarding both cone and rod photoreceptors.[13–18]

A recently developed device, the Foveal Reflection Analyzer,[19] simultaneously measures cone photoreceptor directionality and foveal spectral reflectance in a few seconds. Directionally reflected light from the foveal cones relates to the optical quality of these photoreceptors. In addition, a procedure of model-fit to the spectral reflection[20] provides information on the optical densities of ocular absorbers: lens, macular pigment (MPOD), melanin (MOD), and blood.

This study was primarily conducted to establish the relation between the stage of AMD and a quantitative measure for the integrity of foveal cones related to the optical SCE. Because MPOD and MOD were also available, we evaluated the distribution of the optical densities of these pigments in the different AMD stages, looking for possible protective effects.

Methods

Participants

The AMD patients who participated in the study were obtained from two sources. One group ($n = 25$) was found by searching the fundus photography and fluorescence angiography database of the Ophthalmology Department of the University Medical Center (UMC) Utrecht for the presence of drusen and/or pigmentary alterations on fundus photographs or fluorescence angiograms (FAs) performed within the past 3 years. The other group ($n = 15$) consisted of patients with AMD who visited the outpatient clinic of the Ophthalmology Department of the UMC Utrecht. Only patients older than 55 years who were able to give informed consent were eligible. In addition, these patients had to have diagnosed AMD in at least one eye and/or the presence of drusen and/or RPE changes in at least one eye on earlier performed FA and/or fundus photograph, or the presence of atrophic or neovascular AMD in one eye on earlier performed FA and/or fundus photograph. Patients with known diabetes mellitus or an ophthalmic history other than

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Table 1. Definitions of the Original AMD Stages and Number of Eyes and Their Ages in the Currently Used AMD Classification

AMD Stage	AMD Stage Definition	Classification Used	Eyes/Persons* (*n*)	Age ± SD (y)†	*P*
0	No signs of AMD at all or only hard drusen (<63 μm)	No AMD	57/54	54.1 ± 17.8	<0.001
1	Soft, distinct drusen (≥63 μm) only or RPE changes only				
2	Soft, indistinct (≥125 μm) or reticular drusen only, or soft, distinct drusen with RPE changes	Early AMD	45/30	70.5 ± 8.0	0.90
3	Soft, indistinct or reticular drusen with RPE changes				
4	Atrophic or neovascular AMD	Late AMD‡	11/10	72.8 ± 6.5	Ref.

ref., reference.

* Total number of persons exceeds earlier mentioned total number of participants because the two individual eyes of one participant might contribute to two different AMD stages.

† Mean age based on eyes.

‡ Fundus photographs showed that in all participants with late AMD, the atrophic or neovascular changes involved the measured area (central 1.5°).

AMD or intraocular lens (IOL) implantation were excluded. We also excluded eyes with a best corrected visual acuity (BCVA) lower than 0.2, to assure stable fixation. Eyes of which a newly made fundus photograph was ungradable (e.g., missing, low quality) or on which retinal disease other than AMD was seen were also excluded. Altogether, 73 eyes of 40 patients with AMD were included.

A group of participants with eyes with no AMD were recruited through an advertisement in a local newspaper. Only persons older than 18 years who were able to give informed consent and who had no diabetes mellitus or any ocular history except IOL implantation were eligible for inclusion. These persons were excluded if BCVA was lower than 0.8 and if the newly made fundus photograph was ungradable or any retinal disease was seen on it. As a routine, only the right eye was measured in these participants. Finally, data from 45 right eyes of 45 healthy subjects were available. Thus, 118 eyes of 85 subjects were initially included in the study. Note that another five eyes of four patients with AMD were subsequently excluded (see Measurement of Optical SCE, MPOD and MOD in this section).

In patients with AMD and healthy subjects, refractional status of the study eye(s) was obtained with an autorefractometer (Auto Refkeratometer Speedy-K; Nikon Corp., Tokyo, Japan). With these data, BCVA was determined with an ETDRS (Early Treatment of Diabetic Retinopathy Study) chart at 4 m. Pupils were dilated with tropicamide 0.5% and phenylephrine 5%. In mydriasis, five fundus reflectance measurements were made in the study eye(s) at the specific pupil plane position with highest reflection (i.e., the position of the optical SCE maximum). Digital stereoscopic 30° color fundus photographs were then made (FF 450 Plus fundus camera; Carl Zeiss Meditec AG, Oberkochen, Germany).

The study protocol adhered to the tenets of the declaration of Helsinki and was approved by the local medical ethics committee. Written informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

AMD Definition

Digital fundus photographs of the macular area were graded according to the International Classification System for ARM and AMD (ICSAMD)[21] by one of the two professional graders from the Rotterdam Study[22] as having disease severity of one of five mutually exclusive stages, 0 to 4.[8]

In the present study, we deviated from the ICSAMD: all age-related maculopathy (ARM) was called AMD. Because the limited number of eyes in each stage, AMD was divided into early and late AMD: stages 0 and 1 were combined as no AMD, stages 2 and 3 were early AMD, and stage 4 was late AMD. A similar AMD classification has been used in other studies.[23,24] Note, that grading was the only criterion used for classification. Thus, 14 eyes of the patient group ended up in the no-AMD group; none of the recruited healthy eyes had AMD. Because the optical model fitted the data very badly in all measurements of two eyes with no AMD, in one eye with early AMD, and in two eyes with late AMD (see Measurement of Optical SCE, MPOD, and MOD in this section), these eyes were finally excluded. AMD stage definitions, the currently used classifications, the final number of eyes/subjects, and mean ages have been summarized in Table 1.

Measurement of Optical SCE, MPOD, and MOD

A prototype Foveal Reflection Analyzer (FRA) was detailed by Zagers et al.[19] The present version was recently described by van de Kraats and Van Norren.[20]

Briefly, a halogen lamp (12V, 30 W, Wotan 64260; Osram, Munich, Germany) illuminated a 1.8° spot on the fovea. Refraction errors were compensated by adjusting a Badal type front lens system. The light was spectrally filtered (6 mm BG26 filter; Schott AG, Mainz, Germany, and 1 mm Schott UG3 filter, Unaxis TL60; Linos Göttingen, Germany) for the comfort and safety of the subject and to prevent overloading the charge-coupled device (CCD) camera (model KX85; Apogee Instruments, Inc. Auburn, CA) that served as the detector for the reflected light. Spot intensity was 6.42 log troland; calculations showed a maximum safe viewing time of 15 minutes.[25] The filament of the halogen lamp was imaged in the pupil plane of the eye defining a 2.6 × 1.3-mm entrance pupil. With a separation of 0.7 mm below this entrance pupil, a slit-shaped exit pupil of 15 × 1 mm formed the input for a prism-based imaging spectrometer. The two-dimensional image projected on the calibrated CCD camera had one directional dimension, resulting from the intensity distribution over the slit-shaped exit pupil. In the other dimension, it contained spectral reflection information. Only light from the central 1.5° foveal spot was used in the analysis. Video observation of pupil plane and retinal plane facilitated proper alignment. A chin rest and temple pads were used to maintain a stable head position. In every included eye, five measurements were performed at the optimal pupil position (a more extensive description of the measurement routine is given in Van de Kraats and Van Norren[20]).

Spectra were evaluated with an updated version[20] of the original[26] Van de Kraats fundus reflectance model. The model describes the spectral aspects of light reflected from the fundus for all positions in the pupil profile (corresponding to angles at the retina), using a limited number of absorbing and reflecting layers. In short, the incoming light is thought to be reflected at the inner limiting membrane, at the discs in the outer segments of the cone photoreceptors (Rd), at the pigment epithelium, and at the choroid. Known spectral characteristics of the different absorbers in the eye (lens, MP, melanin, and blood) were used to optimize the density of the absorbers and the reflectance at the interfaces to fit the measured data. The recent version of the model simultaneously fits the optical SCE from the cone photoreceptors and the nondirectional reflection from more posterior layers in an extended wavelength range of 400 to 950 nm. In addition, the model incorporates the most recent spectral shapes of lens absorption templates.[27] MPOD is fitted by using the absorption curves for zeaxanthin and lutein published by Handelman et al.[28] The relative contributions of zeaxanthin and lutein to central MP are set at 70% and 30%, respectively. For the blood layer, a linear thickness gradient is used

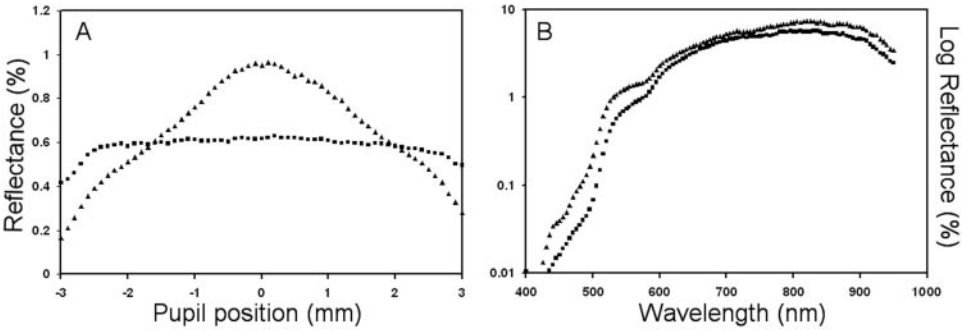

FIGURE 1. Typical spatial and spectral reflection curves of a healthy subject and a patient with early AMD. (**A**) Pupil profile at 540 nm of a 64-year-old healthy male (*triangles*) compared with the pupil profile of a 66-year-old woman with early AMD (*squares*). The pupil profile of the healthy subject shows a Gaussian-shaped reflection originating from the foveal cone photoreceptors (~optical SCE), on a diffuse background. In the patient with early-AMD, only a nondirectional diffuse background reflection was seen. (**B**) Spectral reflection curves from the same subjects as described in (**A**), measured on top of the optical SCE. At the short wavelengths, reflection was very low because of the absorption in the aging lens. At ~460 nm, the macular pigment reduced reflection. At longer wavelengths (> 550 nm) reflection was seen to increase because of decreasing absorption by melanin and blood. At still longer wavelengths, water absorption reduced reflection.

instead of a homogeneous layer of constant thickness, mimicking the range of pathways through the center or edge of small and large blood vessels. This thickness gradient varies from 0 to a certain maximum value, found with the model fit procedure. To use the model fit in advanced AMD (with noisy, or absent SCE), we made it more robust by fixing a number of non–age-dependent parameters. This involved ρ, a measure for the steepness of the optical SCE in the pupil plane, set at a mean value of 0.149 based on data of 102 healthy subjects.[20] In addition, two parameters of the eye media that showed minimal changes with age were set to the values corresponding to those of a subject of 65 years: d_{LY}, density of the young lens component was fixed at 1.58, and d_{RL}, a measure for the amount of Rayleigh scatter losses was set at 0.58.[20] The fundus reflectance model also provided information on the goodness of fit of each measurement by means of a χ^2 value. The mean χ^2 value ± SD of the healthy subjects was 13.2 ± 6.6. This was 24.9 ± 57.6 for the patients with AMD, indicating more noisy measurements in this group, mainly caused by spatially irregular lens densities together with AMD-related foveal disturbances. To limit the number of parameters generated by data that showed a very bad fit, measurements with a χ^2 exceeding 33.1 (mean of normal subjects + 3 SD) were discarded. This resulted in the loss of 1 of 230 healthy subject measurements (0.4%) and 55 of 388 AMD patient measurements (14.2%). In five eyes, all measurements were thus discarded. After this correction, the mean χ^2 ± SD was 13.0 ± 6.0 for the healthy control subjects and 12.7 ± 5.6 for the patients with AMD.

The main parameter in this study was Rd, the reflection at the cone photoreceptor layer. To avoid confusion about the origin of this reflection (i.e., discs in outer segments of foveal cone photoreceptors, rather than the optic disc) the term R_{disc} of the original model[20] was changed to Rd. Likewise, the other two model parameters of interest were called MPOD and MOD, rather than d_{MP} and d_{MEL}.

Statistical Analysis

Because both eyes of several patients with AMD were used, we clustered them (Complex-Samples feature; SPSS; SPSS, Chicago, IL). For this reason we also used the complex-samples' general linear model (GLM) analysis to compare Rd, MPOD, and MOD between the different AMD stages. Since Rd strongly varies with age,[29] we used age as a covariate in analyzing the effect of AMD classification on this parameter. In a recent study,[20] MOD showed no decrease with age. Because some other studies[30–32] point to a slight decrease in melanin with age, we also used age as a covariate for this parameter. MPOD is not thought to change with age.[33] Analysis of variance, together with a post hoc Scheffé test, was used to test for differences in age between no, early, and late AMD. All statistical analyses were performed with commercial software (SPSS for Windows, release 15.0) by SPSS.

Results

Examples of single measurements of a healthy 64-year-old eye and a typical early AMD eye of similar age are presented in Figure 1A. Such measurements take about 1 second each. The optical SCE in the healthy eye showed a Gaussian-shaped light output across the pupil. The distribution in the early-AMD eye was essentially flat, except where the pupil edge cut off the light. The spectral reflection of these eyes is shown in Figure 1B. At short wavelengths, reflection was very low because of absorption by the lens and the macular pigment. At longer wavelengths (>550 nm), reflection was increased by decreasing absorption of melanin and blood and finally was decreased again by the absorption of water.

In Table 2, mean BCVA, Rd, MPOD, and MOD are compared between the different AMD stages.

To investigate whether the FRA can detect foveal cone photoreceptor disturbances very early in the assumed process of AMD, we compared Rd in stage 0 AMD with Rd in stage 1 AMD, after having corrected for age and the (infrequent) use of two eyes of the same subject (not shown in Table 2). Stage 0 AMD contained 43 eyes of 42 subjects; mean eye age ± SD was 48.5 ± 16.4 years. Stage 1 AMD contained 14 eyes of 12 subjects with a mean age ± SD of 71.3 ± 8.55 years. After correcting for age and the use of both eyes in two subjects, Rd

TABLE 2. Estimates of BCVA, Rd, MPOD, and MOD per AMD Category

AMD Stage	LogMAR BCVA (95% CI)*	*P*	Rd % (95% CI)*†	*P*	MPOD (95% CI)*	*P*	MOD (95% CI)*†	*P*
No AMD	0.00 (−0.06–0.05)	Ref.	1.76 (1.53–1.99)	Ref.	0.42 (0.37–0.46)	Ref.	1.23 (1.17–1.30)	Ref.
Early AMD	0.14 (0.08–0.20)	<0.001	0.92 (0.74–1.11)	<0.001	0.53 (0.43–0.63)	0.05	1.09 (1.03–1.14)	0.001
Late AMD	0.71 (0.49–0.93)	<0.001	0.86 (0.55–1.18)	<0.001	0.19 (0.09–0.29)	<0.001	1.01 (0.86–1.15)	0.004

* Estimated means of the different parameters are presented together with their 95% CIs. Standard errors are adjusted by clustering eyes of individual subjects.

† Additional adjustment for age, because Rd[29] and, to a lesser extent, MOD,[30–32] show a decrease with age. MPOD is not thought to change with age.[33]

(95% CI) was 1.84% (1.53–2.16) in stage 0 AMD and 1.60% (1.18–2.03) in stage 1 AMD. This difference was not significant ($P = 0.41$), nor were the differences in MPOD ($P = 0.62$) and MOD ($P = 0.45$).

Our dataset contained only 11 eyes with late AMD, of which 5 eyes had the atrophic form and 6 eyes had the neovascular form. After correction for age and the use of both eyes of one subject, Rd (95% CI) was 0.38% (0.00–0.87) in atrophic AMD and 0.56% (0.22–0.90) in neovascular AMD ($P = 0.54$).

Discussion

In our study, the amount of directionally reflected light from the foveal cone photoreceptors (Rd) was halved in early AMD compared with that in eyes without AMD. DeLint et al.[2] have argued that the optical SCE may replace cone visual pigment kinetics as a sensitive measure for detecting cone photoreceptor disturbances. Thus, our results are in line with those in a study by Elsner et al.,[36] who demonstrated that subjects with early ARM had a significantly lower optical cone photopigment density compared with healthy control subjects. The lower directional cone reflex in AMD found in this study is the result of a decreased number of directionally reflecting units in the retina. The orderly alliance of the foveal cones is disturbed, or the total number of cones is decreased, or the number of photopigment containing discs in the cone outer segments, which we assume to be responsible for the guided reflection, is decreased in AMD. A combination of these processes may also be possible. Using histopathological and histochemical techniques, Curcio et al.[37,38] found that in different stages of ARM the foveal cone mosaic of eyes with large drusen and thick basal deposits (i.e., early AMD in this study) appear surprisingly similar to that in age-matched control eyes, and the total number of foveal cones is also normal. Thus, it is probable that a combination of shortened outer segments (less cone pigment)[36] with, in the case of large drusen, cone disarray plays a major role in the decline of Rd in early AMD. Between AMD stages 0 and 1, changes in foveal cones were absent or too subtle to be picked up by the FRA. Only a longitudinal study can answer the interesting question of whether low Rd in stage 1 is predictive of progression to later stages of AMD.

In late AMD, with severe central foveal disease and low VA, we expected foveal cone reflectance to be lower than in early AMD. Surprisingly, that was not the case in this study (no significant difference in Rd between early and late AMD; not shown in Table 2). The optical model divides light reflected back from the fovea in a large nondirectional background part that originates from the deeper layers and a relatively small directional part originating from the foveal cone photoreceptors (~Rd). Severe central retinal changes in advanced AMD generate noisy reflections in the spatial dimension that may show up as a (limited) directional component (~optical SCE). Most, but apparently not all, of these artifactual fits were removed on the basis of their higher χ^2 values (see the Methods section), inhibiting a further decrease in Rd from a certain stage on in advancing AMD. Because the estimation of MPOD and MOD depends on both directional and background reflectance, these parameters are far less susceptible to the pathologic changes in late AMD.

We found no significant difference in the MPOD between the no-AMD and the early-AMD stages, but a significant decline was seen in late AMD. Whether MP plays a protective role in AMD is a subject for debate. Evidence in favor of such a hypothesis[34,35,39] is counterbalanced by evidence against.[23,30,40] The present study may suggest some protective effect of MP in the later stages of AMD. However, causal relationships cannot be established with a cross-sectional study. With a firm protective role, MPOD was expected to be lower in early AMD compared with the no-AMD stage, which was not the case in this study. The hypothesis that the process of AMD itself had reduced the amount of MP in late AMD is also quite plausible. Macular pigment is concentrated in the central area of the retina along the axons of the cone photoreceptors. In addition, MP has been detected in the photoreceptor outer segment layer in the central fovea.[41] In late AMD, large areas of RPE atrophy and/or neovascularization are present in the central retinal area, leading to advanced parafoveal rod loss and eventually to foveal cone degeneration. In the end, all photoreceptors may disappear,[37] causing a decrease of central MP in late AMD.

MOD, representing the sum of the optic density of RPE and choroidal melanin, was lower in early and late AMD than in the no-AMD stage. Our findings might point to a protective effect of melanin in AMD, possibly by reducing backscattering and exerting antioxidative action. Again, the AMD-related process of RPE degeneration itself may also have led to the smaller amount of melanin measured. Changes in choroidal melanin are less likely, because its central MOD is 2.4 times higher than the central RPE melanin in white persons.[31] In line with our MOD findings, a higher prevalence of all forms of AMD was found in a white population compared with the more pigmented populations.[42] In other studies,[43–45] a higher prevalence of late AMD was found only in whites. Data derived from a longitudinal study recently performed in our laboratory do not suggest a protective effect of melanin on the incidence of early AMD.[23]

In conclusion, foveal cones show signs of misalignment and/or deterioration of their outer segments in early AMD, with soft, indistinct, or reticular drusen with or without RPE changes, or soft, distinct drusen with RPE changes, but not in the no-AMD stage. Apparently hard drusen, soft distinct drusen, or RPE changes only are insufficient to change parameters measured with the FRA. We found no clear evidence of a protective role of macular pigment in AMD. The role of melanin in protecting against AMD may be underestimated and needs further research.

References

1. Stiles WS, Crawford BH. The luminous efficiency of rays entering the eye pupil at different points. *Proc R Soc Lond* [*Biol*]. 1933; 112:428–450.
2. DeLint PJ, Berendschot TTJM, van Norren D. A comparison of the optical Stiles-Crawford effect and retinal densitometry in a clinical setting. *Invest Ophthalmol Vis Sci*. 1998;39(8):1519–1523.
3. de Jong PTVM. Mechanisms of disease: age-related macular degeneration. *N Engl J Med*. 2006;355(14):1474–1485.
4. Jackson GR, Owsley C, Curcio CA. Photoreceptor degeneration and dysfunction in aging and age-related maculopathy. *Ageing Res Rev*. 2002;1(3):381–396.
5. Bressler NM, Bressler SB, Congdon NG, et al. Potential public health impact of Age-Related Eye Disease Study results: AREDS Report No. 11. *Arch Ophthalmol*. 2003;121(11):1621–1624.
6. Wang JJ, Rochtchina E, Lee AJ, et al. Ten-year incidence and progression of age-related maculopathy: The Blue Mountains Eye Study. *Ophthalmology*. 2007;114(1):92–98.
7. Miyazaki M, Kiyohara Y, Yoshida A, Iida M, Nose Y, Ishibashi T. The 5-year incidence and risk factors for age-related maculopathy in a general Japanese population: The Hisayama Study. *Invest Ophthalmol Vis Sci*. 2005;46(6):1907–1910.
8. Klaver CC, Assink JJ, Wolfs RC, et al. Incidence and progression rates of age-related maculopathy. The Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2001;42:2237–2241.
9. Klein R, Klein BEK, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy. *Ophthalmology*. 2002;109(10):1767–1779.

10. Johnson PT, Lewis GP, Talaga KC, et al. Drusen-associated degeneration in the retina. *Invest Ophthalmol Vis Sci*. 2003;44(10): 4481–4488.
11. Kanski JJ. *Clinical Ophthalmology.* Fifth ed. Edinburgh, UK: Butterworth-Heinemann; 2004.
12. Tolentino MJ, Miller S, Gaudio AR, Sandberg MA. Visual-field deficits in early age-related macular degeneration. *Vision Res*. 1994; 34(3):409–413.
13. Birch DG, Fish GE. Focal cone electroretinograms: aging and macular disease. *Doc Ophthalmol*. 1988;69(3):211–220.
14. Owsley C, Jackson GR, Cideciyan AV, et al. Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2000;41(1):267–273.
15. Phipps JA, Guymer RH, Vingrys AJ. Loss of cone function in age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2003;44(5): 2277–2283.
16. Scholl HPN, Bellmann C, Dandekar SS, Bird AC, Fitzke FW. Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2004;45(2):574–583.
17. Walter P, Widder RA, Luke C, Konigsfeld P, Brunner R. Electrophysiological abnormalities in age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol*. 1999;237(12):962–968.
18. Weiter JJ, Delori FC, Dorey CK. Central sparing in annular macular degeneration. *Am J Ophthalmol*. 1988;106(3):286–292.
19. Zagers NPA, van de Kraats J, Berendschot TTJM, van Norren D. Simultaneous measurement of foveal spectral reflectance and cone-photoreceptor directionality. *Appl Opt*. 2002;41(22):4686–4696.
20. van de Kraats J, van Norren D. Modeling the directional and non-directional spectral reflection from the human fovea. *J Biomed Opt*. 2008;13(2):024010.
21. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol*. 1995;39(5):367–374.
22. Hofman A, Grobbee DE, de Jong PTVM, Vandenouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol*. 1991;7(4):403–422.
23. Kanis MJ, Berendschot TTJM, van Norren D. Influence of macular pigment and melanin on incident early AMD in a white population. *Graefes Arch Clin Exp Ophthalmol*. 2007;245(6):767–773.
24. Boekhoorn SS, Vingerling JR, Uitterlinden AG, et al. Estrogen receptor alpha gene polymorphisms associated with incident aging macula disorder. *Invest Ophthalmol Vis Sci*. 2007;48(3):1012–1017.
25. Health Council of the Netherlands, c.o.o.r. Optical radiation: health-based exposure limits for electromagnetic radiation in the wavelength region from 100 nanometer to 1 millimeter. The Hague, The Netherlands; 1993.
26. van de Kraats J, Berendschot TTJM, van Norren D. The pathways of light measured in fundus reflectometry. *Vision Res*. 1996; 36(15):2229–2247.
27. van de Kraats J, van Norren D. Optical density of the aging human ocular media in the visible and the UV. *J Opt Soc Am A*. 2007;24: 1842–1857.
28. Handelman GJ, Snodderly DM, Adler AJ, Russett MD, Dratz EA. Measurement of carotenoids in human and monkey retinas. *Methods Enzymol*. 1992;213:220–230.
29. Zagers NPA, van Norren D. Absorption of the eye lens and macular pigment derived from the reflectance of cone photoreceptors. *J Opt Soc Am A Opt Image Sci Vis*. 2004;21(12):2257–2268.
30. Berendschot TTJM, Willemse-Assink JJM, Bastiaanse M, de Jong PTVM, van Norren D. Macular pigment and melanin in age-related maculopathy in a general population. *Invest Ophthalmol Vis Sci*. 2002;43(6):1928–1932.
31. Weiter JJ, Delori FC, Wing GL, Fitch KA. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. *Invest Ophthalmol Vis Sci*. 1986;27(2):145–152.
32. Delori FC, Burns SA. Fundus reflectance and the measurement of crystalline lens density. *J Opt Soc Am A*. 1996;13(2):215–226.
33. Berendschot TTJM, van Norren D. On the age dependency of the macular pigment optical density. *Exp Eye Res*. 2005;81(5):602–609.
34. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci*. 2001;42(2):439–446.
35. Delcourt C, Carriere I, Delage M, Barberger-Gateau P, Schalch W. Plasma lutein and zeaxanthin and other carotenoids as modifiable risk factors for age-related maculopathy and cataract: The POLA Study. *Invest Ophthalmol Vis Sci*. 2006;47(6):2329–2335.
36. Elsner AE, Burns SA, Weiter JJ. Cone photopigment in older subjects: decreased optical density in early age-related macular degeneration. *J Opt Soc Am A Opt Image Sci Vis*. 2002;19(1):215–222.
37. Curcio CA, Medeiros NE, Millican CL. Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 1996; 37(7):1236–1249.
38. Curcio CA, Owsley C, Jackson GR. Spare the rods, save the cones in aging and age-related maculopathy. *Invest Ophthalmol Vis Sci*. 2000;41(8):2015–2018.
39. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration: Eye Disease Case-Control Study Group. *JAMA*. 1994; 272(18):1413–1420.
40. Jahn C, Wustemeyer H, Brinkmann C, Trautmann S, Mossner A, Wolf S. Macular pigment density in age-related maculopathy. *Graefes Arch Clin Exp Ophthalmol*. 2005;243(3):222–227.
41. Snodderly DM, Auran JD, Delori FC. The macular pigment. II. Spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25(6):674–685.
42. Ambati J, Ambati BK, Yoo SH, Ianchulev S, Adamis AP. Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. *Surv Ophthalmol*. 2003;48(3):257–293.
43. Klein R, Clegg L, Cooper LS, et al. Prevalence of age-related maculopathy in the Atherosclerosis Risk in Communities Study. *Arch Ophthalmol*. 1999;117(9):1203–1210.
44. Klein R, Klein BE, Jensen SC, Mares-Perlman JA, Cruickshanks KJ, Palta M. Age-related maculopathy in a multiracial United States population: the National Health and Nutrition Examination Survey III. *Ophthalmology*. 1999;106(6):1056–1065.
45. Klein R, Klein BEK, Marino EK, et al. Early age-related maculopathy in the cardiovascular health study. *Ophthalmology*. 2003;110(1): 25–33.