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加齢黄斑変性に対する個別化医療実現のための前向き臨床研究にもとづくゲノムワイド関連解析

平成２３年度～平成２５年度　総合研究報告書

研究代表者　吉村　長久

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目　　次

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加齢黄斑変性に対する個別化医療実現のための前向き臨床研究にもとづくゲノムワイド関連解析

研究代表者　吉村　長久　京都大学医学研究科教授

研究要旨
加齢黄斑変性（AMD）は先進国における社会的中途失明の最大の原因となっており、複数ある治療方法から最適の治療方法を選択できずに試行錯誤を繰り返す数ヶ月の間に進行してしまうことが多い。各患者に最適な治療方法を選択し、早期にその治療を開始することができれば、重症化の防止、QOLの向上・自立支援につながるだけではなく、医療経済上の問題の改善にもつながる。
このような個別化医療の実現のために、遺伝子多型が活用できる。本研究では日本人独自の遺伝子多型を用いた個別化医療を実現するために多施設前向き研究を行う。平成23年度には国内の15の施設と共同で抗VEGF治療の前向き研究を開始するとともに、これまでに光線力学療法を行ってきた症例を集めて、光線力学療法の治療効果に相関する遺伝子多型を解明するためのゲノムワイド関連解析を開始した。平成24年度以降には上記前向き研究の結果をもとに抗VEGF治療の効果に相関を認める遺伝子多型をゲノムワイド関連解析によって解明し、AMDの個別化医療に活用できることが判明した遺伝子多型の迅速検出キットを作成して、精度の高い個別化医療を実践していく。

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A．研究目的

加齢黄斑変性（AMD）は先進国における社会的中途失明の最大の原因となっており、我が国でも近年急速に増加している疾患である。最近になって光線力学療法や硝子体注射薬といった治療方法が開発され、加療可能な疾患となってきたが、これらの治療方法の効果は視力を回復させるには十分ではない。患者ごとに各治療方法の効果が異なることから、これら複数の治療方法から各患者に最適な治療方法を選択することができれば、各患者における重症化の防止、ＱＯＬの向上・自立支援につながり、さらに医療費の抑制にもつながると考えられる。

近年、AMDの発症や治療結果に遺伝子（CFH, ARMS2等）の多型が関与することが分かってきたため、この遺伝子多型を用いて個別化医療を実現しようという気運が高まってきた。本研究では遺伝子多型を用いた個別化医療の精度の向上によって適切なAMDの治療を目指し、同時に不必要な治療や通院の抑制を測りたい。

我々のこれまでの研究から、欧米人と日本人とでは関与する遺伝子多型の強弱に差があることが分かりつつある。つまり遺伝子多型を用いて個別化医療を実現するためには、日本人におけるAMDに関与する遺伝子多型の研究が不可欠であると考えられる。

我々は既にARMS2遺伝子のA69S多型の迅速キットを作成し、実際の臨床治療に利用し始めている。また、これまでの我々の研究ではARMS2、CFH、VEGF、PEDFといった遺伝子の多型が様々な治療による予後に影響していることが分かってきた。しかし、他にも治療効果に影響する遺伝子多型は複数存在するはずで、本研究ではこれまでの研究成果を前向き研究を行うことによって確固としたものにしていくと同時に、ゲノムワイド関連解析を行うことによって新たな相関遺伝子多型を明らかにしていく。

平成23年度には国内約15施設でこれまでに光線力学療法を施行されてきた患者の治療経過を参考に、血液サンプルを用いてゲノムワイド関連解析を行うことによって、光線力学療法の治療結果に相関する遺伝子多型を解明し、また抗VEGF薬による治療結果に相関する遺伝子多型を検出するための前向き研究を開始した。平成24年度には前向き研究の治療結果を参考にゲノムワイド関連解析を行って、抗VEGF薬による治療結果に相関する遺伝子多型を明らかにした。平成25年度には治療方針を決定するために有用な遺伝子多型について再現性を確認し、その有用性を検証した。さらに遺伝子多型の迅速検出キットを作成し、個別化医療の精度の向上を目指した。

B．研究方法

AMD個別化医療研究会参加施設（京大、神戸大、埼玉医大、東京医療センター）および協力施設（福島医大、群馬大、山梨大、東大、名市大、阪大、関西医大、島根大、聖隷浜松病院、大塚眼科、宮田眼科）で臨床研究を進めてきた。

これまで主力の治療方法であった光線力学療法および、平成22年春頃から使用が開始されてきた抗VEGF薬による治療後の反応・予後と相関する遺伝子多型をゲノムワイド関連解析の手法を用いて解明した。光線力学療法についてはこれまでに多数例の治療データが各施設に蓄積されているため、まず始めに後ろ向き研究を行うことで、相関遺伝子多型を解明した。抗VEGF薬治療については各施設から患者登録を行い、前向き研究を行い、ゲノムワイド関連解析の結果、抗VEGF治療の反応性を予測出来る可能性のある候補遺伝子の発見に成功した。各治療方法を行った際の反応・予後を予測するために有用な遺伝子多型を検出する迅速キットを作成し、キットを用いて各患者に最適な治療方法を選択し、実際に個別化医療を行ったうえで、その成果の検証を試みた。

【前向き研究 - 抗VEGF治療】

すべての施設での倫理委員会の承認を得た後、各施設で20-30例程度のエントリーを行い、通常の抗VEGF治療を順次行った。治療開始前の必須検査は視力検査、眼底検査、造影検査、光干渉断層計検査とし、治療後は毎月視力検査、眼底検査、光干渉断層計検査を行い臨床経過を記録した。末梢血サンプルは、連結可能匿名化を行ったあとにDNA抽出を行い、ゲノムワイド関連解析の準備を行った。

平成２４年度内には、１年以上の経過が追えた患者が256例を超えたため、その256例のDNAサンプルと臨床経過（治療の有効性、視力予後、再発の有無）を用いてゲノムワイド関連解析を行った。有力な候補遺伝子が発見できたため、平成25年度には残りの登録済みサンプルを用いてその関連を検証した。またARMS2遺伝子のA69S多型を検出するキットを改良し、すべての施設で検査可能にした。

【後ろ向き研究 - 光線力学療法】

各施設で光線力学療法を施行し、１年以上の経過観察が可能であった患者に対して、本研究の説明を行い、協力が得られた患者から同意のうえで血液サンプルを採取し、連結可能匿名化を行ったあとにAMD個別化医療研究会参加施設（京大、神戸大、埼玉医大、東京医療センター）に血液サンプルを送付し、DNA抽出を行い、抽出されたDNAを京大眼科に送付した。上述の抗VGF治療の反応性に関連を認めた遺伝子について、各施設から得られた治療結果（有効性、視力予後、再発の有無）を参考に、治療結果に影響を与えうるかどうかを検証した。

（倫理面への配慮）

平成23年度中に本研究の協力施設15施設のすべてにおいて、倫理委員会の承認が得られた。

末梢血の採取は通常の臨床静脈採血と同様の方法で行われるので、それに伴う身体的・精神的不利益は小さいと考えられるが、採血時の身体的損傷・反応（まれにみられる迷走神経反射など）に対しては臨床的に適切とみなされる処置を遅滞なく行うことにしている。

遺伝子解析結果自体による倫理的・法的・社会的不利益は匿名化・情報管理の体制により防止する。また遺伝子解析を行うことに伴う心理的不利益に対してはヒト由来試料等採取機関により相談・情報提供の機会を提供する。

C．研究結果

【前向き研究 - 抗VEGF治療】

すべての施設での倫理委員会の承認を得た後、web上に作成した症例登録用ページに登録を開始し、256例を用いた研究で有力な候補遺伝子が発見できた。H25年度には再現性確認用に登録された200例を用いて再現性を確認したところ、７番染色体上の遺伝子について、統計学的に有意な関連は認めなかったが、同様の関連傾向を認めており、AMDの個別化医療に有用である可能性が高いと考えられる。

【後ろ向き研究 - 光線力学療法】

光線力学療法を施行済みの96例からDNAを抽出し、イルミナ社の2.5Mチップを用いた一塩基多型の検出が可能であった。現在、治療反応性・治療予後との相関を認める遺伝子を解明するために、ゲノムワイド関連解析を行っているが症例数が少なく、有意な遺伝子多型が発見出来なかった。抗VEGF治療の研究から得られた候補遺伝子についても有意な関連を認めなかった。

D．考察

抗VEGF治療の治療反応性・治療予後との相関を認める遺伝子の候補がみつかったと考えている。一方、光線力学療法については上述の遺伝子と治療反応性・治療予後との関連は認められなかったため、光線力学療法については既に知られているARMS2遺伝子の活用した個別化医療を行っていくべきであろう。

E．結論

光線力学療法および抗VEGF抗体治療の治療反応性・治療予後との相関を認める遺伝子が解明され、加齢黄斑変性の個別化医療の実現が近づいた。

F．健康危険情報

健康危険なし

G．研究発表

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H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
なし

2. 実用新案登録
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3.その他
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研究成果の刊行に関する一覧表

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CLINICAL INVESTIGATION

Multimodal evaluation of macular function in age-related macular degeneration

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Abstract
Objective To evaluate macular function using multimodality in eyes with age-related macular degeneration (AMD) at various stages.
Methods Macular function in 20 control eyes (20 subjects), 17 eyes (17 patients) with large drusen, 18 eyes (18 patients) with drusenoid pigment epithelial detachment (PED), and 19 eyes (19 patients) with neovascular AMD was examined using a Landolt chart for visual acuity; retinal sensitivity was measured by microperimetry; and focal macular electroretinography (fmERG) was performed. In all of these eyes, retinal morphology was examined using optical coherence tomography.
Results Eyes with neovascular AMD showed morphologic changes in the neurosensory retina as well as marked deterioration of macular function in all parameters measured with a Landolt chart, fmERG, and microperimetry. Eyes with large drusen showed only minimal morphologic changes in the neurosensory retina. In this large drusen group, although retinal sensitivity at the central point was significantly decreased ($P = 0.0063$), the other parameters of macular function were well preserved. In eyes with drusenoid PED, the structure of the neurosensory retina was well preserved, while the foveal thickness was significantly increased ($P = 0.013$). The macular function of these eyes was significantly deteriorated, with the VA, amplitude of the a-wave and b-wave, and retinal sensitivity being markedly decreased. In addition, the area of PED correlated with the latency of the a-wave and b-wave and with the retinal sensitivity within the central 4° or 8° region.
Conclusion Multimodal evaluation demonstrated a significant decrease in macular function in drusenoid PED and in neovascular AMD.

Keywords Age-related macular degeneration · Drusenoid pigment epithelial detachment · Drusen · Focal macular electroretinography · Microperimetry

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Introduction

Age-related macular degeneration (AMD) is one of the leading causes of visual impairment and an intensive therapeutic target in developed countries [1–6]. Drusen or drusenoid pigment epithelium detachment (PED), which is a prodrome lesion of advanced AMD, does not usually cause severe loss of visual acuity (VA), but it is the subsequent development of choroid neovascularization (CNV) that so often causes the central visual disturbance. So far, however, visual impairment due to AMD has been evaluated primarily by VA measurement alone. Indeed, VA measurement is essential to evaluate visual function, but it reflects only foveal function. Lesions of AMD, including drusen, CNV, serous retinal detachment, subretinal hemorrhage, and PED, are seen not only beneath the fovea, but also in the larger macular area, which leads to the macular dysfunction [7].

To evaluate visual function of the entire macular area, simultaneous use of focal macular electroretinography (fmERG) and of microperimetry have recently been reported [8, 9]. fmERG enables measurement of macular

function throughout its entirety, even in patients with poor fixation, by monitoring through an infrared camera and manual adjustment of the stimulus to the macular area [10]. Microperimetry allows functional evaluation of selected points throughout the macular area [11, 12]. During this test, the autotracking function corrects for shifts in the measurement position caused by small, involuntary movements. Recent studies using microperimetry have shown that early or advanced AMD often accompanies the severe reduction in sensitivity of the macular area [13–21]. With the use of microperimetry, Yodoi et al. [22] reported a functional reduction in the macular area of eyes with subfoveal polypoidal choroidal vasculopathy, which is a variant of neovascular AMD. In their report, macular function improved after photodynamic therapy with concomitant recovery of the subjective symptoms, despite there being no improvement in VA.

Other recent studies with microperimetry or ERG have evaluated macular function in eyes with AMD and have reported that it is impaired—even in eyes with drusen alone [23, 24]. Indeed, each modality has both advantages and limitations. To evaluate visual function effectively, it would be of help to measure retinal function within the macular area using the multimodality approach. So far, however, little information is available on the multimodal evaluation of visual function in eyes with AMD. Therefore, this study was designed to evaluate the macular function using multimodality in eyes with AMD at various stages, including those with large drusen, drusenoid PED, and neovascular AMD.

Patients and methods

In this prospective study, we performed multimodal evaluation of macular function in eyes with AMD at various stages; the eyes comprised 17 (17 patients) with large drusen, 18 (18 patients) with drusenoid PED, and 19 (19 patients) with neovascular AMD (8 eyes with typical AMD and 11 eyes with polypoidal choroidal vasculopathy). Eyes with large drusen were judged by the presence of multiple large drusen (>125 μm) within 3,000 μm of the center of the macula on fundus photographs. The diagnostic criteria of drusenoid PED were confluent drusen, with a focal area of PED involving the macular area, with a minimum size of 1/2 disc diameter [25], and without CNV detected on ophthalmoscopy or fluorescein and indocyanine green angiography. Neovascular AMD was diagnosed on the basis of fluorescein and indocyanine green angiography, which showed an exudative change with CNV. In the current study, eyes with central geographic atrophy were excluded. We also recruited 20 eyes (20 subjects) as an age-adjusted control group. The criteria for the eyes, including for the control eyes, were as follows: ≥1.0 VA on a Landolt chart, <10 small drusen (<63 μm) within 3,000 μm of the center of the macula on the fundus photograph, normal morphology of the fovea as seen with optical coherence tomography (OCT), and absence of central geographic atrophy or CNV.

This study was approved by the institutional review board of Kyoto University Graduate School of Medicine and adhered to the tenets of the Declaration of Helsinki. Written informed consent for research participation was obtained from each subject before examination.

Each subject underwent a comprehensive ophthalmologic examination, including measurement of best-corrected VA on a Landolt chart, determination of intraocular pressure, indirect ophthalmoscopy, and slit-lamp biomicroscopy with a contact lens. In each subject, 45° digital fundus photographs were obtained using a digital fundus camera (TRC-50LX; Topcon, Tokyo, Japan; 3,216 × 2,136 pixels) after pupil dilatation, and the macular area was examined with a Spectralis HRA + OCT device (Heidelberg Engineering, Heidelberg, Germany). Each patient with large drusen, drusenoid PED, or neovascular AMD underwent fluorescein and indocyanine green angiography with a confocal laser scanning system (HRA-2; Heidelberg Engineering). In each eye, macular function was examined by fundus-monitored microperimetry and fmERG recording.

Retinal sensitivity within the macular area was examined with a fundus-monitored microperimeter [Micro Perimeter 1 (MP1); Nidek, Gamagori, Japan]. A 4-2-staircase strategy with Goldmann III-sized stimuli was used, and 57 stimulus locations within a 10° radius were examined by microperimetry. Each stimulus was located according to the measurement points on the Humphrey 10-2, with some additional points. The white background illumination was set at 1.27 cd/m^2. The differential luminance, defined as the difference among the stimulus luminance, and background luminance, was 127 cd/m^2 at 0-dB stimulation, and the maximum stimulus attenuation was 20 dB. The stimulus duration was 200 ms (ms), and the fixation target varied in size according to the VA of the patient. There were 17 and 37 measurement points within the central circles with radii of 4° and 8°, respectively.

The fmERG recording procedure has been previously described in detail [8, 9]. Briefly, after maximal dilatation of the pupils of both eyes, a Burian–Allen bipolar contact lens electrode (Hansen Ophthalmic Laboratories, Iowa City, IA, USA) was placed in the conjunctival sac of each eye under topical anesthesia. A chloride silver electrode was attached to the left earlobe to serve as the ground electrode. The fmERG was elicited by circular stimuli positioned on the macular area, using a prototype of the ER-80 (Kowa, Tokyo, Japan), which consisted of an

Table 1 Background, foveal thickness, and macular function of control eyes, eyes with large drusen, eyes with drusenoid pigment epithelial detachment, and eyes with neovascular age-related macular degeneration

	Controls	Large drusen	Drusenoid PED	Neovascular AMD	*P* value
Sex (male/female)	16/4	11/6	18/0	16/3	0.054
Phakia/pseudophakia	14/6	9/8	13/5	12/7	0.627
Age, years	82.0 ± 3.2	80.7 ± 5.2	78.9 ± 5.0	77.3 ± 6.9	0.040
Visual acuity, logMAR	−0.07 ± 0.07	0.05 ± 0.14	0.16 ± 0.18	0.42 ± 0.42	<0.0001
Foveal thickness, μm					
ILM to RPE	224 ± 27	196 ± 40	200 ± 49	384 ± 256	<0.0001
ILM to Bruch membrane	224 ± 27	231 ± 36	377 ± 164	533 ± 263	<0.0001
Amplitude of fmERG, μV					
a-wave	1.73 ± 0.65	1.35 ± 0.49	1.21 ± 0.67	0.87 ± 0.58	0.0005
b-wave	3.14 ± 0.89	2.55 ± 0.91	2.20 ± 1.09	1.37 ± 1.04	<0.0001
Latency of fmERG, ms					
a-wave	23.18 ± 1.28	23.67 ± 1.58	24.39 ± 1.77	25.76 ± 3.39	0.040
b-wave	42.05 ± 2.27	45.44 ± 3.87	45.22 ± 3.71	48.87 ± 7.38	0.0005
Retinal sensitivity, dB					
Center point	14.78 ± 3.52	9.94 ± 3.86	3.82 ± 3.43	5.37 ± 6.31	<0.0001
Within 4°	16.50 ± 2.01	13.35 ± 3.57	6.83 ± 4.39	5.78 ± 6.27	<0.0001
Within 8°	16.13 ± 2.10	13.66 ± 3.32	9.19 ± 3.94	6.76 ± 6.23	<0.0001

PED pigment epithelium detachment, *AMD* age-related macular degeneration, *fmERG* focal macular electroretinography, *ILM* internal limiting membrane, *RPE* retinal pigment epithelium

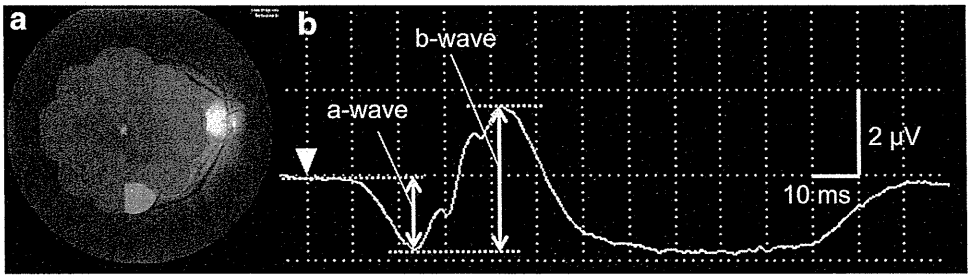

Fig. 1 Macular function in a healthy control eye. Retinal sensitivity map obtained by microperimetry (**a**) and focal macular electroretinography (**b**). *White arrowhead* beginning of stimulus; *yellow arrow* amplitude of each wave of focal macular electroretinogram

infrared camera (Kowa) and a stimulation system (Mayo Corp., Nagoya, Japan). The luminance values of the white stimulus light and the background illumination were 181.5 and 6.9 cd/m^2, respectively. The stimulus within the 7.5°-radius circle was centered on the fovea, as observed through the infrared camera. The fmERG was recorded using 5-Hz rectangular stimuli (100 ms with the light on and 100 ms with the light off). The recording (200 responses) was carried out in triplicate to confirm the reproducibility of the results; thus, a total of 600 responses were averaged by the signal processor (Neuropack MEB-2204; Nihon Kohden, Tokyo, Japan). The fmERG response was digitized at 10 kHz with a band-pass filter of 5–500 Hz for the a-wave and the b-wave. The amplitudes of the a- and b-waves were measured from baseline to the peak of the a-wave and from the trough of the a-wave to the peak of the b-wave, respectively. Latency was defined as the time from the beginning of stimulation to the peak of each component.

For the OCT images, the foveal thickness in each eye was determined in the following two ways: the distance between the internal limiting membrane (ILM) and the outer border of the RPE or the distance between the ILM and the Bruch membrane. In eyes with drusenoid PED, we also measured the height and area of the PED. For the sequential OCT images, the height of the PED was defined as the maximal distance between the outer border of the RPE and the Bruch membrane (sometimes outside the fovea). For the late-phase indocyanine green angiogram, the area of the PED was measured using software built into the HRA-2. Briefly, drusenoid PED was observed as a dark area on the late-phase indocyanine green angiogram, and

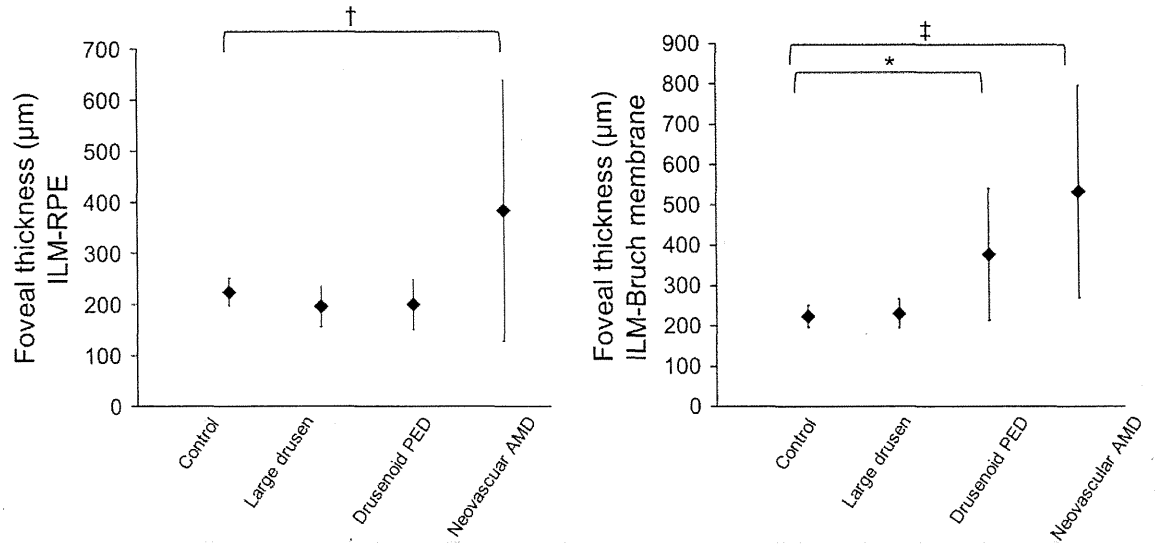

Fig. 2 Foveal thickness of control eyes, eyes with large drusen, eyes with drusenoid pigment epithelial detachment, and eyes with neovascular age-related macular degeneration. $^{*}P < 0.05$, $^{\sqcup}P < 0.01$, $^{\sqcup}P < 0.0001$, as compared with control eyes. *P* values were calculated by the Dunnet test. *ILM* internal limiting membrane, *RPE* retinal pigment epithelium, *PED* pigment epithelium detachment, *AMD* age-related macular degeneration

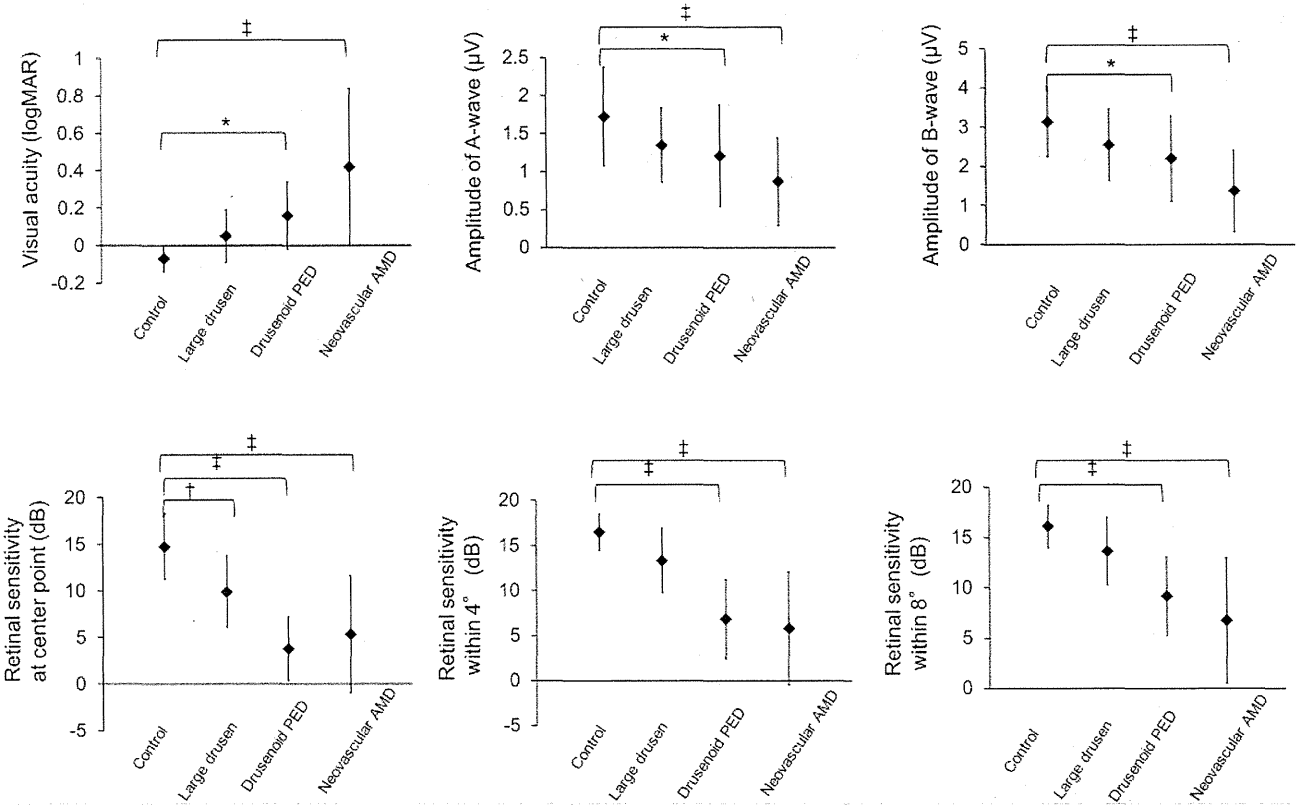

Fig. 3 Macular function measured with multimodality in control eyes, eyes with large drusen, eyes with drusenoid pigment epithelial detachment, and eyes with neovascular age-related macular degeneration. $^{*}P < 0.05$, $^{\sqcup}P < 0.01$, $^{\sqcup}P < 0.0001$, as compared with control eyes. *P* values were calculated by the Dunnet test. *LogMAR* logarithm of the minimum angle of resolution, *PED* pigment epithelium detachment, *AMD* age-related macular degeneration

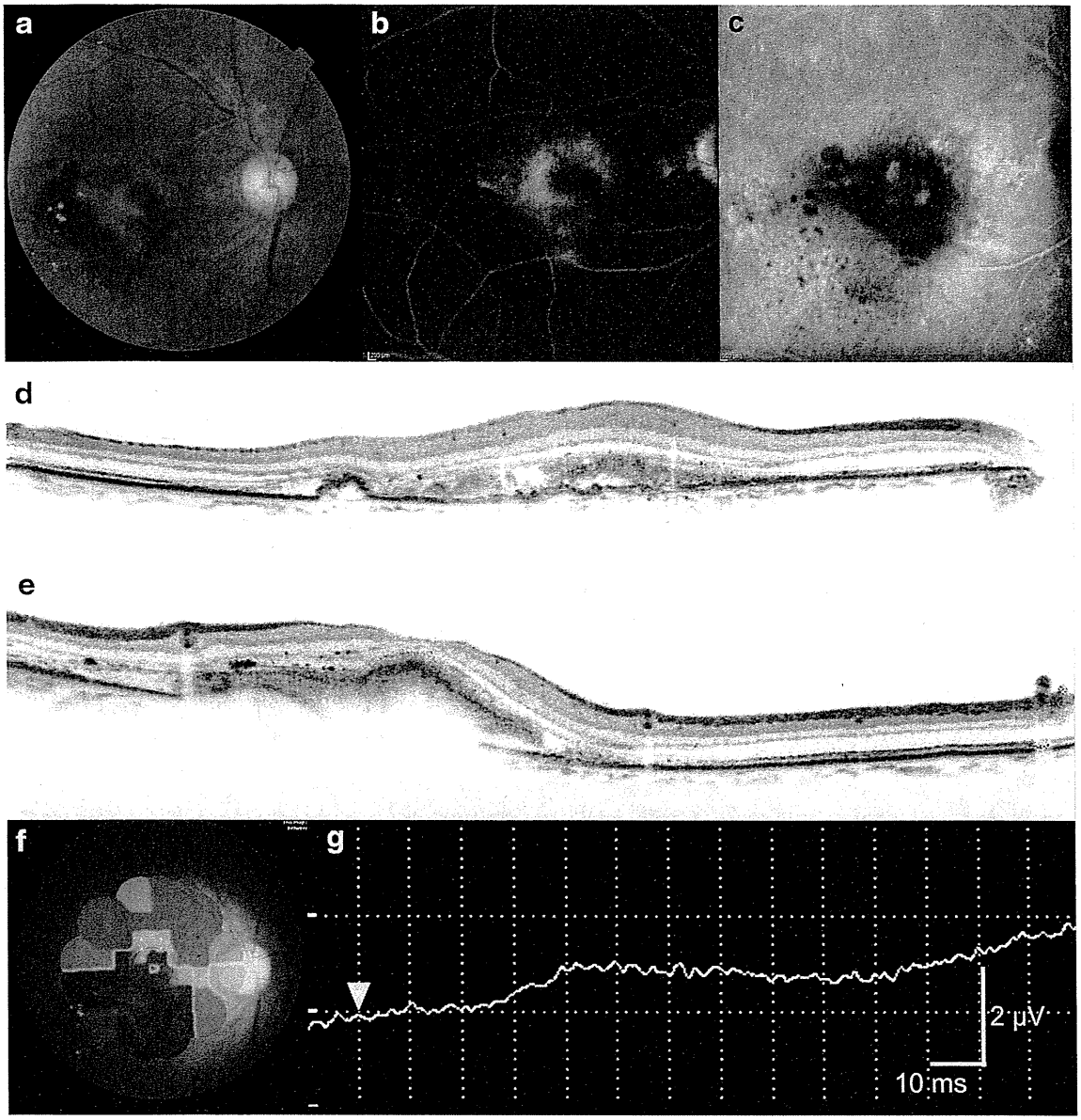

Fig. 4 Macular function in an eye with neovascular age-related macular degeneration. **a** Fundus photograph shows submacular hemorrhage (0.15 on a Landolt chart, OD). **b**, **c** *Fluorescein* and *indocyanine green* angiograms reveal subfoveal choroidal neovascularization. *Horizontal* **d** and *vertical* **e** sections obtained with OCT show subretinal fluid. **f** Retinal sensitivity map obtained with microperimetry shows a substantial reduction of retinal sensitivity in the macular function. **g** Focal macular electroretinogram shows a substantial reduction in amplitude of all waves. *Arrowhead* beginning of stimulus

the edge of this central dark area was traced manually. The surrounding small dark lesions (drusen) isolated from the central PED were not included.

Statistical analysis was performed using PASW Statistics version 17.0 software (SPSS, Chicago, IL, USA). All values were expressed as means ± standard deviations. The best-corrected VA was measured using a Landolt chart and converted to the logarithm of the minimum angle of resolution (logMAR). To clarify differences from the healthy controls, all mean values between groups were compared using one-way analysis of variance and post hoc Dunnet tests. Bivariate analysis was done with the Pearson product moment correlation.

Results

Table 1 shows the characteristics of the study populations. Although the controls (82.0 ± 3.2 years) were significantly older than the patients with neovascular AMD (77.3 ± 6.9 years, $P = 0.019$), there was no significant difference in the gender or lens status of groups. In the

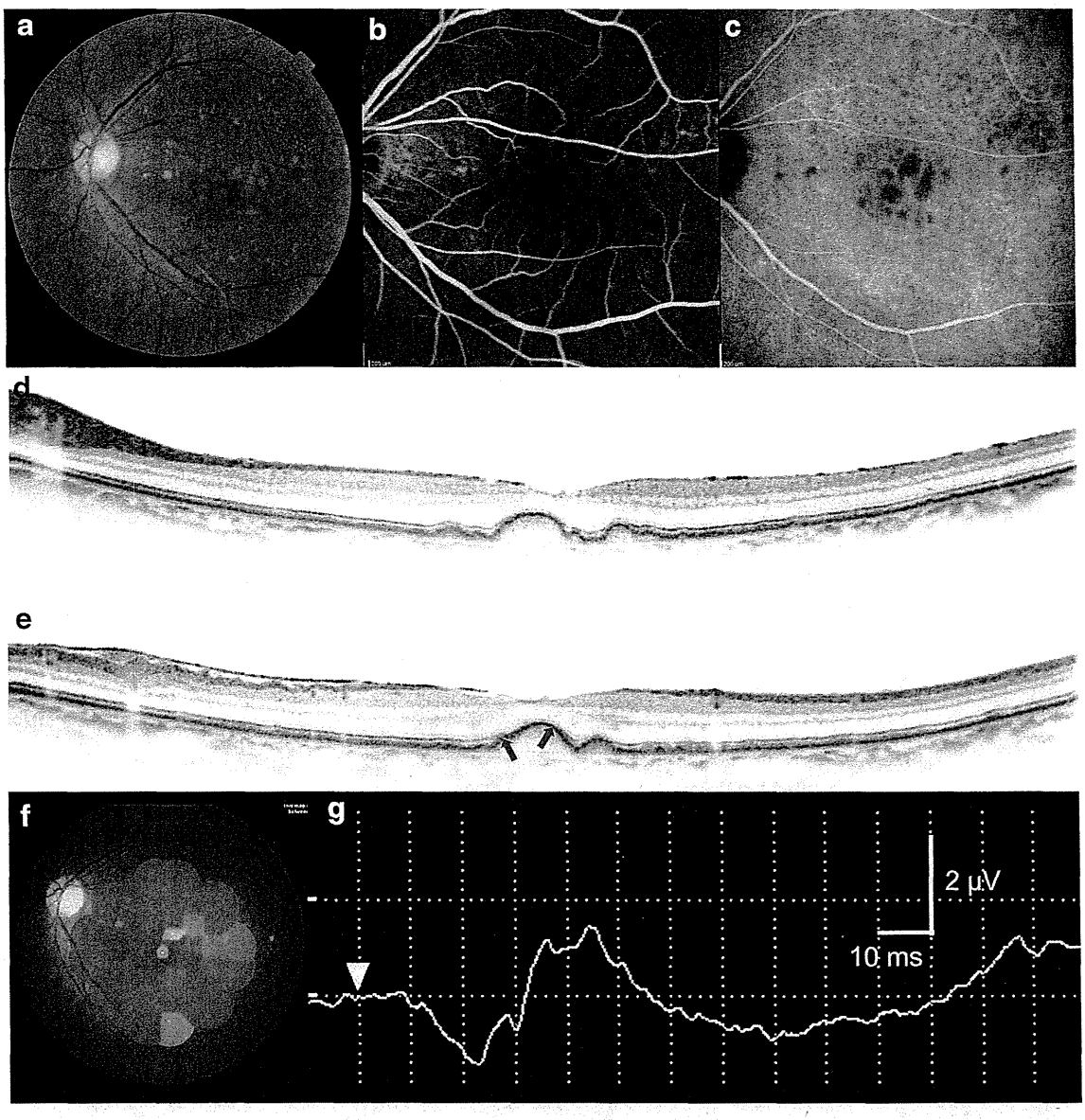

Fig. 5 Macular function in an eye with large drusen. **a** Fundus photograph shows multiple large drusen in the macular area (1.0 on a Landolt chart, OD). **b**, **c** *Fluorescein* and *indocyanine green* angiograms reveal no choroidal neovascularization. *Horizontal* **d** and *vertical* **e** sections obtained with OCT show multiple large drusen beneath and affecting the fovea. The junction of the inner and outer segments of photoreceptors (between the *arrows*) was discontinued. **f** Microperimetry shows preserved retinal sensitivity within the macular area except for the fovea. **g** Focal macular electroretinogram shows that the amplitude of all of the waves was relatively preserved. *Arrowhead* beginning of stimulus

control group, 13 eyes had small drusen in the macular area and 7 had no drusen. All eyes showed good macular function (Fig. 1).

All functional parameters were measured, with VA, fmERG, and microperimetry showing significant variation between the groups (Table 1). All eyes with neovascular AMD showed marked morphologic changes in the neurosensory retina. In this group, cystoid macular edema was seen in 4 eyes (21 %), serous retinal detachment in 14 eyes (74 %), and PED in 17 eyes (89 %); foveal thickness of the neurosensory retina (384 ± 256 μm) was significantly increased compared with the control eyes (224 ± 27 μm) (Fig. 2). Consistent with these morphologic changes, macular function (VA, fmERG, and microperimetry) was significantly deteriorated in the neovascular AMD group (Figs. 3, 4).

In the large drusen group, all eyes showed multiple large drusen in the macular area; the mean number of drusen measuring 125–250 μm was 10.3 ± 4.2 and that of drusen measuring at least 250 μm was 3.1 ± 2.1. These eyes showed minimal morphologic changes in the neurosensory retina. No eyes in this group showed cystoid macular

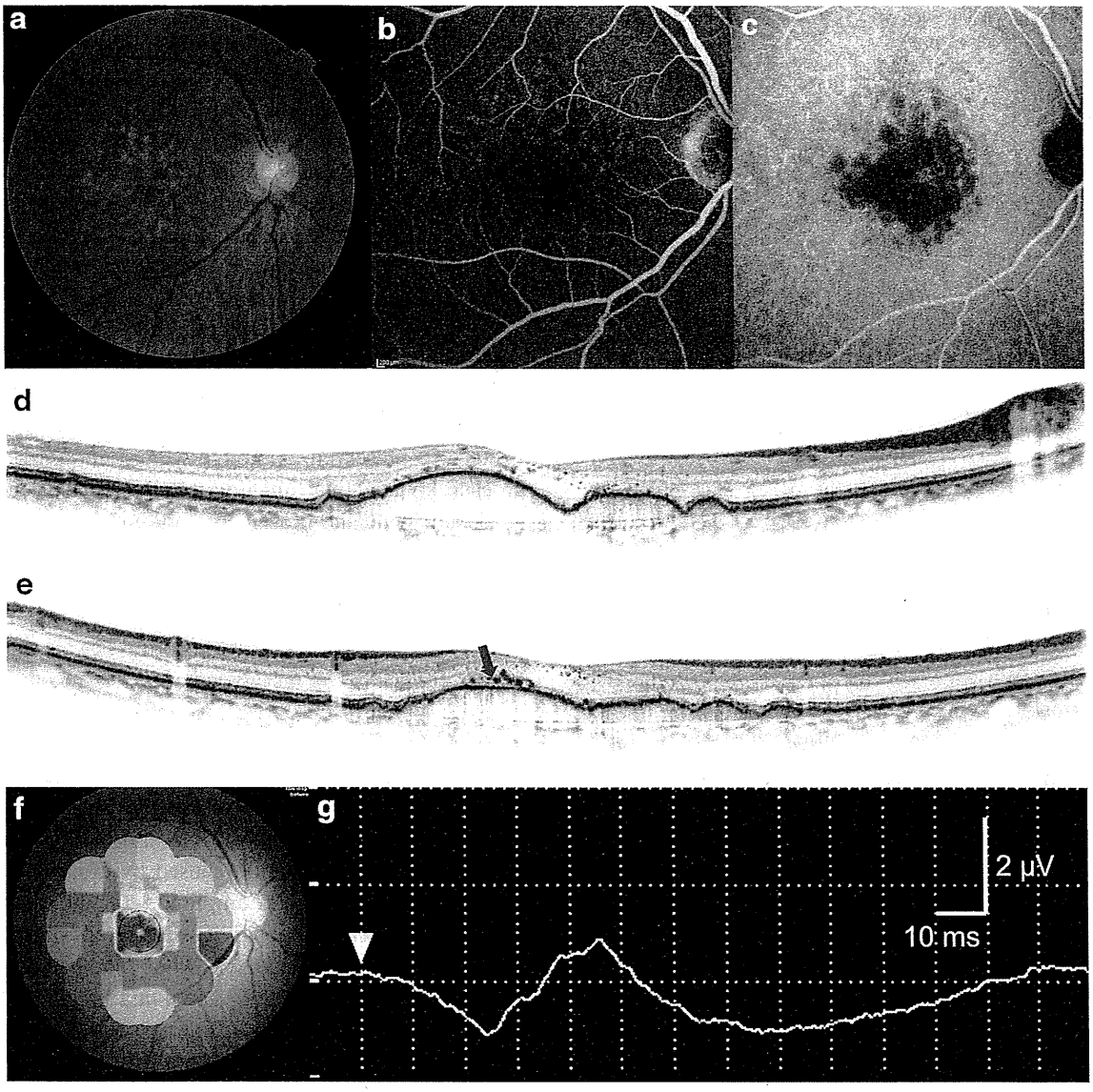

Fig. 6 Macular function in an eye with drusenoid pigment epithelial detachment (PED). **a** Fundus photograph of drusenoid PED under the fovea (0.7 on a Landolt chart, OD). **b** *Fluorescein* angiogram reveals no choroidal neovascularization. **c** From the late-phase *indocyanine green* angiogram, the area of drusenoid PED was calculated as 6.26 mm^2. *Horizontal* **d** and *vertical* **e** sections obtained with OCT show drusenoid PED. The height of the PED was 258 μm. The *red arrow* indicates hyperreflective foci. **f** Retinal sensitivity map obtained with microperimetry shows a marked reduction in retinal sensitivity consistent with drusenoid PED. **g** In the focal macular electroretinogram, the amplitude of each wave was reduced to 60–75 % of normal amplitudes. *Arrowhead* beginning of stimulus

edema, serous retinal detachment, or a vitelliform lesion. Foveal thickness of the neurosensory retina (196 ± 40 μm) was no different from that in the control group (Fig. 2). In this large drusen group, while retinal sensitivity at the central point was significantly decreased, the other parameters of macular function (VA, fmERG, and microperimetry) were preserved (Figs. 3, 5).

In the drusenoid PED group, all eyes had drusenoid PED of at least 1/2 disc diameter within the macular area. The mean area of the PED was 4.78 ± 3.74 mm^2, and the mean height was 266 ± 178 μm. In eyes with drusenoid PED, the foveal thickness between the ILM and the Bruch membrane (377 ± 164 μm) was significantly greater than that in the control eyes (224 ± 27 μm, $P = 0.013$). However, the structure of the neurosensory retina was well preserved, and the foveal thickness between the ILM and RPE (200 ± 49 μm) did not differ from that in the control group (Fig. 2). On the other hand, the macular function of these eyes was significantly deteriorated. VA, amplitude of the a-wave and of the b-wave, and retinal sensitivity measured with the MP1 were significantly decreased when compared with the control eyes (Figs. 3, 6). Table 2 shows the correlation between the size of the drusenoid PED and macular function and between the area of the PED and the

Table 2 Correlation between size of drusenoid pigment epithelium detachment and macular function

	Area of drusenoid PED		Height of drusenoid PED	
	r	*P* value	*r*	*P* value
Visual acuity in logMAR	0.058	0.820	0.432	0.074
Amplitude of fmERG				
a-wave	−0.427	0.077	−0.118	0.642
b-wave	−0.445	0.067	−0.312	0.207
Latency of fmERG				
a-wave	0.635	0.006	−0.090	0.732
b-wave	0.530	0.029	0.100	0.702
Retinal sensitivity				
Center point	−0.472	0.056	−0.423	0.091
Within 4°	−0.682	0.003	−0.625	0.007
Within 8°	−0.761	0.0004	−0.533	0.028

PED pigment epithelium detachment, *logMAR* logarithm of the minimum angle of resolution, *fmERG* focal macular electroretinography

latencies of the a-wave and the b-wave, and retinal sensitivity within the central 4° or 8°. The height of the PED was negatively correlated with retinal sensitivity within the central 4° and 8° areas (Fig. 7).

Discussion

Eyes with neovascular AMD often have a severe decrease in VA. In addition, because such eyes often show serous retinal detachment, subretinal hemorrhage, retinal edema, or PED in the macular area, they may well have a reduction in function in the macular area. With the use of fmERG, Nishihara et al. [26] reported that in eyes with neovascular AMD, the amplitude of each wave was reduced to 29 to 35 % of that of the control eyes. With the use of microperimetry, Sulzbacher et al. [24] and Hautamäki et al. [27] more recently reported that retinal sensitivity was markedly decreased within the area of CNV, macular edema, hemorrhage, subretinal fluid, and PED in eyes with neovascular AMD. In our patients with neovascular AMD, cystoid macular edema was seen in 21 %, serous retinal detachment in 74 %, and PED in 89 % of the patients, and the thickness of the fovea in the neurosensory retina was significantly increased. In eyes with neovascular AMD, severe macular dysfunction is based on the morphologic changes caused by the exudative change resulting from the CNV.

Eyes with drusen often maintain good VA. However, as the number or size of the drusen increases, they may cause a functional disturbance in the macular area. So far, several electrophysiologic assessments have been performed to study the macular function in eyes with drusen [23, 28–33]. Falsini et al. [33] documented an abnormality of the focal ERG threshold in eyes with more than 20 soft drusen, although they did not investigate the correlation between each drusen and the local sensitivity loss. With the use of microperimetry, Midena et al. [16] reported that retinal sensitivity in eyes with large drusen (>125 μm) was severely deteriorated. Iwama et al. [34] reported that eyes with confluent soft drusen often show focal areas with reduced retinal function consistent with irregularity of the RPE line or of the junction between the inner and outer segments of the photoreceptors. In the current study, while retinal sensitivity at the central point was significantly decreased in eyes with large drusen, the other parameters of macular function (VA, fmERG, and microperimetry) were well preserved. Although we did not assess function at each point, retinal function may be focally deteriorated, consistent with the drusen. In addition, the area in which drusen are seen may be involved in the reduction of macular function.

Drusenoid PED refers to a fairly well-circumscribed, shallow elevation of the RPE formed by confluent soft drusen, often located in the center of the macula [35]. VA in eyes with drusenoid PED is reported to be relatively good. In fact, in a recent report from the Age-Related Eye Disease Study, baseline VA in eyes with drusenoid PED was * 20/32, with * 90 % of eyes having VA better than 20/40 [35]. So far, however, little information is available on the macular dysfunction caused by drusenoid PED. In the current study, VA, amplitude of the a-wave and b-wave, and retinal sensitivity measured with the MP1 were significantly decreased when compared with the control eyes. In addition, the area and height of the PED were correlated with the fmERG and with the retinal sensitivity within the macular area—correlations that are consistent with the previously mentioned report of confluent drusen [34]. Photoreceptor damages, which could be observed as discontinuity of the junction of the inner and outer segments and as presence of hyper reflective foci in the OCT image (Fig. 6) [36, 37], might result in decreased macular function in eyes with drusenoid PED. Falsini et al. [33] also discussed that focal ERG sensitivity loss in eyes with drusen might result from photoreceptor drop out, as could be slightly seen in the OCT images of eyes with large drusen in our study (Fig. 5).

The prognosis of drusenoid PED was initially thought to be relatively good [38, 39]; however, a recent cohort study reported a high rate of progression to more advanced AMD [35]. Roquet et al. [25] documented that presence of metamorphopsia and drusenoid PED of greater than two disc diameters were risk factors of CNV occurrence within 2 years. Recently, other research groups have reported results of pilot studies on the early treatment of drusenoid PED without CNV by photodynamic therapy or by