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Associations of Cigarette Smoking But Not Serum Fatty Acids with Age-related Macular Degeneration in a Japanese Population

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Purpose: To assess modifiable environmental risk factors and protective factors for age-related macular degeneration (AMD) in a native Japanese population.

Design: A case-control study.

Participants: We included 422 case-control samples composed of 279 consecutive AMD cases and 143 controls.

Methods: Information regarding systemic conditions and lifestyle were documented in each subject by standardized questionnaire including age, gender, smoking history, body mass index (BMI), and history of cardiovascular disease, hypertension, and diabetes. Serum fatty acids profiles were analyzed by gas chromatography performed on blood samples taken from each study participant. Logistic regression and multiple comparison analyses were utilized in this study.

Main Outcome Measures: Population-specific information assessing systemic conditions, lifestyle, and serum fatty acid profiles.

Results: Among environmental factors analyzed cigarette smoking showed the most significant association with development of all AMD ($P<0.00001$; odds ratio [OR], 4.06; 95% confidence interval [CI], 2.22–7.43), typical neovascular AMD ($P<0.0001$, OR, 4.59; 95% CI, 2.29–9.18), and polypoidal choroidal vasculopathy ($P<0.001$; OR, 4.87; 95% CI, 1.96–12.1). Hypertension and BMI showed a mild association with AMD. Although male prevalence was significantly higher in all case groups than in controls with conventional Scheffe correction, there was no association of gender with AMD development when logistic regression analysis was used to adjust for cigarette smoking. There was no difference in fatty acid profiles, except for a mild association of eicosapentaenoic acid concentration in the all AMD group.

Conclusions: In the Japanese population studied, cigarette smoking influenced the risk of AMD but fractionated serum fatty acid levels did not. Although prior reports indicate a male predominance in Japanese patients with AMD, this study demonstrates that cigarette smoking accounts for this confounding bias. In addition, our population-specific data do not demonstrate significant differences in serum fatty acid composition, including ω-3 and ω-6 long chain polyunsaturated fatty acids, in Japanese patients with and without AMD. These results are consistent with the high proportion of smokers in aged Japanese men and the high fish oil intake in this population.

Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries, estimated to affect >50 million people worldwide.[1] A lower prevalence of AMD is reported to occur in non-Caucasian than Caucasian populations.[2,3] However, the disorder has become more common in the last 2 decades in Singapore, China, and Japan.[4–8] Recent population-based studies have reported that the prevalence of early AMD in the Japanese population is similar to that found in a Caucasian population,[9,10] and incidence of late AMD lies between that in Caucasian and African populations.[11]

Several other pertinent phenotypic and epidemiologic characteristics of Japanese AMD have been reported. In Japanese population-based and case-control studies, AMD is significantly more prevalent in men than women.[12,13] In contrast, AMD in the Caucasian population is reported to be either slightly more prevalent in women than men[14–16] or the incidence is equivalent.[17] Other differences, such as unilaterality at presentation, a comparatively low incidence of drusen and geographic atrophy, and a high incidence of polypoidal choroidal vasculopathy (PCV) have been reported.[18–21] It has been proposed that these phenotypic and epidemiologic differences suggest a difference in pathogenesis between Japanese and Caucasian AMD. Studies by our group and others have demonstrated that gene variants in both compliment factor H and high-temperature requirement factor A1/age-related maculopathy susceptibility 2, contribute significantly to development of AMD in these

East Asian populations and is comparable with that seen in Caucasian populations.[22-25] In the present work, we have described modifiable environmental risk factors and protective factors for AMD development in East Asian populations.

Several environmental factors for development of AMD have been previously reported in the Caucasian population. Reported risk factors include ocular pigmentation, light exposure, dietary factors, a positive family history for AMD, high blood pressure, and smoking.[26-29] Recent epidemiologic data suggest that increased dietary intake of the ω-3 long chain polyunsaturated fatty acids (LCPUFAs) is associated with a reduced risk of progression of advanced AMD in Caucasian patients.[30-33] In this study, we assess modifiable environmental risk factors and protective factors including cigarette smoking and serum ω-3 LCPUFA levels in association with Japanese.

Methods

Subjects

The case-control samples were composed of 279 consecutive patients with AMD ranging in age from 50 to 90 years (71.2±8.7; mean value ± standard deviation), 212 men and 67 women, and 143 controls without AMD ranging in age from 52 to 88 years (68.1±10.0), 73 men and 70 women recruited from outpatient visits in the Department of Ophthalmology, Saitama Medical University Hospital in Saitama prefecture, Japan. All case-control subjects were unrelated Japanese Asians. The study was approved by the Ethical Committee of Saitama Medical University (approved on December 9, 2003; approval number 03-262) and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. Each individual was fully informed of the purpose of, and the procedures involved in, the study. Informed written consent was obtained for each patient.

Ophthalmic Examination, Definition, and Subtype Classification of Age-related Macular Degeneration

All patients with AMD and control subjects underwent full ophthalmologic examination, including slit-lamp biomicroscopy, funduscopy, and contact lens biomicroscopic examinations of the retina. Inclusion criteria were (1) age ≥50, (2) diagnosis of AMD in 1 or both eyes, (3) no association with other retinochoroidal diseases such as angioid streaks, high myopia (>6 diopters of myopic refractive error), central serous chorioretinopathy, presumed ocular histoplasmosis, or retinal angiomatous proliferation (type 3 choroidal neovascularization). The AMD subtypes were diagnosed and classified based on the classification described in the clinical age-related maculopathy staging system (grade 1–5).[34] Furthermore, neovascular AMD (nAMD; grade 5) was defined by ophthalmoscopic and angiographic findings of classic or occult choroidal neovascularization, hemorrhagic RPE detachments, serous or hemorrhagic retinal detachment, or fibrovascular disciform scarring. Nonneovascular AMD (nnAMD) includes ≥1 large drusen or extensive (approximately ≥15) intermediate drusen (grade 3) and geographic atrophy (grade 4) in ≥1 eye. In the study group of AMD patients, there were 248 patients with nAMD (grade 5) and 31 patients with nnAMD (grade 3 or 4). The control subjects (grade 1) were confirmed not to have clinical evidence of AMD by the same complete ophthalmologic examination and the same criteria that were used to identify the study cohort of AMD patients.

We classified nAMD into 2 subtypes: Typical nAMD and PCV. The diagnosis of PCV was made based on fundus examination and indocyanine green angiography at the initial examination. We defined PCV as having ≥1 of the following criteria[35]: Orange, bulging polypoidal dilations in the peripapillary and/or macular area by fundus examination, or characteristic polypoidal lesions delineated by indocyanine green angiography. One patient who had both typical choroidal neovascularization and polypoidal lesions was excluded from this study.

Table 1. Age, Gender and Potential Risk Factors for Age-Related Macular Degeneration (AMD) Development of the Study Subjects

| | Cases | | | | |
	All AMD	Typical nAMD	PCV	nnAMD	Controls
n	279	166	82	31	143
Age (mean ± SD)	71.2±8.7	71.9±9.1	70.6±7.7	68.9±9.3	68.1±10.0
P*	0.031	0.010	0.421	0.998	NA
Gender (male/female)	212/67	117/49	68/14	27/4	73/70
P†	<0.001	<0.001	<0.00001	<0.001	NA
Hypertension (%)	46.2	45.8	48.8	41.9	30.1
P†	<0.001	0.005	0.007	0.200	NA
Heart disease (%)	19.7	20.5	17.1	22.6	16.8
P†	0.465	0.411	0.966	0.444	NA
BMI (mean ± SD)	23.2±3.2	23.3±3.4	22.9±2.9	23.2±2.7	22.5±3.3
P*	0.457	0.367	0.957	0.884	NA
Smoking habits (%)	74.9	72.5	79.2	76.7	40.1
P†	<0.0000000001	<0.0000001	<0.0000001	<0.001	NA

BMI = body mass index; NA = not available; nAMD = neovascular AMD; nnAMD = non-neovascular AMD; PCV = polypoidal choroidal vasculopathy.
Data are expressed as the number of subject, percent of the entire group, or mean values ± standard deviation (SD), except the P-values.
*Scheffe correction.
†Chi-square test.

Risk Factors Questionnaires and Analyses of Fatty Acid Profiles

We administered questionnaires to obtain information regarding age, gender, height, weight, blood pressure, history of smoking and medical history including current medication by interviewing all subjects. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Smokers were defined as having had a smoking habit in the past.

Peripheral blood was collected from consecutive 264 individuals (142 cases and 122 controls) while fasting, who agreed to provide a blood sample after providing informed consent and these samples were submitted for serum fatty acids profile analysis. The samples were centrifuged at 1500 rpm for 10 minutes, and the supernatant serum was stored at $-20°C$ until measurement of 24 fatty acids fractionations was performed using gas chromatography.

Statistical Analysis

Age, gender, and potential risk factors for AMD development between cases and controls were compared using multiple comparison analysis (Scheffe correction) and the chi-square test for quality of proportions. Logistic regression analysis was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (95% CI) for the effect of environmental risk factors (age, gender, hypertension, heart disease, and smoking history) on the

risk of AMD development by adjusting covariate effects for each factor. The concentration of each fatty acid was analyzed by multiple comparison analysis (Scheffe correction).

$P<0.05$ was considered significant. All analyses were performed using commercially available software (SSRI ver. 1.20, SSRI, Tokyo, Japan).

Results

Among the 279 consecutive patients with AMD, 248 (88.9%) patients had nAMD and 31 (11.1%) had nnAMD. Of the patients with nAMD, 166 (66.9%) had typical nAMD and 82 (33.1%) had PCV. There was a small difference in age between all AMD/typical nAMD groups and controls ($P<0.05$). Male prevalence in all AMD, typical nAMD, PCV, and nnAMD groups was significantly higher than in controls ($P<0.001$). Hypertension and a smoking habit were more prevalent in each AMD case group except for hypertension in nnAMD development (Table 1).

Because of significance with regard to age, gender distribution, and several risk factors, we estimated ORs and 95% CI adjusted by age, age/gender, and multivariates using logistic regression analysis (Table 2). Among the environmental factors analyzed, cigarette smoking showed the greatest association with development of all AMD, typical nAMD, and PCV. The multivariate-adjusted

Table 2. Age-, Age-/Gender-, and Multivariate-Adjusted Odds Ratios of Risk Factors of Age-Related Macular Degeneration (AMD) Development

AMD	Age-Adjusted		Age-/Gender-Adjusted		Multivariate-Adjusted	
	P*	OR (95% CI)	P*	OR (95% CI)	P*	OR (95% CI)
All AMD						
Age	NA		NA		0.003	1.04 (1.01–1.07)
Gender	<0.000001	3.11 (2.01–4.81)	NA		0.539	1.21 (0.66–2.24)
Hypertension	0.009	1.79 (1.16–2.78)	0.020	1.71 (1.09–2.69)	0.022	1.80 (1.09–2.97)
Heart disease	0.785	1.08 (0.63–1.85)	0.969	0.99 (0.57–1.73)	0.666	0.88 (0.48–1.60)
BMI	0.026	1.08 (1.01–1.16)	0.086	1.06 (0.99–1.14)	0.090	1.07 (0.99–1.15)
Smoking	<0.0000000001	4.58 (2.90–7.25)	<0.00001	3.94 (2.19–7.09)	<0.00001	4.06 (2.22–7.43)
Typical nAMD						
Age	NA		NA		0.001	1.05 (1.02–1.08)
Gender	<0.001	2.36 (1.46–3.81)	NA		0.707	0.88 (0.44–1.76)
Hypertension	0.031	1.70 (1.05–2.76)	0.045	1.66 (1.01–2.72)	0.066	1.69 (0.97–2.94)
Heart disease	0.744	1.10 (0.61–2.00)	0.901	1.04 (0.57–1.91)	0.763	0.90 (0.47–1.75)
BMI	0.021	1.09 (1.01–1.17)	0.048	1.08 (1.00–1.16)	0.038	1.09 (1.00–1.18)
Smoking	<0.0000001	4.05 (2.43–6.73)	<0.0001	4.21 (2.16–8.23)	<0.0001	4.59 (2.29–9.18)
PCV						
Age	NA		NA		0.079	1.03 (1.00–1.07)
Gender	<0.00001	4.60 (2.36–8.95)	NA		0.373	1.52 (0.60–3.88)
Hypertension	0.024	1.99 (1.10–3.61)	0.036	1.96 (1.05–3.66)	0.031	2.13 (1.07–4.24)
Heart disease	0.930	0.97 (0.47–2.01)	0.698	0.86 (0.40–1.85)	0.299	0.64 (0.28–1.48)
BMI	0.315	1.05 (0.96–1.15)	0.471	1.03 (0.94–1.14)	0.484	1.04 (0.93–1.16)
Smoking	<0.0000001	6.40 (3.24–12.7)	<0.001	5.05 (2.09–12.2)	<0.001	4.87 (1.96–12.1)
nnAMD						
Age	NA		NA		0.737	1.01 (0.96–1.06)
Gender	<0.001	6.47 (2.15–19.4)	NA		0.124	3.20 (0.72–14.1)
Hypertension	0.220	1.72 (0.72–4.08)	0.187	1.86 (0.74–4.65)	0.245	1.76 (0.68–4.59)
Heart disease	0.471	1.42 (0.55–3.71)	0.626	1.28 (0.47–3.48)	0.964	1.02 (0.36–2.90)
BMI	0.239	1.07 (0.95–1.22)	0.332	1.07 (0.93–1.22)	0.217	1.10 (0.95–1.27)
Smoking	<0.001	5.02 (1.99–12.7)	0.153	2.39 (0.72–7.93)	0.149	2.58 (0.71–9.31)

BMI = body mass index; NA = not available; nAMD = neovascular AMD; nnAMD = non-neovascular AMD; PCV = polypoidal choroidal vasculopathy; OR (95% CI) = odds ratio (95% confidence interval).
Multivariate adjustment: adjusted for age, gender, hypertension, heart disease, BMI, and smoking.
*Logistic regression analysis.

Table 3. Probabilities and Odds Ratios of Gender Adjusted by Each Covariate in All Age-related Macular Degeneration (AMD) Group

Covariates	P*	OR (95% CI)
Age	<0.000001	3.11 (2.01–4.81)
Hypertension	<0.000001	2.97 (1.92–4.57)
Heart disease	<0.000001	3.02 (1.97–4.63)
BMI	<0.00001	2.97 (1.89–4.65)
Smoking	0.348	1.32 (0.74–2.37)
Multivariates	0.539	1.21 (0.66–2.24)

AMD = age-related macular degeneration; BMI = body mass index; NA = not available; OR (95% CI) = odds ratio (95% confidence interval).
Multivariate adjustment: adjusted for age, gender, hypertension, heart disease, BMI, and smoking.
*Logistic regression analysis.

probabilities and ORs of all AMD, typical nAMD, and PCV were $P<0.00001$ (OR, 4.06; 95% CI, 2.22–7.43), $P<0.0001$ (OR, 4.59; 95% CI, 2.29–9.18), and $P<0.001$ (OR, 4.87; 95% CI, 1.96–12.1), respectively. Hypertension showed a significant association with PCV development ($P = 0.031$; OR, 2.13; 95% CI, 1.07–4.24), but not with typical nAMD development. In contrast, BMI was associated with typical nAMD ($P = 0.038$; OR, 1.09; 95% CI, 1.00–1.18) development, but not with PCV. Although male prevalence was significantly greater in all case groups than in controls, after conventional Scheffe correction (Table 1), there was no association of gender with AMD development when adjusted for multivariates using logistic regression analysis (Table 2).

To determine which covariates played a role in confounding the relationship between gender and AMD development, probabilities and ORs were adjusted by each covariate. Although gender was associated with AMD development when adjusted by age, hypertension, heart disease, or BMI; smoking- and multivariate-adjusted probabilities and ORs demonstrated no association (Table 3).

The concentration of 24 fatty acids in the serum lipids fraction was evaluated by Scheffe multiple comparison analysis in all AMD, typical nAMD, PCV, nnAMD, and control groups. There was no significant difference in the concentration of any of the 24 fatty acids tested except for a small association of eicosapentaenoic acid (EPA) concentration with the all AMD group ($P = 0.031$). The representative 11 fatty acids concentration, ω-6/ω-3 ratio and arachidonic acid/EPA ratio are summarized in Table 4.

Discussion

A number of prior reports have demonstrated that Japanese AMD is significantly more prevalent in men than women,[12,13] whereas AMD in the Caucasian population is reported to be either slightly more prevalent in women than men[14–16] or nearly equivalent.[17] Our data are consistent with these findings. Male prevalence in all AMD, typical nAMD, PCV, and nnAMD are 76.0%, 70.5%, 82.9%, and 87.1%, respectively, which are higher than that in the Caucasian population (40%–51%).[36,37] However, there was no association of gender with AMD development in any case group when adjusted for a smoking habit as a covariate. This finding is consistent with the high proportion of smokers among older Japanese men.[38] According to the Nationwide Cigarette Smoking Survey by the Japan Tobacco Industry Incorporated, Japanese Ministry of Health and Welfare (available at: http://www.health-net.or.jp/tobacco/front.html [in Japanese], accessed July 5, 2009), smoking prevalence in Japanese peaked in 1966, and was 83.7% for men and 17.7% for women. Since that time, the prevalence has decreased but is currently still much higher for men (45.8%) than for women (13.8%). The results of the present study is the first to indicate that the predominance of AMD

Table 4. Fatty Acid Fractionation

Fatty Acid	Cases				Controls
	All AMD	Typical nAMD	PCV	nnAMD	
Saturates					
PA (16:0)	26.4±2.46 (0.992)	26.1±2.05 (0.995)	27.1±2.98 (0.290)	25.4±1.92 (0.829)	26.3±2.12
SA (18:0)	7.45±0.80 (0.952)	7.42±0.83 (0.925)	7.58±0.78 (0.995)	7.06±0.64 (0.338)	7.53±0.69
Monounsaturates					
OA (18:1ω9)	20.6±2.56 (0.999)	20.6±2.40 (0.995)	20.8±2.69 (0.999)	20.6±3.18 (0.999)	20.7±3.38
ω-3 Polyunsaturates					
ALA (18:3ω3)	0.72±0.22 (0.226)	0.72±0.22 (0.395)	0.68±0.21 (0.066)	0.89±0.21 (0.653)	0.79±0.23
EPA (20:5ω3)	2.04±1.06 (0.031)	2.07±1.12 (0.053)	1.97±1.01 (0.359)	2.06±0.87 (0.677)	1.63±0.90
DPA (22:5ω3)	0.52±0.14 (0.931)	0.52±0.13 (0.993)	0.53±0.15 (0.823)	0.51±0.12 (0.999)	0.51±0.13
DHA (22:6ω3)	3.33±0.87 (0.215)	3.33±0.86 (0.379)	3.37±0.90 (0.354)	3.20±0.83 (0.994)	3.09±0.70
ω-6 Polyunsaturates					
LA (18:2ω6)	26.5±4.40 (0.976)	26.8±4.01 (0.999)	25.5±5.05 (0.481)	27.8±3.43 (0.958)	26.8±3.80
AA (20:4ω6)	4.06±0.88 (0.999)	4.09±0.90 (0.999)	3.98±0.79 (0.987)	4.18±1.12 (0.995)	4.07±1.01
DTA (22:4ω6)	0.12±0.04 (0.999)	0.12±0.04 (0.996)	0.12±0.04 (0.999)	0.13±0.04 (0.968)	0.12±0.03
ω-6/ω-3 ratio	5.29±1.86 (0.431)	5.33±1.80 (0.667)	5.16±1.87 (0.446)	5.59±2.24 (0.999)	5.71±1.38
AA/EPA ratio	2.55±1.47 (0.057)	2.55±1.47 (0.139)	2.57±1.44 (0.295)	2.52±1.66 (0.736)	3.13±1.64

AA = arachidonic acid; ALA = α-linolenic acid; AMD = age-related macular degeneration; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; nAMD = neovascular AMD; nnAMD = non-neovascular AMD; OA = oleic acid; PA = palmitic acid; PCV = polypoidal choroidal vasculopathy; SA = stearic acid; VA = vaccenic acid.
Data are expressed as %mol or mean values ± SD (P-values, Scheffe correction).

found in aged Japanese men is accounted for by confounding bias, derived from the high incidence of cumulative smoking habits. The significant contribution of cigarette smoking in gender predominance may or may not be found in other countries where male smoking prevalence is high, including other Asian populations.

In addition to the strong association of smoking habits with all subtypes of AMD, BMI is associated with typical nAMD but not with PCV. Conversely, hypertension showed an association with PCV development but not with typical nAMD development. The associations of BMI and hypertension are not strong; however, the data presented here are the first to demonstrate this correlation in the various subtypes of AMD examined herein. Several studies have attempted to show genetic differences between typical nAMD and PCV.[24,39,40] To date, a common genetic background among these subtypes has been reported with the exception of a study presenting elastin gene polymorphism differences between PCV and typical nAMD.[40] Because the phenotypic characteristics, response to treatment, and prognosis for PCV differ from that of typical nAMD, further studies regarding the elucidation of genetic and environmental origin of these phenotypic variations are indicated.[41–43]

The LCPUFA are essential fatty acids classified into 2 groups; namely, ω-3 and ω-6 LCPUFAs based on their chemical structures. The representatives of ω-3 LCPUFAs are EPA and docosahexaenoic acid, found mainly in marine oils, and have a range of biological activities including a reduction in circulating triacylglycerol levels that may attenuate both atherosclerosis and thrombosis.[44–47] An inverse association between ω-3 LCPUFA and risk of coronary artery disease is a consistent finding of observational studies.[48–50] Similarly, several lines of evidence support a reduced likelihood of AMD in subjects who reported high levels of ω-3 LCPUFA consumption.[30–33] A recently published systematic review with meta-analysis of 9 studies indicates that a high dietary intake of ω-3 LCPUFA was associated with a 38% reduction in the risk of late AMD.[51] The results presented here, however, demonstrate no significant difference in blood fatty acids after fractionation in Japanese patients with and without AMD. The only difference in the 24 lipid fractions examined was a mild association of EPA concentration in the all AMD group.

Fish intake in Japan, where sushi is a staple, is among the highest of any population group in the world.[52,53] In a population-based, cross-sectional study in Japan and the United States, native Japanese living in Japan had 2-fold higher serum levels of ω-3 LCPUFA than Caucasians and Japanese Americans.[54] Figure 1 provides comparison of serum levels of representative ω-3 and ω-6 LCPUFAs (EPA and arachidonic acid) in our study and in a population-based study in Japan and the United States.[54] Although the present study was designed as a hospital-based, case-control study, higher levels of EPA and lower levels of arachidonic acid were demonstrated. Although EPA should inversely associate with AMD development based on prior hypotheses,[30–33,51] our data demonstrated a paradoxically mild association with AMD development. The difference in EPA
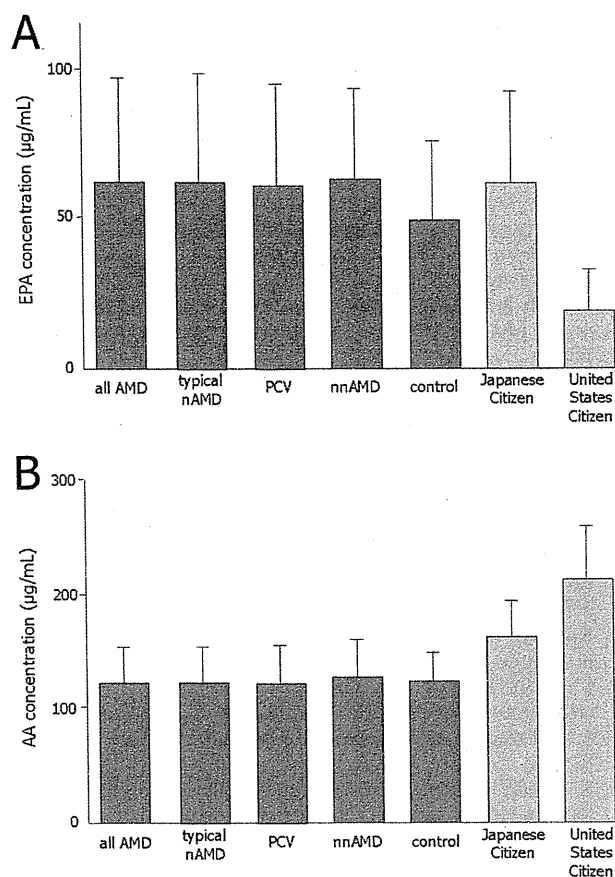

Figure 1. Comparison of serum levels of EPA (A) and AA (B) in our study and a population-based study in Japan and the United States.[55] AA = arachidonic acid; AMD = age-related macular degeneration; EPA = eicosapentaenoic acid; nAMD = neovascular AMD; nnAMD = nonneovascular AMD; PCV = polypoidal choroidal vasculopathy.

levels between Japanese and American residents is much greater than those measured in AMD cases and controls in this study. Therefore, it is possible that this slight difference in serum EPA concentration may in turn play little or no role in Japanese AMD development.

In conclusion, cigarette smoking influenced the risk of Japanese AMD, but fractionated fatty acid levels did not. Although prior reports indicate a male predominance among Japanese AMD, this study demonstrates that cigarette smoking is a confounding bias of the reported male predominance in this population. This work indicates little difference in fractionated fatty acid levels, including ω-3 and ω-6 LCPUFA, between AMD cases and controls in a Japanese population. These results are consistent with the high proportion of smokers among older Japanese men and the frequent fish oil intake. Several lines of evidence indicate the nicotine-mediated cell proliferation and angiogenesis[55,56] and LCPUFAs effects on inflammations and angiogenesis.[57] These characteristics along with other environmental risks and protective factors may play a role in the molecular pathogenesis of Japanese AMD, which is ethnically unique and distinctive.

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Footnotes and Financial Disclosures

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A new mutation in the *RP1L1* gene in a patient with occult macular dystrophy associated with a depolarizing pattern of focal macular electroretinograms

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Purpose: To determine whether a mutation in the RP1-like protein 1 (*RP1L1*) gene is present in a Japanese patient with sporadic occult macular dystrophy (OMD) and to examine the characteristics of focal macular electroretinograms (ERGs) of the patient with genetically identified OMD.
Methods: An individual with OMD underwent detailed ophthalmic clinical evaluations including focal macular ERGs. Mutation screening of all coding regions and flanking intron sequences of the *RP1L1* gene were performed with DNA sequencing analysis in this case with OMD.
Results: A new *RP1L1* mutation (c.3596 C>G in exon 4) was identified. The variant c.3596 C>G in exon 4 resulted in the substitution of cysteine for serine at amino acid position 1199. The serine at position 1199 is well conserved among the RP1L1 family in other species. Four out of five computational assessment tools predicted that this mutation is damaging to the protein function. This mutation was not present in 294 control alleles. The waveform of focal macular ERGs recorded from the patient with OMD had a depolarizing pattern, simulating the ERG waveforms observed after the hyperpolarizing bipolar cell activity is blocked.
Conclusions: We have demonstrated in a Japanese patient the possibility that sporadic OMD may also be caused by an *RP1L1* mutation. The waveform of focal macular ERGs elicited from the OMD patient with the *RP1L1* mutation showed a depolarizing pattern. This characteristic is the same as reported for the focal macular ERGs of OMD.

Occult macular dystrophy (OMD; OMIM 613587) is an inherited macular dystrophy characterized by a progressive decrease in visual acuity with an essentially normal fundus and normal fluorescein angiograms [1,2]. The full-field electroretinograms (ERGs) are normal; however, the focal macular ERGs and multifocal ERGs (mfERGs) recorded from the macular area are abnormal [1-3]. Despite normal ophthalmoscopic findings, spectral domain-optical coherence tomography (SD-OCT) has shown morphological changes in the retina in the macular area [4-8]. Several studies have reported various degrees of disruption of the inner segment/ outer segment (IS/OS) junction and the cone outer segment tip (COST) line [4-8].

The hereditary form of OMD is an autosomal dominant trait; however, sporadic patients have also been reported [3, 9]. The gene responsible for the disease was recently identified as the RP1-like protein 1 (*RP1L1*) in four families

with autosomal dominant OMD [10]. The *RP1L1* gene has been identified through sequence analyses of human and mouse genomes [11,12]. The human *RP1L1* gene is encoded in four exons that span 50 kb on chromosome 8p. The length of the mRNA of *RP1L1* is more than 7 kb, but the exact length varies among individuals because of the presence of several length polymorphisms. *RP1L1* encodes a protein with a minimal length of 2,400 amino acids and a predicted weight of 252 kDa.

The expression of RP1L1 is limited to the retina, and appears to be specific to photoreceptors [12]. The *RP1L1* gene was also found to be conserved in distant vertebrates [11]. Knockout mice lacking the RP1L1 protein have reduced ERG amplitudes and progressive photoreceptor degeneration [13]. The study of *RP1L1*$^{-/-}$ mice also showed that the RP1L1 protein is located in the axoneme of the outer segments and connecting cilia exclusively in rod photoreceptors. The RP1L1 protein appears not to be expressed in cone photoreceptors in mice, although more than 97% of the photoreceptors in mice are rods [13]. However, immunohistochemical analysis of the RP1L1 of Cynomolgus monkeys with the human RP1L1 antibody showed that RP1L1

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was expressed in rod and cone photoreceptors [10]. Because the amino acid sequence of human RP1L1 is only 39% identical to that of the mouse, researchers have suggested that the primate RP1L1 might have different functional roles in the cone photoreceptors of the retina than that of other species [10].

We have identified a new mutation in the *RP1L1* gene in a patient with clinical characteristics of OMD: abnormal focal macular ERGs and blurring of the IS/OS junction and the disappearance of the COST line in SD-OCT images. The fundus examination, fluorescein angiograms, and full-field ERGs were normal in this case. The mutation is an amino acid substitution of cysteine for serine in exon 4 of the *RP1L1* gene that has not been reported in the Single Nucleotide Polymorphism (SNP) database and was also not detected in any of the 294 normal control alleles. The serine at position 1199 is well conserved among the RP1L1 family in other species. Four out of five computational assessment tools (PolyPhen-2, SIFT, PMut, Align GVGD, and MutationTaster) predicted that this mutation is damaging to the protein function. A segregation of the mutation and the disease was found in one affected member and one unaffected member of the same family.

METHODS

The protocol conformed to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Nippon Medical School and the ethics review committees of the National Hospital Organization Tokyo Medical Center. Written informed consent was obtained from all patients after the nature and possible consequences of the study were explained.

Clinical studies: The ophthalmological examinations included best-corrected visual acuity (BCVA) measurements, refraction, slit-lamp biomicroscopy, ophthalmoscopy, fundus photography, perimetry, SD-OCT, fluorescein angiography (FA), full-field ERGs, focal ERGs, and mfERGs. The visual fields were determined with the Goldman perimetry and the Humphrey Visual Field Analyzer (model 745i; Carl Zeiss Meditec, Inc., Dublin, CA). The Swedish interactive threshold algorithm standard strategy was used with program 30–2 of the Humphrey Visual Field Analyzer. The OCT images were recorded using a SD-OCT (Carl Zeiss Meditec) on this patient and normal controls. Full-field scotopic and photopic ERGs were recorded using an extended testing protocol incorporating the International Society for Clinical Electrophysiology of Vision standards [14]. The full-field ERGs were used to assess retinal function under scotopic and photopic states.

Focal macular electroretinograms: Focal macular ERGs were recorded with a commercial Focal Macular ERG system (ER80; Kowa Company, Tokyo, Japan, and PuREC; Mayo Company, Nagoya, Japan) using a bipolar contact lens electrode (MY type Electrode; Mayo Company). The stimulus and background lights were integrated into an infrared fundus camera [15-17]. The size of the stimulus spot was 15° in diameter and was placed on the macula by observing the infrared image of the retina on a monitor. The white stimulus and background illumination were generated by light-emitting diodes that had maximal spectral emissions at 440 to 460 nm and 550 to 580 nm, respectively. The luminances of the stimuli and background were 115.7 cd/m^2 and 8.0 cd/m^2. The duration of the stimulation was 100 ms. The responses were amplified and filtered with digital band pass filters from 5 to 200 Hz. Three hundred responses were summed with a stimulus frequency of 5 Hz. The a-wave, b-wave, d-wave, and oscillatory potentials (OPs) were evaluated.

Multifocal electroretinograms: The mfERGs were recorded using a commercial mfERG system (LE-4000, Tomey, Nagoya, Japan; LE4100; Mayo Company, Inazawa, Japan). This system uses basically the same technology as the Visual Evoked Response Imaging System [18]. The visual stimuli consisted of 37 hexagonal elements with an overall subtense of approximately 50°. The luminance of each hexagon was independently modulated between black (2.47 cd/m^2) and white (200.4 cd/m^2) according to a binary m-sequence at 75 Hz. The surround luminance was set at 75.4 cd/m^2.

Mutation analysis: Blood samples were collected from the patient, and genomic DNA was isolated from peripheral white blood cells using a blood DNA isolation kit (NucleoSpin Blood XL; Macherey Nagel, Düren, Germany). The DNA was used as the template to amplify the *RP1L1* gene. Coding regions and flanking introns of the *RP1L1* gene were amplified with polymerase chain reaction (PCR) using primers produced by Greiner Bio-One (Tokyo, Japan). Primer sequences are listed in Table 1. The PCR products were purified (ExoSAP-IT; USB Corp., Cleveland, OH) and were used as the template for sequencing. Both strands were sequenced on an automated sequencer (Bio Matrix Research; Chiba, Japan). The identified mutations and coding polymorphisms were assayed in 294 control chromosomes from 147 healthy Japanese individuals with direct sequencing except the length polymorphism region. To sequence the length polymorphism region of the *RP1L1* gene, the amplified PCR products were subcloned into the StrataClone PCR cloning vector (Stratagene; La Jolla, CA). At least five cloned products from this case and 20 control individuals were sequenced on an automated sequencer.

Computational assessment of missense mutation: The effect of a missense mutation on the encoded protein was predicted with the PolyPhen-2, SIFT, PMut, Align GVGD, and MutationTaster online tools [19-24]. PolyPhen-2 is a software tool that predicts the possible impact of amino acid substitutions on the structure and function of human proteins using straightforward physical and evolutionary comparative

TABLE 1. SEQUENCES OF OLIGONUCLEOTIDE PRIMERS USED IN THIS STUDY AND PCR PRODUCT SIZE.

| Fragment name	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)
RP1L1-2A	GAGACAGGAAATGCCAATCC	CCGCAACTGCTGAGCAGTGG	471
RP1L1-2B	CCTCTGCTCTGATAAGAAGC	TCCATGTGAGTATTTTGACC	373
RP1L1-3	CCTCCAGCTAGTGATAGAGG	GATTGACAGTACTGAGAAGG	498
RP1L1-4A	TTCCTTTATCCTGATGCTGC	CCAAAGACTTCCCTGCATCC	509
RP1L1-4B	TGTGGGAGGGCTACCCTTGG	GCTGACGAGTCCGAAGAAGC	508
RP1L1-4C	CTATGCATAGATGGAGCAGG	GTTACAGAGGAGTCCAGTGG	536
RP1L1-4D	CAATGTCCTCACCCAGCAGC	TCCAACCTGCAGAACCAAGG	494
RP1L1-4E	GACTCCTGCTCAAAATCTGG	GGACACCCTCTCCTGATTGG	784
RP1L1-4F	GGACAGCAGTCCCTGGAAGG	ACTGCACCGCCTCTTCTTGC	937
RP1L1-4G	AAACACAGTGCAAGAAGAGG	AGGCTCAAGCTGGGAGCCACTCTGC	variable
RP1L1-4H	GGGAAAGGCTCCCAGGAAGATGACC	TTCTGCACCTTCTGACTCTGGCTGG	1470
RP1L1-4I	CACAGAGGAACCCACAGAGC	GAGAAGGCCGAGAGGTTTCG	522
RP1L1-4J	CAAGAGAGAGCTCCAGAAGC	TCTGTTGAGTCTCTGGCTCC	547
RP1L1-4K	GACAAAGATCCCAAACTCGG	AGAGTCAGAAGATGTAGAGG	836
RP1L1-4L	TGAAGGGGAGATGCAAGAGG	GAGTGGGCCTGTCCTCAGGGACTGG	821
RP1L1-4M	AGGCTTCTGAAAGCAGCAGC	ACTATGGACATCTCCAGTGG	517
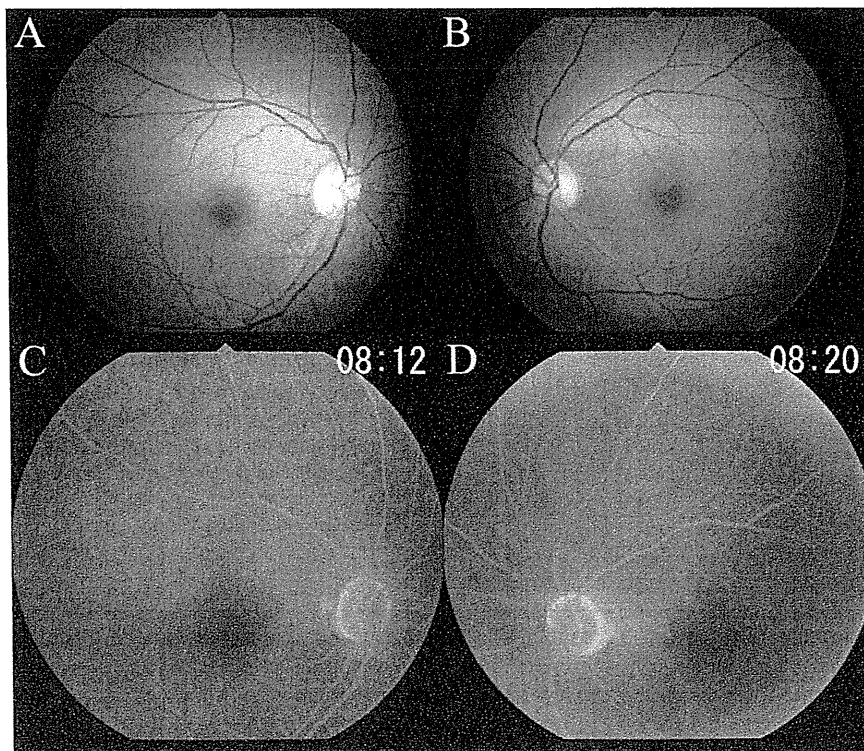
Figure 1. Fundus photographs (**A**, **B**) and fluorescein angiograms (**C**, **D**) of this case showing no abnormal findings.

considerations. SIFT generates multiple alignments of the sequence over different species to look at the conserved sequences of a gene; it assesses the conserved amino acid positions and analyzes the effect of missense changes on the conserved structure of proteins over the course of evolution. The SIFT tool assigns a score to the mutations, and a score of <0.05 is considered potentially damaging. PMut is software aimed at annotating and predicting pathological mutations. Align GVGD combines the biophysical characteristics of amino acids and protein multiple sequence alignments to predict where missense substitutions in genes of interest fall in a spectrum from enriched deleterious to enriched neutral. MutationTaster evaluates the disease-causing potential of sequence alterations.

Statistical analysis: We calculated the 95% confidence intervals (CI) of the results of the focal macular ERGs of normal controls. There were 25 men and 21 women whose age ranged from 23 to 60 years (mean, 38.04±8.33 years) in
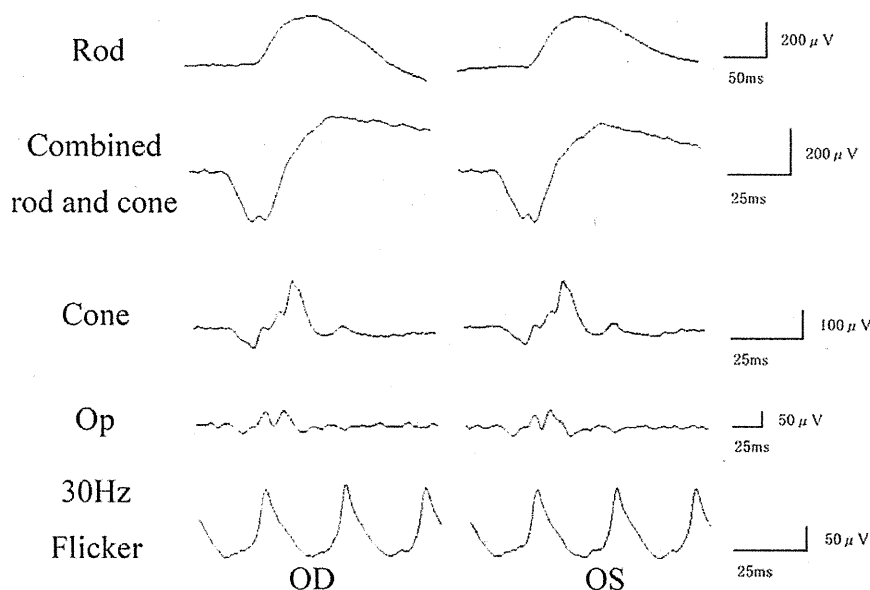
Figure 2. Full-field electroretinograms (ERGs) recorded according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards protocol in this case. The rod, combined rod-cone, cone, oscillatory potentials, and 30-Hz flicker full-field ERGs are shown. The results of full-field ERGs are within the normal limits in this case.
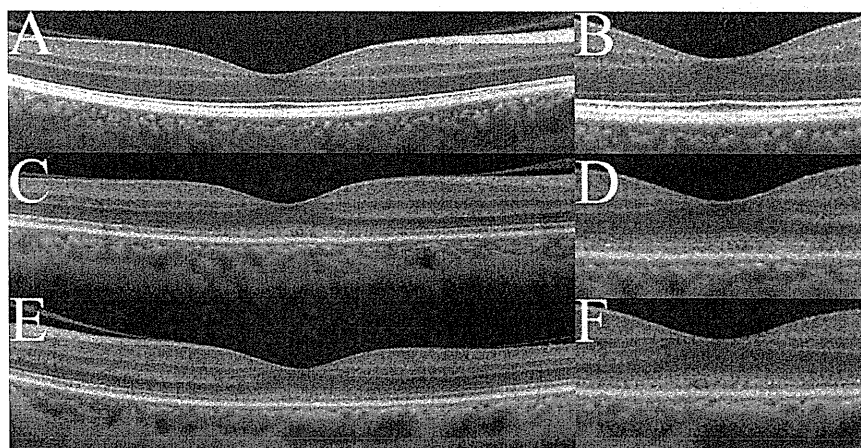

Figure 3. Spectral-domain optical coherence tomography (SD-OCT) findings of the eyes in normal controls (**A, B**) and in this case (**C-F**). Images from right eyes (**C, D**) and left eyes (**E, F**) are shown. Images at lower magnification (**A, C, E**) and higher magnification (**B, D, F**) are shown. The SD-OCT findings for the eyes in this case show obvious blurring of the IS/OS junction and the COST line. The COST line disappeared in the peripheral macula area in this case.

this control group. We recorded focal macular ERGs from either of the eyes of normal controls and calculated the 95% CI of the amplitudes of the a-waves and the b-waves, the implicit time of the a-waves and b-waves, the potentials at 70 ms after the stimulus was turned on, and the time of the recovery of the b-wave to the baseline.

RESULTS

Case report: A 52-year-old woman complained of a gradual decrease in vision in both eyes during the past two to three years. Family history revealed no other members with any eye diseases, including her parents who were deceased. Her BCVAs were 20/63 in the right eye and 20/50 in the left eye. The fundus examination, fluorescein angiography, and full-field ERG results were within the normal limits (Figure 1A-

D and Figure 2). The visual fields were full with the Goldman perimetry, but a relative central scotoma was detected in both eyes with the Humphrey Visual Field Analyzer.

Spectral domain optical coherence tomography: The SD-OCT images of this case showed a blurred IS/OS junction and COST line at the foveal center (Figure 3D,F). In the peripheral macula area, the COST line was absent, and only the blurred IS/OS junction was visible in this case (Figure 3C,E).

Focal macular electroretinograms and multifocal electroretinograms: A severe reduction in the a-waves of the focal macular ERGs was found in this case (Figure 4). Although the b-waves were large, their shapes were abnormal. The b-waves rose to a peak, and the potential was maintained longer than normal. The plateau region of the b-wave was significantly elevated above the baseline potential (Figure 4,
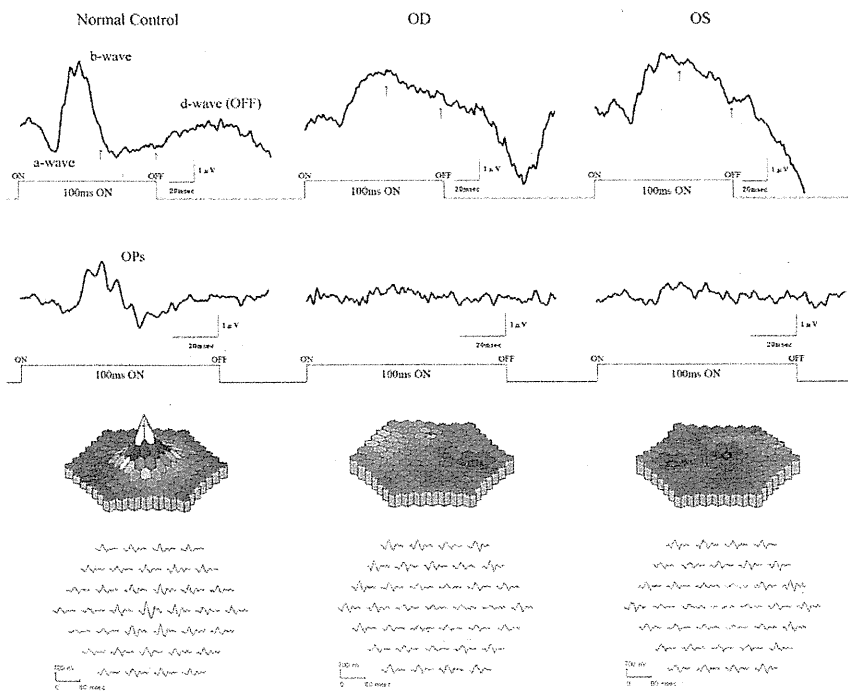
Figure 4. Results of focal macular electroretinograms (ERGs) and multifocal ERGs. Focal macular ERGs and oscillatory potentials recorded from a normal subject and this case are shown (top). The amplitude of the a-wave of this case was severely reduced, and the plateau region was significantly elevated (arrows). The topographic map and the local responses of multifocal ERGs recorded from the normal subject and this case are shown (bottom). The amplitudes in the foveal area were severely reduced in this case.

arrow). To analyze this characteristic, we quantified the potentials at 70 ms after the stimulus was turned on, and the recovery time of the descending slope of b-wave to the baseline from the peak of the b-wave. We calculated the 95% confidence intervals (CI) for the amplitudes of the a-waves and b-waves, the implicit times of the a-waves and b-waves, the potentials at 70 ms after the stimulus turns on, and the time of the recovery of the b-waves to the baseline obtained from the normal controls (Figure 5). Among these six parameters, the amplitudes of the a-waves, the implicit times of the b-waves, the potentials at 70 ms after the stimulus was turned on, and the time of the recovery of the descending slope of the b-wave to the baseline obtained from both eyes of this case were outside the range of the standard deviation and the 95% CI of the normal controls (Figure 5). Especially, the amplitudes of the a-waves, the potentials at 70 ms after the stimulus was turned on, and the time of the recovery of the descending slope of the b-wave to the baseline obtained from this case were severely affected. The amplitudes of the mfERGs in the foveal area were severely reduced in this case (Figure 4).

Molecular genetic findings: Mutation analysis of the *RP1L1* gene in this case showed three missense mutations. There was a c.2578 C>T in exon 4 with a substitution of tryptophan (TGG) for arginine (CGG) at amino acid position 860, a c. 3596 C>G in exon 4 with a substitution of cysteine (TGT) for serine (TCT) at amino acid position 1199, and a c. 4484 C>G

in exon 4 with a substitution of arginine (CGC) for proline (CCC) at amino acid position 1495. The amino acid substitution at position 860 and 1495 has already been reported in the SNP database and is found in a high percentage of the normal population. A mutation at amino acid position 1199 has not been reported in the SNP database or in earlier reports (Figure 6A). The serine at position 1199 is well conserved among the RP1L1 family in other species (Figure 6B). This mutation was predicted to be probably damaging with a score of 0.999 by PolyPhen-2. The SIFT tool analysis revealed a score of 0 and predicted that the replaced amino acid is potentially damaging and would not be tolerated. PMut predicted that this mutation is pathological. Align GVGD predicted this mutation as class C65, which means it most likely interferes with the protein function. Out of five computational assessments, only MutationTaster predicted this mutation as a polymorphism. We confirmed that the mutation in this case was segregated with the disease in one affected member and one unaffected member of the family (Figure 6C). The unaffected member of the family in Case 1 underwent clinical examination, including BCVAs, slit-lamp biomicroscopy, fundus ophthalmoscopy, OCT, and focal ERGs. All examination findings were normal. This mutation was not present in 300 control alleles. This mutation p.S1199C has been registered in GenBank with accession number AB684329.
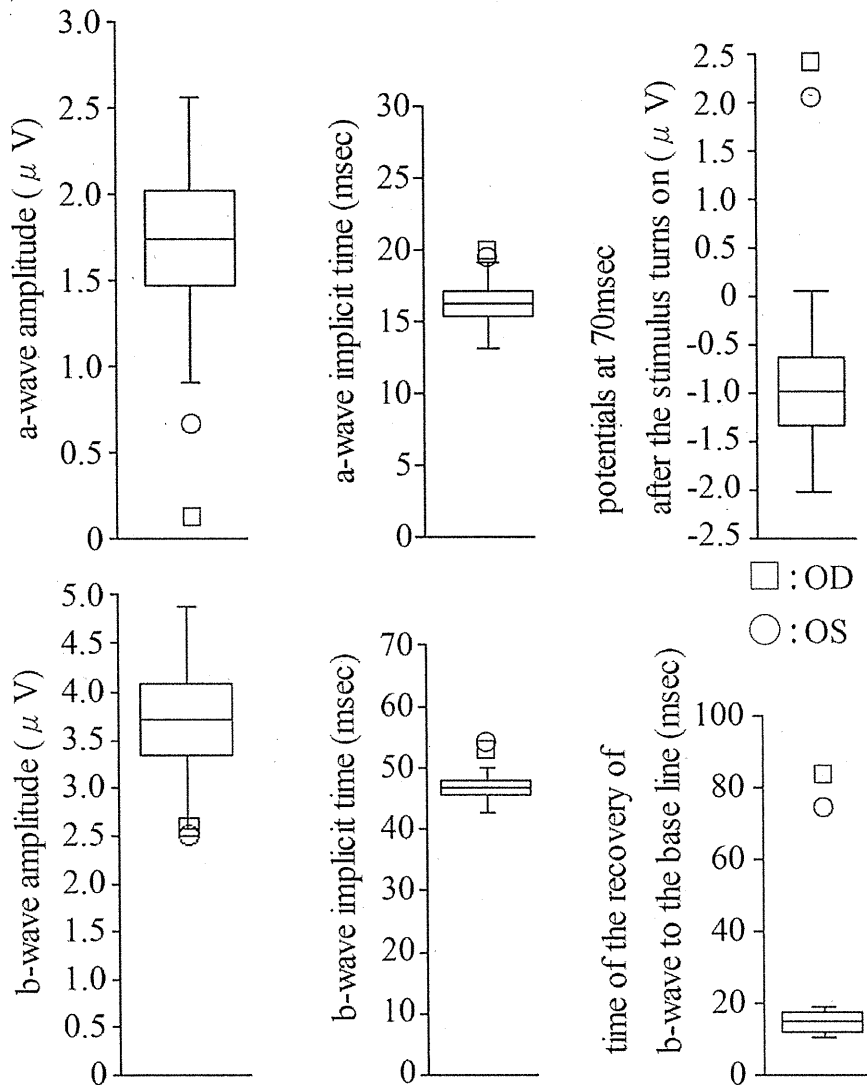
Figure 5. Plot of the amplitudes of the a-waves, b-waves, and the implicit time of the a-waves, b-waves, the potentials at 70ms after the stimulus turns on, and the time of the recovery of b-wave to the baseline for normal controls. There were 25 men and 21 women whose age ranged from 23 to 60 years (mean, 38.04±8.33 years) in this control group. The boxes represent the 95% confidence interval ranges, the horizontal line represents mean values, and the bars represent standard deviation. Data recorded from this case are plotted at indicated mark.

Bowne et al. [11] reported that *RP1L1* mRNA is variable due to the presence of a 48 bp polymorphic coding repeat. They reported that as many as six 48 bp repeats have been observed in normal controls. In this case, one allele contains a 48 bp repeat, and the other allele contains three 48 bp repeats (Figure 6D). There are variations of only two amino acids in the length polymorphism region from this case compared to the reference sequence (NP_849188). One variation with the substitution of E to G in the 14th amino acid of the length polymorphism region was in a previous report [12] (AAN86962, AAN86963, and AAN86964). The other variation with the substitution of G to V in the ninth amino acid of the length polymorphism region was found in more than 10 normal control alleles from a Japanese population. These variations of the length polymorphisms of *RP1L1* with

one and three repeats have been registered in GenBank with accession numbers AB684331 and AB684332, respectively.

DISCUSSION

The mutation found in the *RP1L1* gene in this case was a missense mutation with cysteine substituted for serine at amino acid position 1199. This residue is well conserved among the RP1L1 family in other species, suggesting the importance of this amino acid residue for RP1L1 function. Four out of five computational analysis tools predicted this mutation is damaging to the protein function. We did not find this mutation in the sister of the patient with normal vision, although she was the only other family member we were able to test. To decide whether this mutation was pathogenic, we need to examine more family members and a larger number
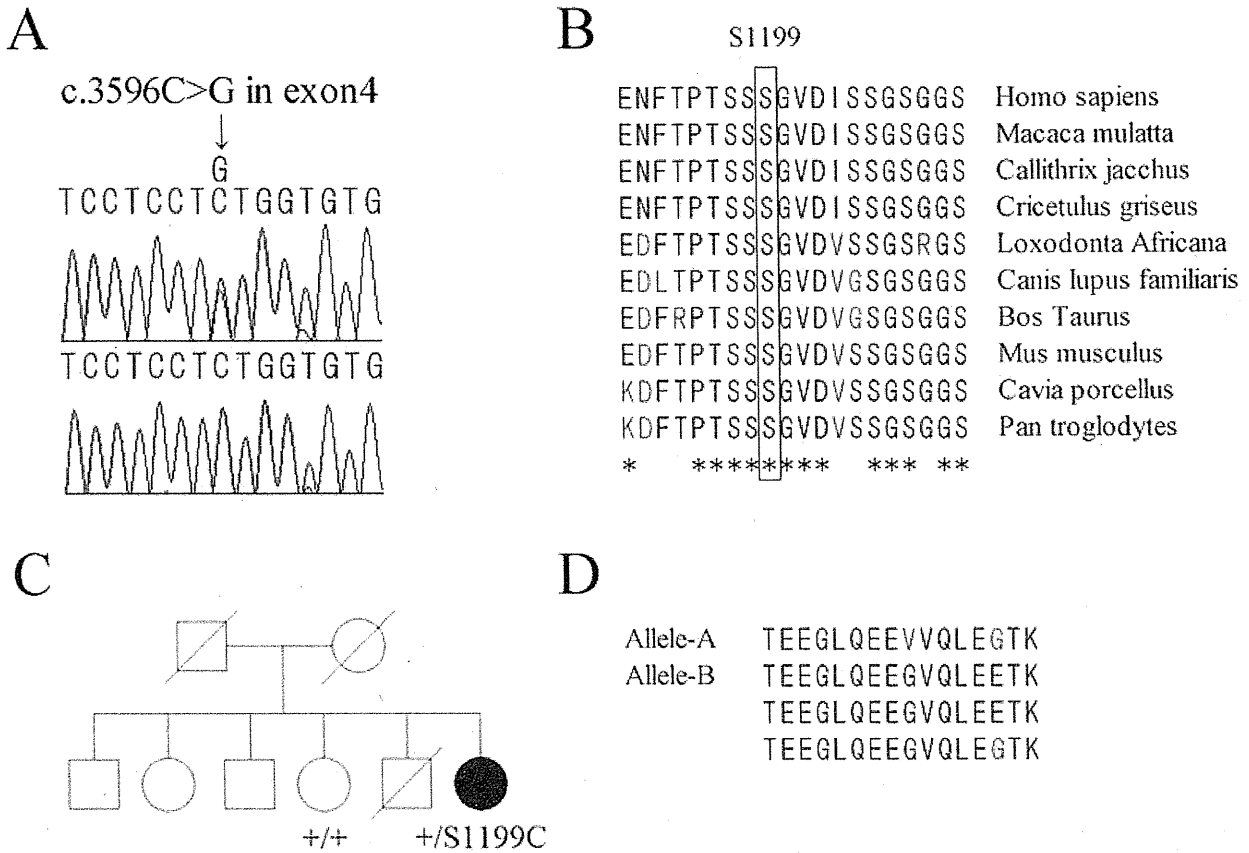
A

c.3596C>G in exon4

↓

G

TCCTCCTCTGGTGTG

TCCTCCTCTGGTGTG

B   S1199

Sequence	Species
ENFTPTSSSGVDISSGSGGS	Homo sapiens
ENFTPTSSSGVDISSGSGGS	Macaca mulatta
ENFTPTSSSGVDISSGSGGS	Callithrix jacchus
ENFTPTSSSGVDISSGSGGS	Cricetulus griseus
EDFTPTSSSGVDVSSGSRGS	Loxodonta Africana
EDLTPTSSSGVDVGSGSGGS	Canis lupus familiaris
EDFRPTSSSGVDVGSGSGGS	Bos Taurus
EDFTPTSSSGVDVSSGSGGS	Mus musculus
KDFTPTSSSGVDVSSGSGGS	Cavia porcellus
KDFTPTSSSGVDVSSGSGGS	Pan troglodytes

*   *******   *** **

C

+/+   +/S1199C

D

Allele-A	TEEGLQEEVVQLEGTK
Allele-B	TEEGLQEEGVQLEETK
	TEEGLQEEGVQLEETK
	TEEGLQEEGVQLEGTK

Figure 6. DNA analysis for c.3596C>G mutation and deduced amino acids of length polymorphism region of the RP1-like protein 1 (*RP1L1*) gene and the pedigree of the family with *RP1L1* gene mutation. **A**: Sequence chromatograms for this case (top) and the normal control (bottom) are shown. This case had a c.3596 C>G mutation in exon 4. **B**: Alignment of S1199 in the RP1L1 family proteins. Amino acid-sequence alignments of RP1L1 from 10 species reported in the NCBI database are shown. Amino acid residues of S1199 in humans and conserved residues from other species are boxed. The asterisks indicate completely conserved residues. S1199 is well conserved in all species reported. **C**: We confirmed that the mutation in Case 1 was segregated with the disease in one affected member and one unaffected member of the family. **D**: Deduced amino acids (AA) of repeated regions of the RP1L1 length polymorphism. In this case, one allele contains a 16 AA, and the other allele contains three 16 AA repeats. Variations of amino acids from reference sequence of RP1L1 are shown in red. Those variations are within normal limits.

of normal controls. However, the phenotype of this case was typical of OMD, and thus the mutation in this case was most likely pathogenic.

The photoreceptor IS/OS junction and the COST line can be detected in the SD-OCT images of normal eyes [25-28]. Recently, several degrees of disruption of the IS/OS junction and/or COST line in the SD-OCT images of patients with OMD have been reported [4-8]. In our case, the IS/OS junction and the COST line appeared blurred in the SD-OCT images similar to previous reports.

Researchers have emphasized that the key to differentiating OMD from other diseases, such as optic neuritis or psychological disorders, is the recording of focal macular ERGs from the central retina [1-3]. Focal macular ERGs have a unique waveform when elicited by long-duration stimuli [29]. As shown in this patient, the waveform of focal macular ERGs recorded from patients with OMD with long-duration stimuli had a depolarizing pattern, simulating the ERG waveforms observed after the hyperpolarizing bipolar cell activity is blocked [30-33]. Researchers have demonstrated that by blocking hyperpolarizing bipolar cells with cis-2,3-piperidine dicarboxylic acid or kynurenic acid in monkeys, the a- and d-waves of photopic ERGs become smaller and the plateau between the b- and d-waves remains elevated above the baseline potential [34]. Full-field cone ERG in some human retinal dystrophies show a similar depolarizing pattern [29,35]. Kondo et al. [29] reported similar focal macular ERGs elicited with 100 ms stimuli from a patient with glittering crystalline deposits in the posterior fundus. The waveform of the focal macular ERGs of this case

was similar to those reported for patients with OMD [31-33]. Because this case had a putative disease-causing mutation of the *RP1L1* gene, we suggest the reduced amplitude of the a-wave and the persistent plateau between the b- and d-waves of the focal macular ERGs elicited with long-duration stimuli might be specific markers that could help diagnose OMD.

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CLINICAL CHARACTERISTICS OF OCCULT MACULAR DYSTROPHY IN FAMILY WITH MUTATION OF *RP1L1* GENE

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Purpose: To report the clinical characteristics of occult macular dystrophy (OMD) in members of one family with a mutation of the *RP1L1* gene.

Methods: Fourteen members with a p.Arg45Trp mutation in the *RP1L1* gene were examined. The visual acuity, visual fields, fundus photographs, fluorescein angiograms, full-field electroretinograms, multifocal electroretinograms, and optical coherence tomographic images were examined. The clinical symptoms and signs and course of the disease were documented.

Results: All the members with the *RP1L1* mutation except one woman had ocular symptoms and signs of OMD. The fundus was normal in all the patients during the entire follow-up period except in one patient with diabetic retinopathy. Optical coherence tomography detected the early morphologic abnormalities both in the photoreceptor inner/outer segment line and cone outer segment tip line. However, the multifocal electroretinograms were more reliable in detecting minimal macular dysfunction at an early stage of OMD.

Conclusion: The abnormalities in the multifocal electroretinograms and optical coherence tomography observed in the OMD patients of different durations strongly support the contribution of *RP1L1* mutation to the presence of this disease.

RETINA 32:1135–1147, 2012

Occult macular dystrophy (OMD) was first described by Miyake et al[1] to be a hereditary macular dystrophy without visible fundus abnormalities. Patients with OMD are characterized by a progressive decrease of visual acuity with normal-appearing fundus and normal fluorescein angiograms (FA). The important signs of OMD are normal full-field electroretinograms (ERGs) but abnormal focal macular ERGs and mul-

tifocal electroretinograms (mfERGs) also exist. These findings indicated that the retinal dysfunction was confined to the macula.[1–5] Optical coherence tomography (OCT) showed structural changes in the outer nuclear and photoreceptor layers.[6–11]

Recently, we found that dominant mutations in the *RP1L1* gene were responsible for OMD.[12] The *RP1L1* gene was originally cloned as a gene derived from common ancestors as a retinitis pigmentosa 1 (*RP1*) gene, which is responsible for 5–10% of autosomal dominant retinitis pigmentosa worldwide, on the same Chromosome 8.[13–17] A number of attempts have been made to identify mutations in *RP1L1* in various retinitis pigmentosa patients with no success. An immunohistochemical study on cynomolgus monkeys showed that *RP1L1* was expressed in rod and cone photoreceptors, and *RP1L1* is thought to play important roles in the morphogenesis of the photoreceptors.[13,18] Heterozygous *RP1L1* knockout mice were reported to be normal, whereas homozygous knockout mice develop subtle retinal degeneration.[18] However, the RP1L1 protein has a very low degree of overall sequence

identity (39%) between humans and mice compared with the average values of sequence similarity observed between humans and mice proteins. The results of linkage studies have strongly supported the contribution of *RP1L1* mutations to the presence of this disease,[12] but the function of *RP1L1* in the human retina has not been completely determined.

A large number of cases of OMD have been reported[7,10,19]; however, we did not always find the same mutations in sporadic cases or in small families, which had less than three affected members. This led us to hypothesize that several independent mutations can lead to the phenotype of OMD, that is, OMD is not a single disease caused by a specific gene mutation, but may represent different diseases with similar retinal dysfunctions.

Thus, the aim of this study was to determine the characteristics of OMD by investigating the phenotypes of patients with the *RP1L1* mutation from a single Japanese family.

Patients and Methods

We investigated 19 members from a single Japanese family. A homozygous mutation, p.Arg45Trp in the *RP1L1* gene, was confirmed in 14 members,[12] and 13 of the 14 were diagnosed with OMD. Among the 14 members with a mutation in the *RP1L1* gene, 11 were followed-up at the Niigata University in Niigata, Japan. The other three were examined at the National Institute of Sensory Organs in Tokyo, Japan. Each member had a complete ophthalmic examination including best-corrected visual acuity (BCVA), refraction, perimetry, fundus photography, FA, full-field ERGs,[20] mfERGs,[21] and OCT. The visual fields were determined by Goldmann perimetry or by Humphrey Visual Field Analyzer (Model 750i; Carl Zeiss Meditec, Inc, Dublin, CA). The SITA Standard strategy was used with the 30-2 program or the 10-2 program for the Humphrey Visual Field Analyzer.

Electroretinograms were used to assess the retinal function under both scotopic and photopic conditions.[22] Full-field ERGs were recorded using the International Society of Clinical Electrophysiology and Vision standard protocol. Multifactorial electroretinograms were recorded with the Visual Evoked Response Imaging System (VERIS science 4.1; EDI, San Mateo, CA). A Burian–Allen bipolar contact lens electrode was used to record the mfERGs. The visual stimuli consisted of 61 or 103 hexagonal elements with an overall subtense of approximately 60°. The luminance of each hexagon was independently modulated between black (3.5 cd/m^2) and white (138.0 cd/m^2) according to

a binary m-sequence at 75 Hz. The surround luminance was 70.8 cd/m^2.

The OCT images were obtained with a spectral-domain OCT (HD-OCT; Carl Zeiss Meditec or a 3D-OCT-1000, Mark II; Topcon) from 21 eyes of 12 cases in the same pedigree.

The procedures used adhered to the tenets of the Declaration of Helsinki and were approved by the Medical Ethics Committee of both the Niigata University and National Institute of Sensory Organs. An informed consent was received from all the subjects for the tests.

Results

The findings of 5 generations of 1 family with OMD are shown in Figure 1. The numbered family members had the same mutation in *RP1L1* (p.Arg45Trp), and family members designated with the filled squares or filled circles were phenotypically diagnosed with OMD by routine examinations including visual field tests, FA, mfERGs, and Fourier-domain OCT. Only Patient 5 (age 60 years) had normal phenotype, although she had the *RP1L1* mutation.

The clinical characteristics and the results of ocular examinations of all the 14 family members with the *RP1L1* mutation (p.Arg45Trp) are listed in Tables 1 and 2. Family Member #5 was diagnosed as normal because she had normal mfERGs.

Among the 13 OMD patients (average age at the final examination, 57.2 ± 22.1 years), 12 complained of disturbances of central vision and 4 complained of photophobia (Table 1). Patient 1 did not report any visual disturbances in the right eye as did Patient 6 for both eyes. The visual dysfunction in these eyes was confirmed by mfERGs. For 13 patients, the age at the onset of visual difficulties varied from 6 years to 50 years with a mean of 27.3 ± 15.1 years.

All the patients were affected in both eyes, and the onset was the same in the 2 eyes except for Patients 1, 11, 12, and 14. Patient 1 first noticed a decrease in her visual acuity in her left eye at age 50 years, and she still did not have any subjective visual disturbances in her right eye 30 years later. However, a clear decrease in the mfERGs in the macular area was detected in both eyes. Patient 11 first noticed a decrease in the visual acuity in her right eye at age 47 years when the BCVA was 0.2 in the right eye and 1.2 in the left eye (Figure 2). Seven years later at age 54 years, she noticed a decrease in the vision in her left eye. Similarly, Patients 12 and 14 did not report any visual disturbances in their right eyes until 2 (Patient 12) or 8 (Patient 14) years after the onset in their left eyes.
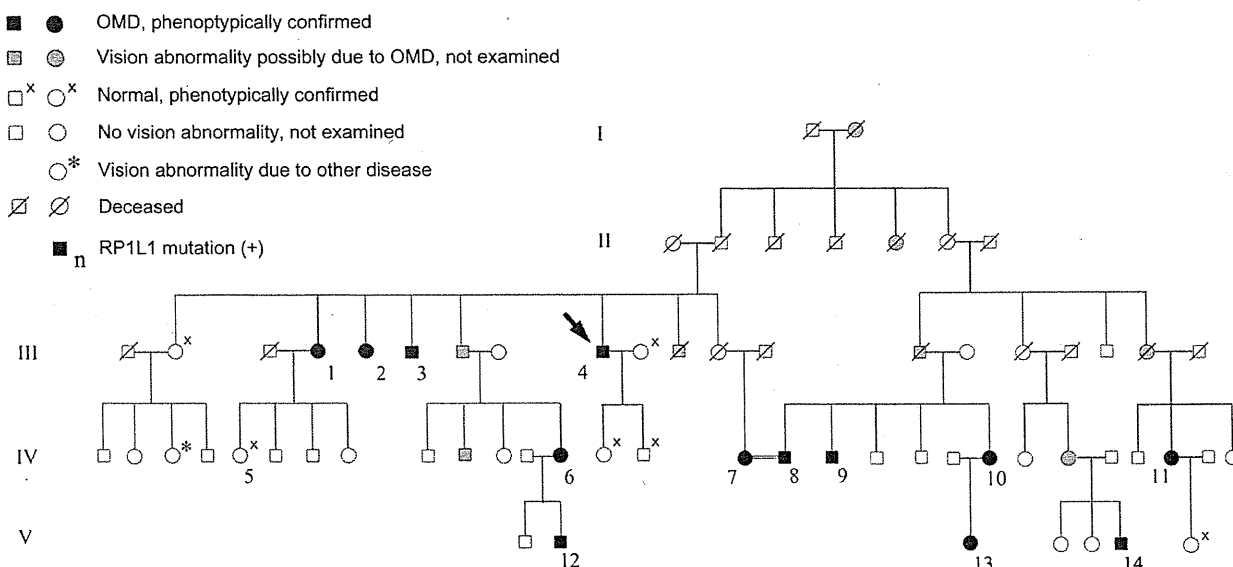
Fig. 1. Pedigree of a family with OMD. The identification number of the patients is marked beside the symbols. The proband is indicated by an arrow. The open squares and circles with crosses are the relatives whose visual function was confirmed to be normal by routine examinations including Humphrey visual field tests, mfERGs, and Fourier-domain OCT. Those designated by hatched squares or circles were reported to have poor vision with similar severity and onset as the other genetically confirmed OMD patients. One relative marked by an asterisk had unilateral optic atrophy because of retrobulbar neuritis.

The duration of the continuous decrease in the BCVA varied from 10 years to 30 years (mean, 15.6 ± 7.7 years) in 16 eyes of 9 adult patients. After this period, these patients reported that their vision did not decrease. Patients 2, 3, 8, and 14 complained of photophobia, and the degree of photophobia remained unchanged after the visual acuity stopped decreasing. Patients 1, 2, 4, 7, and 9 had additional disturbances of vision because of senile cataracts, and Patients 2 and 4 had bilateral cataract surgery. The visual disturbances because of the OMD were still progressing at the last examination in the left eye of Patient 11 (age 57 years), and both eyes of Patient 12 (age 20 years), Patient 13 (age 18 years), and Patient 14 (age 28 years).

Different systemic disorders were found in some of the patients; however, there did not seem to be a specific disorder, which was common to all of them (Table 1).

In the 16 eyes of 9 patients whose BCVA had stopped decreasing, the BCVA varied from 0.07 to 0.5 (Table 2). The BCVA of the left eye of Patient 6 was 0.07 because of an untreated senile cataract. If this eye is excluded, the final BCVAs of all the stationary eyes range from 0.1 to 0.5. Patient 2 had photophobia, and her BCVA measured by manually presenting Landolt rings on separate cards under room light was 0.4 in the right eye and 0.5 in the left eye, which was better than that measured by a Landolt chart of 0.3 in the right eye and 0.3 in the left eye with background illumination.

For the 13 patients whose original refractions were confirmed, 11 of 26 eyes were essentially emmetropic

($<\pm$ 0.5 diopters). Both eyes of Patients 1, 3, 4, 6, and 8 and the left eye of Patient 5 were hyperopic (+0.675 to +4.625 diopters). The right eye of Patient 7, the left eye of Patient 12, and both eyes of Patient 13 were moderately myopic (−0.625 to −2.75 diopters). These results indicate that there is no specific refraction associated with OMD patients in this family.

The visual fields were determined by Goldmann perimetry or Humphrey Visual Field Analyzer. All the patients had a relative central scotoma in both eyes except for Patient 1 whose right eye was normal by Goldmann perimetry. In all cases, no other visual field abnormalities were detected during the entire course of the disease. In the patients examined shortly after the onset, a relative central scotoma was not detected by Goldman perimetry and was confirmed by static perimetry.

The fundus of all except one eye was normal. The left eye of Patient 9 had background diabetic retinopathy. At the first consultation at age 46 years, Patient 9 did not have diabetes, and the funduscopic examination and FA revealed no macular abnormalities. At the age 66 years, there were few microaneurysms in the left macula away from the fovea; however, OCT did not show any diabetic changes such as macular edema. The OMD was still the main cause of visual acuity reduction in this patient.

Six patients consented to FA, and no abnormality was detected in the entire posterior pole of the eye. It is noteworthy that both the fundus and FA of Patient 4 were normal at the age 73 years, which was >50 years