with the discovery of an association between polymorphic variation in the complement factor H gene (*CFH*) and AMD. Following this, other loci at 10q26, *ARMS2/HTRA1* (8–12), were implicated, in addition to several genes involved in the complement pathway. The discovery of *CFH* variants and the alternative complement pathway in the pathophysiology of AMD subsequently led to the investigation of other complement factors, such as complement component 3 (*C3*) (13–30), complement component 2 (*C2*), and complement factor B (*CFB*) (13, 31–37). We previously performed a systematic review of *C3* (38). The current review focuses on the *C2* and *CFB* variants.

The *C2* gene, located on 6p21.33, encodes a serum glycoprotein that functions as part of the classical complement pathway, which is involved in innate immunity and inflammation (Online Mendelian Inheritance in Man (OMIM) number 613927). Two polymorphisms (rs9332739 G > C and rs547154 G > T) have been implicated in AMD. The *C2* polymorphisms may be associated directly with AMD or indirectly through the high level of linkage disequilibrium (LD) that exists between *C2* and *CFB*, which is located downstream on the same chromosome (OMIM number 138470) and which contains additional variants that are also highly associated with AMD (33, 36), rs4151667 T > A and rs641153 G > A. Therefore, we conducted a systematic review to pool the results of all available population-based association studies on *C2* (rs547154 and rs9332739), *CFB* (rs4151667 and rs641153), and AMD, with the following objectives:

To estimate the prevalence of the minor alleles of *C2* and *CFB*.

To ascertain whether there are genetic associations with AMD susceptibility and, if so, to estimate the magnitude of those associations and the possible genetic modes of action.

MATERIALS AND METHODS

Search strategy

Studies were identified from the MEDLINE (US National Library of Medicine), EMBASE (Excerpta Medica Database; Elsevier B.V., Amsterdam, the Netherlands), and Scopus (SciVerse Scopus; Elsevier B.V.) databases using the PubMed, Ovid, and Scopus search engines up to June 18, 2011, by 1 reviewer (A. T.). Search strategies used for PubMed were as follows: (gene *or* allele *or* polymorphism) *and* (macular degeneration) *and* ("complement component 2" *or* "C2" *or* "complement factor 2") *or* ("CFB" *or* "complement factor B"). Where there were multiple publications with the same subjects, the most complete and recent results were used. The reference lists of the selected articles were also reviewed to identify additional relevant publications. Details of other search strategies are described in the Appendix.

Inclusion criteria

Two reviewers (A. T. and M. M.) independently went through all titles and abstracts of the identified studies. Any human population-based association study, regardless of sample size, was included if it met the following criteria:

Genotyped *C2* (rs547154 G > T and rs9332739 G > C) or *CFB* (rs4151667 T > A and rs641153 G > A) polymorphisms.

The outcome was AMD, and there was at least 1 comparison/control group.

There was sufficient description of the results—that is, numbers of subjects in genotype and outcome groups. Where eligible, the authors of articles with insufficient information were contacted, with a request for additional information. If they did not provide data after 2 contacts, those studies were excluded from our review.

Data extraction

Summary data for *C2* and *CFB* were extracted independently by 2 reviewers (A. T. and M. M.) using a standardized data extraction form. Data on covariables such as mean age, percentage of males, percentage of smokers, and ethnicity were also extracted. Any disagreement was resolved by consensus.

Risk of bias assessment

The quality of studies was independently assessed by 2 reviewers (A. T. and M. M.) using a risk of bias assessment for genetic association studies, described in detail previously (38). Briefly, the assessment considered 5 domains: selection bias, information bias, confounding bias, multiple tests and selective reports, and assessment of Hardy-Weinberg equilibrium (HWE). Each item was classified with regard to risk of bias ("yes/no") or as unclear if there was insufficient information to assess risk of bias ("unclear").

Statistical analysis

Data in the control group of each study were used to assess HWE using an exact test. Genetic effects were stratified by ethnicity (Caucasian or Asian) and analyzed using 2 approaches, as described below (38, 39).

Per-allele approach. Suppose that g and G are minor and major alleles, respectively, and gg, Gg, and GG are minor homozygous, heterozygous, and common homozygous genotypes, respectively, for each polymorphism. A minor g allele frequency was estimated for each study, and data were then pooled using meta-analysis for pooling prevalence (40). Odds ratios for g alleles versus G alleles, along with 95% confidence intervals, were estimated. Heterogeneity of odds ratios across studies was assessed using a Q test, and the degree of heterogeneity was quantified using I^2. If heterogeneity was present (i.e., if the Q test was significant or I^2 was greater than 25%), the cause of heterogeneity was explored by fitting a covariable (e.g., age, percent male, or percent smokers) in a meta-regression model, when the data for these covariables were available (41–44).

Per-genotype approach. Two odds ratios (gg vs. GG, denoted odds ratio 1 (OR_1), and Gg vs. GG, denoted odds

ratio 2 (OR_2)) were estimated for each study. Heterogeneity of odds ratios was assessed using the method mentioned previously. If there was heterogeneity in at least 1 of these odds ratios, the cause of heterogeneity was explored using meta-regression analysis. A mixed-effects hierarchical model with a logit link function (40) was applied to determine the overall gene effect using the xtmelogit command in STATA (StataCorp LP, College Station, Texas). The genotypes were considered in the model as fixed effects, whereas the study was considered a random effect. A likelihood ratio test was used to assess whether an overall gene effect was significant. Pooled odds ratios and 95% confidence intervals were then estimated from the mixed model.

The mode of genetic effect, measured by the parameter lambda (λ), which is defined as the ratio of log OR_2 to log OR_1, was then estimated using the model-free Bayesian approach (45). The value of lambda ranges from 0 to 1. If $\lambda = 0$, a recessive model is suggested; if $\lambda = 1$, a dominant model is suggested; and if $\lambda = 0.5$, a codominant model is suggested. If $\lambda > 1$ or $\lambda < 0$, then a homozygous or heterosis model is likely, although this is rare.

Sensitivity analyses were performed by including and excluding studies not in HWE. Publication bias (study-size effect) was assessed using the Egger test and contour-enhanced funnel plots (46–48). Trim-and-fill meta-analysis was applied to impute unidentified studies (49). The population attributable risk (PAR) for genotypes was calculated as in the papers by Hayden et al. (50) and Rossman et al. (51). Analyses were performed using STATA, version 11.1 (52), and WinBUGS 1.4.2 (53), with normal vague prior distributions for estimation of parameters (i.e., lambda and the odds ratio). The analyses were run with a burn-in of 1,000 iterations, followed by 10,000 iterations for parameter estimates. A P value less than 0.05 was considered statistically significant, except for tests of heterogeneity, where a level of 0.10 was used.

RESULTS

Identifying studies

A total of 59, 87, and 319 studies were located from MEDLINE, EMBASE, and Scopus (Figure 1), respectively. After removal of 110 duplicates, 355 titles or abstracts were screened, with 23 determined to be eligible. The full articles on the 23 remaining studies were reviewed; 4 studies were further excluded, leaving 19 studies for data extraction. Among the 19 included studies, 11 (57.9%) were identified in all 3 databases, 5 (26.3%) were identified through both MEDLINE and EMBASE, 2 (10.5%) were identified only in Scopus, and 1 (5.3%) was identified only in EMBASE. Sixteen studies had data on rs9332739 polymorphisms, 13 studies had data on rs547154, 14 studies had data on rs4151667, and 14 studies had data on rs641153. The characteristics of these 19 studies are given in Table 1.

Risk of bias assessment

As is shown in Web Table 1 (available on the *Journal*'s website (http://aje.oxfordjournals.org/)), the criteria for diagnosis of early and late AMD and controls were clearly described for all included studies, and therefore the risk of ascertainment bias was low. The risk of bias was highest in the quality control for genotyping (unclear or not mentioned in 8 out of 19 studies, or 42.1%), followed by selective reporting (7/19, 36.8%) and not assessing HWE (5/19, 26.3%).

C2 rs9332739. In 16 studies, investigators assessed the association between rs9332739 and AMD (see Web Table 2). Among these, 14 studies were carried out among persons of European descent (13, 15, 19, 22, 24, 26, 27, 31–36, 54) and 2 were carried out in Asian populations (37, 55). HWE was assessed in the control groups and was met in all studies. Among the Caucasian studies, the pooled frequency of minor allele C was lower in AMD cases than in non-AMD populations, with frequencies of 2.5% (95% confidence interval (CI): 2.0, 3.0) and 4.8% (95% CI: 3.9, 5.6), respectively. The odds ratios were mildly heterogeneous ($\chi^2 = 17.46$ (14 df), $P = 0.233$, $I^2 = 19.8\%$), with a pooled odds ratio of 0.55 (95% CI: 0.46, 0.65), suggesting that the C allele was approximately half as frequent in the AMD group as in controls. The frequency of the C allele in the single Chinese population was very similar to that in Caucasians (approximately 2%), but it was the major allele in the single Indian population, at approximately 96%, and was more prevalent in cases than in controls.

Genotype frequencies in the AMD and control groups are shown in Table 2. The gene effects for OR_1 (CC vs. GG) and OR_2 (GC vs. GG), along with 95% confidence intervals, were plotted across studies in Caucasian populations (see Web Figure 1, parts A and B). OR_1 was homogenous ($\chi^2 = 2.33$ (14 df), $P = 1.00$, $I^2 = 0\%$), whereas OR_2 showed mild heterogeneity across studies ($\chi^2 = 18.69$ (14 df), $P = 0.177$, $I^2 = 25.1\%$). The mixed logit model yielded pooled estimates for OR_1 and OR_2 of 0.38 (95% CI: 0.14, 1.08) and 0.52 (95% CI: 0.45, 0.61), respectively, which suggested that persons with CC and GC genotypes had approximately 62% and 48% lower risks of AMD than persons with the GG genotype.

The estimated lambda value was 0.69 (95% CI: 0.37, 0.97), suggesting that a dominant or additive mode of effect was most likely. Publication bias was assessed for OR_1 and OR_2 using funnel plots, which suggested symmetry of gene effects for both odds ratios (see Web Figure 1, parts C and D) (for OR_1, Egger test coefficient = 0.92 (standard error (SE), 0.66), $P = 0.188$; for OR_2, Egger test coefficient = 0.23 (SE, 0.85), $P = 0.789$). Adding the 2 Asian studies yielded very similar results, with a lambda value of 0.71 (95% CI: 0.34, 0.99). Despite the C allele's being the major allele in the Indian population (37, 55), the direction of the association was still protective. Pooling only advanced AMD cases in 6 Caucasian studies yielded summary estimates of OR_1 and OR_2 of 0.22 (95% CI: 0.04, 1.10) and 0.52 (95% CI: 0.43,0.63), respectively.

C2 rs547154. Thirteen studies (13, 15, 19, 20, 22, 26, 31–33, 35–37, 55) were eligible for pooling of gene effects of the rs547154 polymorphism (see Web Table 3). Ten studies (15, 19, 22, 26, 31–33, 35, 36) were in Caucasians, and 3 studies (20, 37, 55) in Asians. The allele frequency

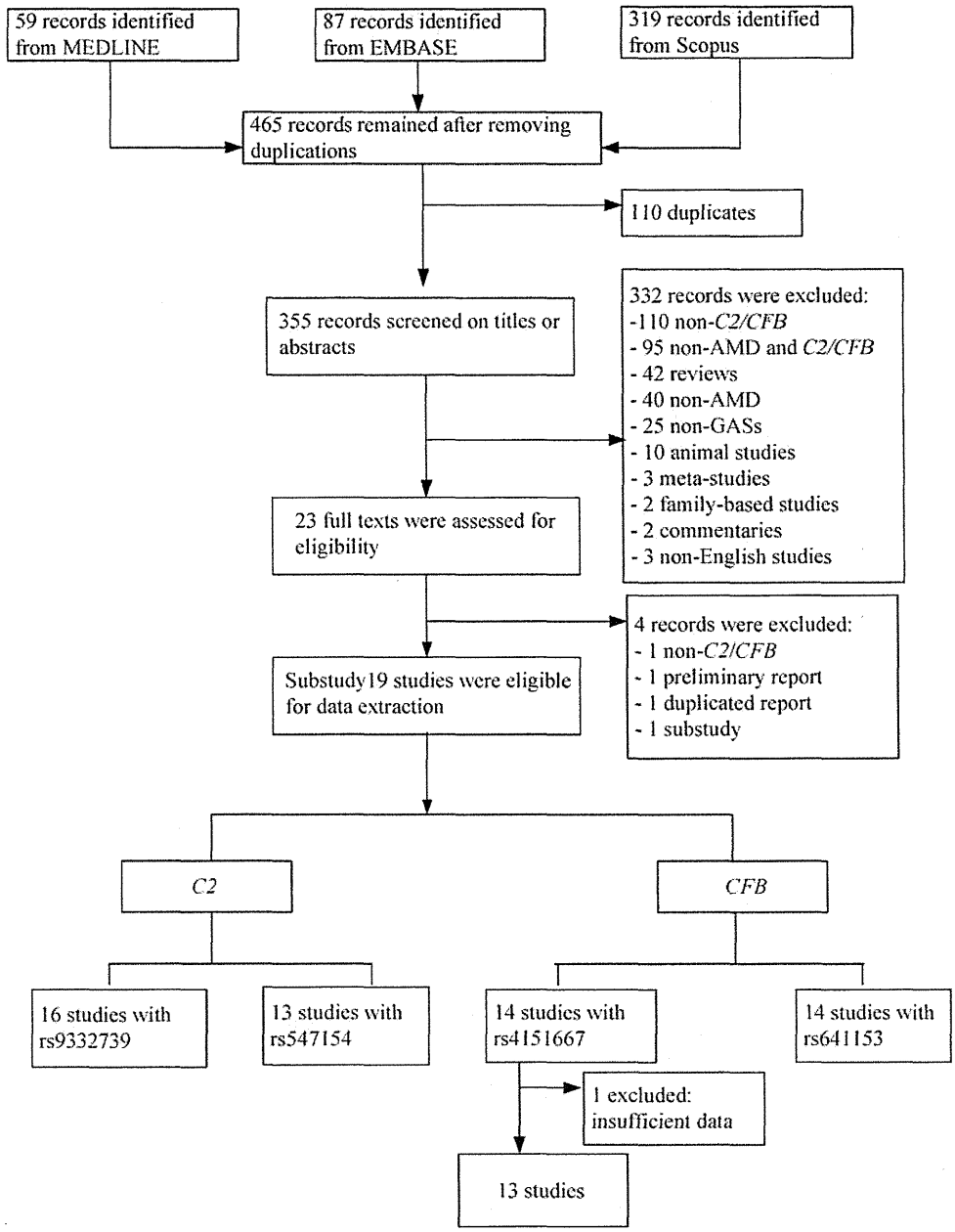

Figure 1. Selection of published studies (2006–2011) for a systematic review and meta-analysis of the association of complement component 2(*C2*)/complement factor B (*CFB*) gene polymorphisms with age-related macular degeneration (AMD). (GAS, genetic association study).

in 1 Caucasian study (19) was not in HWE and was excluded from pooling. The pooled frequencies of the T allele in AMD and non-AMD populations were 4.6% (95% CI: 4.0, 5.2) and 9.0% (95% CI: 7.3, 10.8), respectively. The odds ratios (T vs. G) were moderately heterogeneous ($\chi^2 = 13.12$ (8 df), $P = 0.108$, $I^2 = 39.0\%$), with a pooled odds ratio of 0.47 (95% CI: 0.37, 0.60). This suggested that the T allele was about half as frequent in AMD cases as in controls. There was no evidence of publication bias (Egger test coefficient = 0.02, $P = 0.986$). Sensitivity analysis was performed by including the study which did not observe HWE; this yielded similar results, with a pooled odds ratio of 0.42 (95% CI: 0.32, 0.55). Subgroup analysis in advanced AMD cases was not performed because of insufficient data.

In Asian studies, the absolute frequency of the T allele in cases and controls was almost double that in Caucasians, with similar relative frequencies (pooled odds ratio = 0.48, 95% CI: 0.22, 1.05).

Genotype frequencies were characterized in the AMD and non-AMD groups separately by ethnicity (see Table 3). OR_1 (TT vs. GG) was homogenous across studies ($\chi^2 =$

Table 1. General Characteristics of Studies Included in a Systematic Review and Meta-Analysis of the Association of Complement Component Polymorphisms With Age-Related Macular Degeneration, 2006–2011[a]

First Author, Year (Reference No.)	Mean Age, years	% Male	% Smokers	Study Design	Type of Case
Maller, 2006 (13)	76.3	45.5		Case-control	Advanced AMD
Gold, 2006 (32)	73.6			Matched case-control	AMD
Spencer, 2007 (36)	73.7	38.8	57.6	Case-control	AMD grades 3–5
Chu, 2008 (56)	67.1	54.8		Matched case-control	Exudative AMD
Jakobsdottir, 2008 (33)	76.2	43.7	43.3	Case-control	56%–66% GA and CN
Scholl, 2008 (26)	73.5	43.6	47.5	Case-control	69.6% CNV
Bergeron-Sawitzke, 2009 (15)	65.4	45.7	46.5	Age-, sex-, and race-matched case-control	AMD grades 3–5
Francis, 2009 (19)—AREDS				Cohort	GA/CNV
Francis, 2009 (19)—CEIMDC	76.7	33.2		Case-control	GA/CNV
Farwick, 2009 (31)	70.9	40.4	40.2	Cross-sectional	30.5% advanced AMD
Goto, 2009 (20)	73	54.8		Matched case-control	Advanced AMD
Park, 2009 (22)				Cohort	Early and late (54.6%)
Pei, 2009 (23)	69.9	53	45.8	Age- and sex-matched case-control	CNV
Reynolds, 2009 (24)		50	54.4	Case-control	Grade 4 (GA)/5(CNV) i or both eyes
Richardson, 2009 (35)	73.1	34.7		Case-control	71.7% advanced AMD
Seddon, 2009 (27)				Case-control	Advanced AMD
Kaur, 2010 (37)				Matched case-control	
Liu, 2010 (55)	64.2	45.4		Age-matched case-control	66.4% CNV and 33.6%
McKay, 2009 (34)	74.9	38.5		Age-matched case-control	GA/CNV
Chen, 2011 (54)	77.1	43.8	35.7	Case-control	38% GA and 72% CN

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; CEIMDC, Casey Eye Institute Mac neovascularization; GA, geographic atrophy.

[a] For details on the AMD grading scale, see the AREDS website (https://web.emmes.com/study/areds/mop.htm) and the article by Seddon et

Table 2. Frequencies of the Complement Component 2 (*C2*) rs9332739 Genotype in AMD and Control Groups and Genotype Effects of Studies Included in the Meta-Analysis, 2006–2011

First Author, Year (Reference No.)	No. of Subjects								Genotype Effect			
	AMD Group				Non-AMD Group				CC vs. GG		GC vs. GG	
	CC	GC	GG	Total	CC	GC	GG	Total	OR_1[a]	95% CI	OR_2	95% CI
Caucasians												
Maller, 2006 (13)	1	63	1,174	1,238	3	95	836	934	0.24	0.03, 2.29	0.47	0.34, 0.66
Gold, 2006 (32)	1	35	861	897	1	40	340	381	0.40	0.03, 6.33	0.19	0.12, 0.29
Spencer, 2007 (36)	1	40	657	698	1	27	254	282	0.39	0.02, 6.20	0.57	0.34, 0.95
Jakobsdottir, 2008 (33)	0	10	172	182	1	9	156	166	0.30	0.01, 7.48	1.01	0.40, 2.54
Scholl, 2008 (26)	0	7	105	112	0	5	62	67	0.59	0.01, 30.23	0.83	0.25, 2.72
Bergeron-Sawitzke, 2009 (15)	0	17	404	421	0	22	193	215	0.48	0.01, 24.20	0.37	0.19, 0.71
Farwick, 2009 (31)	2	35	767	804	0	7	95	102	0.62	0.03, 13.06	0.62	0.27, 1.43
Francis, 2009 (19)—AREDS	0	37	484	521	1	37	370	408	0.25	0.01, 6.28	0.76	0.48, 1.23
Francis, 2009 (19)—CEIMDC	0	6	392	398	0	20	256	276	0.65	0.01, 33.04	0.20	0.08, 0.49
Park, 2009 (22)	0	9	114	123	0	10	138	148	1.21	0.02, 61.44	1.09	0.43, 2.77
Reynolds, 2009 (24)	0	8	96	104	0	9	48	57	0.50	0.01, 25.72	0.44	0.16, 1.22
Richardson, 2009 (35)	0	23	494	517	0	11	146	157	0.30	0.01, 15.00	0.62	0.29, 1.30
Seddon, 2009 (27)	0	8	272	280	2	90	1,075	1,167	0.79	0.04, 16.55	0.35	0.17, 0.74
McKay, 2009 (34)	1	29	395	425	0	45	383	428	2.91	0.12, 71.63	0.62	0.38, 1.02
Chen, 2011 (54)	1	78	1,256	1,335	1	48	460	509	0.37	0.02, 5.87	0.60	0.41, 0.87
Pooled data	7	405	7,642	8,054	10	475	4,812	5,297	0.38	0.14, 1.08	0.52	0.45, 0.61
Asians												
Kaur, 2010 (37)	164	11	2	177	154	20	1	175	0.53	0.05, 5.93	0.28	0.02, 3.39
Liu, 2010 (55)	0	10	228	238	0	10	210	220	0.92	0.02, 46.64	0.95	0.39, 2.34
Pooled data	164	21	230	415	154	30	211	395	0.77	0.43, 1.38	0.54	0.46, 0.63

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; CEIMDC, Casey Eye Institute Macular Degeneration Center; CI, confidence interval; OR, odds ratio.

[a] Continuing correction was performed by adding 0.5 in all cells for OR_1.

1.38 (8 df), $P = 0.994$, $I^2 = 0\%$), but OR_2 (GT vs. GG) was moderately heterogeneous ($\chi^2 = 13.47$ (8 df), $P = 0.097$, $I^2 = 40.6\%$) (see Web Figure 2, parts A and B). A mixed-effects model was applied and resulted in pooled OR_1 and OR_2 estimates of 0.23 (95% CI: 0.11, 0.48) and 0.48 (95% CI: 0.42, 0.56), respectively, indicating that persons with the TT and GT genotypes had approximately 77% and 52% significantly lower risks of having AMD compared with persons with the GG genotype, respectively. The estimated lambda value was 0.53 (95% CI: 0.30, 0.93), which suggested that an additive model was most likely. Neither the Egger test nor the funnel plot suggested asymmetry of the funnel plot for OR_1 (coefficient = 0.33 (SE, 29), $P = 0.347$) or OR_2 (coefficient = −0.16 (SE, 1.14), $P = 0.892$) (see Web Figure 2, parts C and D). The gene effects in the 3 Asian studies were moderately to highly heterogeneous, with I^2 values of 52.3% ($\chi^2 = 4.19$ (2 df), $P = 0.123$) and 82.8% ($\chi^2 = 11.65$ (2 df), $P = 0.003$) for OR_1 and OR_2, respectively. The pooled OR_1 and OR_2 were 0.32 (95% CI: 0.12, 0.83) and 0.40 (95% CI: 0.28, 0.56), respectively, which were similar to the associations in Caucasians.

CFB rs4151667. Fourteen studies (13, 15, 19, 22, 23, 26, 31–35, 37, 55, 56) assessed the association between rs4151667 and AMD. After unsuccessful attempts to contact the authors, 1 study (56) was excluded because of insufficient data. Allele frequency data for the remaining 13 studies were characterized by ethnicity (see Web Table 4), and all studies observed HWE. The pooled frequencies of the A allele in the 10 Caucasian studies were 2.4% (95% CI: 2.1, 2.7) and 4.7% (95% CI: 4.4, 5.1) in AMD and non-AMD groups, respectively. The allele-effect odds ratios (A vs. T) were homogeneous across studies ($\chi^2 = 7.20$ (9 df), $P = 0.616$, $I^2 = 0\%$), with a pooled odds ratio of 0.54 (95% CI: 0.45, 0.64), suggesting that the A allele was approximately half as frequent in the AMD group as in controls. Allele frequencies in Asians were 2.4% (95% CI: 1.1, 3.6) and 3.5% (95% CI: 0.9, 6.0) in AMD and non-AMD groups, respectively—largely similar to Caucasians.

Genotype frequencies from the 13 studies are shown in Table 4. In the 10 Caucasian studies, genotypic effects for OR_1 (AA vs. TT) and OR_2 (AT vs. TT) were homogenous, with I^2 values of 0% for both OR_1 ($\chi^2 = 3.16$ (9 df), $P = 0.957$) and OR_2 ($\chi^2 = 7.19$ (9 df), $P = 0.618$). The mixed-effects logit model yielded pooled estimates for OR_1 and OR_2 of 0.99 (95% CI: 0.28, 3.58) and 0.50 (95% CI: 0.42, 0.61), respectively, which suggested a nonsignificant

Table 3. Frequencies of the Complement Component 2 (*C2*) rs547154 Genotype in AMD and Control Groups and Genotype Effects of Studies Included in the Meta-Analysis, 2006–2011

First Author, Year (Reference No.)	No. of Subjects								Genotype Effect			
	AMD Group				Non-AMD Group				TT vs. GG		GT vs. GG	
	TT	GT	GG	Total	TT	GT	GG	Total	OR_1[a]	95% CI	OR_2	95% CI
Caucasians												
Maller, 2006 (13)	4	126	1,108	1,238	9	164	761	934	0.31	0.09, 0.99	0.53	0.41, 0.68
Gold, 2006 (32)	2	86	806	894	5	75	302	382	0.15	0.03, 0.78	0.43	0.31, 0.60
Spencer, 2007 (36)	2	66	630	698	4	55	223	282	0.18	0.03, 0.97	0.42	0.29, 0.63
Jakobsdottir, 2008 (33)	0	9	170	179	0	31	130	161	0.77	0.02, 38.83	0.22	0.10, 0.48
Scholl, 2008 (26)	0	6	106	112	0	10	57	67	0.54	0.01, 27.57	0.32	0.11, 0.93
Bergeron-Sawitzke, 2009 (15)	0	51	379	430	0	39	176	215	0.47	0.01, 23.53	0.61	0.39, 0.96
Farwick, 2009 (31)	0	60	609	669	0	5	83	88	0.14	0, 6.95	1.64	0.64, 4.19
Francis, 2009 (19)[b,c]	0	14	184	198	0	139	167	306	0.91	0.02, 46.01	0.09	0.05, 0.16
Park, 2009 (22)	1	31	354	386	1	26	133	160	0.38	0.02, 6.05	0.45	0.26, 0.78
Richardson, 2009 (35)	2	54	469	525	3	41	156	200	0.22	0.04, 1.34	0.44	0.28, 0.68
Pooled data	11	489	4,631	5,131	22	446	2,022	2,490	0.23	0.11, 0.48	0.48	0.42, 0.56
Asians												
Goto, 2009 (20)	2	7	89	98	4	28	158	190	0.89	0.16, 4.94	0.44	0.19, 1.06
Kaur, 2010 (37)	2	26	149	177	11	74	90	175	0.11	0.02, 0.51	0.21	0.13, 0.36
Liu, 2010 (55)	2	28	208	238	2	32	186	220	0.89	0.12, 6.41	0.78	0.45, 1.35
Pooled data	6	61	446	513	17	134	434	585	0.32	0.12, 0.83	0.40	0.28, 0.56

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.

[a] Continuing correction was performed by adding 0.5 in all cells for OR_1.

[b] Both subsamples (Age-Related Eye Disease Study and Casey Eye Institute Macular Degeneration Center) were included.

[c] Not included in pooling because of departure from Hardy-Weinberg equilibrium.

risk association for the AA genotype (likely due to the outlier study by McKay et al. (34)) but a significant preventive association for the AT genotype when compared with the TT genotype (see Web Figure 3, parts A and B). The estimated lambda value was 0.70 (95% CI: 0.37, 0.98), suggesting that a dominant or additive effect was most likely. Neither the Egger test not the funnel plot suggested asymmetry of the funnel for either OR_1 (coefficient = −0.14 (SE, 1.12), $P = 0.509$) or OR_2 (coefficient = 0.51 (SE, 0.78), $P = 0.530$) (see Web Figure 3, parts C and D).

Only 4 studies (13, 19, 22, 34) had data on advanced AMD cases. The AA and AT effects were homogeneous (for OR_1, $\chi^2 = 2.49$ (3 df), $P = 0.477$, $I^2 = 0$; for OR_2, $\chi^2 = 0.45$ (3 df), $P = 0.929$, $I^2 = 0$), with the pooled OR_1 and OR_2 being equal to 0.53 (95% CI: 0.41, 0.68) and 1.66 (95% CI: 0.30, 9.09), respectively; the discrepancy in the pooled OR_2 was probably due to the outlier study by McKay et al. (34).

The genotyping effects in the 4 Asian studies were homogenous for both OR_1 and OR_2, with an I^2 value of 0%. The pooled OR_1 and OR_2 were 0.96 (95% CI: 0.06, 15.31) and 0.68 (95% CI: 0.40, 1.16), respectively.

CFB rs641153. Fourteen studies (13, 23, 24, 26, 27, 31, 32, 34–37, 54–56) had data for the *CFB* rs641153 polymorphism. Of these, 10 studies (13, 24, 26, 27, 31, 32, 34–36, 54) were conducted in Caucasians, and 4 (23, 37, 55, 56) were conducted in Asians (see Web Table 5). All control groups were in HWE. Among the Caucasian studies, the pooled frequency of the A allele was 4.1% (95% CI: 3.1, 5.2) in AMD groups and 9.6% (95% CI: 7.9, 11.3) in non-AMD groups. The allele-effect odds ratios were moderately heterogeneous across studies ($\chi^2 = 22.44$ (8 df), $P = 0.004$, $I^2 = 59.9\%$). The pooled odds ratio (A vs. G) was 0.40 (95% CI: 0.31, 0.52); that is, having the A allele was less than half as frequent in AMD cases as in controls. The pooled absolute frequency of the A allele within the 4 Asian studies was slightly higher than that in Caucasians, but the relative frequency was very similar (OR = 0.55, 95% CI: 0.30, 1.02).

The odds ratios for genotypic effects, OR_1 (AA vs. GG) and OR_2 (GA vs. GG), were estimated for each study (see Table 5). Pooled estimates were homogenous for OR_1 ($\chi^2 = 1.42$ (9 df), $P = 0.998$, $I^2 = 0\%$) but highly heterogeneous for OR_2 ($\chi^2 = 25.96$ (9 df), $P = 0.002$, $I^2 = 65.3\%$) (see Web Figure 4, parts A and B). The mixed logit model yielded pooled OR_1 and OR_2 estimates of 0.26 (95% CI: 0.14, 0.48) and 0.42 (95% CI: 0.37, 0.48), respectively, indicating that persons with the AA and GA genotypes were at 74% and 58% lower risk of AMD, respectively, than those with the GG genotype. The estimated lambda value was 0.72 (95% CI: 0.44, 0.98), which suggested that a dominant or additive effect was more likely. The Egger test found no evidence of asymmetry of the funnels for either OR_1 (coefficient = −0.10 (SE, 0.37), $P = 0.790$) or OR_2 (coefficient = −1.87 (SE, 1.42), $P = 0.226$) (see Web

Table 4. Frequencies of the Complement Factor B (*CFB*) rs4151667 Genotype in AMD and Control Groups and Genotype Effects of Studies Included in the Meta-Analysis, 2006–2011

First Author, Year (Reference No.)	No. of Subjects								Genotype Effect			
	AMD				Non-AMD Group				AA vs. TT		AT vs. TT	
	AA	AT	TT	Total	AA	AT	TT	Total	OR_1[a]	95% CI	OR_2	95% CI
Caucasians												
Maller, 2006 (13)	1	70	1,167	1,238	2	89	843	934	0.36	0.03, 3.99	0.57	0.41, 0.79
Gold, 2006 (32)	1	35	867	903	1	41	341	383	0.39	0.02, 6.31	0.34	0.21, 0.54
Jakobsdottir, 2008 (33)	0	10	168	178	1	10	156	167	0.31	0.01, 7.66	0.93	0.38, 2.29
Scholl, 2008 (26)	0	7	105	112	0	5	62	67	0.59	0.01, 30.23	0.83	0.25, 2.72
Bergeron-Sawitzke, 2009 (15)	0	17	404	421	0	22	193	215	0.48	0.01, 24.20	0.37	0.19, 0.71
Farwick, 2009 (31)	2	35	765	802	0	7	95	102	0.62	0.03, 13.09	0.62	0.27, 1.44
Francis, 2009 (19)[b]	0	6	191	197	0	11	150	161	0.79	0.02, 39.84	0.43	0.15, 1.18
Park, 2009 (22)	0	19	367	386	0	15	145	160	0.40	0.01, 20.05	0.50	0.25, 1.01
Richardson, 2009 (35)	0	23	497	520	0	12	150	162	0.30	0.01, 15.31	0.58	0.28, 1.19
McKay, 2009 (34)	3	23	399	425	0	45	383	428	6.72	0.35, 130.53	0.49	0.29, 0.83
Pooled data	7	245	4,930	5,182	4	257	2,518	2,779	0.99	0.28, 3.58	0.50	0.42, 0.61
Asians												
Pei, 2009 (23)	0	5	118	123	0	8	122	130	1.03	0.02, 52.53	0.65	0.21, 2.03
Kaur, 2010 (37)	1	12	164	177	1	20	154	175	0.94	0.06, 15.14	0.56	0.27, 1.19
Liu, 2010 (55)	0	8	230	238	0	7	213	220	0.93	0.02, 46.89	1.06	0.38, 2.97
Pooled data	1	25	512	538	1	35	489	525	0.96	0.06, 15.31	0.68	0.40, 1.16

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.

[a] Continuing correction was performed by adding 0.5 in all cells for OR_1.

[b] Both subsamples (Age-Related Eye Disease Study and Casey Eye Institute Macular Degeneration Center) were included.

Figure 4, parts C and D). The genotypic effects in advanced AMD cases were determined within 5 studies (13, 24, 27, 34, 54), which suggested a homogenous effect for OR_1 ($\chi^2 = 1.02$ (4 df), $P = 0.907$, $I^2 = 0\%$) but a moderately heterogeneous effect for OR_2 ($\chi^2 = 7.60$ (4 df), $P = 0.107$, $I^2 = 47.4\%$); the corresponding OR_1 and OR_2 were 0.27 (95% CI: 0.12, 0.59) and 0.45 (95% CI: 0.38, 0.53), respectively. There was no evidence of publication bias.

Pooling genotypic effects within the 4 Asian studies yielded estimates for OR_1 and OR_2 of 0.17 (95% CI: 0.05, 0.59) and 0.55 (95% CI: 0.41, 0.74), respectively—largely consistent with those seen in Caucasians.

DISCUSSION

We performed a systematic review and meta-analysis of the associations between *C2* (rs9332739, rs547154) and *CFB* (rs4151667, rs641153) polymorphisms and AMD, including Caucasian subjects numbering 7,121–13,351 and Asian subjects numbering 810–1,301. The results suggest robust associations in Caucasians; that is, carriage of a minor allele of C or T in the *C2* rs9332739 and *C2* rs547154 polymorphisms decreases the risks of having AMD by approximately 45% and 53% relative to carriage of G and G major alleles, respectively. A similar trend was found for the *CFB* polymorphisms; carrying a minor allele A in rs4151667 and rs641153 decreased the risks of AMD by approximately 46% and 59%, respectively, relative to a major allele of T and G. The genetic mode of action could be additive or dominant for all polymorphisms. Sensitivity analyses, including and excluding studies not observing HWE, yielded similar results.

The minor C and T protective alleles of the *C2* polymorphisms investigated here are quite rare in Caucasians, with frequencies of 4.8% and 9.0%, respectively. The minor protective alleles for the 2 *CFB* polymorphisms are equally rare, with frequencies of 4.7% and 9.6%, respectively. The pooled odds ratios for AMD for these corresponding alleles were 0.55, 0.47, 0.54, and 0.41, respectively, and the PARs were 2.0%, 5.0%, 2.2%, and 6.0%. This does not imply that these alleles are causally responsible for the association with AMD and, given the LD in this region, they are probably overlapping effects. Nevertheless, we can say that these *C2*/*CFB* polymorphisms together probably serve as a marker for an absolute lowering of the risk of all AMD in Caucasians by 2.0%–6.0%.

Genetic effects for both sets of polymorphisms were very similar across Caucasian and Asian ethnic groups represented in this meta-analysis, and is in accord with the findings of Ioannidis et al. (57). Allele frequencies differed only slightly across ethnic groups, except for the *C2* rs9332739 polymorphism, in which the minor C allele frequency was dramatically higher in Indians than in Caucasians (37) (96% vs. 3%). Kaur et al. (37) confirmed that these results were verified by sequencing and hence do not represent a miscalled strand. This raises the possibility of

Table 5. Frequencies of the Complement Factor B (*CFB*) rs641153 Genotype in AMD and Control Groups and Genotype Effects of Studies Included in the Meta-Analysis, 2006–2011

First Author, Year (Reference No.)	No. of Subjects								Genotype Effect			
	AMD				Non-AMD Group				AA vs. GG		GA vs. GG	
	AA	GA	GG	Total	AA	GA	GG	Total	OR_1[a]	95% CI	OR_2	95% CI
Caucasians												
Maller, 2006 (13)	3	106	1,129	1,238	10	171	753	934	0.20	0.06, 0.73	0.41	0.32, 0.53
Gold, 2006 (32)	2	52	497	551	3	53	213	269	0.29	0.05, 1.72	0.42	0.28, 0.64
Spencer, 2007 (36)	2	66	630	698	3	50	229	282	0.24	0.04, 1.46	0.48	0.32, 0.71
Scholl, 2008 (26)	0	6	106	112	0	10	57	67	0.54	0.01, 27.57	0.32	0.11, 0.93
Farwick, 2009 (31)	0	26	750	776	0	26	93	119	0.12	0.002, 6.32	0.12	0.07, 0.22
Reynolds, 2009 (24)	0	6	97	103	0	11	46	57	0.48	0.01, 24.41	0.26	0.09, 0.74
Richardson, 2009 (35)	2	54	473	529	3	41	155	199	0.22	0.04, 1.32	0.43	0.28, 0.67
Seddon, 2009 (27)	0	23	256	279	6	138	1,023	1,167	0.31	0.02, 5.47	0.67	0.42, 1.06
McKay, 2009 (34)	3	33	389	425	5	86	337	428	0.52	0.12, 2.19	0.33	0.22, 0.51
Chen, 2011 (54)	3	128	1,204	1,335	4	83	422	509	0.26	0.06, 1.18	0.54	0.40, 0.73
Pooled data	15	500	5,531	6,046	34	669	3,328	4,031	0.26	0.14, 0.48	0.42	0.37, 0.48
Asians												
Chu, 2008 (56)	1	30	113	144	4	32	90	126	0.20	0.02, 1.81	0.75	0.42, 1.32
Pei, 2009 (23)	0	18	105	123	0	18	112	130	1.07	0.02, 54.23	1.07	0.53, 2.16
Kaur, 2010 (37)	2	18	142	162	10	53	95	158	0.13	0.03, 0.62	0.23	0.13, 0.41
Liu, 2010 (55)	0	17	221	238	1	25	194	220	0.29	0.01, 7.23	0.60	0.31, 1.14
Pooled data	3	83	581	667	15	128	491	634	0.17	0.05, 0.59	0.55	0.41, 0.74

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.
[a] Continuing correction was performed by adding 0.5 in all cells for OR_1.

the "flip-flop" phenomenon, in which varying LD structure between different populations leads to a flip in the direction of the allelic effect, presumably because the genotyped SNP is tagging the causative allele, and different marker alleles are in LD with the causative allele across different populations (58–60). However, the C allele in the Indian population was consistent in having a protective association, similar to other ethnic groups, which did not fit with the "flip-flop" phenomenon.

These genetic associations are very similar to the ones recently described in a meta-analysis of genome-wide association studies for AMD (61); the allele effect for *C2* rs9332739 was 0.46, and the allele effect for *CFB* rs641153 was 0.54. These pooled estimates were derived from over 2,500 cases and over 4,100 controls, and the consistency of the results shows that this effect size is robust.

Multilocus associations

Although some studies had assessed compound genotype effects of the 2 SNPs in *C2* and *CFB*, the way in which investigators had reported their data did not allow us to pool haplotype effects. Previous reports show nearly complete LD between *C2* rs9332739 and *CFB* rs4151667 (r = 0.91–1.00) (32–34) and separately between *C2* rs547154 and *CFB* rs641153 (r = 0.92–0.96) (35, 36), indicative of dependent genetic effects. Given that all 4 SNPs showed similar magnitudes of genetic effects, identification of functional causal variants from the existing data would be difficult and might require very diverse populations with smaller LD blocks to isolate functional regions. This is a timely reminder that distance is a poor proxy for LD; the 2 SNPs examined here in *CFB* are only 156 base pairs apart and are not in LD ($r^2 = 0.004$), yet rs641153 in *CFB* is in complete LD with rs547154 in *C2*, which is 3,242 base pairs away (http://hapmap.ncbi.nlm.nih.gov/). Likewise, the 2 SNPs in *C2*, which are 7,134 base pairs apart, are not in LD ($r^2 = 0.004$), but rs9332739 in *C2* is in complete LD with rs 4151667 in *CFB*, which is 10,220 base pairs away.

The fact that 2 LD blocks are equally powerful markers for AMD risk but are independent of each other leads to the possibility that they are both tagging a causative SNP that is not in either LD block. Fine mapping or next-generation sequencing may shed more light on this possibility.

Burden of disease

The *C2* and *CFB* polymorphisms analyzed here contribute only 2%–6% of the population risk of AMD. In terms of public health prevention, focusing on smoking cessation would carry a much greater benefit, with a PAR of 36.9% (34), and stronger genetic loci, such as *CFH*, carry a much greater PAR (i.e., 58.9%) (11). Some groups of researchers have combined the PAR of the 14 variants identified to obtain much larger and clinically useful estimates (61) in

an attempt to develop a genetic risk score (27). Others have generated haplotypes, which is concordant with the evolving view that this could represent a more robust method of analysis (35).

Strengths and weaknesses

This study had a number of strengths. We followed a rigorous protocol of systematic review, identifying data from 3 different databases. Data extraction was carried out in duplicate. We pooled allele frequencies and genetic effects separately, as suggested by the guidelines of the Human Genome Epidemiology Network (62). We pooled effects using a model-free method, which allows the data to suggest which genetic mode of action might be at work. We thoroughly investigated heterogeneity and study-size effects and estimated the PAR. However, we could not assess haplotype effects, which would have required individual patient data or compound genotype summary data. Another potential drawback is that the majority of the studies were clinic-based case-control studies, which might have produced overestimation of the genetic association. This bias could be avoided through the use of population-based nested case-control studies, but these types of studies are few, because it is costly to perform examinations and fundus photographs on thousands of people to determine who has early signs of AMD. In addition, few people would have advanced AMD in such studies.

In summary, our meta-analysis provides evidence for an association between *C2/CFB* polymorphisms and AMD. Carriage of preventive alleles for *C2* rs9332739 and rs547154 would decrease the risk of AMD in Caucasians by approximately 45% and 53%, respectively; carriage of preventive alleles for *CFB* rs415667 and rs641153 would decrease it by approximately 46% and 59%. These allele effects contribute to an absolute lowering of the risk of all AMD in general Caucasian populations by 2.0%–6.0%. Although these associations appear consistent in Caucasian and Asian ethnic groups, the data are still sparse, and further studies are required to estimate the effects in non-Caucasian ethnic groups with more precision. Early work indicates that these polymorphisms may affect binding affinities (e.g., between *CFB* and *C3b* (63, 64)), promoting or retarding the complement cascade; however, better understanding of the full functional implications of these alleles will require more research.

ACKNOWLEDGMENTS

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Conflict of interest: none declared.

REFERENCES

1. Klein ML, Schultz DW, Edwards A, et al. Age-related macular degeneration: clinical features in a large family and linkage to chromosome 1q. *Arch Ophthalmol.* 1998;116(8):1082–1088.
2. Mitchell P, Smith W, Attebo K, et al. Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study. *Ophthalmology.* 1995;102(10):1450–1460.
3. Pang CP, Baum L, Chan WM, et al. The apolipoprotein E ε4 allele is unlikely to be a major risk factor of age-related macular degeneration in Chinese. *Ophthalmologica.* 2000;214(4):289–291.
4. VanNewkirk MR, Nanjan MB, Wang JJ, et al. The prevalence of age-related maculopathy: the Visual Impairment Project. *Ophthalmology.* 2000;107(8):1593–1600.
5. Evans J, Wormald R. Is the incidence of registrable age-related macular degeneration increasing? *Br J Ophthalmol.* 1996;80(1):9–14.
6. Schmidt S, Klaver C, Saunders A, et al. A pooled case-control study of the apolipoprotein E (*APOE*) gene in age-related maculopathy. *Ophthalmic Genet.* 2002;23(4):209–223.
7. Vingerling JR, Dielemans I, Hofman A, et al. The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology.* 1995;102(2):205–210.
8. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical *LOC387715* is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet.* 2005;14(21):3227–3236.
9. Conley YP, Jakobsdottir J, Mah T, et al. *CFH, ELOVL4, PLEKHA1* and *LOC387715* genes and susceptibility to age-related maculopathy: AREDS and CHS cohorts and meta-analyses. *Hum Mol Genet.* 2006;15(21):3206–3218.
10. Despriet DD, Klaver CC, Witteman JC, et al. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *JAMA.* 2006;296(3):301–309.
11. Thakkinstian A, Han P, McEvoy M, et al. Systematic review and meta-analysis of the association between complement factor H *Y402H* polymorphisms and age-related macular degeneration. *Hum Mol Genet.* 2006;15(18):2784–2790.

12. Kaur I, Katta S, Hussain A, et al. Variants in the 10q26 gene cluster (*LOC387715* and *HTRA1*) exhibit enhanced risk of age-related macular degeneration along with *CFH* in Indian patients. *Invest Ophthalmol Vis Sci.* 2008;49(5):1771–1776.
13. Maller J, George S, Purcell S, et al. Common variation in three genes, including a noncoding variant in *CFH*, strongly influences risk of age-related macular degeneration. *Nat Genet.* 2006;38(9):1055–1059.
14. Maller JB, Fagerness JA, Reynolds RC, et al. Variation in complement factor 3 is associated with risk of age-related macular degeneration. *Nat Genet.* 2007;39(10):1200–1201.
15. Bergeron-Sawitzke J, Gold B, Olsh A, et al. Multilocus analysis of age-related macular degeneration. *Eur J Hum Genet.* 2009;17(9):1190–1199.
16. Cui L, Zhou H, Yu J, et al. Noncoding variant in the complement factor H gene and risk of exudative age-related macular degeneration in a Chinese population. *Invest Ophthalmol Vis Sci.* 2010;51(2):1116–1120.
17. Despriet DD, van Duijn CM, Oostra BA, et al. Complement component C3 and risk of age-related macular degeneration. *Ophthalmology.* 2009;116(3):474.e2–480.e2.
18. Edwards AO, Fridley BL, James KM, et al. Evaluation of clustering and genotype distribution for replication in genome wide association studies: the Age-Related Eye Disease Study. *PLoS One.* 2008;3(11):e3813. (doi:10.1371/journal.pone.0003813).
19. Francis PJ, Hamon SC, Ott J, et al. Polymorphisms in *C2*, *CFB* and *C3* are associated with progression to advanced age related macular degeneration associated with visual loss. *J Med Genet.* 2009;46(5):300–307.
20. Goto A, Akahori M, Okamoto H, et al. Genetic analysis of typical wet-type age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese population. *J Ocul Biol Dis Infor.* 2009;2(4):164–175.
21. Gu J, Pauer GJ, Yue X, et al. Assessing susceptibility to age-related macular degeneration with proteomic and genomic biomarkers. *Mol Cell Proteomics.* 2009;8(6):1338–1349.
22. Park KH, Fridley BL, Ryu E, et al. Complement component 3 (*C3*) haplotypes and risk of advanced age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2009;50(7):3386–3393.
23. Pei XT, Li XX, Bao YZ, et al. Association of c3 gene polymorphisms with neovascular age-related macular degeneration in a Chinese population. *Curr Eye Res.* 2009;34(8):615–622.
24. Reynolds R, Hartnett ME, Atkinson JP, et al. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. *Invest Ophthalmol Vis Sci.* 2009;50(12):5818–5827.
25. Scholl HP, Fleckenstein M, Fritsche LG, et al. *CFH*, *C3* and *ARMS2* are significant risk loci for susceptibility but not for disease progression of geographic atrophy due to AMD. *PLoS One.* 2009;4(10):e7418. (doi:10.1371/journal.pone.0007418).
26. Scholl HP, Charbel Issa P, Walier M, et al. Systemic complement activation in age-related macular degeneration. *PLoS One.* 2008;3(7):e2593. (doi:10.1371/journal.pone.0002593).
27. Seddon JM, Reynolds R, Maller J, et al. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest Ophthalmol Vis Sci.* 2009;50(5):2044–2053.
28. Seitsonen SP, Onkamo P, Peng G, et al. Multifactor effects and evidence of potential interaction between complement factor H *Y402H* and *LOC387715* A69S in age-related macular degeneration. *PLoS One.* 2008;3(12):e3833. (doi:10.1371/journal.pone.0003833).
29. Spencer KL, Olson LM, Anderson BM, et al. *C3* R102G polymorphism increases risk of age-related macular degeneration. *Hum Mol Genet.* 2008;17(12):1821–1824.
30. Yates JR, Sepp T, Matharu BK, et al. Complement *C3* variant and the risk of age-related macular degeneration. *N Engl J Med.* 2007;357(6):553–561.
31. Farwick A, Dasch B, Weber BH, et al. Variations in five genes and the severity of age-related macular degeneration: results from the Muenster Aging and Retina Study. *Eye (Lond).* 2009;23(12):2238–2244.
32. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (*BF*) and complement component 2 (*C2*) genes is associated with age-related macular degeneration. *Nat Genet.* 2006;38(4):458–462.
33. Jakobsdottir J, Conley YP, Weeks DE, et al. *C2* and *CFB* genes in age-related maculopathy and joint action with *CFH* and *LOC387715* genes. *PLoS One.* 2008;3(5):e2199. (doi:10.1371/journal.pone.0002199).
34. McKay GJ, Silvestri G, Patterson CC, et al. Further assessment of the complement component 2 and factor B region associated with age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2009;50(2):533–539.
35. Richardson AJ, Amirul Islam FM, Guymer RH, et al. Analysis of rare variants in the complement component 2 (*C2*) and factor B (*BF*) genes refine association for age-related macular degeneration (AMD). *Invest Ophthalmol Vis Sci.* 2009;50(2):540–543.
36. Spencer KL, Hauser MA, Olson LM, et al. Protective effect of complement factor B and complement component 2 variants in age-related macular degeneration. *Hum Mol Genet.* 2007;16(16):1986–1992.
37. Kaur I, Katta S, Reddy RK, et al. The involvement of complement factor B and complement component C2 in an Indian cohort with age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2010;51(1):59–63.
38. Thakkinstian A, McKay GJ, McEvoy M, et al. Systematic review and meta-analysis of the association between complement component 3 and age-related macular degeneration: a HuGE review and meta-analysis. *Am J Epidemiol.* 2011;173(12):1365–1379.
39. Thakkinstian A, Thompson JR, Minelli C, et al. Choosing between per-genotype, per-allele, and trend approaches for initial detection of gene-disease association. *J Appl Stats.* 2009;36(6):633–646.
40. Thakkinstian A, McEvoy M, Minelli C, et al. Systematic review and meta-analysis of the association between β_2-adrenoceptor polymorphisms and asthma: a HuGE review. *Am J Epidemiol.* 2005;162(3):201–211.
41. Thompson JR, Minelli C, Abrams KR, et al. Meta-analysis of genetic studies using Mendelian randomization—a multivariate approach. *Stat Med.* 2005;24(14):2241–2254.
42. Thompson SG. Why sources of heterogeneity in meta-analysis should be investigated. *BMJ.* 1994;309(6965):1351–1355.
43. Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. *Stat Med.* 1999;18(20):2693–2708.
44. Thompson SG, Smith TC, Sharp SJ. Investigating underlying risk as a source of heterogeneity in meta-analysis. *Stat Med.* 1997;16(23):2741–2758.

45. Minelli C, Thompson JR, Abrams KR, et al. The choice of a genetic model in the meta-analysis of molecular association studies. *Int J Epidemiol.* 2005;34(6):1319–1328.
46. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629–634.
47. Palmer TM, Peter JL, Sutton AJ, et al. Contour-enhanced funnel plots in meta-analysis. *STATA J.* 2008;8(2):242–254.
48. Peters JL, Sutton AJ, Jones DR, et al. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J Clin Epidemiol.* 2008; 61(10):991–996.
49. Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics.* 2000;56(2):455–463.
50. Hayden KM, Zandi PP, Lyketsos CG, et al. Apolipoprotein E genotype and mortality: findings from the Cache County Study. *J Am Geriatr Soc.* 2005;53(6):935–942.
51. Rossman MD, Thompson B, Frederick M, et al. HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. *Am J Hum Genet.* 2003;73(4):720–735.
52. StataCorp LP. *Stata Statistical Software: Release 11.0.* College Station, TX: StataCorp LP; 2009.
53. Spiegelhalter D, Thomas A, Best N, et al. *WinBUGS User Manual.* Cambridge, United Kingdom: MRC Biostatistics Unit, Institute of Public Health, University of Cambridge; 2007.
54. Chen Y, Zeng J, Zhao C, et al. Assessing susceptibility to age-related macular degeneration with genetic markers and environmental factors. *Arch Ophthalmol.* 2011;129(3): 344–351.
55. Liu X, Zhao P, Tang S, et al. Association study of complement factor H, *C2*, *CFB*, and *C3* and age-related macular degeneration in a Han Chinese population. *Retina.* 2010;30(8):1177–1184.
56. Chu J, Zhou CC, Lu N, et al. Genetic variants in three genes and smoking show strong associations with susceptibility to exudative age-related macular degeneration in a Chinese population. *Chin Med J (Engl).* 2008;121(24): 2525–2533.
57. Ioannidis JP, Ntzani EE, Trikalinos TA. 'Racial' differences in genetic effects for complex diseases. *Nat Genet.* 2004;36(12):1312–1318.
58. Clarke GM, Cardon LR. Aspects of observing and claiming allele flips in association studies. *Genet Epidemiol.* 2010;34 (3):266–274.
59. Zaykin DV, Shibata K. Genetic flip-flop without an accompanying change in linkage disequilibrium [letter]. *Am J Hum Genet.* 2008;82(3):794–796.
60. Lin PI, Vance JM, Pericak-Vance MA, et al. No gene is an island: the flip-flop phenomenon. *Am J Hum Genet.* 2007;80(3):531–538.
61. Yu Y, Bhangale TR, Fagerness J, et al. Common variants near *FRK/COL10A1* and *VEGFA* are associated with advanced age-related macular degeneration. *Hum Mol Genet.* 2011;20(18):3699–3709.
62. Little J, Higgins J, eds. *The HuGENet™ HuGE Review Handbook, Version 1.0.* Ottawa, Ontario, Canada: University of Ottawa; 2006.
63. Heurich M, Martínez-Barricarte R, Francis NJ, et al. Common polymorphisms in *C3*, factor B, and factor H collaborate to determine systemic complement activity and disease risk. *Proc Natl Acad Sci U S A.* 2011;108(21):8761–8766.
64. Montes T, Tortajada A, Morgan BP, et al. Functional basis of protection against age-related macular degeneration conferred by a common polymorphism in complement factor B. *Proc Natl Acad Sci U S A.* 2009;106(11):4366–4371.
65. Seddon JM, Sharma S, Adelman RA. Evaluation of the Clinical Age-Related Maculopathy Staging System. *Ophthalmology.* 2006;113(2):260–266.

APPENDIX

Search strategy used for EMBASE (Ovid)

1. Gene
2. Allele
3. Polymorphism
4. Macular degeneration
5. Complement component 2
6. Complement factor 2
7. Component 2
8. C2
9. Complement factor B
10. Component B
11. CFB
12. FB
13. (1 *OR* 2 *OR* 3)
14. (5 *OR* 6 *OR* 7 *OR* 8)
15. (9 *OR* 10 *OR* 11 *OR* 12)
16. 13 *AND* 4 *AND* (14 *OR* 15)

Search strategy used for Scopus

[(ALL("gene") *OR* ALL("allele") *OR* ALL("polymorphism")] *AND* [ALL("macular degeneration")] *AND* [(ALL ("complement component 2") *OR* ALL("complement factor 2") *OR* ALL("c2") *OR* ALL("component 2")] *OR* [ALL("complement factor B") *OR* ALL("component B") *OR* ALL("cfb") *OR* ALL("bf")] *AND* [LIMIT-TO(SUBJAREA, "MEDI") *OR* LIMIT-TO(SUBJAREA, "BIOC")] *AND* [EXCLUDE(SUBJAREA, "NEUR") *OR* EXCLUDE (SUBJAREA, "IMMU") *OR* EXCLUDE(SUBJAREA, "AGRI")] *AND* [EXCLUDE(SUBJAREA, "MULT") *OR* EXCLUDE(SUBJAREA, "PHAR") *OR* EXCLUDE(SUBJAREA, "CHEM")].

Molecular Vision 2012; 18:1031-1039 <http://www.molvis.org/molvis/v18/a109>
Received 27 November 2011 | Accepted 20 April 2012 | Published 24 April 2012

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

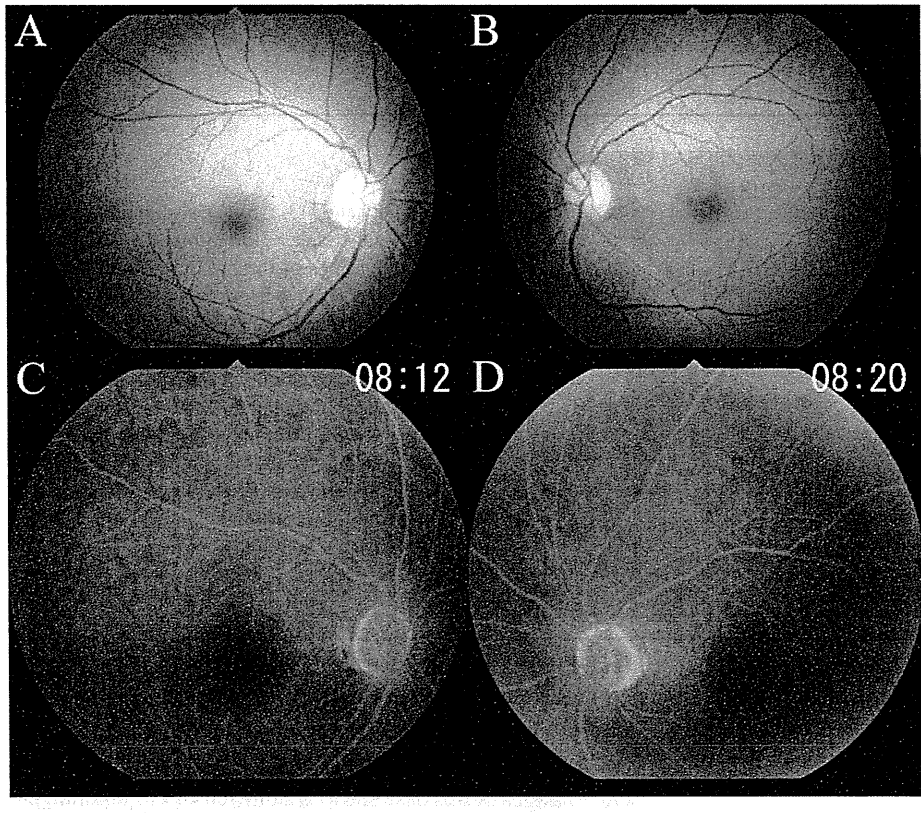

Figure 1. Fundus photographs (**A**, **B**) and fluorescein angiograms (**C**, **D**) of this case showing no abnormal findings.

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

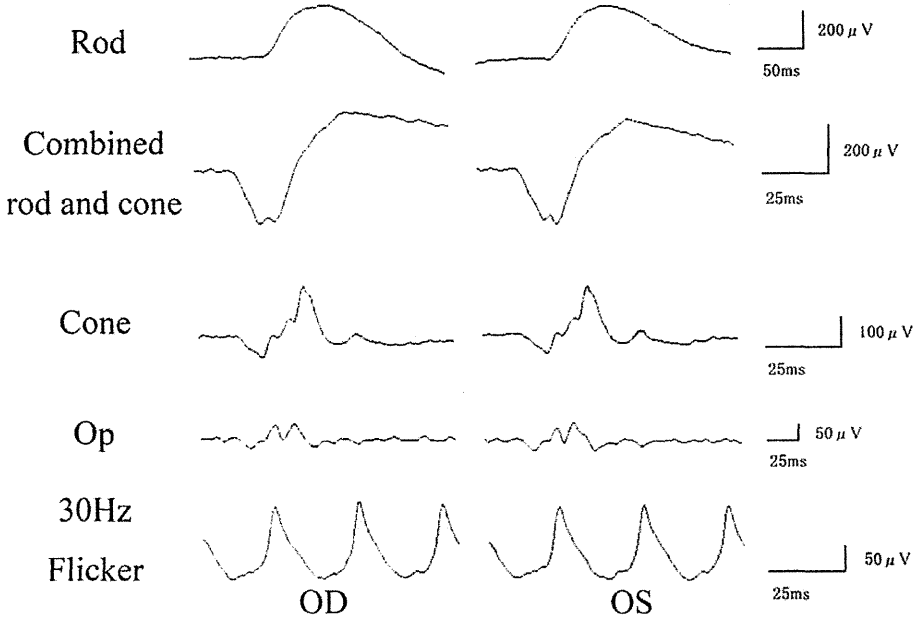

Figure 2. Full-field electroretinograms (ERGs) recorded according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards protocol in this case. The rod, combined rod-cone, cone, oscillatory potentials, and 30-Hz flicker full-field ERGs are shown. The results of full-field ERGs are within the normal limits in this case.

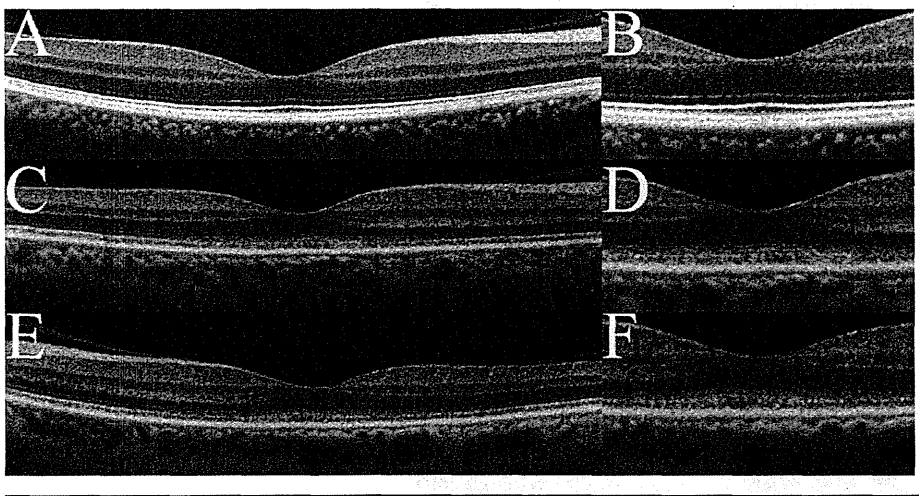

Figure 3. Spectral-domain optical coherence tomography (SD-OCT) findings of the eyes in normal controls (**A**, **B**) and in this case (**C-F**). Images from right eyes (**C**, **D**) and left eyes (**E**, **F**) are shown. Images at lower magnification (**A**, **C**, **E**) and higher magnification (**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,

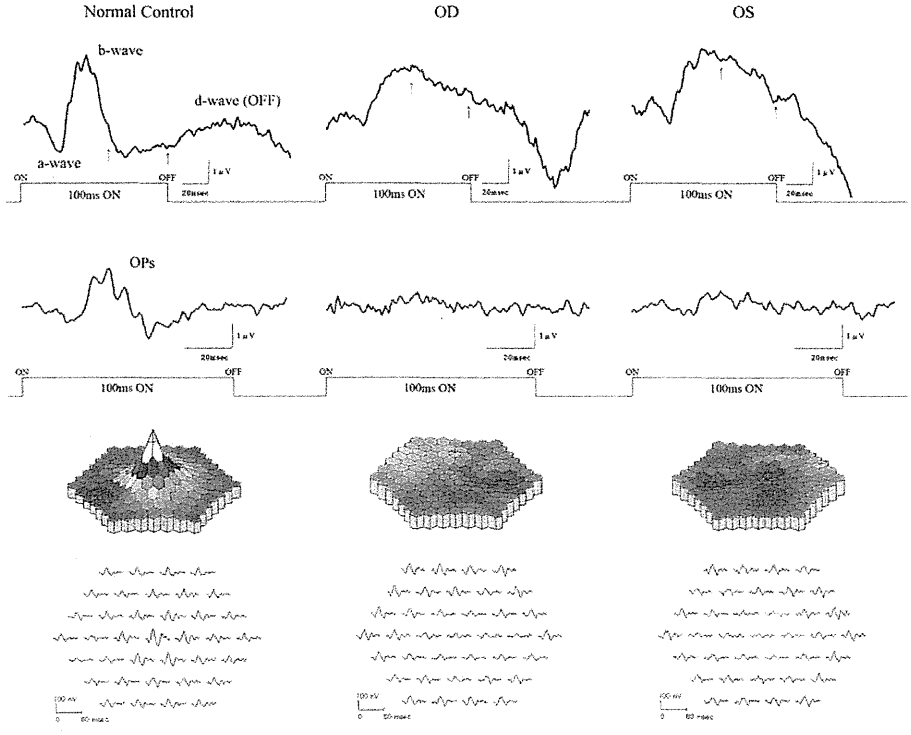

Figure 4. Results of focal macular electroretinograms (ERGs) and multifocal ERGs. Focal macular ERGs and oscillatory potentials recorded from a normal subject and this case are shown (top). The amplitude of the a-wave of this case was severely reduced, and the plateau region was significantly elevated (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.

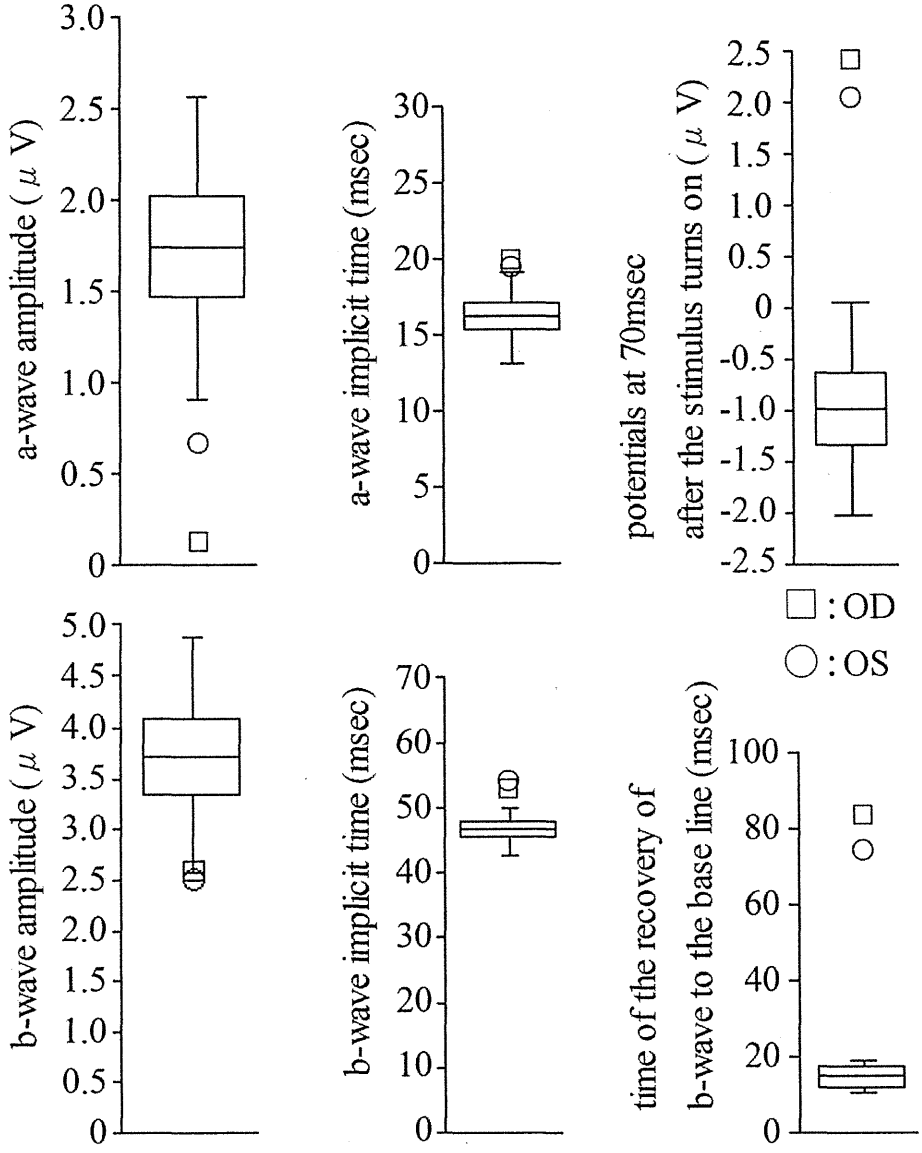

Figure 5. Plot of the amplitudes of the a-waves, b-waves, and the implicit time of the a-waves, b-waves, the potentials at 70ms after the stimulus turns on, and the time of the recovery of b-wave to the baseline for normal controls. There were 25 men and 21 women whose age ranged from 23 to 60 years (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

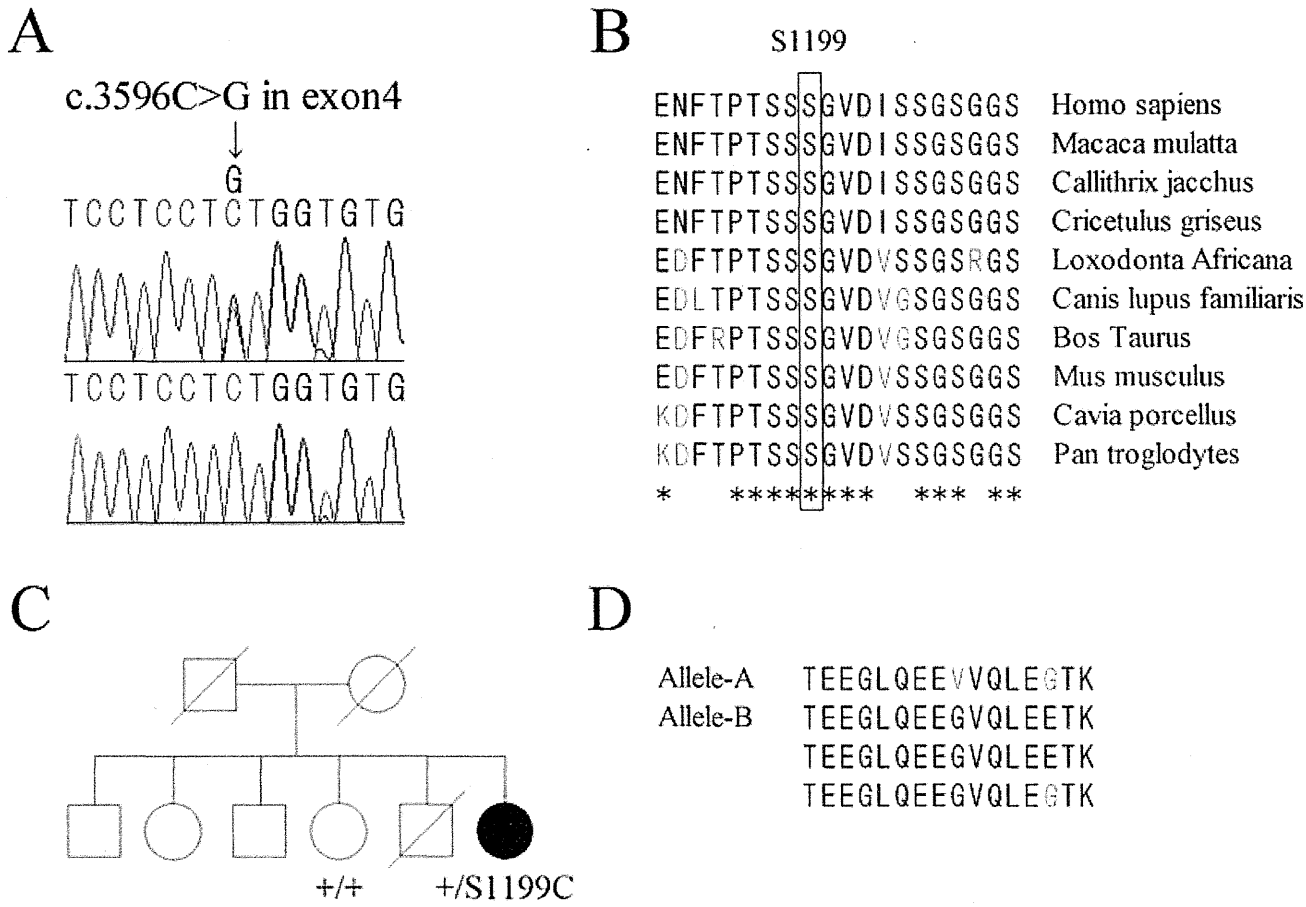

Figure 6. DNA analysis for c.3596C>G mutation and deduced amino acids of length polymorphism region of the RP1-like protein 1 (*RP1L1*) gene and the pedigree of the family with *RP1L1* gene mutation. **A**: Sequence chromatograms for this case (top) and the normal control (bottom) are shown. This case 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.

ACKNOWLEDGMENTS

We thank Dr. Duco Hamasaki (Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL) for proofreading of our manuscript.

REFERENCES

1. Miyake Y, Ichikawa K, Shiose Y, Kawase Y. Hereditary macular dystrophy without visible fundus abnormality. Am J Ophthalmol 1989; 108:292-9. [PMID: 2774037]
2. Miyake Y, Horiguchi M, Tomita N, Kondo M, Tanikawa A, Takahashi H, Suzuki S, Terasaki H. Occult macular dystrophy. Am J Ophthalmol 1996; 122:644-53. [PMID: 8909203]
3. Matthews GP, Sandberg MA, Berson EL. Foveal cone electroretinograms in patients with central visual loss of unexplained etiology. Arch Ophthalmol 1992; 110:1568-70. [PMID: 1444913]
4. Park SJ, Woo SJ, Park KH, Hwang JM, Chung H. Morphologic photoreceptor abnormality in occult macular dystrophy on spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci 2010; 51:3673-9. [PMID: 20164460]
5. Sisk RA, Berrocal AM, Lam BL. Loss of Foveal Cone Photoreceptor Outer Segments in Occult Macular Dystrophy. Ophthalmic Surg Lasers Imaging 2010; 9:1-3. [PMID: 20337322]
6. Kim YG, Baek SH, Moon SW, Lee HK, Kim US. Analysis of spectral domain optical coherence tomography findings in occult macular dystrophy. Acta Ophthalmol 2011; 89:e52-6. [PMID: 20560888]
7. Fujinami K, Tsunoda K, Hanazono G, Shinoda K, Ohde H, Miyake Y. Fundus autofluorescence in autosomal dominant occult macular dystrophy. Arch Ophthalmol 2011; 129:597-602. [PMID: 21555613]
8. Tsunoda K, Usui T, Hatase T, Yamai S, Fujinami K, Hanazono G, Shinoda K, Ohde H, Akahori M, Iwata T, Myake Y. Clinical characteristics of occult macular dystrophy in family with mutation of RP1L1 gene. Retina. 2012 [PMID: 22466457]In press
9. Lyons JS. Non-familial occult macular dystrophy. Doc Ophthalmol 2005; 111:49-56. [PMID: 16502307]
10. Akahori M, Tsunoda K, Miyake Y, Fukuda Y, Ishiura H, Tsuji S, Usui T, Hatase T, Nakamura M, Ohde H, Itabashi T, Okamoto H, Takada Y, Iwata T. Dominant mutations in RP1L1 are responsible for occult macular dystrophy. Am J Hum Genet 2010; 87:424-9. [PMID: 20826268]
11. Conte I, Lestingi M, den Hollander A, Alfano G, Ziviello C, Pugliese M, Circolo D, Caccioppoli C, Ciccodicola A, Banfi S. Identification and characterization of the retinitis pigmentosa 1-like1 gene (RP1L1): a novel candidate for retinal degenerations. Eur J Hum Genet 2003; 11:155-62. [PMID: 12634863]
12. Bowne SJ, Daiger SP, Malone KA, Heckenlively JR, Kennan A, Humphries P, Hughbanks-Wheaton D, Birch DG, Liu Q, Pierce EA. Characterization of RP1L1, a highly polymorphic paralog of the retinitis pigmentosa 1 (RP1) gene. Mol Vis 2003; 9:129-37. [PMID: 12724644]
13. Yamashita T, Liu J, Gao J, LeNoue S, Wang C, Kaminoh J, Bowne SJ, Sullivan LS, Daiger SP, Zhang K. Essential and synergistic roles of RP1 and RP1L1 in rod photoreceptor axoneme and retinitis pigmentosa. J Neurosci 2009; 29:9748-60. [PMID: 19657028]
14. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M, International Society for Clinical Electrophysiology of Vision. ISCEV Standard for full-field clinical electroretinography (2008 update). Doc Ophthalmol 2009; 118:69-77. [PMID: 19030905]
15. Miyake Y, Shiroyama N, Horiguchi M, Ota I. Asymmetry of focal ERG in human macular region. Invest Ophthalmol Vis Sci 1989; 30:1743-9. [PMID: 2759790]
16. Miyake Y. Macular oscillatory potentials in humans: macular OPs. Doc Ophthalmol 1990; 75:111-24. [PMID: 2276312]
17. Miyake Y. Focal macular electroretinography. Nagoya J Med Sci 1998; 61:79-84. [PMID: 9879190]
18. Bearse MA Jr, Sutter EE. Imaging localized retinal dysfunction with the multifocal electroretinogram. J Opt Soc Am A Opt Image Sci Vis 1996; 13:634-40. [PMID: 8627420]
19. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 2010; 7:248-9. [PMID: 20354512]
20. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003; 31:3812-4. [PMID: 12824425]
21. Ferrer-Costa C, Orozco M, de la Cruz X. Characterization of disease-associated single amino acid polymorphisms in terms of sequence and structure properties. J Mol Biol 2002; 315:771-86. [PMID: 11812146]
22. Tavtigian SV, Deffenbaugh AM, Yin L, Judkins T, Scholl T, Samollow PB, de Silva D, Zharkikh A, Thomas A. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet 2006; 43:295-305. [PMID: 16014699]
23. Mathe E, Olivier M, Kato S, Ishioka C, Hainaut P, Tavtigian SV. Computational approaches for predicting the biological effect of p53 missense mutations: a comparison of three sequence analysis based methods. Nucleic Acids Res 2006; 34:1317-25. [PMID: 16522644]
24. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010; 7:575-6. [PMID: 20676075]
25. Srinivasan VJ, Monson BK, Wojtkowski M, Bilonick RA, Gorczynska I, Chen R, Duker JS, Schuman JS, Fujimoto JG. Characterization of outer retinal morphology with high-speed, ultrahigh-resolution optical coherence tomography. Invest Ophthalmol Vis Sci 2008; 49:1571-9. [PMID: 18385077]
26. Marmor MF, Choi SS, Zawadzki RJ, Werner JS. Visual insignificance of the foveal pit: reassessment of foveal

hypoplasia as fovea plana. Arch Ophthalmol 2008; 126:907-13. [PMID: 18625935]

27. Byeon SH, Kang SY. Interpretation of outer retina appearance in high-resolution optical coherence tomography. Am J Ophthalmol 2009; 147:185-6. [PMID: 19100358]
28. Lim JI, Tan O, Fawzi AA, Hopkins JJ, Gil-Flamer JH, Huang D. A pilot study of Fourier-domain optical coherence tomography of retinal dystrophy patients. Am J Ophthalmol 2008; 146:417-26. [PMID: 18635153]
29. Kondo M, Miyake Y. Assessment of local cone on- and off-pathway function using multifocal ERG technique. Doc Ophthalmol 2000; 100:139-54. [PMID: 11142743]
30. Miyake Y. What can we know from focal macular ERG? Jpn J Clin Ophthalmol. 2002; 56:680-8.
31. Okuno T, Oku H, Kondo M, Miyake Y, Sugasawa J, Utsumi T, Ikeda T. Abnormalities of visual-evoked potentials and pupillary light reflexes in a family with autosomal dominant occult macular dystrophy. Clin Experiment Ophthalmol 2007; 35:781-3. [PMID: 17997791]
32. Hanazono G, Ohde H, Shinoda K, Tsunoda K, Tsubota K, Miyake Y. Pattern-reversal visual-evoked potential in patients with occult macular dystrophy. Clin Ophthalmol. 2010; 4:1515-20. [PMID: 21191449]
33. Miyake Y. Occult macular dystrophy. Elctrodiagnosis of retinal diseases. Tokyo, Japan: springer-Verlag; 2006:153–159
34. Sieving PA, Murayama K, Naarendorp F. Push-pull model of the primate photopic electroretinogram: a role for hyperpolarizing neurons in shaping the b-wave. Vis Neurosci 1994; 11:519-32. [PMID: 8038126]
35. Sieving PA. 'Unilateral cone dystrophy': ERG changes implicate abnormal signaling by hyperpolarizing bipolar and/or horizontal cells. Trans Am Ophthalmol Soc 1994; 92:459-71. [PMID: 7886877]

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 20 April 2012. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.