Table 2. (Continued)

Case	Age and Gender	BCVA at Final Visit		Refraction (D)*		Visual Field	Fundus Appearance	FA	Full-Field ERG	Relative Amplitude in mfERG at Fovea (Ring 1/Ring 5 or Ring 6)†	Other Ocular Disorders
		OD	OS	OD	OS						
9	66, M	0.2	0.3	+0.125	+0.125	Relative central scotoma, OU	Normal, OD Background diabetic retinopathy with microaneurysm, OS	Normal, OU	Normal mixed rod–cone responses, OU	1.21, OD1.59, OS	Senile cataract, OU
10	58, F	0.1	0.1	+0.5	+0.375	Relative central scotoma, OU	Normal, OU	NE	Normal cone responses, OU	Not measurable, OU	—
11	57, F	0.1	0.4	+0.5	0.0	Relative central scotoma, OU	Normal, OU	Normal, OU	Normal ISCEV standard protocol ERG, OU	Not measurable, OU	—
12	20, M	0.3	0.3	−0.375	−0.75	Relative central scotoma, OU	Normal, OU	Normal, OU	Normal ISCEV standard protocol ERG, OU	0.98, OD1.03, OS	—
13	18, F	0.2	0.15	−1.625¶	−2.75¶	Relative central scotoma, OU	Normal, OU	Normal, OU	Normal ISCEV standard protocol ERG, OU	Not measurable, OU	—
14	28, M	1.0	0.6	−0.25	−0.25	Relative central scotoma, OU	Normal, OU	NE	Normal ISCEV standard protocol ERG, OU	1.63, OD, 0.66, OS	—

D, diopter; ISCEV, International Society of Clinical Electrophysiology and Vision; NE, not examined.
*Spherical equivalents at the initial visit.
†The responses of Ring 1 were extinguished and the N1–P1 amplitudes were not measurable in Cases 2, 3, 4, 7, 10, 11, and 13.
‡This patient had already undergone cataract surgeries for both eyes at the initial visit, and no data could be obtained about the original refraction.
§This patient's visual acuity was reduced also by senile cataract.
¶The refraction of this patient was measured after instillation of cycloplegics.

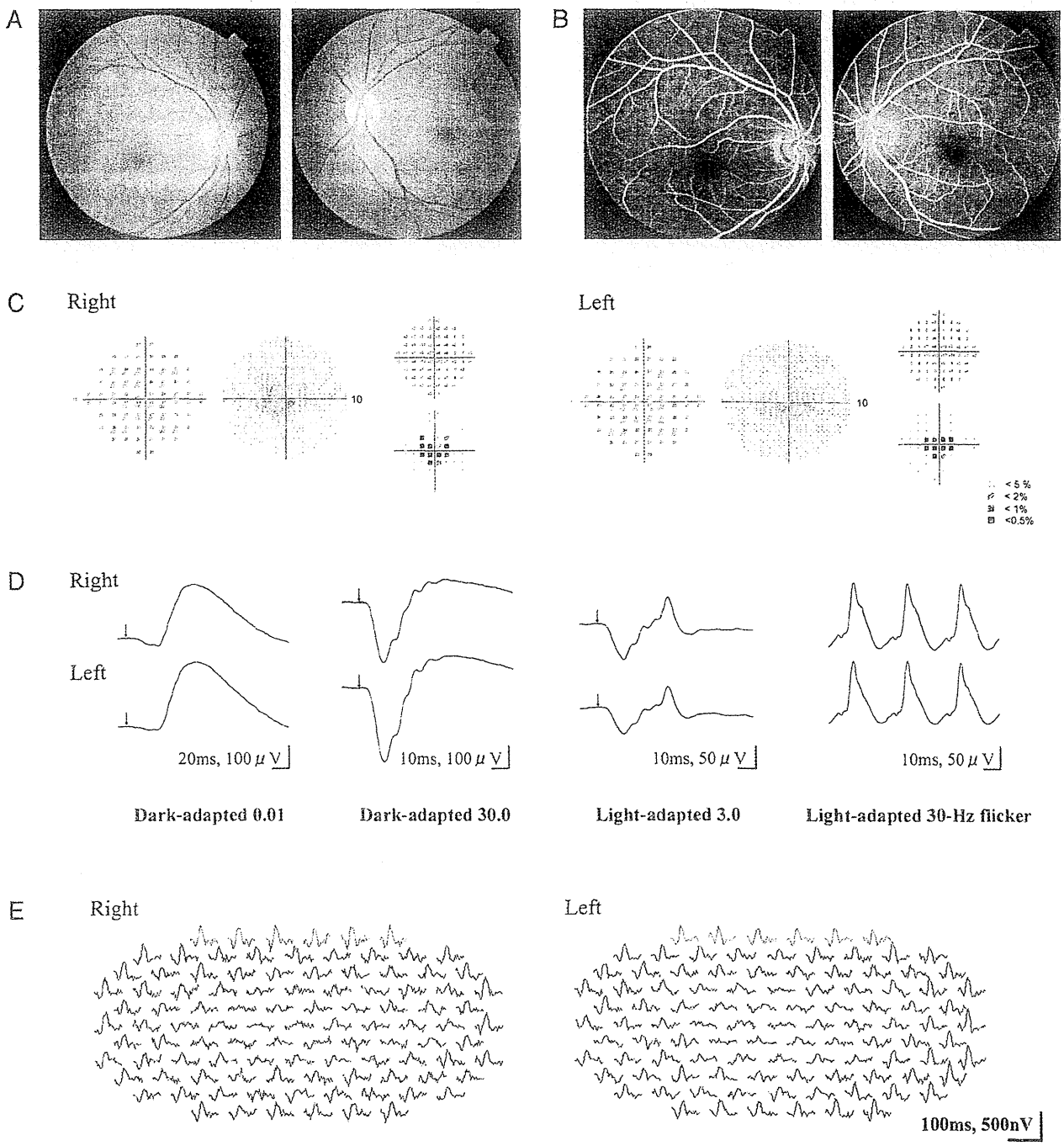

Fig. 2. Results of ocular examination of Patient 11. The data in (**A**) to (**E**) were collected 3 years after the onset of the visual disturbance at age 50 years. At this time, the patient had not noticed a decrease in the visual acuity in her left eye. The BCVA was 0.1 in the right eye and 1.2 in the right eye. **A**, and **B**. Fundus photographs and FAs showing no abnormal findings. **C**. Static visual field test (Humphrey Visual Field Analyzer, 10-2) showing relative central scotoma in both eyes. **D**. Full-field rod, mixed rod–cone, cone ERGs, and 30-Hz flicker responses. All the responses are normal in both eyes. **E**. Trace arrays of mfERGs tested with 103 hexagonal stimuli shown without spatial averaging. The responses of the central locus are extinguished in both eyes.

after the onset. This patient first noticed visual disturbances at age 20 years and was diagnosed with OMD at age 73 years. The appearance of the macula and optic disk at age 83 years was still normal >60 years after the onset of the symptoms.

Rod, mixed rod–cone, and cone full-field ERGs were recorded from 7 patients using the International Society of Clinical Electrophysiology and Vision standard protocol, and all of them showed normal rod and cone responses as in the representative case shown in

Figure 2. Only the mixed rod–cone responses were recorded from Patient 9, and only the cone responses were recorded from Patient 10, and these responses were also normal.

The amplitudes of the mfERGs were reduced in the central region of both eyes in all the 13 patients. We quantified the relative mfERG responses at the fovea by dividing the N1–P1 amplitudes of the central ring (Ring 1) by those in the outermost eccentric ring (Ring 5 in cases of 61 stimuli and Ring 6 in cases of 103 stimuli) in 13 OMD patients and 1 normal family member (Case 5) with the *RP1L1* mutation (Table 2).[4] Among the 26 eyes of the 13 OMD patients, the N1–P1 amplitudes of the central locus were measurable in 12 eyes in 6 cases tested with the 61 stimuli. The ratio of the amplitudes of Ring 1/Ring 5 in these OMD patients ranged from 0.60 to 2.74 (average of normals: 4.34 ± 0.67, n = 20). In 6 eyes tested with 61 stimuli and all the 8 eyes tested with 103 stimuli, the responses in the central locus were extinguished and the amplitudes were not measurable (see examples in Figure 2E). The ratio of the amplitudes of Ring 1/Ring 5 in a normal family member (Case 5, right eye) was 4.24, which was within the normal range.

The results of routine ocular examinations in Patient 11 at the age 50 years, when she did not have any visual disturbances in her left eye, are shown in Figure 2. The BCVA was 0.1 in the right eye and 1.2 in the left eye. The fundus and FA were normal in both eyes. Humphrey visual field tests (SITA Standard and pattern deviation 10-2) showed a relative central scotoma in both eyes. The full-field rod, mixed rod–cone, cone, and 30-Hz flicker ERGs were normal in both eyes. The mfERGs were reduced in and around the region of the central scotoma in both eyes. The Humphrey visual field test (30-2) did not detect a central scotoma in either eye (data not shown). The findings in the left eye of this patient are typical of the early stage of the OMD, where the dysfunction of the foveal region could be clearly detected in the mfERGs even though the subjective visual disturbance was almost undetectable.

Spectral-domain OCT images were recorded from 11 family members with the *RP1L1* mutation. The outer retinal structure was considered to be normal when the external limiting membrane, photoreceptor inner/outer segment (IS/OS) line, cone outer segment tip (COST) line, and retinal pigment epithelium (RPE) were clearly detected in the OCT images (Figure 3A).[11,23]

The OCT images of 5 representative OMD patients are aligned in the order of years after the onset in Figure 3B. The right eye of Case 1, which had electrophysiologically confirmed macular dysfunction but did not have subjective visual disturbances, showed a normal IS/OS line and COST line but only at the foveal center (asterisk in Figure 3B, ①). However, in the parafoveal region, the IS/OS line was blurred and the COST line could not be observed (arrowheads in Figure 3B, ①).

In the right eye of Case 11, the OCT images which were taken 10 years after the onset showed that the IS/OS line at the fovea was very blurred and thick but not disrupted. The COST line could not be observed in the macular area. In the perimacular region that had normal visual function, all the outer retinal structures were seen to be normal (Figure 3B, ②). Similar findings were observed in the left eye of Case 1 and the right eye of Case 8 (Figure 3B, ③ and ④).

In the right eye of Case 4, which was examined 63 years after the onset, the IS/OS line was disrupted at the fovea. The COST line could not be observed in the macula but was still visible in the perimacular region. The external limiting membrane and RPE could be observed to be normal over the entire region (Figure 3B, ⑤).

The OCT images of 2 sporadic cases of OMD without the *RP1L1* mutation are shown in Figure 3C. Both patients had a progressive central scotoma with normal-appearing fundus and normal FA. The full-field ERGs were normal but the focal macular ERGs elicited with a 10° spot were not recordable. Their OCT images, however, were not similar to those in patients with *RP1L1* mutation; the IS/OS line could be clearly observed at the fovea (Figure 3C, ①and ②), and the COST line could also be observed at the fovea, although it was slightly more blurred than in the normal cases. There was a minute disruption of the IS/OS line at the foveola in 1 case (asterisk in Figure 3C, ①).

The OCT findings in 21 eyes of 11 cases with the *RP1L1* mutation are summarized in Table 3. The examined eyes are listed in the order of years after the onset. Case 5, who was diagnosed as not having the typical characteristics of OMD, had completely normal retinal structures. In the case of OMD without subjective visual disturbances, the COST line and IS/OS line were normally observed only at the very center of the fovea (Case 1, right eye, Figure 3B, ①). In other affected cases, the COST line was not present and the IS/OS line appeared blurred in the entire fovea (Cases 14, right eye to 8). In patients with longer duration OMD, the IS/OS line was disrupted or not present as in Cases 2 and 4.

The retinal thickness at the foveola was measured as the distance from the internal limiting membrane to the inner border of the RPE. Considering the variation in the thickness in normals, we classified that the retina at the foveola was abnormally thin when the thickness was <160 μm. All the affected eyes with disease duration ≤ 12 years had normal foveal thickness (right

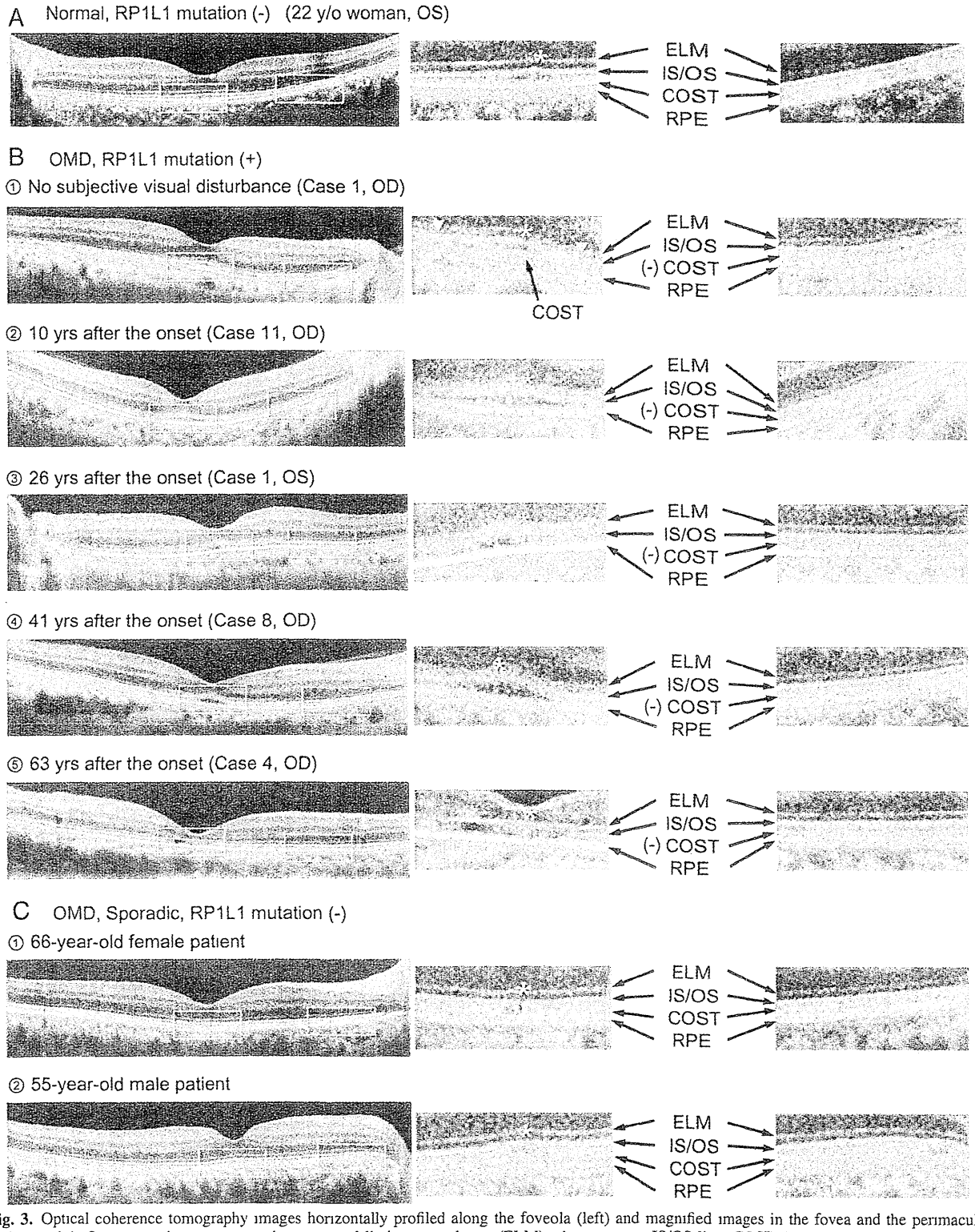

Fig. 3. Optical coherence tomography images horizontally profiled along the foveola (left) and magnified images in the fovea and the perimacular region (right) Outer retinal structures, such as external limiting membrane (ELM), photoreceptor IS/OS line, COST line, and RPE, are indicated by arrows. The foveal center is indicated by an asterisk. All the OCT images were taken with the HD-OCT (Carl Zeiss). **A.** Optical coherence tomography image of a normal control without the *RP1L1* mutation (22-year-old woman). All the outer retinal structures, for example, external limiting membrane, IS/OS line, COST line, and RPE, are clearly observed both in the fovea and the perimacular region. **B.** Optical coherence tomography images of patients affected by OMD with the *RP1L1* mutation. ① Optical coherence tomography image of the right eye of Case 1, which did not have subjective visual disturbances The COST line is present in the foveal center (black arrow), but not in the parafoveal region (arrowheads). The IS/OS line is clearly

eye of Cases 1 to Case13), whereas the fovea of all the affected eyes with durations ≥20 years were classified as thin (Case 7 to Case 4).

To determine whether a significant correlation existed between the results of mfERGs and OCT, the relative amplitudes of the mfERGs at the fovea (Ring 1/Ring 5 or 6) are listed in Table 3. In cases where the disease durations was ≥3 years, the relative amplitude at the fovea was approximately 1.0 or nonrecordable because the responses of the central locus were extinguished. Only cases with very short durations had mildly reduced mfERGs in the fovea (2.34 in the right eye of Case 1 and 1.63 in the right eye of Case 14).

Discussion

Course of OMD Patients with RP1L1 Mutation

Our results confirmed that all the patients with the *RP1L1* mutation had similar phenotypes; slowly progressive visual disturbances of both eyes, normal-appearing fundus, normal FA and full-field ERGs during the entire course of the disease, selective dysfunction at the macula detected by focal macular ERGs and mfERGs, selective abnormality of the photoreceptor layer in the macula revealed by OCT, and a final BCVA not poorer than 0.1. The age at the onset of OMD was, however, very variable among the family members and varied from 6 years to 50 years.

Our study also confirmed that there are patients with OMD who have normal visual acuity and no subjective visual disturbances until the disease progressed to a more advanced stage. Similar findings have been reported for other patients with OMD,[1,2,24] although the etiology of these patients was not confirmed by genetic analyses. For such patients, the function of the small region in the foveola of these eyes has probably been spared so that the BCVA was normal. This was morphologically confirmed by the OCT; in the right eye of Case 1, the BCVA of which was 1.2, the OCT image showed that photoreceptor structures were spared only at the foveal center.

Among the 14 family members with the *RP1L1* mutation, only Case 5 (60-year-old woman) did not show any signs of macular dysfunction in both subjective and objective tests. Thus, this woman may be a carrier of a mutated gene, but we cannot exclude the possibility that macular dysfunction may appear later. In our genetic study of 4 other OMD families, 2 brothers (58 and 55 years old) were not diagnosed with OMD, although both had the *RP1L1* mutation (p.Arg45Trp).[12] In all the OMD patients with the *RP1L1* mutation, the visual dysfunction was detected no later than 50 years of age.[12]

Occult macular dystrophy has been reported to be a slowly progressive disease; however, there were no patients whose BCVA became worse than 0.1 except for Patient 7 who had an untreated senile cataract. Our results confirmed that once the BCVA is reduced to 0.1 to 0.2, the disease becomes stationary and both the subjective and objective visual functions do not deteriorate thereafter. Similarly, in 3 other families with the *RP1L1* mutation, the final BCVA was not worse than 0.15 in any member.[12]

There was 1 family member (asterisk, Figure 1) who had a sudden decrease of vision in the left eye at age 49 years, but she was diagnosed with retrobulbar neuritis at the Niigata University. Her vision did not recover after steroid pulse therapy, and the optic disk gradually became atrophic. The BCVA 1 year later was 1.2 in the right eye and 0.07 in the left eye. We concluded that the vision reduction was not related to the OMD. Nakamura et al[25] reported a case of OMD that had normal-tension glaucoma with abnormal cupping of the optic disk. To date, the relationship between OMD and optic disk diseases has not been determined. In our family, the optic disks of all the OMD patients appeared normal, and OCT did not show any thinning of the nerve fiber layer or ganglion cell layer in any of the patients.

Diagnostic Reliabilities of mfERGs and OCT

There were patients, such as Case 6 (both eyes), Case 1 (right eye), and Case 11 (left eye), with OMD from an *RP1L1* mutation who did not have any subjective visual disturbances and whose diagnosis were only confirmed by the electrophysiologic tests. These

Figure 3. (continued) observed at the foveal center (asterisk) but appears blurred in the parafoveal region (arrowheads). ②, ③, and④. Optical coherence tomography image of the right eye of Case 11, the left eye of Case 1, and the right eye of Case 8, which show typical signs of OMD. The COST line is not present over the entire macula but is present in the perimacular regions. The IS/OS line is blurred and thick in the fovea. ⑤ Optical coherence tomography image (vertical section) of the right eye of Case 4. This image was obtained 63 years after the onset of visual symptoms. The IS/OS line is disrupted at the fovea. The COST line cannot be seen in the macula but is still visible in the perimacular region. There is an apparent thinning of the photoreceptor layer at the fovea. **C.** Optical coherence tomography images of sporadic cases of OMD without the *RP1L1* mutation. ① and ②. Both patients had progressive central scotoma with normal-appearing fundus and normal FA. The full-field ERGs were normal but focal macular ERGs elicited by a 10° spot were not recordable. The IS/OS line could be clearly observed at the fovea in both cases, except in minute disruption at the foveola in ① (asterisk). The COST line could be observed at the fovea in both cases, although slightly more blurred than in the normal case.

Table 3. Optical Coherence Tomography Findings in 21 Eyes of 11 Family Members with RP1L1 Mutation in the Order of Years After the Onset

Years After the Onset (Years)	Case	OD/ OS	BCVA	Relative Amplitude in mfERG at fovea (Ring 1/Ring 5 or 6)	OCT Findings at Fovea				Other Findings
					Disappearance of COST at fovea	Blurring of IS/OS Junction at Fovea	Abnormality of RPE	Thinning of Fovea (Thickness <160 μm)	
None	5	OD	1.2	4.24	–	–	–	–(217)	Not diagnosed as OMD
Unknown	1	OD	1.2	2.34	±*	±*	–	–(200)	No subjective visual disturbance
2	14	OD	1.0	1.63	+	+	–	–(160)	
3	11	OS	0.4	Not measurable	+	+	–	–(168)	
6	12	OD	0.3	0.98	+	+	–	–(174)	
		OS	0.3	1.03	+	+	–	–(168)	
10	14	OS	0.6	0.66	+	+	–	–(160)	
10	11	OD	0.1	Not measurable	+	+	–	–(164)	
12	13	OD	0.2	Not measurable	+	+	–	–(181)	
		OS	0.15	Not measurable	+	+	–	–(177)	
20	7	OD	0.1	Not measurable	+	+	–	+(134)	
		OS	0.07	Not measurable	+	+	–	+(142)	
31	1	OS	0.1	0.60	+	+	–	+(150)	
38	10	OD	0.1	Not measurable	+	+	–	+(150)	
		OS	0.1	Not measurable	+	+	–	+(153)	
41	8	OD	0.1	1.01	+	+	–	+(148)	
		OS	0.1	1.30	+	+	–	+(148)	
46	2	OD	0.4	Not measurable	+	+†	–	+(156)	
		OS	0.5	Not measurable	+	+†	–	+(154)	
63	4	OD	0.2	Not measurable	+	+†	–	+(77)	
		OS	0.2	Not measurable	+	+†	–	+(76)	

*The COST and IS/OS junction were normal only at the foveal center. In the parafovea, the COST could not be observed and the IS/OS junction was blurred.
†The IS/OS junction was disrupted at the fovea.

findings indicate that mfERGs or focal macular ERGs are sensitive enough to detect very early macular dysfunction in OMD.

Similarly, OCT could be another sensitive tool for the detection of early OMD because an abnormality of the COST line and the IO/OS line in the macula was observed in all the affected cases. However, we believe that the mfERG is more sensitive than OCT in detecting early dysfunctions of the macula in eyes with OMD. For example, Case 14 was a 28-year-old man whose BCVA was 1.0 (right eye) and 0.6 (left eye), but his fundus and visual field tests did not show any differences between the 2 eyes. He did notice a visual disturbance in his left eye 8 years before the onset in his right eye. In the OCT images, both the COST line and the IS/OS line were similarly affected for both eyes at the fovea, and the retinal thickness at the fovea was 160 μm in both eyes (Table 3). The mfERGs, on the other hand, were different in the 2 eyes; the relative amplitude of mfERG at the fovea (Ring 1/Ring 5) was 1.63 (38.2/23.5) in his right eye and 0.66 (15.8/23.8) in his left eye (Table 3). Thus, we believe that both the mfERGs and OCT can be useful in the diagnosis of OMD, but mfERGs are more reliable in detecting and evaluating minimal macular dysfunction at the early stage of the disease. The abnormalities in the OCT, however, progress slowly and continuously until the late stage, and thus they may be more useful for following the long-term progression of OMD.

Roles of RP1L1 Gene and Occurrence of OMD

Our study confirmed that all the affected patients with *RP1L1* mutation had abnormalities of the photoreceptor structures; the IS/OS line was very blurred and thick and the COST line could not be observed in the macula (Figure 2). But in the perimacular region, which had normal visual function, all the outer retinal structures were seen to be normal. During the whole disease process, neither the external limiting membrane nor the RPE had any significant changes and remained normal. In some of sporadic cases of the OMD, similar abnormalities in the OCT could not be observed, although localized macular dysfunction was confirmed electrophysiologically (Figure 3C).

The location of COST line coincided with the location where the outer segment disks are renewed in the cones.[23,26] The disappearance of the COST line indicates an early stage of dysfunction of the cone photoreceptors as has been found in acute zonal occult outer retinopathy.[11] Recently, ultrahigh-resolution OCT with adaptive optics has revealed that the IS/OS line corresponds to the ellipsoids of the photoreceptor inner segments, which are rich in mitochondria and play important roles in cellular metabolism.[27]

Immunohistochemistry for the *RP1L1* gene in retinal section of cynomolgus monkeys showed that it was expressed in both the inner and outer segments of the rod and cone photoreceptors, although the exact site within the photoreceptor has not been confirmed.[12] *RP1L1* is believed to play important roles in the morphogenesis of photoreceptors, and once the function of *RP1L1* is disrupted by a mutation, both the electrophysiologic responses and structures of the photoreceptor can be altered. Cellular dysfunction because of an *RP1L1* mutation affects either the inner or outer segment, or both, of the photoreceptors, which first becomes apparent as an abnormality of both the COST line and IS/OS line in the OCT images.

Considering that the OCT abnormalities in sporadic cases did not show similar pattern as patients with the *RP1L1* mutation, the phenotypically confirmed OMD surely consists of diseases caused by several independent etiologies. In any case, the abnormalities in the mfERGs and OCT observed in OMD in this family strongly support the contribution of *RP1L1* mutation to the presence of this disease.

There are still some important questions of the disease process in OMD that are unsolved. First, why is only the macular region affected while the perimacular region remains intact both functionally and morphologically even at a very advanced stage? Second, why do OMD patients have normal fundus appearance until the end stage, and why does the RPE remain intact until the end stage when the photoreceptor structures are markedly damaged (Figure 3B, ⑤)? Fujinami et al[28] demonstrated that the fundus autofluorescence images in the macula of OMD patients are normal, indicating that the RPE is normal. Third, why does the disease progression stop when the BCVA decreases to 0.1 to 0.2?

These characteristics in the disease process are peculiar to the OMD and not observed in other macular dystrophies. More detailed investigations on the function of *RP1L1* should provide information to answer these questions.

We suggest that OMD is not a single disease caused by a specific gene mutation, *RP1L1*, but may represent different disease entities with similar retinal dysfunctions. Considering all our findings on OMD, we can phenotypically define the OMD as a slowly progressing bilateral dysfunction of the photoreceptors located in the macula, not accompanied by either vascular or RPE damage. The etiology of OMD cases without the *RP1L1* mutation is now under investigation with large number of cases and some of them might be found to be because of other autosomal recessive mutations.

Key words: electroretinography, focal macular ERG, multifocal ERG, occult macular dystrophy, optical coherence tomography, *RP1L1*.

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Human Genome Epidemiology (HuGE) Review

The Association Between Complement Component 2/Complement Factor B Polymorphisms and Age-related Macular Degeneration: A HuGE Review and Meta-Analysis

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The authors performed a systematic review of the association of complement component 2(*C2*)/complement factor B (*CFB*) gene polymorphisms with age-related macular degeneration (AMD). In total, data from 19 studies published between 2006 and 2011 were pooled for 4 polymorphisms: rs9332739 and rs547154 in the *C2* gene and rs4151667 and rs641153 in the *CFB* gene. Data extraction and assessments for risk of bias were independently performed by 2 reviewers. Allele frequencies and allele and genotypic effects were pooled. Heterogeneity and publication bias were explored. Pooled minor allele frequencies for all 4 SNPs were between 4.7% and 9.6% for all polymorphisms, except for an Indian population in which the C allele at rs9332739 was the major allele. For the *C2* polymorphisms, the minor C allele at rs9332739 and the minor T allele at rs547154 carried estimated relative risks (odds ratios) of 0.55 (95% confidence interval (CI): 0.46, 0.65) and 0.47 (95% CI: 0.39, 0.57), respectively. For the *CFB* polymorphisms, the minor A alleles at rs4151667 and rs614153 carried estimated risks of 0.54 (95% CI: 0.45, 0.64) and 0.41 (95% CI: 0.34, 0.51), respectively. These allele effects contributed to an absolute lowering of the risk of all AMD in Caucasian populations by 2.0%–6.0%. This meta-analysis provides a robust estimate of the protective association of *C2/CFB* with AMD.

complement component factor 2; complement factor B; genetic association studies; genetics; genome, human; macular degeneration; meta-analysis; molecular epidemiology

Abbreviations: AMD, age-related macular degeneration; C2, complement component 2; C3, complement component 3; CFB, complement factor B; CFH, complement factor H; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; OMIM, Online Mendelian Inheritance in Man; OR, odds ratio; PAR, population attributable risk; SE, standard error.

Editor's note: This article also appears on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/default.htm).

Age-related macular degeneration (AMD) is one of the leading causes of blindness worldwide (1–4), accounting for half of all new registered cases of blindness (5). With the increase in longevity, the burden of AMD is set to grow, with almost 30% of persons older than 75 years showing early signs of the disease (1, 6, 7). The pathologic hallmark of the disease is drusen, deposits of proteins and lipids, in the retinal pigment epithelium; these deposits, along with pigmentary irregularities, constitute early AMD. Progression to late AMD involves geographic atrophy, in which there is loss of retinal pigment epithelium and photoreceptors and/or neovascularization.

Genome-wide association studies have had considerable success in identifying genetic contributions to complex disorders. The first success in ocular diseases came in 2005,

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

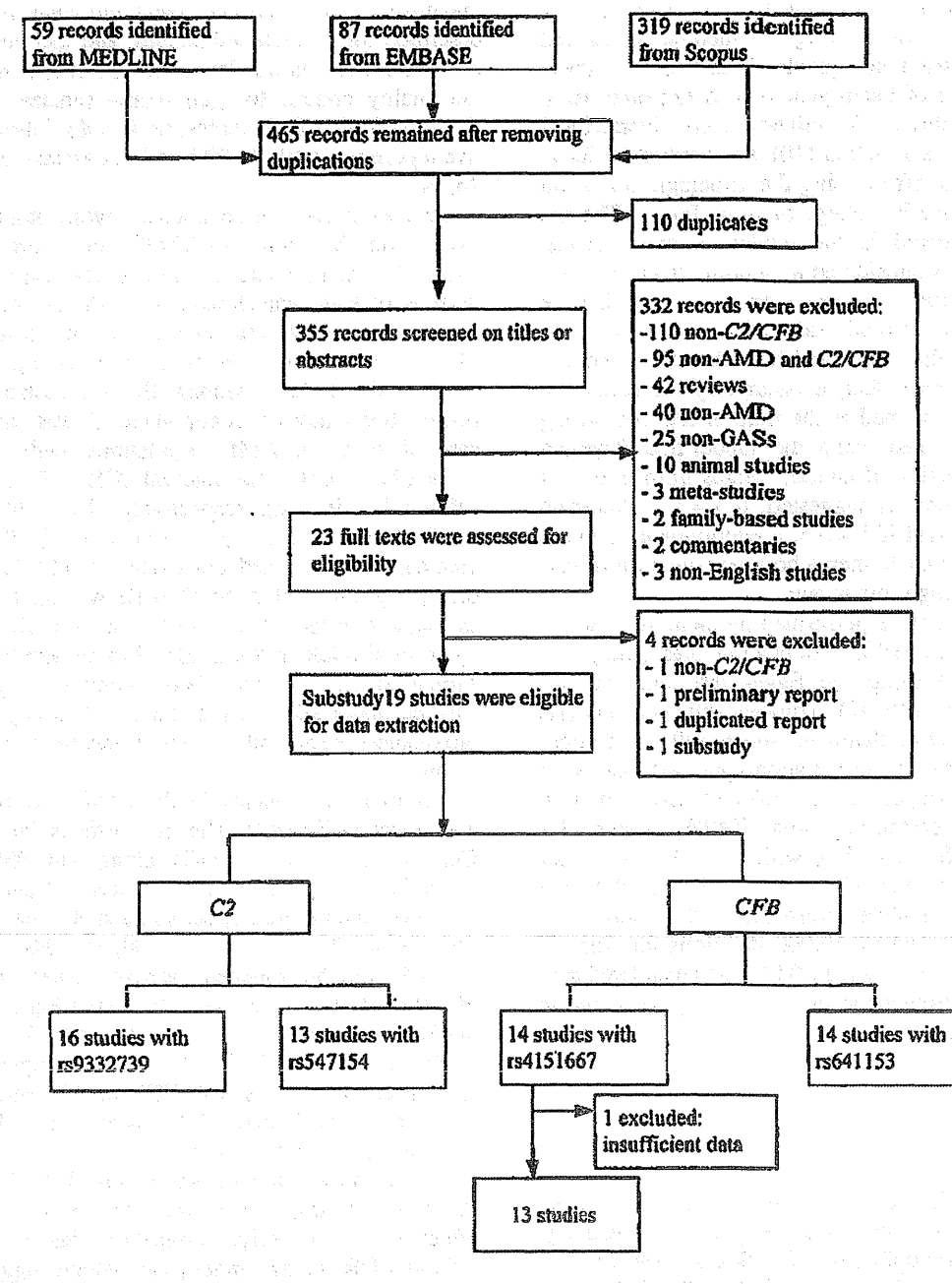

Figure 1. Selection of published studies (2006–2011) for a systematic review and meta-analysis of the association of complement component 2(*C2*)/complement factor B (*CFB*) gene polymorphisms with age-related macular degeneration (AMD). (GAS, genetic association study).

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 2(*C2*)/Complement Factor B (*CFB*) Gene Polymorphisms With Age-Related Macular Degeneration, 2006–2011[a]

First Author, Year (Reference No.)	Mean Age, years	% Male	% Smokers	Study Design	Type of Case	Type of Control
Maller, 2006 (13)	76.3	45.5		Case-control	Advanced AMD	Non-AMD
Gold, 2006 (32)	73.6			Matched case-control	AMD	Non-AMD
Spencer, 2007 (36)	73.7	38.8	57.6	Case-control	AMD grades 3–5	AMD grade 1-2
Chu, 2008 (56)	67.1	54.8		Matched case-control	Exudative AMD	Non-AMD
Jakobsdottir, 2008 (33)	76.2	43.7	43.3	Case-control	56%–66% GA and CNV	Non-AMD
Scholl, 2008 (26)	73.5	43.6	47.5	Case-control	69.6% CNV	Non-AMD
Bergeron-Sawitzke, 2009 (15)	65.4	45.7	46.5	Age-, sex-, and race-matched case-control	AMD grades 3–5	Non-AMD
Francis, 2009 (19)—AREDS				Cohort	GA/CNV	AMD grade 1
Francis, 2009 (19)—CEIMDC	76.7	33.2		Case-control	GA/CNV	Drusen, <63 μm in diameter
Farwick, 2009 (31)	70.9	40.4	40.2	Cross-sectional	30.5% advanced AMD	Non-AMD
Goto, 2009 (20)	73	54.8		Matched case-control	Advanced AMD	Non-AMD
Park, 2009 (22)				Cohort	Early and late (54.6%)	AMD grade 1
Pei, 2009 (23)	69.9	53	45.8	Age- and sex-matched case-control	CNV	Non-AMD
Reynolds, 2009 (24)		50	54.4	Case-control	Grade 4 (GA)/5(CNV) in one or both eyes	AMD grade 1 in both eyes
Richardson, 2009 (35)	73.1	34.7		Case-control	71.7% advanced AMD	Drusen, <63 μm in diameter
Seddon, 2009 (27)				Case-control	Advanced AMD	AMD grade 1
Kaur, 2010 (37)				Matched case-control		
Liu, 2010 (55)	64.2	45.4		Age-matched case-control	66.4% CNV and 33.6% drusen	Non-AMD
McKay, 2009 (34)	74.9	38.5		Age-matched case-control	GA/CNV	Non-AMD
Chen, 2011 (54)	77.1	43.8	35.7	Case-control	38% GA and 72% CNV	Non-AMD

Abbreviations: AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; CEIMDC, Casey Eye Institute Macular Degeneration Center; CNV, choroidal 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 al. (65).

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
Fariwick, 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.

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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")].