ONLINE FIRST

Risk Alleles in *CFH* and *ARMS2* and the Long-term Natural History of Age-Related Macular Degeneration

The Beaver Dam Eye Study

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Objective: To describe the relationships of risk alleles in complement factor H (*CFH*, rs1061170) and age-related maculopathy susceptibility 2 (*ARMS2*, rs10490924) to the incidence and progression of age-related macular degeneration (AMD) during a 20-year period.

Methods: There were 4282 persons aged 43 to 86 years at the baseline examination in 1988-1990 enrolled in a population-based cohort study who participated in at least 1 examination spaced 5 years apart during a 20-year period and had gradable fundus photographs for AMD and genotype information on *CFH* and *ARMS2*. Low, intermediate, and high genetic risk for AMD was defined by the presence of 0 to 1, 2, or 3 to 4 risk alleles for *CFH* and *ARMS2*, respectively. Multistate models were used to estimate the progression of AMD throughout the entire age range.

Results: There were 2820 (66%), 1129 (26%), and 333 persons (8%) with low, intermediate, and high genetic risk for AMD, respectively. The 5-year incidences of early and late AMD were 9.1% and 1.6%, respectively, and increased with age but did not differ significantly by sex. Using the multistate model, of persons aged 45 years with no AMD in the low, intermediate, and high AMD genetic risk groups, 33.0%, 39.9%, and 46.5%, respectively, were estimated to develop early AMD, and 1.4%, 5.2%, and 15.3% were estimated to develop late AMD by age 80 years.

Conclusions: These population-based data provide estimates of the long-term risk of the incidence and progression of AMD and its lesions by age and genetic risk alleles for *CFH* and *ARMS2*. They also show that when early AMD is present, knowing the phenotype contributes more to risk assessment than knowing the genetic risk based on these 2 AMD genes.

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Following the observations of the associations of specific single-nucleotide polymorphisms (SNPs) in the complement factor H region (*CFH*, rs1061170) and in the age-related maculopathy susceptibility 2 region (*ARMS2*, rs10490924) with late age-related macular degeneration (AMD), there has been a growing number of studies examining the relationships of these and other AMD candidate genes and their interactions with environmental and host risk factors.[1-21] Most of these investigations have been either clinical case series or case-control studies and have focused largely on late AMD. Few long-term studies have examined the relationships of these genetic risk factors along the continuum of the disease from its earliest to its most advanced stages.[2,22] The purpose of this report is to describe the relationships of age and *CFH* and *ARMS2* risk alleles to the incidence of AMD during a 20-year period, building on previous reports in the Beaver Dam Eye Study (BDES) cohort.[23-26]

METHODS

POPULATION

Methods used to identify the population and descriptions of the population in the BDES have appeared in previous reports.[27-31] A private census of the population of Beaver Dam, Wisconsin, was performed from fall 1987 to spring 1988.[27] There were 5924 eligible individuals, 4926 of whom participated in the examination phase between March 1, 1988, and September 15, 1990; 3721 participated in the 5-year follow-up examination phase between March 1, 1993, and June 15, 1995; 2962 participated in the 10-year follow-up examination phase between March 16, 1998, and June 9, 2000; 2375 participated in the 15-year follow-up exami-

nation phase between March 31, 2003, and June 1, 2005; and 1913 participated in the 20-year follow-up examination phase between November 5, 2008, and November 16, 2010. Ninety-nine percent of the population was white.

Approval for this study was granted by the institutional review board at the University of Wisconsin, Madison. Informed consent was obtained from each participant before every examination. The tenets of the Declaration of Helsinki were observed.

Comparisons between participants and nonparticipants at each examination have appeared elsewhere.[28-31] In general, those who participated at the 20-year follow-up were more likely to be younger than nonparticipants who were alive or those who died before follow-up and, with adjustment for age, were less likely to have AMD. The mean (SD) and median times between the baseline and 20-year follow-up examinations were 20.4 (0.6) years and 20.3 years.

PROCEDURES

Similar procedures were used at baseline and follow-up examinations.[23-26,32-37] A standardized interview and examination were administered at each visit. Information on demographic characteristics was obtained from the questionnaire used in the interview. Stereoscopic 30° color fundus photographs centered on the disc (Diabetic Retinopathy Study standard field 1) and macula (Diabetic Retinopathy Study standard field 2) and a nonstereoscopic color fundus photograph temporal to but including the fovea of each eye were obtained.

Details of the grading procedure have been described.[23,36,37] In brief, a circular grid was placed on 1 photographic slide of the stereoscopic pair, which divided the macular area into 9 subfields consisting of a central circle (a single subfield), inner ring (comprising the 4 inner subfields), and outer ring (comprising the 4 outer subfields). Some lesions were graded in each subfield, other lesions only in Diabetic Retinopathy Study field 2 as a whole, and still others in additional fields. For the purpose of this report, measurements made only within the 9 subfields defined by the grid are given. Circles of defined diameter (63, 125, 175, 250, 325, 350, and 650 μm) printed on clear acetate were used to estimate drusen size and areas involved by drusen, increased retinal pigment, and retinal pigment epithelial (RPE) depigmentation.

Two gradings were performed on photographs of each eye at each examination, with graders masked to any information about the fellow eye and the participant.[23-26,36,37] First, 1 of 2 senior graders (S.M.M.) performed a preliminary grading. Next, a detailed grading was performed by 1 of 3 other experienced graders. The assessment consisted of a subfield-by-subfield, lesion-by-lesion evaluation of each photograph set using the Wisconsin Age-Related Maculopathy Grading System.[36,37] Next, a series of edits and reviews was performed. The presence and severity of specific lesions of AMD (eg, maximum drusen size/type/area and pigmentary abnormalities) at the fifth examination as determined by detail grading were compared with those of the preliminary grading. Standardized edit rules were used to adjudicate disagreements.[23-26] Finally, the detail graders were asked to make side-by-side comparisons between the 15- and 20-year follow-up photographs randomly ordered so that photography dates were masked for eyes that showed changes for AMD lesions between these 2 examinations; in cases with no photographs at the 15-year examination, those from the next most recent examination were used for comparison. After this masked longitudinal review of 15- and 20-year photographs was complete, the senior grader (S.M.M.) and principal investigator (R.K.) performed a final unmasked review of all 5 visits for progression and regression. All new classification of late AMD was confirmed at this time. Additional information on gradability at previous examinations has been reported.[23-26]

GENETIC MEASUREMENTS

Samples of DNA were extracted from buffy coat specimens collected at the baseline examination. The 2 most common AMD-associated SNPs, Y402H in *CFH* (OMIM 134370 [general ID] and 610698 [AMD phenotype]) and A69S in *ARMS2* (OMIM 611313), were used in this study. The A69S variant was genotyped in 5188 individuals using 2 different platforms (Taqman, Applied Biosystems; and iSelect array, Illumina, Inc). Assays were performed at 2 separate times in 2248 and 2940 samples. Five hundred eighty-eight samples were genotyped with both platforms, with a genotype concordance rate of 99.7%. The genotype calls from each assay were combined to create a single data set for analysis. The Y402H variant was directly genotyped using a Taqman assay in 3015 samples in the BDES.[38,39] To increase the sample size for the Y402H variant, we used data imputation techniques (Markov chain haplotyping algorithm, version 1.032) on 2940 samples genotyped for 70 markers in the *CFH* region using a custom Illumina array. Using the surrounding linkage disequilibrium structure at *CFH*, we inferred genotypes at Y402H in both typed and untyped samples, keeping only genotypes that could be imputed with a high probability ($r^2 \geq 0.9$). A concordance rate of 99.8% was observed among 1476 samples for which genotyped and imputed data were available.[40]

DEFINITIONS

Age was documented at each participant visit and treated categorically in the following age groups: 43 to 54, 55 to 64, 65 to 74, 75 to 84, and 85 years or older. Individuals ranged in age from 43 to 86 years at the time of the baseline examination.

Three genetic risk groups were defined on the basis of distributions of late AMD by risk allele status. The groups were identified as follows: low genetic risk, persons with 0 to 1 risk allele (no risk alleles or 1 risk allele for either *CFH* or *ARMS2*); intermediate genetic risk, persons with 2 risk alleles (2 risk alleles for either *CFH* or *ARMS2* but none for the other or 1 risk allele for each); and high genetic risk, persons with 3 to 4 risk alleles (2 risk alleles for either *CFH* or *ARMS2* and at least 1 risk allele for the other, or 2 risk alleles for both).

The severity of AMD was determined using the 5-step BDES AMD Severity Scale (eFigure 1; http://www.archophthalmol.com). The definitions of each level are as follows.

- *10 (No AMD)*. Hard drusen or small soft drusen (<125 μm diameter) regardless of the area of involvement and no pigmentary abnormalities (defined as increased retinal pigment or RPE depigmentation present) or no definite drusen with any pigmentary abnormality.
- *20 (Minimally severe early AMD)*. Hard drusen or small soft drusen (<125 μm diameter) regardless of the area of involvement and with any pigmentary abnormality or soft drusen (≥125 μm diameter) with a drusen area of less than 331 820 μm^2 (equivalent to O_2, a circle with a diameter of 650 μm) and no pigmentary abnormalities.
- *30 (Moderately severe early AMD)*. Soft drusen (≥125 μm diameter) with a drusen area of less than 331 820 μm^2 (equivalent to O_2) and with any pigmentary abnormality or soft drusen (≥125 μm in diameter) with a drusen area of 331 820 μm^2 or greater (equivalent to O_2), with or without increased retinal pigment but no RPE depigmentation.
- *40 (Severe early AMD)*. Soft drusen (≥125 μm diameter) with drusen area of 331 820 μm^2 or greater (equivalent to O_2) and RPE depigmentation present, with or without increased retinal pigment.

• *50 (Late AMD)*. Pure geographic atrophy in the absence of exudative macular degeneration or exudative macular degeneration, with or without geographic atrophy.

As noted, there were additional severity classifications based on scales for retinal drusen size, drusen type, and pigmentary abnormalities. Details of these scales appear in eFigures 3-5.

Persons at risk for developing early AMD were those without any lesion defining early or late AMD at baseline or the beginning of a 5-year examination interval. Incidence of early AMD in the worse eye was defined by developing level 20, 30, or 40 in at least 1 eye when both eyes had been level 10 at the previous examination. When 1 eye was ungradable, it was assumed to have the same AMD level as the fellow eye. Incidence was determined for the presence of signs of early AMD, such as large drusen (≥125 μm diameter), drusen type (soft indistinct/reticular), and pigmentary abnormalities. The incidence of a specific lesion was defined by its presence at follow-up when it was not present at the previous examination in any of the subfields. Similarly, persons at risk for developing late AMD (level 50) were those without late AMD at the beginning of a 5-year examination interval who developed late AMD in 1 or both eyes at follow-up.

The progression or regression of AMD during the 20-year period was analyzed in the worse eye and evaluated using a multistate model (MSM). Progression of AMD was defined for an individual as either eye transitioning to a more severe AMD level, and regression of AMD was defined as either eye transitioning to a less severe AMD level. In the MSM, regression from level 50 to less severe levels is not believed to be biologically possible and, therefore, was not included in the model. Progression and regression along the pigment, drusen type, and drusen size scales were defined similarly (eFigures 3-5) using data from the right eye. For most analyses, age and other characteristics were defined at the beginning of an examination interval.

STATISTICAL ANALYSIS

Incidence analyses were conducted using χ^2 tables with commercial software (SAS, version 9.2; SAS Institute Inc). The incidence of AMD was calculated for each 5-year period and accumulated during the 20 years of the study. The values of the incident outcomes were updated for each consecutive 5-year period. Once a person developed an incident outcome, their data were no longer considered for analysis. Relationships were further stratified by age group and genetic risk. Incidence was evaluated in the worse eye in all analyses.

Next, MSM analyses were conducted in R[41] using the msm package (http://www.jstatsoft.org/v38/i08).[42] Covariate effects on transition intensities were summarized as hazard ratios. Using matrix exponentiation, we obtained annual transition matrices for each initial state, age, sex, and genetic risk group as described in the "Definitions" subsection. From these transition matrices, we calculated estimated transition probabilities to each drusen, pigment, or AMD state (and death) after 5 years and the cumulative incidence of each AMD state by genetic risk group. Cumulative incidence calculations were based on model estimates, not individual data. Estimated cumulative incidence calculations used annual assessments of AMD status; participants were assigned to the most severe AMD state observed at or before their current age. Confidence intervals for these nonlinear functions of the transition intensity variables were obtained from a parametric bootstrap.[43] Transitions along the drusen size and type and pigment scales were analyzed in the right eye, and transitions along the AMD severity scale were analyzed in the worse eye.

Population-attributable risk was defined as the portion of the incidence of a disease in the population that is due to exposure and was calculated by subtracting the incidence in the unexposed group (no risk alleles) from the incidence in the total population. Change in the area under the receiver operating characteristic curve (AUC) was used to measure improvement in prediction when traditional AMD risk factors and *CFH* and *ARMS2* were added to the model based on AMD severity. The AUC was calculated for each set of predictors using the receiver operating characteristic curve statement (Proc Logistic; SAS Institute Inc) and was plotted (ODS Graphics statement; SAS Institute Inc).

RESULTS

THE COHORT

There were 4362 individuals who participated in at least 1 BDES examination and had genotype data for *CFH* and *ARMS2*; 4282 of these participants had at least 1 eye gradable for AMD lesions during at least 1 BDES examination. Person-specific analyses were based on the worse eye at each interval. Reliable AMD data for at least 1 eye were available for 13 721 person-visits: 4232 at baseline, 3217 at the 5-year follow-up, 2565 at the 10-year follow-up, 2068 at the 15-year follow-up, and 1639 at the 20-year follow-up. eFigure 2 shows the number of individuals who were included in the analyses at each pair of examinations. There were 4270, 4262, and 4266 unique individuals in whom the right eye was gradable for transitions along scales of retinal pigment (eFigure 3), drusen size (eFigure 4), and drusen type (eFigure 5), respectively, and 4282 unique individuals in whom the worse eye was gradable for transitions along the 5-step AMD scale (eFigure 1).

INCIDENCE AND RATE OF PROGRESSION RELATIONSHIPS

The overall 5-year incidence of early AMD during the 20-year period was 9.1% and for late AMD was 1.6%. There were 2820 (66%), 1129 (26%), and 333 (8%) persons with low, intermediate, and high genetic risk for AMD, respectively. The genotype distribution for *CFH* was TT, 39.4%; TC, 46.8%; and CC, 13.8%; for *ARMS2* it was GG, 60.3%; GT, 35.0%; and TT, 4.7%. The incidence of early and late AMD increased with increasing number of risk alleles for *CFH* and *ARMS2* (**Table 1**).

The 5-year incidence of early and late AMD and specific AMD lesions increased with age during the 20-year period (**Table 2**). The 5-year incidence of early AMD was 8.4%, 9.7%, and 14.0% and for late AMD was 0.9%, 2.1%, and 5.9% for low, intermediate, and high genetic risk groups, respectively. With adjustment for age, there were associations of increasing genetic risk with all incident AMD outcomes.

CUMULATIVE INCIDENCE OF AMD IN TWO AGE GROUPS AND GENETIC RISK

Figure 1 shows the MSM-determined estimated cumulative incidence of increasing severity of early AMD (level 20 to 40) and late AMD (level 50) through age 100 years based on assessments of AMD severity state for persons free of AMD at ages 45 and 65 years for the 3 genetic risk groups.

Table 1. Five-Year Incidence of Early and Late AMD by *CFH* and *ARMS2* Genotype in the Beaver Dam Eye Study, 1988-2010

	CFH Genotype, No. at Risk (% Event)					
	Early AMD			Late AMD		
ARMS2 Genotype	TT	CT	CC	TT	CT	CC
GG	1784 (7.3)	1873 (9.4)	558 (11.1)	2184 (0.5)	2432 (1.4)	718 (1.1)
GT	886 (8.5)	1168 (8.9)	341 (11.4)	1145 (0.7)	1520 (2.4)	441 (3.4)
TT	122 (11.5)	80 (18.8)	28 (32.1)	160 (3.6)	48 (12.1)	55 (10.9)

Abbreviations: AMD, age-related macular degeneration; *ARMS2*, age-related maculopathy susceptibility 2; *CFH*, complement factor H.

Table 2. Incidence of AMD Outcomes by Age and Genetic Risk in the Beaver Dam Eye Study, 1988-2010

			Age, y									
	Overall		43-54		55-64		65-74		75-84		≥85	
Outcome and Genetic Risk[a]	No. at Risk	Events, No. (%)	No. at Risk	Events, No. (%)	No. at Risk	Events, No. (%)	No. at Risk	Events, No. (%)	No. at Risk	Events, No. (%)	No. at Risk	Events, No. (%)
Early AMD												
Overall	6840	624 (9.1)	1567	62 (4.0)	2503	147 (5.9)	2018	251 (12.4)	700	143 (20.4)	52	21 (40.4)
Low	4543	381 (8.4)	998	40 (4.0)	1621	76 (4.7)	1383	155 (11.2)	502	96 (19.1)	39	14 (35.9)
Intermediate	1848	180 (9.7)	458	19 (4.2)	704	45 (6.4)	509	74 (14.5)	166	37 (22.3)	11	5 (45.5)
High	449	63 (14.0)	111	3 (2.7)	178	26 (14.6)	126	22 (17.5)	32	10 (31.3)	2	2 (100.0)
Drusen size >125 μm												
Overall	7396	646 (8.7)	1648	56 (3.4)	2680	153 (5.7)	2187	261 (11.9)	813	153 (18.8)	68	23 (33.8)
Low	4887	386 (7.9)	1047	39 (3.7)	1717	74 (4.3)	1483	152 (10.3)	588	105 (17.9)	52	16 (30.8)
Intermediate	2011	189 (9.4)	485	14 (2.9)	765	52 (6.8)	562	83 (14.8)	185	35 (18.9)	14	5 (35.7)
High	498	71 (14.3)	116	3 (2.6)	198	27 (13.6)	142	26 (18.3)	40	13 (32.5)	2	2 (100.0)
Soft indistinct drusen												
Overall	7627	473 (6.2)	1664	33 (2.0)	2768	98 (3.5)	2275	194 (8.5)	845	129 (15.3)	75	19 (25.3)
Low	5017	269 (5.4)	1058	21 (2.0)	1766	46 (2.6)	1535	109 (7.1)	602	80 (13.3)	56	13 (23.2)
Intermediate	2091	149 (7.1)	487	10 (2.1)	798	32 (4.0)	591	68 (11.5)	198	35 (17.7)	17	4 (23.5)
High	519	55 (10.6)	119	2 (1.7)	204	20 (9.8)	149	17 (11.4)	45	14 (31.1)	2	2 (100.0)
Any pigmentary abnormality												
Overall	7893	548 (6.9)	1638	33 (2.0)	2755	105 (3.8)	2436	206 (8.5)	966	176 (18.2)	98	28 (28.6)
Low	5207	315 (6.1)	1046	18 (1.7)	1776	55 (3.1)	1638	113 (6.9)	678	113 (16.7)	69	16 (23.2)
Intermediate	2132	164 (7.7)	475	11 (2.3)	775	31 (4.0)	627	71 (11.3)	232	43 (18.5)	23	8 (34.8)
High	554	69 (12.5)	117	4 (3.4)	204	19 (9.3)	171	22 (12.9)	56	20 (35.7)	6	4 (66.7)
Late AMD												
Overall	8814	140 (1.6)	1741	2 (0.1)	2992	9 (0.3)	2745	47 (1.7)	1204	66 (5.5)	132	16 (12.1)
Low	5772	52 (0.9)	1109	1 (0.1)	1908	0	1832	12 (0.7)	832	30 (3.6)	91	9 (9.9)
Intermediate	2398	50 (2.1)	509	0	851	5 (0.6)	710	21 (3.0)	296	20 (6.8)	32	4 (12.5)
High	644	38 (5.9)	123	1 (0.8)	233	4 (1.7)	203	14 (6.9)	76	16 (21.1)	9	3 (33.3)
Pure geographic atrophy												
Overall	8775	49 (0.6)	1743	0	2992	3 (0.1)	2730	15 (0.6)	1179	22 (1.9)	131	9 (6.9)
Low	5770	21 (0.4)	1109	0	1910	0	1832	4 (0.2)	826	11 (1.3)	93	6 (6.5)
Intermediate	2383	17 (0.7)	512	0	851	1 (0.1)	704	9 (1.3)	286	6 (2.1)	30	1 (3.3)
High	622	11 (1.8)	122	0	231	2 (0.9)	194	2 (1.0)	67	5 (7.5)	8	2 (25.0)
Exudative AMD												
Overall	8867	91 (1.0)	1742	2 (0.1)	2998	6 (0.2)	2753	32 (1.2)	1234	44 (3.6)	140	7 (5.0)
Low	5798	31 (0.5)	1109	1 (0.1)	1911	0	1835	8 (0.4)	846	19 (2.3)	97	3 (3.1)
Intermediate	2416	33 (1.4)	510	0	854	4 (0.5)	713	12 (1.7)	306	14 (4.6)	33	3 (9.1)
High	653	27 (4.1)	123	1 (0.8)	233	2 (0.9)	205	12 (5.9)	82	11 (13.4)	10	1 (10.0)

Abbreviation: AMD, age-related macular degeneration.
[a] Outcomes were determined using the worse eye. Genetic risk groups: low, 0-1 risk alleles; intermediate, 2 risk alleles; and high, 3-4 risk alleles.

For individuals in the low genetic risk group who were free of AMD at age 45 years (Figure 1A), the cumulative incidence of late AMD (level 50) was estimated to increase to 9.9% and the cumulative incidence of early AMD to 41.6% by age 100 years. The estimated cumulative incidences were increasingly higher at a given age for early and late AMD in the intermediate- and high-risk groups (Figure 1B and C compared with Figure 1A). At age 80 years, the estimated cumulative incidence of late AMD in individuals with no AMD at age 45 years was 1.4% (Figure 1A), 5.2% (Figure 1B), and 15.3% (Figure 1C) in the low-, intermediate-, and high-risk groups, respectively.

The estimated cumulative incidence of early and late AMD was smaller in individuals who survived to age 65 years without signs of AMD than in individuals who had no signs of AMD at age 45 years (Figure 1D compared with A, E compared with B, and F compared with C). For example, in persons with no evidence of early AMD at ages 45 and 65 years, the estimated cumulative incidence of late AMD by age 80 years in those with high genetic risk was 15.3% (Figure 1C) and 7.9% (Figure 1F), respectively. For those aged 45 and 65 years with no evidence of AMD and low genetic risk, the estimates for developing late AMD were lower (1.7% [Figure 1A] and 0.6% [Figure 1D], respectively).

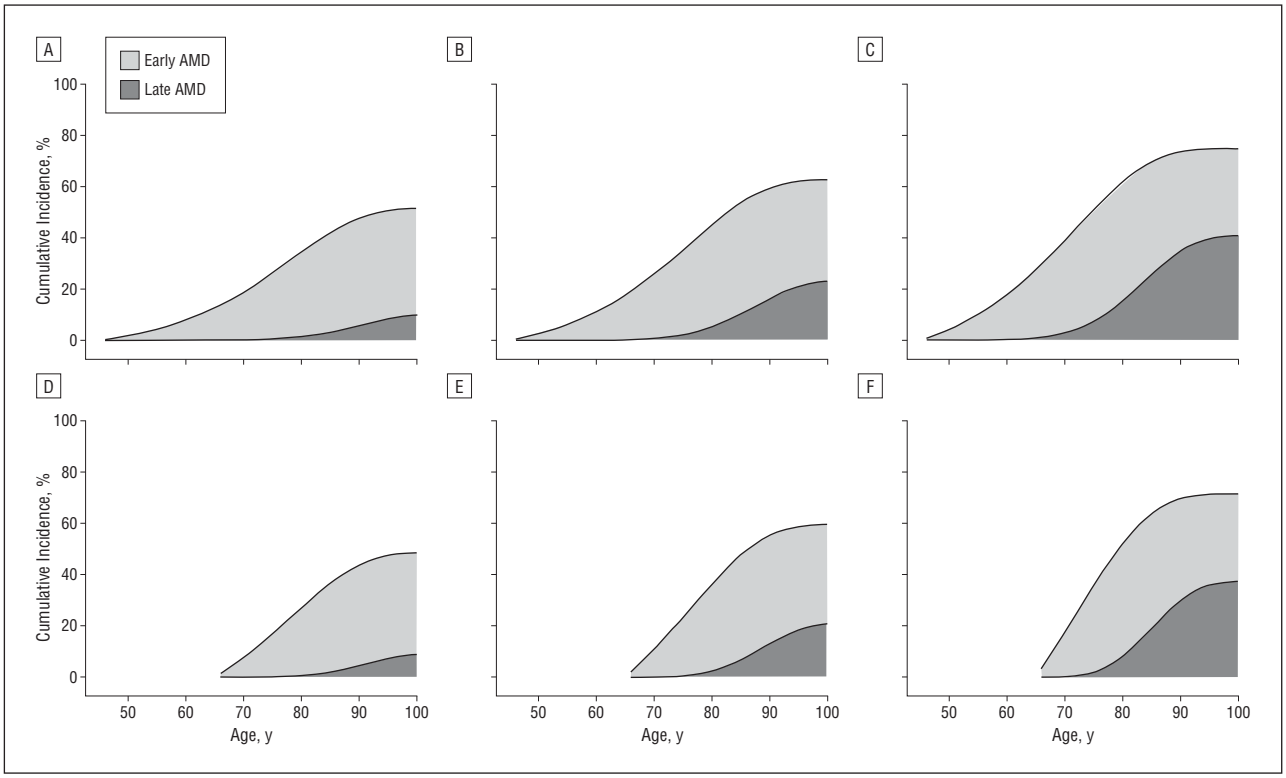

Figure 1. Estimated cumulative incidence of early (height of light gray area) and late (height of dark gray area) age-related macular degeneration (AMD) by genetic risk and age (assuming no AMD at the starting age) in the Beaver Dam Eye Study, 1988-2010. Rows are ordered by increasing starting age (top, age 45 years; bottom, age 65 years), and columns are ordered by increasing genetic risk. A and D, Low risk. B and E, Intermediate risk. C and F, High risk. For example, A shows the cumulative incidence of AMD in individuals assuming no AMD at age 45 years and low genetic risk, and E shows the cumulative incidence of AMD in individuals assuming no AMD at age 65 years and intermediate genetic risk.

CUMULATIVE INCIDENCE OF LATE AMD WITH INCREASING SEVERITY OF AMD BY GENETIC RISK

Figure 2 shows the estimated cumulative incidence of more severe stages of AMD in persons who, at age 45 years, had AMD levels 10, 20, 30, and 40. Individuals with low genetic risk who had early AMD at age 45 years were estimated to have a higher cumulative incidence of late AMD by age 80 years compared with individuals without AMD at age 45 years (Figure 2D, G, and J compared with A). This was also true for the intermediate-risk (Figure 2E, H, and K compared with B) and high-risk (Figure 2F, I, and L compared with C) groups. In those in the low genetic risk group at age 45 years, 1.4%, 7.0%, 15.0%, and 33.4% of individuals with AMD levels 10 (Figure 2A), 20 (Figure 2D), 30 (Figure 2G), and 40 (Figure 2J), respectively, were estimated to have developed late AMD by age 80 years.

CUMULATIVE INCIDENCE OF AMD WITH INCREASING SEVERITY OF DRUSEN SIZE, TYPE, AND PIGMENTARY ABNORMALITIES BY INCREASING GENETIC RISK

Figure 3 shows the MSM estimates of cumulative incidence of increasing size and severity of retinal drusen and increasing severity of pigmentary abnormalities in individuals with low, intermediate, and high genetic risk who were free from those lesions at age 45 years. For example, Figure 3A shows that the estimated cumulative incidence of intermediate-sized drusen (≥63 to <125 μm diameter, height of area shaded light gray) was 23.8%, 24.4%, and 23.3%, and large drusen (≥125 μm diameter, height of areas shaded medium and dark gray) was 11.0%, 15.8%, and 18.6% at age 80 years in individuals with low (Figure 3A), intermediate (Figure 3B), and high (Figure 3C) genetic risk, respectively, who had no or small drusen at age 45 years. Figure 3D-F shows that the estimated cumulative incidence of hard distinct drusen (height of the light gray area) was 68.3%, 57.6%, and 43.6%; for soft distinct drusen (height of the medium gray area) it was 13.6%, 15.4%, and 17.0%; and for soft indistinct/reticular drusen (height of the dark gray area) it was 7.3%, 13.4%, and 18.8% at age 80 years in individuals with low (Figure 3D), intermediate (Figure 3E), and high (Figure 3F) genetic risk, respectively, who had no or hard indistinct drusen at age 45 years. Because individuals with higher genetic risk are more likely to develop a more severe drusen type, there is an inverse association of genetic risk groups with the cumulative incidence of hard distinct drusen at age 80 years (Figure 3D-F). Figure 3G-I shows the estimated cumulative incidence of increased retinal pigment was 13.9%, 21.4%, and 28.9%; for RPE depigmentation it was 1.8%, 3.5%, and 8.1%; and for geographic atrophy it was 0.1%, 0.4%, and 1.8% at age 80 years in individuals with low (Figure 3G), intermediate (Figure 3H), and high (Figure 3I) genetic risk, respectively, who had no pigmentary abnormalities at age 45 years.

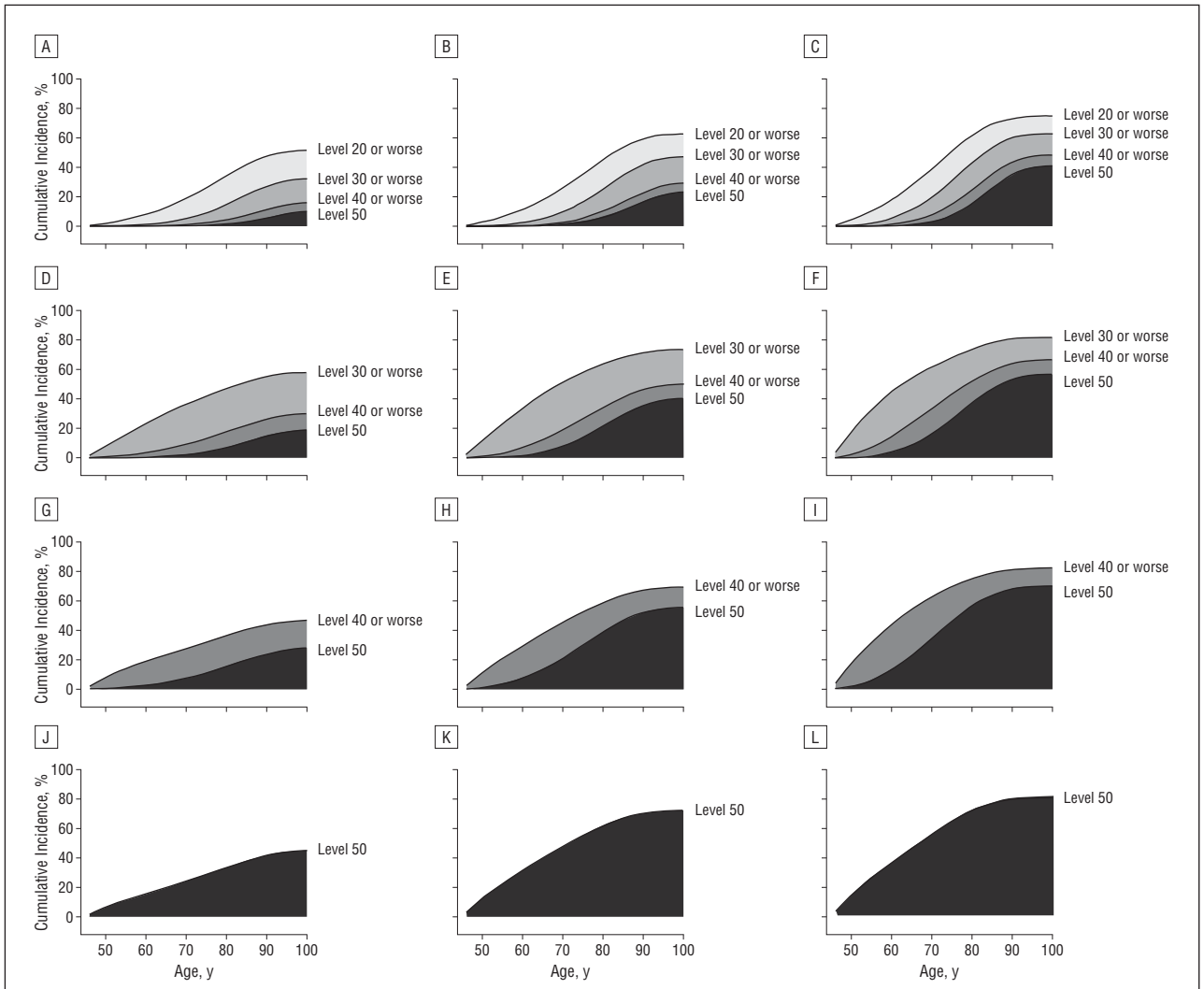

Figure 2. Estimated cumulative incidence of more severe age-related macular degeneration (AMD) in persons at age 45 years by increasing beginning AMD level (rows top [level 10] to bottom [level 40]) and increasing genetic risk (columns left [low] to right [high]) in the Beaver Dam Eye Study, 1988-2010. For example, A shows the cumulative incidence of AMD levels 20 to 50 (20 indicates height of light gray area; 30, height of medium gray area; 40, height of dark gray area; and 50, height of black area) for an individual whose AMD level is 10 at age 45 years and who has low genetic risk, and panel I shows the cumulative incidence of AMD levels 40 and 50 for an individual who had AMD level 30 at age 45 years and who has high genetic risk. Numbers to the right of lines indicate AMD severity level on the Beaver Dam Study AMD severity scale.

POPULATION-ATTRIBUTABLE RISK AND PROGNOSTIC ASSESSMENT

The estimated population-attributable risk fraction for early and late AMD was 9.6% and 53.2%, respectively, when at least 1 *CFH* risk allele was present and 5.0% and 43.0% when at least 1 *ARMS2* risk allele was present.

The AUC for progression from no or early AMD to late AMD for a model that included only AMD severity level was 0.9316 (**Figure 4**). Adding traditional risk factors (eg, age, sex, history of smoking, hypertension, history of physical activity, and history of multivitamin use) showed an incremental gain of 0.0280 and a further incremental gain of 0.0066 with the addition of *CFH* and *ARMS2* to the model. The *ARMS2* and *CFH* genes added a small incremental gain after AMD severity level and traditional risk factors had been added to a model with geographic atrophy (0.0019) or exudative AMD (0.0099) as the end point.

COMMENT

We examined the relationships between genetic risk, defined by the number of allelic variants of SNPs of 2 AMD candidate genes, *CFH* (rs1061170) and *ARMS2* (rs10490924), and the estimated cumulative incidence and progression of AMD in the population-based BDES cohort during a 20-year period. Using MSMs, the estimated cumulative incidences of early AMD at age 80 years in persons without AMD at age 45 years in the genetically low, intermediate, and high risk groups were 33.0%, 39.9%, and 46.5%, respectively, and for late AMD were 1.4%, 5.2%, and 15.3%.

Genetic risk group status was directly associated with the incidence of more severe drusen type, large drusen, and pigmentary abnormalities. The association of genetic risk with late AMD (odds ratio per increasing genetic risk group, 2.93) was stronger than for early AMD (odds ratio, 1.38).

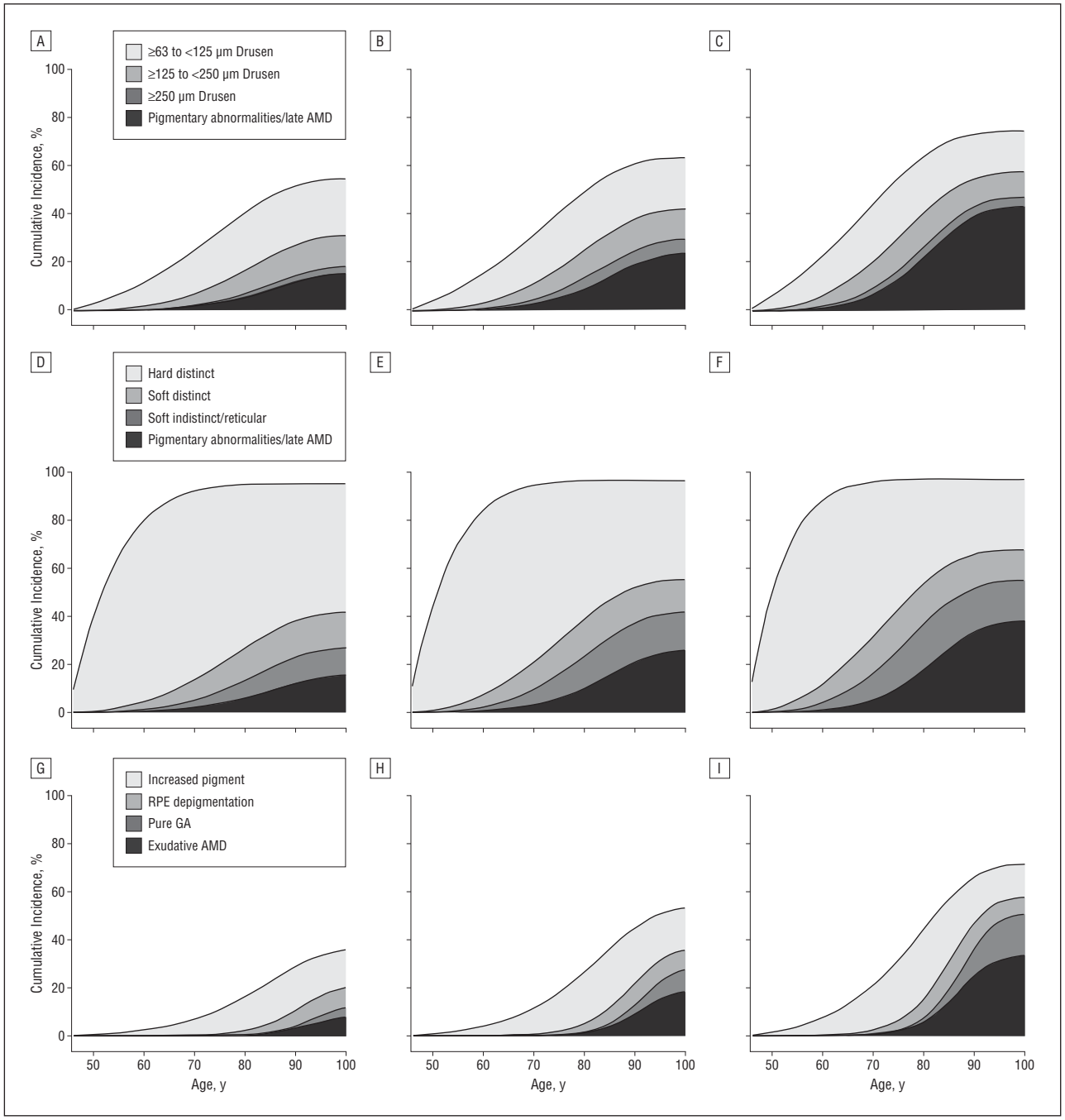

Figure 3. Estimated cumulative incidence of age-related macular degeneration (AMD) by increasing genetic risk level (from low [left] to intermediate [middle] to high [right]). A-C, Intermediate-sized and large drusen in individuals with no or small drusen at age 45 years. D-F, Hard distinct, soft distinct, and soft indistinct/reticular drusen in individuals with no or hard indistinct drusen at age 45 years. G-I, Increased retinal pigment, retinal pigment epithelial (RPE) depigmentation, pure geographic atrophy (GA), and exudative AMD in individuals with no pigmentary abnormalities at age 45 years.

The population-attributable risk for late AMD was 53% when at least 1 *CFH* risk allele was present and 43% when at least 1 *ARMS2* risk allele was present.

Using 20 years of BDES data and MSMs, we estimated the cumulative incidence for developing late AMD in persons aged 45 years without AMD who survive to age 80 years, the current estimated life expectancy for a 45-year-old in the United States, to vary from 1.4% in persons with low genetic risk to 15.3% in those with high genetic risk. These findings are consistent with those of virtually all earlier studies[1-26,44-48] showing a strong association of increasing age and genetic risk with high long-term incidence of AMD. The relatively high estimated overall cumulative incidence in persons with high genetic risk and the availability of preventive approaches have led others[2,22,49,50] to develop risk assessment models based on genetic and environmental exposures. The rationale for such screening is that earlier detection of individuals at high risk of developing late AMD may lead to changes in behaviors, such as cessation of smoking, changes in diet (eg, eating more foods containing omega-3 fatty acids and more leafy green vegetables), and in-

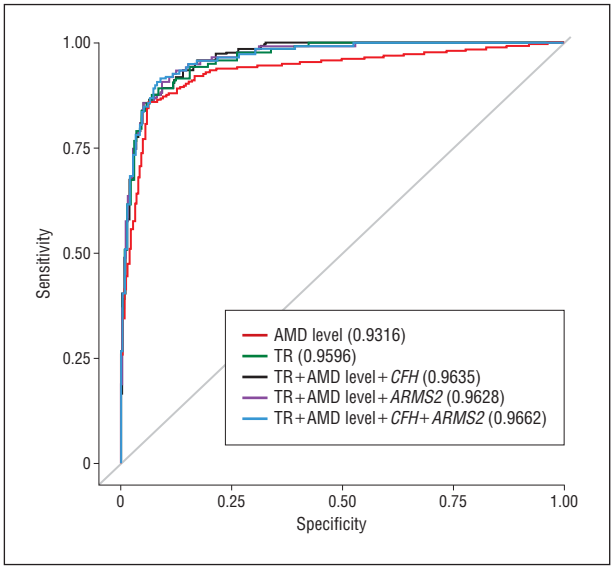

Figure 4. Area under the receiver operating characteristic curves (AUC) for various models of progression from no or early age-related macular degeneration (AMD) to late AMD during a 5-year interval in the Beaver Dam Eye Study, 1988-2010. *ARMS2* indicates age-related maculopathy susceptibility 2; *CFH*, complement factor H; and TR, traditional risk.

creased physical activity levels that might, in part, prevent or reduce the incidence and progression of AMD. For example, data from a pilot study[51] showed that smokers reported they would be more likely to quit smoking if told they were at high genetic risk of developing late AMD. However, in the BDES, smoking status has not proven to be a risk factor for the cumulative incidence of late AMD,[52] and to date, it has not been demonstrated that interventions to alter behaviors (eg, change in diet, smoking cessation) affect the incidence of late AMD.

We have demonstrated the effects of the 2 most common, and arguably strongest, genetic correlates of AMD in this study; however, approximately 80% of individuals at high genetic risk for late AMD are estimated to be free of this level of severity of disease if they live until age 80 years. For every 1000 persons in the cohort screened for genetic risk, only 78 persons aged 45 years would be expected to have the high-risk genotype and, of those, only 12 are estimated to develop late AMD if they live to age 80; 66 will not. Thus, data from our study and others suggest that such screening using genotyping in young persons without early AMD is not indicated.[53] Once early AMD is present, defining genetic risk using *CFH* and *ARMS2* status, although statistically significantly contributing to the prediction of the risk for late AMD, the addition is small and contributes little beyond that of knowing the phenotypic stage of the disease (Figure 4). It has also been shown by Klein and colleagues,[22] using data from the Age-Related Eye Disease Study, that genotyping adds little to the AUC analyses beyond that of knowing the phenotype once signs of early AMD are present.

Genetic risk of the 2 candidate SNPs was shown for early and late AMD lesions. Candidate SNPs from *CFH* and *ARMS2* have consistently shown stronger associations with exudative AMD and geographic atrophy than with signs of early AMD.* Our results are consistent with cross-sectional findings from the Blue Mountains Eye Study,[63] the Rotterdam Study,[55] and the ALIENOR (Antioxydants, Lipides, Essentiels, Nutrition et maladies Oculaires) study,[54] in which persons who were homozygous for the *CFH* variant had increased odds, ranging from 1.2 to 2.4 for early AMD compared with no AMD. In addition, in the Rotterdam Study, the association became stronger with increasing severity of early AMD. Our results are also consistent with the findings of Yu and colleagues,[2] who used a similar MSM in data from 2560 patients without late AMD in the Age-Related Eye Disease Study and found that *CFH* and *ARMS2* were related to the development of large drusen but not intermediate-sized drusen (≥63 to <125 μm diameter). They did not examine the relationship of these genes to pigmentary abnormalities.

The reason for the weaker relationship of genetic risk to early than to late AMD is not understood. Delcourt and colleagues[54] speculated that the weaker odds of approximately 2 found between individuals homozygous for the C allele of the *CFH* Y402H polymorphism and early AMD compared with odds of 16 to 23 for late AMD may be the result of lesions considered to be specific for early AMD being a heterogeneous group of abnormalities, some of which bear a very low risk for late AMD. Another possibility is that environmental factors may have more effect on the lesions now considered to be characteristic of early AMD compared with late AMD.

The biological mechanisms of the *CFH* gene in the pathogenesis of AMD have been well described and include changes in *CFH* protein levels that lead to alterations in the regulation of complement activation in response to inflammation. The *CFH* gene also affects the metabolism of lipids, such as malondialdehyde, that accumulate in response to oxidative stress and are thought to contribute to RPE cell death.[67] The role of *ARMS2* is also uncertain. It may work through different mechanisms, such as stabilization of the extracellular matrix in the Bruch's membrane and in protecting against oxidative stress.[68-70] Smailhodzic and colleagues[71] reported complement deregulation to be associated not only with *CFH* high-risk alleles but also with *ARMS2* high-risk alleles, suggesting that *ARMS2* may be involved in the activation of the complement system. We did not find an interaction between *CFH* and *ARMS2* and incident early AMD (R.K., unpublished data, March 2012), and our study was underpowered to examine such an interaction for late AMD.

We used the MSM in the reported analyses in this article, which permits staged modeling of AMD progression, incorporating all facets of the natural history of AMD as well as death into a single, biologically plausible model rather than modeling aspects of the disease process in isolation. It uses a biological meaningful time scale (participant age) rather than an artificial time scale (time of study) and incorporates time-varying covariates by updating covariate values at observation times. The MSM accounts for the correlation between past outcomes and future outcomes by conditioning on the current state (the Markov assumption). That is, a participant's AMD state at the next scheduled visit given the current AMD state is assumed to be independent of the history of AMD. This assumption is fairly standard in survival analysis applications (key for the Cox regression model). An important advantage of the MSM over the Cox model is the ability to more fully use the available information on AMD progression. The primary dis-

*References 1-5, 10-16, 18-22, 39, 49-51, 53-66.

advantage of the MSM is computational complexity; the assumptions underlying the MSM are either the same or less restrictive than the alternatives.

Caution should be observed in interpreting our data. First, we used SNPs from only 2 AMD candidate genes; it is possible that inclusion of all the identified possible loci, many of which are likely to be unknown, might have influenced our findings. Second, the racially/ethnically homogeneous population (99.6% white) limits our inferences. Third, power to examine relationships for some infrequent end points (eg, reticular drusen, geographic atrophy, and exudative AMD) was limited, as well as our ability to assess the possibility of gene × gene and gene × age interactions. Mortality may limit the interpretation of associations because of selective survival. On the other hand, the BDES has many strengths, including repeated examinations during a 20-year period using standardized detailed procedures for obtaining stereoscopic color fundus photographs of the macula and an objective system for grading those photographs for AMD phenotypes.

This report provides long-term population-based observations regarding the relationships of genetic risk as defined by the number of *CFH* and *ARMS2* risk alleles and age with the natural history of AMD from its earliest to its latest stages. The high incidence of AMD at older ages and increased survival suggest a growing burden of this disease. The value of risk assessment will be determined as the pathogenesis of the disease becomes better understood and new evidence emerges to support cost-effective interventions before onset or at the earliest stages of the disease. For now, knowing the phenotype when early AMD is present contributes more to risk assessment than knowing the genetic risk based on the 2 AMD candidate genes with the largest attributable risk.

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REFERENCES

1. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. 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.
2. Yu Y, Reynolds R, Rosner B, Daly MJ, Seddon JM. Prospective assessment of genetic effects on progression to different stages of age-related macular degeneration using multistate Markov models. *Invest Ophthalmol Vis Sci*. 2012;53(3):1548-1556.
3. Edwards AO, Ritter R III, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308(5720):421-424.
4. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308(5720):419-421.
5. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308(5720):385-389.
6. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. *Eur J Hum Genet*. 2009;17(1):100-104.
7. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. *Nat Genet*. 2007;39(10):1200-1201.
8. Gold B, Merriam JE, Zernant J, et al; AMD Genetics Clinical Study Group. 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.
9. Nozaki M, Raisler BJ, Sakurai E, et al. Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci U S A*. 2006;103(7):2328-2333.
10. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am J Hum Genet*. 2005;77(3):389-407.
11. Fisher SA, Abecasis GR, Yashar BM, et al. Meta-analysis of genome scans of age-related macular degeneration. *Hum Mol Genet*. 2005;14(15):2257-2264.
12. Weeks DE, Conley YP, Tsai HJ, et al. Age-related maculopathy: a genomewide scan with continued evidence of susceptibility loci within the 1q31, 10q26, and 17q25 regions. *Am J Hum Genet*. 2004;75(2):174-189.
13. Iyengar SK, Song D, Klein BE, et al. Dissection of genomewide-scan data in extended families reveals a major locus and oligogenic susceptibility for age-related macular degeneration. *Am J Hum Genet*. 2004;74(1):20-39.
14. Seddon JM, Santangelo SL, Book K, Chong S, Cote J. A genomewide scan for age-related macular degeneration provides evidence for linkage to several chromosomal regions. *Am J Hum Genet*. 2003;73(4):780-790.
15. Majewski J, Schultz DW, Weleber RG, et al. Age-related macular degeneration—a genome scan in extended families. *Am J Hum Genet*. 2003;73(3):540-550.
16. Yu Y, Reynolds R, Fagerness J, Rosner B, Daly MJ, Seddon JM. Association of variants in the *LIPC* and *ABCA1* genes with intermediate and large drusen and advanced age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52(7):4663-4670.
17. Seddon JM, Reynolds R, Rosner B. Associations of smoking, body mass index, dietary lutein, and the *LIPC* gene variant rs10468017 with advanced age-related macular degeneration. *Mol Vis*. 2010;16:2412-2424.
18. 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.
19. Schaumberg DA, Hankinson SE, Guo Q, Rimm E, Hunter DJ. A prospective study of 2 major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors. *Arch Ophthalmol*. 2007;125(1):55-62.
20. Schmidt S, Hauser MA, Scott WK, et al. Cigarette smoking strongly modifies the association of *LOC387715* and age-related macular degeneration. *Am J Hum Genet*. 2006;78(5):852-864.
21. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical *LOC387715* is a second major susceptibility gene for age-related macular degeneration, contributing inde-

pendently of complement factor H to disease risk. *Hum Mol Genet*. 2005;14 (21):3227-3236.
22. Klein ML, Francis PJ, Ferris FL III, Hamon SC, Clemons TE. Risk assessment model for development of advanced age-related macular degeneration. *Arch Ophthalmol*. 2011;129(12):1543-1550.
23. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 1992;99(6):933-943.
24. Klein R, Klein BE, Jensen SC, Meuer SM. The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 1997;104(1):7-21.
25. Klein R, Klein BE, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology*. 2002;109(10):1767-1779.
26. Klein R, Klein BE, Knudtson MD, Meuer SM, Swift M, Gangnon RE. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology*. 2007;114(2):253-262.
27. Linton KL, Klein BE, Klein R. The validity of self-reported and surrogate-reported cataract and age-related macular degeneration in the Beaver Dam Eye Study. *Am J Epidemiol*. 1991;134(12):1438-1446.
28. Klein R, Klein BE, Linton KL, De Mets DL. The Beaver Dam Eye Study: visual acuity. *Ophthalmology*. 1991;98(8):1310-1315.
29. Klein R, Klein BE, Lee KE. Changes in visual acuity in a population: the Beaver Dam Eye Study. *Ophthalmology*. 1996;103(8):1169-1178.
30. Klein R, Klein BE, Lee KE, Cruickshanks KJ, Chappell RJ. Changes in visual acuity in a population over a 10-year period: the Beaver Dam Eye Study. *Ophthalmology*. 2001;108(10):1757-1766.
31. Klein R, Klein BE, Lee KE, Cruickshanks KJ, Gangnon RE. Changes in visual acuity in a population over a 15-year period: the Beaver Dam Eye Study. *Am J Ophthalmol*. 2006;142(4):539-549.
32. Klein R, Klein BE. *The Beaver Dam Eye Study: Manual of Operations: Revised*. Springfield, VA: National Technical Information Service; 1991. NTIS Publication PB91-149823.
33. Klein R, Klein BE. *The Beaver Dam Eye Study II: Manual of Operations*. Springfield, VA: National Technical Information Service; 1995. NTIS Publication PB95-273827.
34. Klein R, Klein BE. *The Beaver Dam Eye Study III: Manual of Operations*. Springfield, VA: National Technical Information Service; 1999. NTIS Publication PB99-137861.
35. Klein BE, Klein R. *The Beaver Dam Eye Study V: Manual of Operations*. Springfield, VA: National Technical Information Service; 2010. NTIS Publication PB2010-114194.
36. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. *The Wisconsin Age-Related Maculopathy Grading System*. Springfield, VA: National Technical Information Service; 1991. NTIS Publication PB91-184267.
37. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin Age-Related Maculopathy Grading System. *Ophthalmology*. 1991;98(7):1128-1134.
38. Sivakumaran TA, Igo RP Jr, Kidd JM, et al. A 32 kb critical region excluding Y402H in *CFH* mediates risk for age-related macular degeneration. *PLoS One*. 2011; 6(10):e25598. doi:10.1371/journal.pone.0025598.
39. Thompson CL, Klein BE, Klein R, et al. Complement factor H and hemicentin-1 in age-related macular degeneration and renal phenotypes. *Hum Mol Genet*. 2007; 16(17):2135-2148.
40. Huang L, Li Y, Singleton AB, et al. Genotype-imputation accuracy across worldwide human populations. *Am J Hum Genet*. 2009;84(2):235-250.
41. R Development Core Team. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2009.
42. Jackson CH. Multi-state models for panel data: the msm package for R. *J Stat Softw*. 2011;38(8):1-28.
43. Efron B, Tibshirani RJ. *An Introduction to the Bootstrap*. Boca Raton, FL: Chapman and Hall/CRC; 1994.
44. Bressler NM, Munoz B, Maguire MG, et al. Five-year incidence and disappearance of drusen and retinal pigment epithelial abnormalities: Waterman study. *Arch Ophthalmol*. 1995;113(3):301-308.
45. Klaver CC, Assink JJ, van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2001; 42(10):2237-2241.
46. Mitchell P, Wang JJ, Foran S, Smith W. Five-year incidence of age-related maculopathy lesions: the Blue Mountains Eye Study. *Ophthalmology*. 2002;109(6): 1092-1097.
47. van Leeuwen R, Klaver CC, Vingerling JR, Hofman A, de Jong PT. The risk and natural course of age-related maculopathy: follow-up at 6½ years in the Rotterdam Study. *Arch Ophthalmol*. 2003;121(4):519-526.
48. Wang JJ, Foran S, Smith W, Mitchell P. Risk of age-related macular degeneration in eyes with macular drusen or hyperpigmentation: the Blue Mountains Eye Study cohort. *Arch Ophthalmol*. 2003;121(5):658-663.
49. Ying GS, Maguire MG; Complications of Age-Related Macular Degeneration Prevention Trial Research Group. Development of a risk score for geographic atrophy in Complications of the Age-Related Macular Degeneration Prevention trial. *Ophthalmology*. 2011;118(2):332-338.
50. Zanke B, Hawken S, Carter R, Chow D. A genetic approach to stratification of risk for age-related macular degeneration. *Can J Ophthalmol*. 2010;45(1):22-27.
51. Rennie CA, Stinge A, King EA, Sothirachagan S, Osmond C, Lotery AJ. Can genetic risk information for age-related macular degeneration influence motivation to stop smoking? a pilot study. *Eye (Lond)*. 2012;26(1):109-118.
52. Klein R, Klein BE, Moss SE. Relation of smoking to the incidence of age-related maculopathy: the Beaver Dam Eye Study. *Am J Epidemiol*. 1998;147(2):103-110.
53. Klein R, Klein BE, Myers CE. Risk assessment models for late age-related macular degeneration. *Arch Ophthalmol*. 2011;129(12):1605-1606.
54. Delcourt C, Delyfer MN, Rougier MB, et al. Associations of complement factor H and smoking with early age-related macular degeneration: the ALIENOR study. *Invest Ophthalmol Vis Sci*. 2011;52(8):5955-5962.
55. 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.
56. Ferris FL, Davis MD, Clemons TE, et al; Age-Related Eye Disease Study (AREDS) Research Group. A simplified severity scale for age-related macular degeneration: AREDS report No. 18. *Arch Ophthalmol*. 2005;123(11):1570-1574.
57. Gangnon RE, Lee KE, Klein BEK, Iyengar SK, Sivakumaran TA, Klein R. Effect of the Y402H variant in the complement factor H gene on the incidence and progression of age-related macular degeneration: results from multistate models applied to the Beaver Dam Eye Study. *Arch Ophthalmol*. 2012;130(9):1169-1176.
58. Hughes AE, Orr N, Patterson C, et al. Neovascular age-related macular degeneration risk based on *CFH, LOC387715/HTRA1,* and smoking. *PLoS Med*. 2007; 4(12):e355. doi:10.1371/journal.pmed.0040355.
59. Klein R, Cruickshanks KJ, Nash SD, et al. The prevalence of age-related macular degeneration and associated risk factors. *Arch Ophthalmol*. 2010;128(6):750-758.
60. Magnusson KP, Duan S, Sigurdsson H, et al. *CFH* Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PLoS Med*. 2006;3(1):e5. doi:10 .1371/journal.pmed.0030005.
61. Ryu E, Fridley BL, Tosakulwong N, Bailey KR, Edwards AO. Genome-wide association analyses of genetic, phenotypic, and environmental risks in the Age-Related Eye Disease Study. *Mol Vis*. 2010;16:2811-2821.
62. Thompson CL, Jun G, Klein BE, et al. Genetics of pigment changes and geographic atrophy. *Invest Ophthalmol Vis Sci*. 2007;48(7):3005-3013.
63. Xing C, Sivakumaran TA, Wang JJ, et al. Complement factor H polymorphisms, renal phenotypes and age-related macular degeneration: the Blue Mountains Eye Study. *Genes Immun*. 2008;9(3):231-239.
64. Munch IC, Ek J, Kessel L, et al. Small, hard macular drusen and peripheral drusen: associations with AMD genotypes in the Inter99 Eye Study. *Invest Ophthalmol Vis Sci*. 2010;51(5):2317-2321.
65. Klein R, Knudtson MD, Klein BE, et al. Inflammation, complement factor H, and age-related macular degeneration: the Multi-ethnic Study of Atherosclerosis. *Ophthalmology*. 2008;115(10):1742-1749.
66. Tedeschi-Blok N, Buckley J, Varma R, Triche TJ, Hinton DR. Population-based study of early age-related macular degeneration: role of the complement factor H Y402H polymorphism in bilateral but not unilateral disease. *Ophthalmology*. 2007;114(1):99-103.
67. Weismann D, Hartvigsen K, Lauer N, et al. Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. *Nature*. 2011;478(7367): 76-81.
68. Fritsche LG, Loenhardt T, Janssen A, et al. Age-related macular degeneration is associated with an unstable *ARMS2* (LOC387715) mRNA. *Nat Genet*. 2008; 40(7):892-896.
69. Kortvely E, Hauck SM, Duetsch G, et al. *ARMS2* is a constituent of the extracellular matrix providing a link between familial and sporadic age-related macular degenerations. *Invest Ophthalmol Vis Sci*. 2010;51(1):79-88.
70. Kanda A, Chen W, Othman M, et al. A variant of mitochondrial protein *LOC387715/ARMS2,* not *HTRA1,* is strongly associated with age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2007;104(41):16227-16232.
71. Smailhodzic D, Klaver CC, Klevering BJ, et al. Risk alleles in *CFH* and *ARMS2* are independently associated with systemic complement activation in age-related macular degeneration. *Ophthalmology*. 2012;119(2):339-346.