Differences in the Genetic Susceptibility to Age-Related Macular Degeneration Clinical Subtypes

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Purpose. We compared across age-related macular degeneration (AMD) subtypes the effect of AMD risk variants, their predictive power, and heritability.

Methods. The prevalence of AMD was estimated among active non-Hispanic white Kaiser Permanente Northern California members who were at least 65 years of age as of June 2013. The genetic analysis included 5,170 overall AMD cases ascertained from electronic health records (EHR), including 1,239 choroidal neovascularization (CNV) cases and 1,060 nonexudative AMD cases without CNV, and 23,130 controls of non-Hispanic white ancestry from the Kaiser Permanente Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort. Imputation was based on the 1000 Genomes Project reference panel.

Results. The narrow-sense heritability due to common autosomal single nucleotide polymorphisms (SNPs) was 0.37 for overall AMD, 0.19 for AMD unspecified, 0.20 for nonexudative AMD, and 0.60 for CNV. For the 19 previously reported AMD risk loci, the area under the receiver operating characteristic (ROC) curve was 0.675 for overall AMD, 0.640 for AMD unspecified, 0.678 for nonexudative AMD, and 0.766 for CNV. The individual effects on the risk of AMD for 18 of the 19 SNPs were in a consistent direction with those previously reported, including a protective effect of the *APOE* ε4 allele. Conversely, the risk of AMD was significantly increased in carriers of the ε2 allele.

Conclusions. These findings provide an independent confirmation of many of the previously identified AMD risk loci, and support a potentially greater role of genetic factors in the development of CNV. The replication of established associations validates the use of EHR in genetic studies of ophthalmologic traits.

Keywords: age-related macular degeneration, prevalence, heritability, ApoE

Age-related macular degeneration (AMD) is a complex, late-onset vision disorder, which is the leading cause of blindness among the elderly in developed countries.[1] As a natural course of the disease, AMD progresses in stages and displays a wide range of clinical features. Early AMD begins with the formation of drusen in the macula and/or irregularities (hypopigmentation and hyperpigmentation) in the RPE.[2,3] The majority of patients with AMD develop the nonexudative (or dry) form, with approximately 10% to 15% advancing to exudative (or wet) AMD.[4] Geographic atrophy (GA) is an advanced form of nonexudative AMD, which is a consequence of drusen accumulation between the RPE and Bruch's membrane that results in the death or degeneration of the RPE cells and outer retinal atrophy. Exudative AMD, also known as choroidal neovascularization (CNV) is the more severe form of advanced AMD associated with rapid progression of vision loss, where blood vessels break through the neural retina.

Heritability estimates from twin studies and risk estimates for single nucleotide polymorphisms (SNPs) identified in genome-wide association studies (GWAS) suggest that genetic factors have an important role in the development of advanced and early AMD. Many AMD risk loci, including those in the *CFH, C2, C3, CFB*, and *ARMS2/HTRA1* gene regions, influence the risk of early and advanced AMD, with smaller effects on the risk of early AMD.[5] Additionally, functional variants in *LIPC*, *ABCA1*, and *CETP* of the HDL pathway, associated with early and/or advanced AMD, may be involved in drusen formation and accumulation, which is a hallmark of AMD.[6,7] As such, genetic factors may modulate the risk of AMD at different stages and subtypes of its development. The AMD Gene Consortium published the largest AMD GWAS meta-analysis of advanced AMD and identified 19 independent loci, including seven loci of small effects that reached genome-wide significance for the first time,[7] which have yet to be replicated in a large, independent study. Differences in the effects of these loci by AMD clinical subtypes have not been examined.

Most prior AMD studies relied on a grading system[2,8–10] to classify subjects as having no signs of AMD (grade 1), early AMD (grade 2), intermediate AMD (grade 3), GA (grade 4), and CNV (grade 5). The grading systems do not, however, align directly with the clinical subtypes diagnosed by ophthalmologists using International Classification of Diseases 9 (ICD9) codes, which

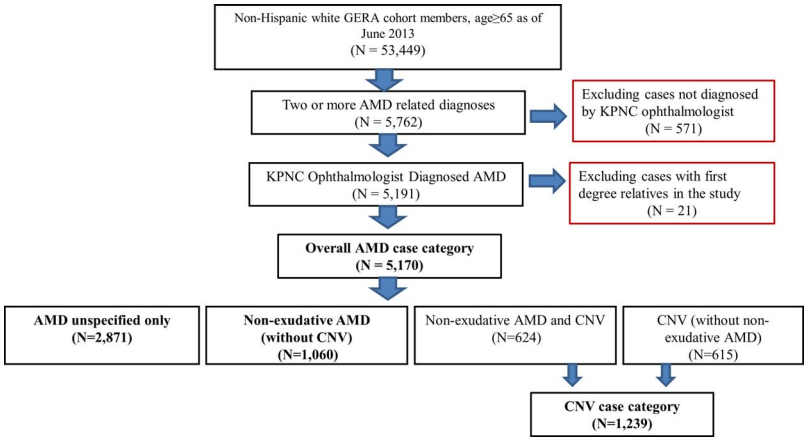

Figure 1. Flowchart for determining AMD case definition in GERA cohort. The final numbers in the three case categories are indicated in bold.

specifies nonexudative AMD and CNV subtypes, but lacks separate codes to distinguish GA from other forms of nonexudative AMD. In this study, we evaluated the validity of ICD9 codes from electronic health records (EHR) to define AMD phenotypes for large scale genetic studies.

The purpose of this study was to investigate the effect of genetic factors on different clinical phenotypes of AMD by estimating the narrow sense heritability, area under the receiver operating characteristic (ROC) curve (AUC), and odds ratios (ORs) at the previously reported risk loci for overall and subtypes of AMD in the Kaiser Permanente Genetic Epidemiology Research Study on Adult Health and Aging (GERA) cohort, nested in a large United States health-system. Examining clinical subtypes of AMD may elucidate the genetic determinants of the different forms of AMD.

Methods

Setting

Kaiser Permanente Medical Care Plan, Northern California Region (KPNC) is a nonprofit integrated health care delivery organization with an active membership of 3.3 million people in 2013. It is the largest healthcare provider in northern California, covering approximately 30% of the population. Comparisons with the general population have shown that the membership is representative of the population of northern California, with the exception of extremes of the socioeconomic spectrum.[11] This medical plan provides comprehensive care, including coverage of optometry and ophthalmologic care. In 1995, KPNC instituted a comprehensive EHR system, which records diagnoses, prescriptions, lab results, and the treating clinicians from all inpatient and outpatient encounters. The plan has high membership retention, with over 90% of those over age 65, and 66% of all active members as of June 2012, having five or more years of retrospective membership.

The GERA cohort

The GERA cohort comprises 110,266 adult members of KPNC who were participants in the Kaiser Permanente Research Program on Genes, Environment and Health (RPGEH). A detailed description of the cohort and study design can be found in the database of genotypes and phenotypes (dbGaP; Study Accession: phs000674.v1.p1; available in the public domain at http://www.ncbi.nlm.nih.gov/gap). Briefly, a total of 437,190 subjects participated in the RPGEH through a mailed survey of adult members of KPNC (~1.9 million) in 2007. Then, survey respondents were asked to provide a written consent. By the end of 2010, 147,498 survey respondents (34%) signed consent authorizing use of biospecimens, survey data, and their EHR data for studies of genetic and environmental influences on health and disease, and sent saliva kits. In total, 110,266 samples were genotyped, forming the GERA cohort. Characteristics of the GERA cohort participants are shown in Supplementary Table S1. The average age of the participants at the time of sample collection was 62.9 years old (SD = 13.8); length of membership in KPNC averaged 23.5 years, indicating the stability of KPNC membership. In this study, we focused on non-Hispanic white subjects in the GERA cohort who were at least 65 years of age as of June 30, 2013 (N = 53,449).

AMD Phenotypes and Control Definitions

Age-related macular degeration was diagnosed by KPNC ophthalmologists after a dilated eye examination of the retina. Optical coherence tomography (OCT) and fluorescein angiography were typically performed when there was evidence of subretinal fluid or hemorrhage in the retina. Ophthalmologists determined AMD subtype based on patient age, drusen number, drusen size, evidence of outer retinal atrophy, and the results from OCT or fluorescein angiography.

Figure 1 shows the hierarchical case classification in the GERA cohort. We established four case categories based on EHR from 1995 to 2013: overall AMD, AMD unspecified, nonexudative AMD, and CNV. Subjects with two or more AMD-related diagnoses – AMD unspecified (ICD9 362.50), nonexudative macular degeneration (ICD9 362.51), or exudative macular degeneration (ICD9 362.52) that characterizes the presence of CNV – with at least one of those diagnoses made by a KPNC ophthalmologist were classified as overall AMD cases. Overall AMD cases then were subcategorized as CNV (if they had any diagnosis of 362.52 by KPNC ophthalmologists) or nonexudative AMD (if they had at least one diagnosis of 362.51, and no diagnoses of 362.52, by KPNC ophthalmologists). To assess the genetic risks associated with these diagnoses and validate the AMD phenotypes defined through EHR data, cases identified through diagnosis of 362.50 alone, who were likely to have mild nonexudative AMD, were designated as AMD unspecified.

Controls were free of any AMD-related diagnoses, including drusen (ICD9 362.57), and were at least 65 years of age as of June 30, 2013. We further restricted to subjects who had visits to an ophthalmology clinic during 2008 through 2013, reasoning that some early indication of AMD might be detected

through a routine dilated eye examination during their recent visits.

Kinship analysis with kinship-based inference for GWAS (KING; available in the public domain at http://people.virginia.edu/~wc9c/KING/) identified 197 overall AMD cases (3.8%) and 879 controls (3.5%) with first-degree relatives in the GERA sample. Our analysis retained one subject from each set of first-degree relatives. Cases with AMD were preferentially included if the relative pair was case and control, and otherwise related individuals in pairs were removed at random, resulting in a total of 5,170 AMD cases and 25,130 controls.

While nonexudative AMD could gradually progress to CNV, a patient could have nonexudative AMD in one eye and CNV in the other, or both forms in the same eye. These scenarios could not be distinguished based on ICD9 codes alone. Among the 624 cases with nonexudative AMD and CNV diagnoses, 564 (90%) had nonexudative AMD diagnosis after their initial CNV diagnosis, indicating that the majority of these patients had both forms of AMD. Nonetheless, these cases were included only in the CNV case category.

On average, the overall AMD cases had 14.7 (SD = 15.7) diagnoses. The consistent diagnoses from repeated examinations support the reliability of the diagnostic categories used by the study. Furthermore, independent, blinded chart review conducted by an ophthalmologist (RBM) confirmed all of the 336 overall AMD cases, including 149 of the 154 CNV (96.8%; 95% confidence interval [CI], 92.6%–98.9%) and 80 of the 82 nonexudative AMD cases without CNV (97.6% CI, 91.5%–99.7%). Eight of the nonexudative AMD cases had progressed to geographic atrophy. The chart audit to confirm the accuracy of the AMD phenotype was based on clinical notes, a history of intraocular injection treatment with ranibizumab or bevacizumab, and a review of imaging studies, including fundus photographs, OCT, or fluorescein angiography. A total of 98% of CNV, 66% of the nonexudative AMD without CNV, and 78% of overall AMD had at least one of the aforementioned studies available for review. Overall, the validation study demonstrated a high positive predictive value for the phenotype definitions in this study.

Genotyping, Quality Control (QC), Imputation, and Genetic Ancestry of the GERA Cohort

DNA was extracted from the Oragene saliva kits, normalized, and plated at KPNC, and then genotyped at the University of California at San Francisco (UCSF) Genomics Core Facility using the Affymetrix Axiom array.[12,13] Samples with a data quality control (DQC) < 0.82 or initial genotype call rate < 0.97 were excluded, resulting in a total of 83,285 individuals of Europeans ancestry in the GERA cohort. To improve genotype calls, SNPs were recalled within packages of plates assayed under similar conditions (array type, reagent, hybridization time, and DNA concentration). The SNPs were removed if either package call rate or overall call rate (across packages) was below 90%. Additional SNP exclusion criteria were: (1) large allele frequency variance across packages – defined as the ratio of overall variance of the SNP allele frequency across packages to the sample SNP heterozygosity (total sample variance: <31); (2) large allele frequency differences between males and females (>0.15) for autosomal SNPs; and (3) poor concordance among duplicates (<0.24). These QC procedures removed a total of 4,431 SNPs.

Following the QC steps, genotypes were prephased with Shape-IT v2.r727,[14] then imputed to a cosmopolitan reference panel consisting of all of the individuals from the 1000 Genomes Project[15] (March 2012 release) using IMPUTE2 v2.3.0 and standard procedures.[16] The info metric from IMPUTE2 is a quality measure, estimating the correlation (r^2) of the imputed genotype to the true genotype.[17] Herein, we report SNP associations with info > 0.8.

We used EIGENSTRAT[18] to compute eigenvectors with 41,228 high quality SNPs that were common among all arrays and the Human Genome Diversity Project (HGDP). These principal components (PCs) were used in the GWAS to adjust for genetic ancestry.

Statistical Analysis

Prevalence Estimates. Point prevalence for each AMD category was calculated among active non-Hispanic white KPNC members who were at least 65 years of age as of June 30, 2013.

Previously Reported AMD Loci. The AMD Gene Consortium published the largest AMD GWAS meta-analysis based on HapMap imputation, identifying 19 SNPs, each representing an independent locus that reached genome-wide significance.[7] Additionally, we included rs1061170, a well-established independent association[19–24] in the *CFH* gene. We used rs429358 to assess the effect of the ε4 allele in the *APOE* locus, which has been shown to be strongly associated with AMD in prior studies.[25–27] One SNP, rs5749482, was neither genotyped nor imputed, because it was not in the reference panel from the 1000 Genomes Project. Thus, in total, we consider 19 SNPs as previously established for subsequent analyses. These 19 SNPs have been directly genotyped or imputed with high quality in the GERA cohort.

Narrow-Sense Heritability Due to Common Alleles. Using AMD prevalence estimates among non-Hispanic whites in KPNC to represent background disease prevalence, we evaluated the narrow-sense heritability explained by common variants tagged by all SNPs on the Axiom EUR array and separately for the 19 previously reported AMD risk variants for each AMD category. These analyses were done using a method as previously described and implemented in GCTA version 1.22.[28] Briefly, we used a linear mixed model to estimate the polygenic additive genetic variance explained by all SNPs on the 0 to 1 scale adjusted for the fixed effects of age, sex, and 10 ancestry PCs. These variance estimates then were transformed to the liability scale in the classical liability threshold model via a probit transformation while correcting for case ascertainment bias, which required specification of the population prevalence of the AMD subphenotypes. To accurately assess the proportion of variance explained within limited computational capacity, we selected three controls for each case matched on sex and age within 3 years. When more than three subjects were available, we chose as controls those subjects with the smallest genetic distances from the cases, defined as the Euclidean distance of the first two principal components.

Area Under the ROC Curve (AUC). The AUC was calculated using SAS (SAS 9.3; SAS, Inc., Cary, NC, USA) to assess the contribution of the previously identified 19 SNPs and nongenetic components for three different combinations of predictors: (1) age and sex; (2) age, sex, smoking status, and hypertension; and (3) age, sex, smoking status, hypertension, and 19 previously identified AMD risk variants. The 19 SNPs were included as covariates in the model. Since age greatly affects the AUC estimates, the matched controls described above also were used to accurately assess the AUC for the previously identified 19 SNPs without covariate adjustment. Since the two SNPs in the *CFH* locus are in moderate LD, they are coded as diplotypes in the model.

Stronger Associations Near Previously Reported SNPs. With a genotyping array designed to optimize genome wide coverage and imputation to the 1000 genome project, we searched for stronger associations within a 1 Mb region of the previously reported SNPs in our study. Analyses were

Table 1. Prevalence and Characteristics of Non-Hispanic White AMD Cases and Controls, Age ≥ 65 as of June 30, 2013

	Overall AMD	AMD Unspecified	Nonexudative AMD	CNV	Controls
Prevalence among non-Hispanic whites in KPNC*	8.02%	4.11%	1.83%	2.08%	–
Number of GERA Subjects†	5,170	2,871	1,060	1,239	25,130
Age, mean ± SD‡	78.56 ± 7.34	78.27 ± 7.47	78.61 ± 7.29	79.54 ± 6.93	72.24 ± 7.14
Sex					
Male (%)	2,260 (42.71)	1,291 (44.97)	447 (42.17)	522 (42.13)	11,263 (44.82)
Female (%)	2,910 (56.29)	1,580 (55.03)	613 (57.83)	717 (57.87)	13,867 (55.18)
Smoker Status§					
Current smoker (%)	201 (4.16)	107 (3.99)	32 (3.26)	62 (5.30)	1,000 (4.18)
Former smoker (%)	2,388 (49.38)	1,284 (47.82)	491 (50.05)	613 (52.39)	11,509 (48.12)
Never smoker (%)	2,247 (46.46)	1,294 (48.19)	458 (46.69)	495 (42.31)	11,408 (47.70)
Hypertension§					
Yes (%)	2,563 (49.57)	1,393 (48.52)	521 (49.15)	649 (52.38)	11,086 (44.11)
No (%)	2,607 (50.24)	1,478 (51.48)	539 (50.85)	590 (47.62)	14,044 (55.89)

* Prevalence estimates were calculated based on 317,340 active non-Hispanic white members in KPNC age ≥ 65 as of June 30, 2013.
† The subjects included in this study were non-Hispanic whites from the GERA cohort who were at least 65 years of age as of June 30, 2013.
‡ Age at biospecimen collection in 2008 through 2009.
§ Based on self-report from the RPGEH survey. The numbers do not add up to the total due to a small fraction of subjects who did not answer the smoking survey questions.

conducted using PLINK[29] v1.07 and R.[14] For each AMD category, we tested single-marker associations in a logistic regression model adjusted for age, sex, and the first 10 ancestry PCs using allele counts for typed SNPs, and imputed dosages for the imputed SNPs and a log-additive genetic model.

Results

We estimated the point prevalence of the three AMD categories among the active non-Hispanic white KPNC members who were at least 65 years old (Table 1). Estimated prevalence was 8.0% for overall AMD, 4.1% for AMD unspecified, 1.8% for nonexudative AMD without CNV, and 2.1% for CNV. Among non-Hispanic white GERA cohort participants, we identified 5,170 overall AMD cases and 25,130 controls. The distribution of AMD subtypes is similar to those in KPNC: 21% had nonexudative AMD without CNV and 24% had CNV. Cases were older and more likely to be female, ever smokers, and have hypertension compared to controls (Table 1).

Using the point prevalence estimates, we estimated the narrow sense heritability, derived from all SNPs on the EUR array, to be 36% for overall AMD: lower for AMD unspecified (19%) and nonexudative AMD (20%) than for CNV (60%; Table 2). The heritability explained by the 19 previously reported SNPs—ranging from 4.4% to 16.3%—was lower in AMD unspecified or nonexudative AMD than CNV. Variants in the *CFH* and *ARMS2/HTRA1* regions explained the majority of the heritability attributable to the 19 previously reported SNPs. The SNP rs10490924 in *ARMS2* accounted for a larger proportion of the heritability in CNV than nonexudative AMD cases or AMD unspecified.

The estimated AUCs for the 19 previously identified SNPs showed a pattern similar to the heritability analyses across AMD categories. Using age and sex matched controls, the AUC was 0.675 for overall AMD. Among the subtypes, the AUC was lower for AMD unspecified (0.640) and nonexudative AMD (0.678), than CNV (0.766; Fig. 2). In Table 3, we present the AUC from models predicting AMD categories with different combination of genetic and nongenetic predictors in the unmatched dataset. Age is the major risk factor for AMD. Comparing model 1 to model 2, inclusion of self-reported smoking status and hypertension yielded similar AUC estimates. The complete model (model 3), with the addition of the 19 previously identified SNPs, showed excellent performance distinguishing cases and controls ($AUC_{overall\text{-}AMD} = 0.798$, $AUC_{AMD\text{-}unspecified} = 0.772$, $AUC_{nonexudative\text{-}AMD} = 0.801$, $AUC_{CNV} = 0.864$).

We calculated ORs for each of the 19 previously reported AMD risk variants by AMD category (Table 4). Observed effects for all 19 SNPs were consistent in direction with those previously

Table 2. Estimated Narrow-Sense Heritabilities of AMD Phenotypes

Proportion of Genome*	Overall AMD h^2 (SE)	AMD Unspecified h^2 (SE)	Nonexudative AMD h^2 (SE)	CNV h^2 (SE)
All EUR array SNPs (MAF ≥ 0.05)	0.360 (0.034)	0.186 (0.041)	0.203 (0.082)	0.598 (0.080)
Previously reported 19 SNPs†	0.081 (0.025)	0.044 (0.014)	0.068 (0.023)	0.163 (0.050)
CFH, ARMS2,C3,C2-CFB‡	0.073 (0.043)	0.031 (0.019)	0.061 (0.038)	0.153 (0.090)
CFH and *ARMS2*§	0.059 (0.046)	0.024 (0.019)	0.048 (0.039)	0.131 (0.100)
CFH (rs1061170)	0.036 (0.050)	0.020 (0.029)	0.029 (0.041)	0.053 (0.074)
CFH (rs10737680)	0.036 (0.050)	0.018 (0.026)	0.026 (0.037)	0.049 (0.068)
ARMS2 (rs10490924)	0.034 (0.047)	0.012 (0.017)	0.025 (0.035)	0.090 (0.122)

* These estimates were calculated using GCTA[28] version 1.22.
† The list of 19 previously published SNPs with $P < 5 \times 10^{-8}$ are in Table 4.
‡ Top index SNPs are rs1061170 and rs10737680 in *CFH*, rs10490924 in *ARMS2*, rs2230199 in *C3* and rs429608 near *C2-CFB*.
§ Top index SNPs are rs1061170 and rs10737680 in *CFH*, rs10490924 in *ARMS2*.

Table 3. AUCs for Predictive Models of AMD Phenotypes in Unmatched Dataset

Predictors	Overall AMD	AMD Unspecified	Nonexudative AMD	CNV
Model 1: age + sex	0.755 ± 0.004	0.740 ± 0.005	0.759 ± 0.008	0.786 ± 0.007
Model 2: age + sex + smoking status + hypertension	0.756 ± 0.004	0.740 ± 0.005	0.759 ± 0.008	0.791 ± 0.006
Model 3: age + sex + smoking status + hypertension + 19 SNPs*	0.798 ± 0.004	0.772 ± 0.005	0.801 ± 0.007	0.864 ± 0.006

* The list of 19 previously published SNPs with $P < 5 \times 10^{-8}$ are in Table 4. Since the two SNPs in the *CFH* locus are in moderate LD, they are coded as diplotypes in the model.

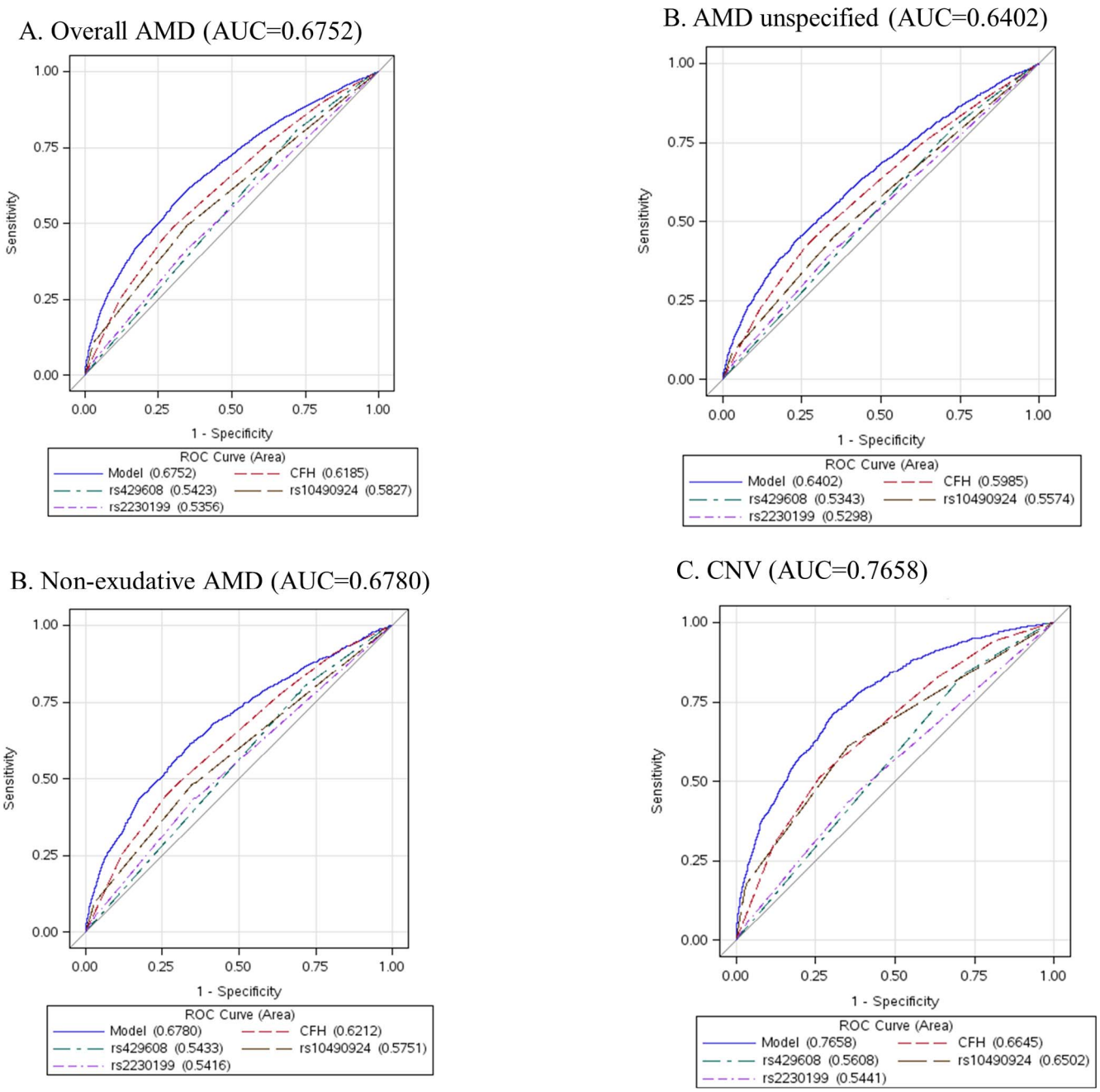

Figure 2. Receiver operating characteristic curves based on the 19 previously reported AMD risk variants for different AMD phenotypes: (**A**) Overall AMD (AUC = 0.675), (**B**) AMD unspecified (AUC = 0.640), (**C**) nonexudative AMD (AUC = 0.678), (**D**) CNV (AUC = 0.766). Since the two SNPs in the *CFH* locus are in moderate linkage disequilibrium (LD), they are coded as diplotypes in the model. Up to three controls were selected for each case matched on sex and age within 3 years. When more than three subjects were available, subjects with the smallest genetic distances from the cases, defined as the Euclidean distance of the first two principal components, were selected.

Table 4. Association Results for the 19 SNPs Previously Reported Associated With Advanced AMD

Nearby Gene	CHR	SNP	Risk Allele	FRQ	Info*	Overall AMD		AMD Unspecified		Nonexudative AMD		CNV		Previously Reported†
						OR	P	OR	P	OR	P	OR	P	OR
ARMS2	10	rs10490924	T	0.21	1	1.85	5.1×10^{-116}	1.56	1.3×10^{-37}	1.87	3.9×10^{-33}	2.84	3.3×10^{-107}	2.76
CFH	1	rs1061170	C	0.38	1	1.77	2.1×10^{-125}	1.59	4.9×10^{-54}	1.79	1.4×10^{-35}	2.28	2.0×10^{-76}	2.41
CFH	1	rs10737680	A	0.58	1	1.83	2.4×10^{-125}	1.61	3.7×10^{-53}	1.90	8.2×10^{-37}	2.5	8.1×10^{-75}	2.43
C2-CFB	6	rs429608	G	0.85	0.99	**1.56**	$\mathbf{3.8 \times 10^{-34}}$	**1.41**	$\mathbf{1.6 \times 10^{-14}}$	**1.61**	$\mathbf{9.7 \times 10^{-11}}$	**2.02**	$\mathbf{4.7 \times 10^{-21}}$	1.74
C3	19	rs2230199	C	0.21	0.99	**1.3**	$\mathbf{1.6 \times 10^{-21}}$	**1.24**	$\mathbf{5.1 \times 10^{-10}}$	**1.41**	$\mathbf{7.7 \times 10^{-11}}$	**1.37**	$\mathbf{3.0 \times 10^{-10}}$	1.42
***APOE*‡**	19	rs429358	C	0.13	0.92	**0.85**	$\mathbf{2.1 \times 10^{-5}}$	**0.90**	**0.029**	**0.83**	**0.018**	**0.76**	$\mathbf{2.7 \times 10^{-4}}$	0.72
	$r^2 = 0.09$§	rs7412	T	0.08	0.93	**1.19**	$\mathbf{4.6 \times 10^{-5}}$	**1.17**	**0.003**	**1.33**	$\mathbf{2.7 \times 10^{-4}}$	1.10	0.224	
COL8A1-FILIP1L	3	rs13081855	T	0.09	0.99	1.06	0.141	1.05	0.308	1.04	0.608	1.12	0.112	1.23
CETP	16	rs1864163	G	0.74	1	**1.1**	$\mathbf{4.4 \times 10^{-4}}$	**1.10**	$\mathbf{4.5 \times 10^{-3}}$	1.10	0.076	1.07	0.19	1.22
	$r^2 = 0.38$§	rs173539	C	0.68	0.94	**1.15**	$\mathbf{2.1 \times 10^{-8}}$	**1.16**	$\mathbf{5.7 \times 10^{-6}}$	**1.16**	$\mathbf{3.7 \times 10^{-3}}$	**1.16**	$\mathbf{1.5 \times 10^{-3}}$	
IER3-DDR1	6	rs3130783	G	0.79	1	1.06	0.052	0.95	0.191	0.93	0.172	1.07	0.193	1.16
	$r^2 = 0.52$§	rs1264376	A	0.14	1	**1.22**	$\mathbf{6.7 \times 10^{-10}}$	**1.17**	$\mathbf{1.7 \times 10^{-4}}$	**1.24**	$\mathbf{6.5 \times 10^{-4}}$	**1.28**	$\mathbf{2.2 \times 10^{-5}}$	
VEGFA	6	rs943080	T	0.51	0.91	**1.08**	$\mathbf{1.3 \times 10^{-3}}$	1.04	0.218	**1.13**	**0.011**	**1.15**	$\mathbf{2.1 \times 10^{-3}}$	1.15
TNFRSF10A	8	rs13278062	T	0.52	0.92	**1.08**	$\mathbf{1.5 \times 10^{-3}}$	1.05	0.136	1.07	0.180	**1.18**	$\mathbf{2.7 \times 10^{-4}}$	1.15
SLC16A8	22	rs8135665	T	0.19	0.66	**1.08**	**0.045**	**1.15**	$\mathbf{2.3 \times 10^{-3}}$	0.98	0.804	0.98	0.735	1.15
CFI	4	rs4698775	G	0.31	0.99	1.05	0.077	1.05	0.132	0.99	0.898	1.07	0.134	1.14
LIPC	15	rs920915	C	0.5	0.99	**1.08**	$\mathbf{1.0 \times 10^{-3}}$	**1.07**	**0.017**	**1.09**	**0.067**	**1.09**	**0.04**	1.13
	$r^2 = 0.07$§	rs2043084	G	0.78	0.99	**1.14**	$\mathbf{9.5 \times 10^{-6}}$	**1.14**	$\mathbf{3.0 \times 10^{-4}}$	**1.13**	**0.028**	**1.18**	$\mathbf{2.6 \times 10^{-3}}$	
TGFBR1	9	rs334353	T	0.75	1	**1.07**	**0.013**	1.06	0.071	1.09	0.105	1.06	0.273	1.13
RAD51B	14	rs8017304	A	0.62	0.99	**1.06**	**0.013**	1.03	0.365	1.06	0.257	**1.15**	$\mathbf{2.7 \times 10^{-3}}$	1.11
	$r^2 = 0.20$§	rs194752	T	0.10	1	**1.18**	$\mathbf{8.2 \times 10^{-6}}$	**1.13**	$\mathbf{9.3 \times 10^{-3}}$	**1.20**	**0.015**	**1.31**	$\mathbf{4.7 \times 10^{-5}}$	
COL10A1	6	rs3812111	T	0.61	0.99	**1.06**	**0.013**	**1.07**	**0.019**	1.04	0.454	1.05	0.266	1.1
ADAMTS9	3	rs6795735	T	0.44	1	1.04	0.080	1.03	0.262	1.01	0.888	**1.09**	**0.039**	1.1
B3GALTL	13	rs9542236	C	0.43	0.99	0.99	0.530	0.98	0.458	0.98	0.612	1.03	0.473	1.1

* The info metric from Impute2 is an estimated correlation of the imputed genotype to the true genotype.

† Loci that were previously associated with advanced AMD with $P < 5 \times 10^{-8}$. Odds ratios were reported in the AMD consortium meta-analysis,[7] except rs1061170, which was previously reported by Yu et al.,[20] and *APOE* variants by McKay et al.[26] The OR estimates for rs429358 and rs7412 by McKay et al.[26] were adjusted for age, sex, and smoking, but not genetic ancestry. The estimated OR of 1.83 for rs7412 by McKay et al.[26] was not comparable here as it was tested in a recessive model.

‡ Missense SNPs define the common forms of *APOE*. The T-allele of rs7412 defines ε2 carriers and the C-allele of rs429358 defines ε4 carriers.

§ These rows indicate SNPs that are not one of the 19 previously reported SNPs, but had stronger associations ($P < 10^{-6}$) in our study compared to the previously reported top SNP in the same locus. The LD with the previously reported SNP was reported as the correlation coefficient (r^2) in the present study.

Table 5. Association of *APOE* Genotypes With AMD Phenotypes

ApoE Gene Forms*		Overall AMD			AMD Unspecified			Nonexudative AMD			CNV		
	FRQ in Controls	FRQ	OR	95% CI	FRQ	OR	95% CI	FRQ	OR	95% CI	FRQ	OR	95% CI
Apo-ε4/ε4	0.014	0.009	0.70	(0.50, 0.98)	0.009	0.68	(0.44, 1.05)	0.011	0.94	(0.51, 1.71)	0.007	0.47	(0.23, 0.95)
Apo-ε3/ε4	0.203	0.165	0.82	(0.74, 0.90)	0.174	0.89	(0.79, 0.99)	0.155	0.78	(0.65, 0.95)	0.153	0.69	(0.58, 0.83)
Apo-ε3/ε3	0.622	0.643	Reference		0.636	Reference		0.633	Reference		0.668	Reference	
Apo-ε2/ε4	0.025	0.023	1.02	(0.81, 1.28)	0.023	0.99	(0.74, 1.32)	0.027	1.29	(0.86, 1.94)	0.020	0.83	(0.53, 1.31)
Apo-ε2/ε3	0.129	0.152	1.17	(1.06, 1.29)	0.150	1.16	(1.03, 1.31)	0.167	1.32	(1.10, 1.59)	0.145	1.03	(0.85, 1.24)
Apo-ε2/ε2	0.007	0.007	1.33	(0.88, 2.00)	0.008	1.48	(0.91, 2.40)	0.007	1.20	(0.51, 2.84)	0.007	1.51	(0.69, 3.28)

The multivariate logistic regression model, including genotypes of the *APOE* gene, adjusted for age, sex, smoking status, 10 ancestry PCs, and top hits as covariates. Top hits were rs10490924, rs1061170, rs10737680, rs429608, and rs2230199.

* The common forms of the *APOE* gene are defined based on the best guess genotypes of rs429358 and rs7412. Apo-ε2/ε3 = rs429358(T;T) rs7412 (C;T); Apo-ε3/ε4 = rs429358(C;T) rs7412 (C;C).

reported. There were 14 SNPs nominally associated ($P < 0.05$) with overall AMD and 16 were nominally associated with at least one of the AMD phenotypes. While rs5729482, near the *TIMP3* gene, was neither in the 1000 genomes pilot data nor genotyped or imputed in our results, we found rs5754227 to be well imputed (info = 0.98), in strong LD with rs5729482 ($r^2 = 0.92$) based on HapMap r22 CEU, that showed evidence of replication ($OR_{overall\text{-}AMD} = 1.09$, $P_{overall\text{-}AMD} = 0.032$; $OR_{AMD\text{-}unspecified} = 1.05$, $P_{AMD\text{-}unspecified} = 0.289$; $OR_{nonexudative\text{-}AMD} = 1.06$, $P_{nonexudative\text{-}AMD} = 0.396$; $OR_{CNV} = 1.16$, $P_{CNV} = 0.019$). Two SNPs, rs13081855 and rs9542236, which were reported to be associated with advanced AMD for the first time in the most recent AMD meta-analysis, did not have nominally significant associations for any AMD phenotype.[7] Most of the previously reported AMD risk variants showed larger effect sizes for nonexudative AMD or CNV compared to overall AMD. In general, effect sizes were greater for CNV than nonexudative AMD. The notable exceptions are rs1864163 in *CETP* and rs8135665 in *SLC16A8*, whose strongest associations were observed in overall AMD. Only one of the seven new loci identified in the recent meta-analysis, rs8017304 in *RAD51B*, was nominally associated with nonexudative AMD and CNV.

Furthermore, we identified SNPs in our analyses with stronger associations in the several gene regions (Table 4, § rows) than those previously reported at the same loci. The SNPs we report here were not reported in the AMD meta-analysis results, presumably due to the more limited coverage of the HapMap reference panel used for imputation. Our analysis used imputation to the 1000 Genomes reference panel, which has the potential to refine further association signals in AMD loci. Additionally, our top associations in *CETP*, *IER3-DDR1*, *LIPC* and *RAD51B* gene regions were in low to moderate LD with the SNPs reported from AMD meta-analysis, $r^2 = 0.38$, 0.52, 0.07, and 0.20, respectively. Our strongest signals appear to be in low to moderate LD in our sample with the previously reported SNPs. As a result, the stronger associations observed in our study may represent better candidate SNPs for follow-up functional analysis.

We also found that the risk of AMD was significantly influenced by the ε2 and ε4 alleles of *APOE* (Table 4). The ε2 defined by T-allele of rs7412 and ε4 allele defined by C-allele of rs429358 are in low LD ($r^2 = 0.09$). In Table 5, we present a detailed *APoE* diplotype analysis with AMD phenotypes adjusting for age, sex, smoking status, ancestry PCs, and AMD risk loci with large effect sizes. Compared to subjects with genotype ε3ε3, having one copy of the ε2 allele significantly increased risk of overall AMD (OR = 1.17, $P = 5.7 \times 10^{-3}$) and nonexudative AMD (OR = 1.32, $P = 0.077$), but not CNV (OR = 1.03, $P = 0.153$). Having two copies of the ε2 allele increased the risk of AMD of all forms, but the association was not statistically significant due to its rarity (frequency [FRQ] < 0.01), suggesting a possible recessive effect on the risk of CNV. Meanwhile, the ε4 allele was protective against all categories of AMD, with the largest effect on CNV.

Discussion

By comparing the narrow sense heritability (h^2), AUC, and ORs of SNPs previously reported to be associated with advanced AMD, we demonstrated evidence for a greater genetic contribution to CNV compared to overall AMD, AMD unspecified, and nonexudative AMD. The heritabilities for nonexudative and unspecified AMD did not appear to be distinctively different. This accorded well with the United States twin study that reported lower heritability for overall AMD (0.46) than intermediate (0.67) or advanced AMD (0.71),[30] and greater than expected sibling concordance ($P = 4.2 \times 10^{-5}$) for the same subtype of advanced AMD among 209 sibling pairs with advanced AMD.[21] The heritability estimates from GCTA analysis for binary traits are robust to moderate misspecification of disease prevalence (0.75–1.5 of the true prevalence).[28] The prevalence of AMD phenotypes was derived from the full adult membership of KPNC age 65 or older. The prevalence estimate for CNV is in line with estimates from other population based studies.[31,32] The prevalence of nonexudative and unspecified AMD in KPNC could be an underestimate. Nonetheless, the same case criteria were applied to GERA cohort and background population, it is unlikely that misspecification of disease prevalence would be responsible for the differences in estimated heritability observed across AMD subtypes.

Previous AMD risk models[33–36] have focused on progression to advanced AMD and demonstrated that AMD risk variants greatly improved model discrimination power. To the best of our knowledge, this is the first study to demonstrate the utility of AMD risk variants in the prediction of overall and subtypes of AMD. The AUC for CNV using age- and sex-matched controls was 0.766 ± 0.008, slightly higher than the AUC (0.734) for advanced AMD reported in the largest meta-analysis of AMD.[7] The AUC for the complete model predicting CNV in our unmatched dataset was 0.864 ± 0.006, comparable to previously reported full model AUC with a similar set of predictors, which ranges from 0.831 to 0.884.[33–36]

Differential genetic susceptibility for overall AMD and subtypes of AMD is in part due to the risk variants with large effect size in *ARMS2/HTRA1*, *CFH*, and *C2-CFB*. Our results appear to replicate the well-accepted stronger association of variant in *ARMS2/HTRA1* with CNV. The pattern of estimated

effect size differences across subgroups was less distinctive for risk variants of small effects. Our results generally were consistent with previous genetic association studies of early or intermediate AMD[5,6,25,37–39] and those that have found a difference in genetic effects associated with the GA and CNV subtypes.[6,7,21,40]

In the present study, the 19 common variants previously identified through studies of advanced AMD also were associated with overall and subtypes of AMD, indicating a shared genetic basis across AMD stages. Conceivably, higher genetic susceptibility leading to a greater risk of progression to advanced stages of AMD also may result in younger age at onset of AMD.

Most previous studies including a meta-analysis of 10 studies,[27] have found a reduced risk of advanced AMD for subjects carrying one or two ε4 alleles and nonsignificant increased risk for ε2 carriers.[41–45] The largest pooled analysis of 15 studies[26] confirmed ε4 alleles to be strongly protective against and showed that ε2 homozygote carriers were at a moderately elevated risk of advanced AMD (OR = 1.83, $P = 0.04$; the latter being consistent with a recessive genetic model). The role of *ApoE* variants in the risk of earlier stage AMD and nonexudative AMD is not well understood. Our finding is consistent with the results from the largest pooled analysis of 15 studies. The increased risk of CNV for subjects with the ε2ε2 genotype (OR = 1.4,; $P = 0.11$) in our study supports a recessive model. In addition, we showed that ε4 alleles reduced the risk of AMD of all subtypes and the ε2 allele increased risk in overall AMD in an additive manner. Protein ApoE is a lipid transport protein for low density lipoprotein (LDL). The isoforms of ApoE differ in binding affinity to the LDL-receptor, which affects total cholesterol levels. Variants in the *LIPC, ABCA1,* and *CETP* genes also have been shown to be associated with AMD and cholesterol levels,[6,19,38] suggesting that variation in genes that modulate retinal cholesterol levels may influence AMD risk. Recently, a number of studies pointed to the involvement of *ApoE* in T cell proliferation, macrophage function regulation, and modulation of inflammation.[46,47] The association of *ApoE* with AMD also could support the role of immunoregulation and cell signaling in AMD pathogenesis.[48] Moreover, while the ε4 allele is protective against AMD, it is associated with an increased risk of cardiovascular disease (CVD), atherosclerosis, and Alzheimer's disease (AD).[49] Amyloid, a major inflammatory component commonly seen in the plaques of Alzheimer patients, also is observed in drusen.[50] Further investigations are needed to understand the mechanisms by which *ApoE* mediates the risks of AMD.

We recognize several potential limitations of this study. First, while our study was well powered to confirm the association of previously reported loci with overall AMD; that is, for an OR of 1.10, we had power of 0.84 for SNPs with a minor allele frequency of 10%, and 0.99 for a minor allele frequency of 30% (α level 0.05, 1-tailed test), we did not confirm the association of several of the AMD consortium risk loci. The lack of replication for these loci could be due to differences in phenotype definition or overestimation of the discovery ORs due to the winner's curse effect. We did, however, observe effects in a consistent direction for 18 of the 19 SNPs. Second, nonexudative AMD presents a wide spectrum of clinical manifestations. The ICD9 codes do not distinguish GA, nor do they differentiate stages or severities of nonexudative AMD. Third, ICD9 codes do not indicate whether the affected eye was the left or right eye. While some eyes with nonexudative AMD may have advanced to CNV, evidence suggested that majority of the cases with nonexudative AMD and CNV diagnoses appeared to have both forms of AMD. Categorizing the overlapping cases as CNV, but not nonexudative AMD, may drive the risk and heritability estimates lower for nonexudative AMD, increasing the observed differences between AMD subtypes. Fourth, CNV may arise from other rare causes, such as angioid streaks, presumed ocular histoplasmosis syndrome, or choroidal rupture. However, nearly every CNV patient in our study had at least one separate diagnosis of AMD, indicating that CNV was likely due to AMD. Fifth, self-reported hypertension and smoking status in AUC model was collected through the RPGEH survey conducted in 2007 and did not necessarily reflect the status before the onset of AMD. Prospective studies could more accurately measure these predictive effects on incident AMD cases. Finally, some early AMD cases may have not been captured in the EHR because the initial changes in the macula could be asymptomatic. Such misclassification would lead to underestimation of narrow-sense heritability for overall AMD and reduced strength of the association of genetic variants with AMD. However, we required controls to have visits to an ophthalmology clinic during 2008 through 2013. The appearance of drusen would likely be detected during these visits and limit misclassification.

This study has several important strengths: First, the membership of KPNC is representative of the population of northern California,[11] enabling generalization of the prevalence estimates to the general population of northern California non-Hispanic whites. Second, we reported, for the first time to our knowledge, the estimated narrow sense of heritability for overall and subtypes of AMD in a large single population in which the case ascertainment scheme and age criteria were applied to the GERA cohort and the background population. Finally, we replicated associations with the most established AMD risk variants with consistent pattern and strength, demonstrating the utility of the EHR data for conducting large scale genetic analyses of complex ophthalmologic phenotypes, such as AMD.

In summary, our results showed the strong genetic component underlying in all forms of AMD and a greater genetic contribution to CNV compared to overall or nonexudative AMD.

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