Human Molecular Genetics, 2011, Vol. 20, No. 18 **3699–3709**
doi:10.1093/hmg/ddr270
Advance Access published on June 10, 2011

Common variants near *FRK/COL10A1* and *VEGFA* are associated with advanced age-related macular degeneration

Yi Yu[1,†], Tushar R. Bhangale[6,†], Jesen Fagerness[8,9,†], Stephan Ripke[8,9,†], Gudmar Thorleifsson[10], Perciliz L. Tan[11,12,13], Eric H. Souied[14,15], Andrea J. Richardson[16], Joanna E. Merriam[17], Gabriëlle H.S. Buitendijk[18,19], Robyn Reynolds[1], Soumya Raychaudhuri[8,9,21,22], Kimberly A. Chin[1], Lucia Sobrin[23], Evangelos Evangelou[24], Phil H. Lee[8,9], Aaron Y. Lee[25,26], Nicolas Leveziel[14,15], Donald J. Zack[27,28,29,30,37], Betsy Campochiaro[27,28,29,30], Peter Campochiaro[27,28], R. Theodore Smith[17], Gaetano R. Barile[17], Robyn H. Guymer[16], Ruth Hogg[31], Usha Chakravarthy[31], Luba D. Robman[16], Omar Gustafsson[10], Haraldur Sigurdsson[32,33], Ward Ortmann[7], Timothy W. Behrens[7], Kari Stefansson[10,33], André G. Uitterlinden[20], Cornelia M. van Duijn[19], Johannes R. Vingerling[18,19], Caroline C.W. Klaver[18,19], Rando Allikmets[17,34], Milam A. Brantley Jr[25,26], Paul N. Baird[16], Nicholas Katsanis[11,12,13], Unnur Thorsteinsdottir[10,33], John P.A. Ioannidis[2,3,4,5,24,35,36], Mark J. Daly[8,9], Robert R. Graham[7] and Johanna M. Seddon[1,5,*]

[1]Ophthalmic Epidemiology and Genetics Service, New England Eye Center, [2]Center for Genetic Epidemiology and Modeling, [3]Institute for Clinical Research and Health Policy Studies, [4]Tufts Clinical and Translational Science Institute and [5]Department of Ophthalmology, Tufts Medical Center, Tufts University School of Medicine, 800 Washington Street, No. 450, Boston, MA 02111, USA, [6]Department of Bioinformatics and Computational Biology and [7]Immunology and Tissue Growth and Repair Department, Human Genetics Group, Genentech, Inc., South San Francisco, CA 94080, USA, [8]Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge Street, Sixth Floor, Boston, MA 02114, USA, [9]Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, 7 Main Street, Cambridge, MA 02142, USA, [10]deCODE genetics, 101 Reykjavik, Iceland, [11]Center for Human Disease Modeling, [12]Department of Cell Biology and [13]Department of Pediatrics, Duke University, Durham, NC 27710, USA, [14]Department of Ophthalmology, University Paris Est Creteil, Hopital Intercommunal de Creteil, Creteil, 94000, France, [15]Department of Ophthalmology, Faculté de Médecine Henri Mondor, UPEC, Créteil, France, [16]Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, University of Melbourne, East Melbourne, Victoria, Australia, [17]Department of Ophthalmology, Columbia University, New York, NY 10032, USA, [18]Department of Ophthalmology, [19]Department of Epidemiology and [20]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands, [21]Division of Genetics and [22]Division of Rheumatology, Brigham and Women's Hospital, Boston, MA 02115, USA and [23]Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114, USA, [24]Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina 45110, Greece, [25]Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St Louis, MO 63110, USA, [26]Barnes Retina Institute, St Louis, MO 63144, USA, [27]Department of Ophthalmology, [28]Department of Neuroscience, [29]Department of Molecular Biology and Genetics, Wilmer Eye Institute and [30]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA, [31]Center for Vision and Vascular Science, The Queen's University,

[*]To whom correspondence should be addressed. Tel: +1 6176369000; Fax: +1 6176365844; Email: jseddon@tuftsmedicalcenter.org
[†]These authors contributed equally to the project.

Belfast, UK, [32]Department of Ophthalmology, National University Hospital, 101 Reykjavik, Iceland, [33]Faculty of Medicine, University of Iceland, 101 Reykjavik, Iceland, [34]Department of Pathology and Cell Biology, Columbia University, New York, NY 10032, USA, [35]Department of Medicine and [36]Department of Health Research and Policy, Stanford Prevention Research Center, Stanford University School of Medicine, Stanford, CA 94305, USA and [37]Institut de la Vision, UPMC, Paris, France

Received April 15, 2011; Revised May 27, 2011; Accepted June 6, 2011

Despite significant progress in the identification of genetic loci for age-related macular degeneration (AMD), not all of the heritability has been explained. To identify variants which contribute to the remaining genetic susceptibility, we performed the largest meta-analysis of genome-wide association studies to date for advanced AMD. We imputed 6 036 699 single-nucleotide polymorphisms with the 1000 Genomes Project reference genotypes on 2594 cases and 4134 controls with follow-up replication of top signals in 5640 cases and 52 174 controls. We identified two new common susceptibility alleles, rs1999930 on 6q21-q22.3 near *FRK*/*COL10A1* [odds ratio (OR) 0.87; $P = 1.1 \times 10^{-8}$] and rs4711751 on 6p12 near *VEGFA* (OR 1.15; $P = 8.7 \times 10^{-9}$). In addition to the two novel loci, 10 previously reported loci in *ARMS2/HTRA1* (rs10490924), *CFH* (rs1061170, and rs1410996), *CFB* (rs641153), *C3* (rs2230199), *C2* (rs9332739), *CFI* (rs10033900), *LIPC* (rs10468017), *TIMP3* (rs9621532) and *CETP* (rs3764261) were confirmed with genome-wide significant signals in this large study. Loci in the recently reported genes *ABCA1* and *COL8A1* were also detected with suggestive evidence of association with advanced AMD. The novel variants identified in this study suggest that angiogenesis (*VEGFA*) and extracellular collagen matrix (*FRK/COL10A1*) pathways contribute to the development of advanced AMD.

INTRODUCTION

Advanced age-related macular degeneration (AMD) (MIM 603075) is a leading cause of visual impairment and blindness in people older than 60 years. AMD is a common, late-onset disease that is modified by covariates including smoking and body mass index and has recurrence ratios for siblings of a case that are 3–6-fold higher than in the general population (1). The burden of this disease is increasing among the growing elderly population. Among individuals aged 75 or older, approximately one in four have some sign of this disease and about one in 15 have the advanced form with visual loss (2). There are two main forms of advanced AMD. The neovascular (NV), or 'wet', form is characterized by in-growth of choroidal vessels under the retina. Geographic atrophy (GA), the advanced 'dry' form of the disease, occurs when there is full thickness loss of the outer retinal layers, retinal pigment epithelium (RPE) and choriocapillaris in the central macula. Although anti-vascular endothelial growth factor (VEGF) therapy has significantly improved the functional and morphological outcomes for patients with NV disease (3), there are currently no effective therapies or preventive strategies for GA.

Several genetic loci have been associated with advanced AMD, including complement pathway genes *CFH* (4–9), *C2* (8,10), *CFB* (8,10), *C3* (11), *CFI* (12) and the *ARMS2/HTRA1* (13,14) region. Recent genome-wide studies in large cohorts have also identified the association between advanced AMD and variants in *LIPC* (15), a gene in the high-density lipoprotein (HDL) pathway, and *TIMP3* (16), and suggested association with other loci in the HDL pathway. The discovery of the multiple associations with complement-related genes revealed an unanticipated central role for this pathway in disease pathogenesis. This has led directly to the initiation of multiple clinical trials of drugs that alter the complement pathway in AMD patients (17). A combined risk score including these multiple genetic loci along with demographic, environmental and macular characteristics which modify risk is highly predictive of progression from the early and intermediate stages of AMD to the advanced stages which cause visual loss (18,19).

The genetic variants known to date are estimated to account for $<50\%$ of the heritability of the disease (8,20). To identify additional loci that contribute to the genetic risk of advanced AMD and to illuminate new candidate physiological processes that might be involved, we performed a meta-analysis of genome-wide association study (GWAS) for advanced AMD that consisted cases/controls from the Tufts/Massachusetts General Hospital (MGH) GWAS Cohort Study (15), the Michigan, Mayo, Age-Related Eye Disease Study (AREDS), Pennsylvania (MMAP—Michigan, Mayo, AREDS, Pennsylvania Cohort Study) Cohort Study (16), as well as controls from the Myocardial Infarction Genetics Consortium (MIGen) (21) and the Genetic Association Information Network (GAIN) Schizophrenia Study (22). We imputed a large number of single-nucleotide polymorphisms (SNPs) using the 1000 Genomes Project reference data to search deeply throughout the genome in this large merged data set of Tufts/MMAP/MIGen/GAIN (TMMG). We then sought direct replication of the top representative SNPs of each clumped region in 10 independent cohorts from Johns Hopkins University (JHU), Columbia University (COL), Genentech, deCODE (Iceland), Washington University (Wash-U), Centre for Eye Research Australia (AUS), the Rotterdam

Study (RS), an independent replication sample from Tufts/MGH, Hopital Intercommunal de Creteil (FR-CRET) and The Queen's University of Belfast (Irish). We also conducted a combined analysis for the results of top SNPs in all participating cohorts using a fixed effects model.

RESULTS

After the quality control analyses (see Materials and Methods; Supplementary Material, Table S1), the TMMG data set consisted of genotype data for 2594 individuals with advanced AMD and 4134 controls, all of European ancestry. A set of 6 036 699 high-quality SNPs from imputation using the 1000 Genomes Project data was tested for the association with advanced AMD. We plotted our meta-analysis of GWAS *P*-values in quantile–quantile plots. The strong associations of previously reported SNPs distorted the *P*-values distribution toward the top-end of the plot (Supplementary Material, Fig. S1A). After removing these well-validated associated loci, we observed little statistical inflation in the remaining distribution of association statistics (inflation factor $\lambda_{gc} = 1.047$; Supplementary Material, Fig. S1B). Since inflation factor scales with sample size, we estimated the value that would be expected in a study of 1000 cases and 1000 controls ($\lambda_{1000} = 1.015$). Again, there was little evidence of any general inflation of the test statistics. As expected, we observed highly statistically significant association signals at SNPs in six previously published loci, including *ARMS2/HTRA1* (rs10490924, $P = 1.2 \times 10^{-144}$), *CFH* (rs1061170, $P = 5.6 \times 10^{-138}$, and rs1410996, $P = 2.1 \times 10^{-134}$), *CFB* (rs641153, $P = 2.9 \times 10^{-22}$), *C3* (rs2230199, $P = 1.4 \times 10^{-18}$), *C2* (rs9332739, $P = 4.3 \times 10^{-12}$), *CFI* (rs10033900, $P = 2.4 \times 10^{-11}$) and *LIPC* (rs1532085, $P = 1.0 \times 10^{-7}$) (Fig. 1).

In addition to the previously identified loci, we detected a region at 6q21–q22.3 (Fig. 2A) that contained 30 SNPs in tight LD ($R^2 > 0.8$) which were strongly associated with AMD status in the TMMG sample ($P < 5 \times 10^{-7}$). The associated region contains the genes *COL10A1* (encoding the alpha chain of type X collagen) and *FRK* (encoding the fyn-related kinase). To confirm the new locus for advanced AMD, we selected two SNPs rs12204816 ($P = 1.73 \times 10^{-7}$, near *COL10A1*) and rs1999930 ($P = 3.1 \times 10^{-7}$, between *FRK* and *COL10A1*) from this block for further replication study. In addition to the *FRK/COL10A1* variants, we also sought to replicate 37 other previously unreported candidate loci ($P < 5 \times 10^{-5}$ in the TMMG meta-analysis), as well as previously reported loci.

In aggregate, the replication data sets consisted of 5640 cases and 52 174 controls from 10 independent cohorts from JHU, COL, Genentech, Iceland, Wash-U, AUS, RS, FR-CRET, Irish and an independent replication sample from Tufts/MGH (Supplementary Material, Table S2). The effective sample sizes of each cohort are noted in Supplementary Material, Table S3. Of the two SNPs we selected for replication in *FRK/COL10A1* locus, rs12204816 failed the genotyping quality criteria in the replication phase, but rs1999930 was successfully genotyped in all 10 replication cohorts. In the TMMG meta-analysis, the minor T allele frequency of rs1999930 was 26% in cases and 30% in controls (Table 1), with an odds ratio (OR) of 0.81 and a 95% confidence interval (CI) range of 0.74–0.88 (Fig. 2B; Supplementary Material, Table S3). Combining the effect sizes of all independent replication cohorts using a fixed effects model confirmed the association (OR = 0.90, $P = 8.3 \times 10^{-4}$). In the combined analysis of all the samples, the T allele of rs1999930 significantly ($P = 1.1 \times 10^{-8}$) reduced the risk of advanced AMD [OR = 0.87 (95% CI: 0.83–0.91)]. There was no significant evidence for heterogeneity under Cochran's *Q*-test ($P = 0.32$, $I^2 = 15\%$) across data sets.

Another previously unreported locus (rs4711751) near *VEGFA* with a suggestive association signal ($P = 2.2 \times 10^{-5}$) in the TMMG meta-analysis was confirmed in our replication study. The T allele of rs4711751, with an allele frequency of 0.54 in cases and 0.50 in controls, was associated with increased risk of advanced AMD [OR = 1.21 (95% CI:1.11–1.32)]. The results were consistent in direct replication genotyping in an independent set of 5419 cases and 47 687 controls [OR = 1.13 (95% CI: 1.06–1.19), $P = 4.3 \times 10^{-5}$]. This SNP reached genome-wide significance [OR = 1.15 (95% CI: 1.10–1.21), $P = 8.7 \times 10^{-9}$] in the combined analysis (Fig. 2C and D; Supplementary Material, Table S4), including all replication cohorts except the Rotterdam Study, in which rs4711751 was not genotyped. We found no significant evidence for heterogeneity ($P = 0.26$, $I^2 = 24\%$) for the rs4711751 association results across the nine cohorts tested.

Besides the two novel *FRK/COL10A1* and *VEGFA* loci, three recently reported loci were also associated with advanced AMD (Table 1). The risk variants in *TIMP3* (rs9621532, $P = 2.2 \times 10^{-15}$) and HDL pathway genes *LIPC* (rs10468017, $P = 2.7 \times 10^{-12}$) and *CETP* (rs3764261, $P = 6.9 \times 10^{-9}$) reached genome-wide significance in the combined analysis. Two other variants in *ABCA1* (rs1883025, $P = 1.2 \times 10^{-7}$) and *COL8A1* (rs13095226, $P = 9.7 \times 10^{-7}$) which were reported in our previous GWAS (15) are also still noteworthy candidates (Supplementary Material, Table S5). Supplementary Material, Table S6, shows other published candidate SNPs which were not associated with advanced AMD in this GWAS meta-analysis.

We also investigated the specific association with GA and NV subtypes of AMD in our TMMG samples. The minor allele (T) of rs1999930 had a similar effect size for GA [OR = 0.78 (0.69–0.89), $P = 1.0 \times 10^{-4}$] and NV [OR = 0.82 (0.75–0.90), $P = 4.1 \times 10^{-5}$]. The risk allele (T) of rs4711751 also had a similar magnitude of effect on GA [OR = 1.23 (1.08–1.40), $P = 2.0 \times 10^{-3}$] and NV [OR = 1.20 (1.09–1.32), $P = 2.5 \times 10^{-4}$]. Association signals at *CFH*, *C2*, *CFB*, *C3*, *CFI* and *ARMS2/HTRA1* were also significant for both GA and NV compared with controls. *ARMS2/HTRA1* was more strongly related to NV compared with GA as previously reported (23).

This study provides an opportunity to establish a prediction model for advanced AMD with all the associated genetic risk factors combined together. We evaluated a risk score based on the sum of the genotype dosage of 14 risk variants (SNPs in Table 1 plus rs1883025 in *ABCA1* and rs13095226 in *COL8A1* in Supplementary Material, Table S5) which were validated or suggested in this study, each weighted by the natural logarithm of OR estimated by a multivariate logistic

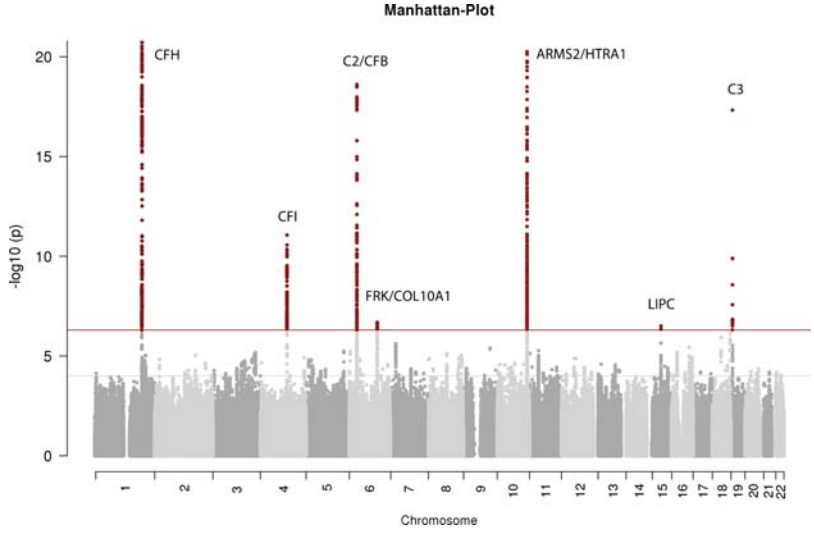

Figure 1. Manhattan plot. Log (*P*) values of association results from the cleaned TMMG data set are plotted for SNPs on each chromosome. SNPs with $P < 5 \times 10^{-7}$ are colored in red and the representative genes for each associated region are labeled.

regression model of these 14 variants in TMMG samples. It is estimated that there is a >50-fold difference in advanced AMD risk between the high-risk individuals (risk score >2) and the low-risk individuals (risk-score <−2) (Supplementary Material, Fig. S2).

DISCUSSION

In this study aiming to find new genetic factors for advanced AMD, we report a genome-wide significant association near *FRK/COL10A1* (rs1999930, $P = 1.1 \times 10^{-8}$), a locus not previously implicated in advanced AMD. We also identified a novel locus (rs4711751, $P = 8.7 \times 10^{-9}$) for advanced AMD near *VEFGA*. In addition, we confirmed strong association with the previously reported genetic variations at 10 loci including *ARMS2/HTRA1* (rs10490924, $P = 3.6 \times 10^{-322}$), *CFH* (rs1061170, $P = 1.3 \times 10^{-261}$, and rs1410996, $P = 7.4 \times 10^{-235}$), *CFB* (rs641153, $P = 5.5 \times 10^{-31}$), *C3* (rs2230199, $P = 4.6 \times 10^{-29}$), *C2* (rs9332739, $P = 2.4 \times 10^{-23}$), *CFI* (rs10033900, $P = 4.1 \times 10^{-10}$), *LIPC* (rs10468017, $P = 2.7 \times 10^{-12}$), *TIMP3* (rs9621532, $P = 2.2 \times 10^{-15}$) and *CETP* (rs3764261, $P = 6.9 \times 10^{-9}$) in the combined analysis. Our analyses also support previously identified loci in *ABCA1* and *COL8A1*.

The estimated heritability based on twin studies is 71% for advanced forms of this disease (24). Using a standard liability threshold model (25), the previously reported loci combined with the new loci discovered in this study explain ~39% of the total variance (or 55% of the heritability) of advanced AMD. Therefore, there are still unidentified genetic variants that may explain the missing heritability. Additional AMD risk variants likely remain to be discovered and will require a combined strategy of larger AMD meta-analyses to detect variants of more modest effect, genome scans using higher density SNP arrays to capture previously missed variants and exome-sequencing studies to identify rare variants.

VEGFA is a member of the VEGF family and functions to increase vascular permeability, angiogenesis, cell growth and migration of endothelial cells. VEGFA is the target for multiple therapies including ranibizumab, a molecule that is FDA-approved for the treatment of wet AMD. It has been hypothesized that activation of VEGFA may induce pathologic angiogenesis beneath the RPE layer. The newly identified SNP (rs4711751) is 60 kb downstream of *VEGFA* and >90 kb away from a SNP (rs2010963) in the *VEGFA* promoter region which has been reported to be associated with AMD (26). However, SNP rs4711751 appears to be independent of the rs2010963 variant ($R^2 = 0.015$, $D' = 0.14$ in samples of European ancestry); therefore, the association we identified near *VEGFA* was in a novel region and is not likely due to LD with SNPs in the *VEGFA* promoter region. Of note, the previously reported rs2010963 SNP showed no evidence of association in the TMMG meta-analysis ($P = 0.26$) (Supplementary Material, Table S6). In addition, rs4711751 is in moderate LD with nearby genome-wide significant variants reported in type 2 diabetes, waist–hip ratio and chronic kidney disease ($R^2 = 0.31$, $D' = 0.91$ to rs881858). However, rs881858 was not significantly associated with advanced AMD in the TMMG meta-analysis ($P = 0.11$) and cannot explain the association we observe in rs4711751.

Finally, we note that the newly identified SNP rs4711751 is in strong LD with rs943080 ($R^2 = 1.0$ in 1000 Genomes CEU data), a variant that resides in a highly evolutionarily conserved region (Fig. 3). The risk allele (T) at rs4711751 is on the same haplotype as the evolutionarily conserved allele (T) at rs943080. Allelic change from T to C on this conserved region may disrupt a putative transcription factor-binding site for cone–rod homeobox (CRX), which is an essential transcription factor highly expressed in RPE and retinal ganglion cells (27). This suggests a possible mechanism for the candidate causal SNP rs943080. Individuals with the protective allele (C) at rs943080 may have decreased binding of CRX at the locus, leading to decreased expression of VEGFA, which in turn protects these individuals from

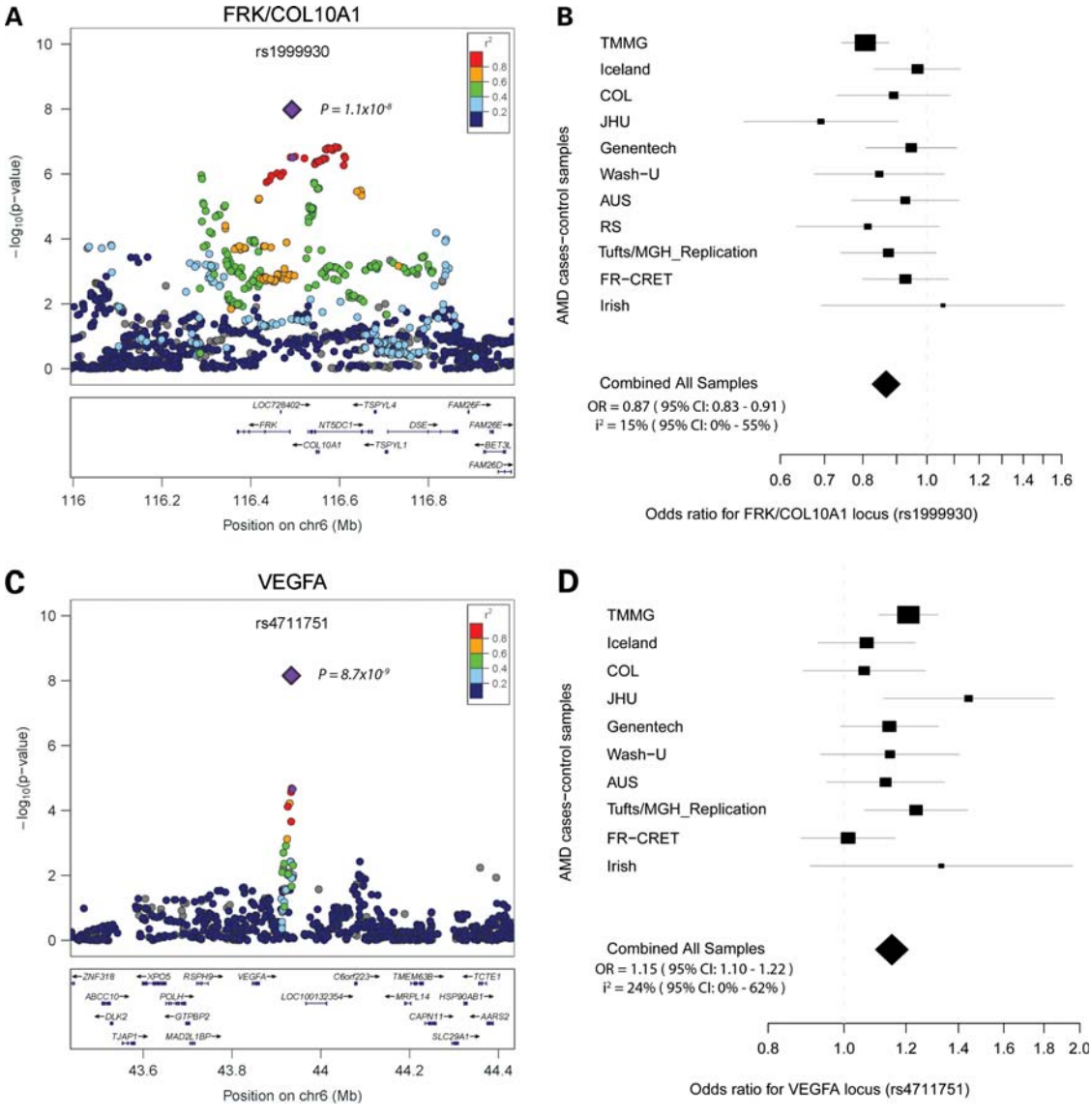

Figure 2. *FRK/COL10A1* and *VEGFA* regions and association with AMD. (**A**) Observed association in the 500 kb region surrounding the *FRK/COL10A1* locus in meta-analysis of TMMG data sets. The representative SNP (rs1999930) for this region with $P = 3.1 \times 10^{-7}$ is shown by a small purple circle. In the combined analysis including all 11 cohorts, this SNP was associated with AMD at $P = 1.1 \times 10^{-8}$ (large purple diamond). (**B**) Forest plot for rs1999930 association across 11 cohorts. (**C**) Observed association in the 500 kb region surrounding the *VEGFA* locus in meta-analysis of TMMG data sets. The represented SNP (rs4711751) for this region of $P = 2.2 \times 10^{-5}$ is shown by a small purple circle. In the combined analysis including all 10 cohorts, this SNP was associated with AMD at $P = 8.7 \times 10^{-9}$ (large purple diamond). (**D**) Forest plot for rs4711751 association across 10 cohorts.

development of neo-vascularization involved in wet AMD. This hypothetical mechanism needs future experimental validation.

COL10A1 encodes the alpha chain of type X collagen, a short-chain collagen expressed by hypertrophic chondrocytes during endochondral ossification. In patients with osteoarthritis, expression of *COL10A1* was significantly downregulated (28). Another collagen matrix pathway gene (*COL8A1*), which was implicated in our previous GWAS (15), also showed suggestive association to advanced AMD in our combined association analysis ($P = 9.7 \times 10^{-7}$). The C-terminal non-collagenous (NC1) domain of the collagen has been reported as an inhibitor of angiogenesis (29–31). *FRK* has also been shown to have negative function on the stimulation of microvascular survival of the developing retina by mediating the downstream signaling of thrombospondin-1 and the thrombospondin receptor (*CD36*), which has been shown to antagonize VEGFA signaling of the Akt pathway (32). The risk locus rs1999930 associated with advanced AMD in our study is in strong LD ($R^2 = 0.81$ in 1000 Genomes CEU data) with a functional variant rs9488843. The allele (G) at rs9488843, which creates a possible transcription factor-binding site for paired box 3 (PAX3) near the promoter region of *COL10A1*, is on the same haplotype as the allele (T) at rs1999930. Individuals with the protective allele (G) at rs9488843 may have increased binding of PAX3 at the locus, leading to elevated expression of *COL10A1* or *FRK* which results in the suppression or

Table 1. Genes associated with AMD in genome-wide meta-analysis and analysis of all samples combined

SNP	Gene	CHR	BP	EA[a]	TMMG meta-analysis					Replication		Combined analysis		
					Frequency		INFO[b]	OR	*P*-value	OR[c]	*P*-value[c]	OR	*P*-value	Samples[d]
					Cases	Controls								
Newly identified SNPs associated with AMD susceptibility														
rs1999930	*FRK/COL10A1*	6	116 387 134	T	0.26	0.30	0.97	0.81	3.1×10^{-7}	0.90	8.3×10^{-4}	0.87	1.1×10^{-8}	abcdefghijK
rs4711751	*VEGFA*	6	43 828 582	T	0.54	0.50	0.68	1.21	2.2×10^{-5}	1.13	4.3×10^{-5}	1.15	8.7×10^{-9}	ABCDEFGIJK
SNPs previously associated with AMD														
rs10490924	*ARMS2/HTRA1*	10	124 214 448	T	0.41	0.21	0.97	3.19	1.2×10^{-144}	2.80	5.0×10^{-181}	2.94	3.6×10^{-322}	ABEFIJK
rs1061170	*CFH*	1	196 659 237	C	0.61	0.37	1.00	2.74	5.6×10^{-138}	2.21	2.3×10^{-129}	2.41	1.3×10^{-261}	ABEFGIJ
rs1410996	*CFH*	1	196 696 933	G	0.80	0.58	1.00	3.12	2.1×10^{-134}	2.43	4.4×10^{-106}	2.71	7.4×10^{-235}	ABEIJK
rs641153	*CFB*	6	31 914 180	A	0.05	0.10	0.91	0.46	2.9×10^{-22}	0.61	7.8×10^{-12}	0.54	5.5×10^{-31}	abeijk
rs2230199	*C3*	19	6 718 387	C	0.24	0.19	0.57	1.68	1.4×10^{-18}	1.43	5.2×10^{-13}	1.53	4.6×10^{-29}	ABIJk
rs9332739	*C2*	6	31 903 804	C	0.02	0.04	0.89	0.45	4.3×10^{-12}	0.46	8.2×10^{-13}	0.46	2.4×10^{-23}	abeijk
rs9621532[e]	*TIMP3*	22	33 084 511	C	0.04	0.05	1.00	0.72	3.7×10^{-4}	0.59	3.0×10^{-13}	0.63	2.2×10^{-15}	abcdefijk
rs10468017	*LIPC*	15	58 678 512	T	0.26	0.29	0.87	0.83	4.6×10^{-5}	0.84	1.3×10^{-8}	0.84	2.7×10^{-12}	abcdefgijk
rs10033900	*CFI*	4	110 659 067	T	0.52	0.46	0.81	1.31	2.4×10^{-11}	1.09	1.3×10^{-2}	1.18	4.1×10^{-10}	ABEIjk
rs3764261	*CETP*	16	56 993 324	A	0.36	0.33	0.98	1.16	1.2×10^{-4}	1.14	1.4×10^{-5}	1.15	6.9×10^{-9}	ABCdEFGIJk

[a]Effective allele (EA): frequency and OR based on this SNP for each locus coded by the plus strand of reference human genome.
[b]INFO: information content, R^2 quality metric for imputation.
[c]Replication *P*-values and ORs were derived from meta-analysis results of all replication samples independent of the TMMG sample.
[d]Samples participated in the combined analysis for each SNP were indicated by letters (A/a to K/k). A capital letter indicates the effective allele of the SNP-increased risk of AMD in the specific sample. A lower case letter indicates the effective allele of the SNP-reduced risk of AMD in the specific sample. 'a' represents Tufts/MMAP/MIGen/GAIN (TMMG) samples; 'b', deCODE genetics sample replication (Iceland); 'c', the Columbia University sample replication (COL); 'd', the Johns Hopkins University sample replication (JHU); 'e', Genentech sample replication (Genentech); 'f', Washington University sample replication (WASH-U); 'g', the Centre for Eye Research Australia sample replication (AUS); 'h', the Rotterdam study sample replication (RS); 'i', the independent replication sample of Tufts/MGH (Tufts/MGH replication); 'j', the Hopital Intercommunal de Creteil sample replication (FR-CRET); 'k', the Queen's University of Belfast sample replication (Irish).
[e]The result of this SNP was from imputation data based on HapMap2 Project; all other SNPs were imputed based on 1000 Genomes Project.

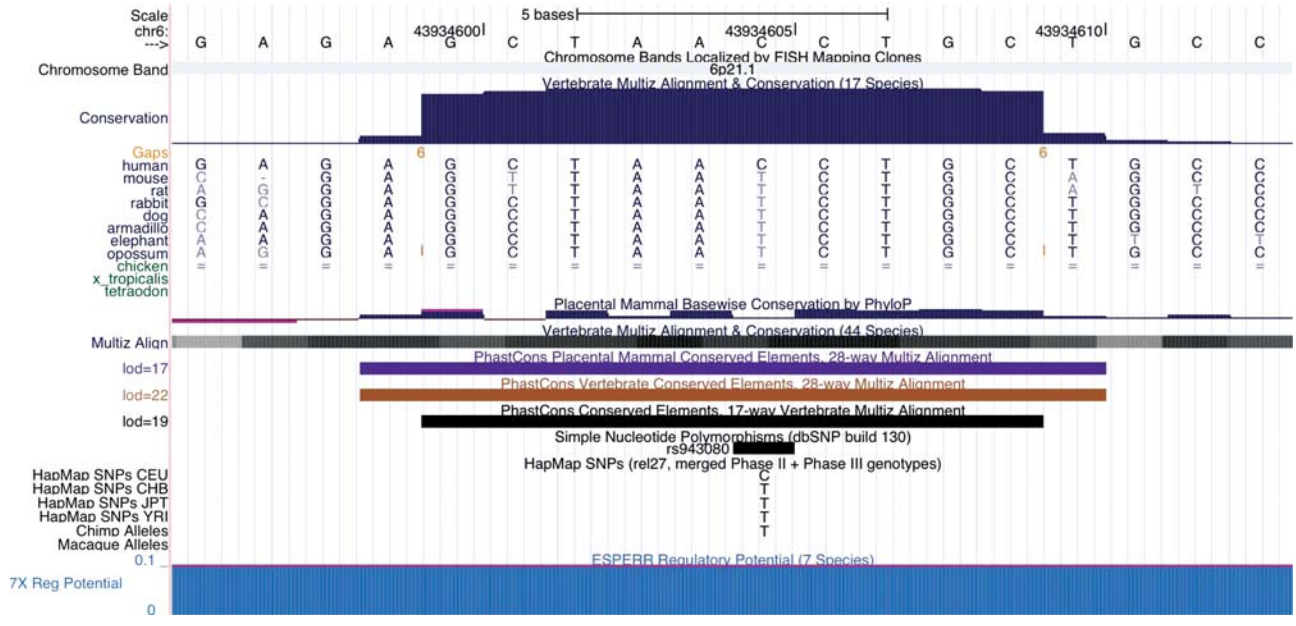

Figure 3. rs934080 in a putative CRX transcription factor-binding site. rs4711751 is in strong LD with rs934080, a variant which resides in a highly evolutionarily conserved region (UCSC genome browser) and disrupts a putative CRX transcription factor-binding site (CAA[T/C]C).

inhibition of angiogenesis. Further experimental work is required to investigate the functional role of rs9488843 in the development of advanced AMD.

The sample size of this study is the largest of all published association studies for advanced AMD to date. A major advantage of the study design is the careful diagnosis of cases across all cohorts. Since we included only subjects with advanced AMD in our study and excluded subjects with intermediate or large drusen, heterogeneity due to phenotype definition is reduced. However, it is possible that associations exist for other endophenotypes, like macular drusen, an early or intermediate stage of the disease, as suggested for loci in the HDL pathway (33).

Our novel findings are not likely caused by population admixture or population substructure, because subjects in all cohorts are of European ancestry, and we adjusted for the genetic ancestry components in our study. The large number of replication cohorts and samples reduced the chance of false-positive findings. The effect sizes of both rs1999930 and rs4711751 in the replication cohorts are smaller than the effect sizes estimated in the TMMG analysis. The larger effect size observed in the discovery cohort (TMMG) could be due to a 'winner's curse' phenomenon where association is often exaggerated relative to the estimated effect in follow-up studies (34).

For this study, we utilized the generally accepted genome-wide level of significance ($P < 5 \times 10^{-8}$) as our threshold for association. However, that threshold assumes a multiple hypothesis testing burden of ~1 000 000 independent SNPs. Indeed, in our study, since we used the 1000 Genomes Project imputation data, there were many more individual SNPs tested. However, many of those SNPs are highly inter-correlated. To our knowledge, there are no empirical studies that address levels of genome-wide significance for the 1000 Genomes Project-derived data.

Our genetic risk score model provides a framework for future research, and the clinical utility of genetic risk profiling of advanced AMD needs to be further evaluated in independent samples. Compared with other complex diseases, the associated risk variants for advanced AMD are more informative in terms of predicting risk of disease. As this prediction model only included genetic risk factors, we expect an improvement of the performance of advanced AMD risk assessment with additional environmental and demographic factors in prospective studies as in our previous calculations (18,19).

In summary, we have identified two novel associations for advanced AMD near *FRK/COL10A1* and *VEGFA*. We also confirmed associations for 10 previously published advanced AMD loci in a combined analysis. The genetic loci associated with AMD suggest that the disease process may be explained in part by dysregulation of the alternative complement pathway (*CFH*, *C2*, *CFB*, *C3*, *CFI*), HDL cholesterol metabolism (*LIPC*, *CETP*, *ABCA1*), angiogenesis (*VEGFA*) and degradation of extracellular matrix (*COL10A1*, *COL8A1*, *FRK*, *TIMP3*, and possibly *ARMS2*).

MATERIALS AND METHODS

The TMMG meta-analysis data set consisted of: (i) 1242 cases and 492 controls from the Tufts/MGH GWAS Cohort Study (15), which were derived from ongoing AMD study protocols as described previously (8,15,24,35–37); (ii) 1355 cases and 1076 controls from the MMAP Cohort Study (16); (iii) 1188 controls from the (MIGen) Consortium Study (21) and (iv) 1378 controls from the GAIN Schizophrenia Study (22). For the Tufts/MGH sample, cases had GA or NV disease based on fundus photography and ocular examination [clinical age-related maculopathy grading system (CARMS) stages

4 and 5] (38). Examined controls were unrelated to cases, 60 years of age or older and were defined as individuals without macular degeneration, categorized as CARMS stage 1, based on fundus photography and ocular examination. MMAP subjects were obtained and selected based on the dbGaP (phs000182.v2.p1) phenotype information (16). We included only MMAP controls and MMAP cases with GA or NV in the analysis; other MMAP subjects with large drusen were excluded. MIGen controls have been included in our previous GWAS study and described in detail (15). Shared controls from the GAIN Schizophrenia Study were obtained from dbGap (phs000021.v3.p2) and described in Manolio *et al.* (22).

The Tufts/MGH and MIGen samples were genotyped at the Broad Institute and National Center for Research Resources (NCRR) Center for Genotyping and Analysis, using the Affymetrix SNP 6.0 GeneChip (AFFY 6.0, 909 622 SNPs) (39). Shared controls from the GAIN study obtained from dbGap were also genotyped by using the Affymetrix SNP 6.0 GeneChip. MMAP samples obtained from dbGap were genotyped on the Illumina HumanCNV370v1 Bead Array (ILMN 370, 370 404 SNPs) (16). All samples included in this study met quality control measures as described previously (15,16). Briefly, individuals with call rates <0.95, SNPs with call rates <0.98, Hardy–Weinberg equilibrium $P < 10^{-6}$ and minor allele frequency (MAF) <0.01 were excluded. Potential relatedness between individuals was identified through a genome-wide identity-by-state (IBS) matrix using PLINK (40). IBS was estimated for each pair of individuals, and one individual from each duplicate pair or related pair (pihat $>$ 0.2) was removed. Ancestry outliers were identified based on principal components analysis using EIGENSOFT (Supplementary Material, Fig. S3) (41). After these quality control analyses (Supplementary Material, Table S1), the merged data set of TMMG contained 6728 samples, of which 4300 were genotyped by AFFY 6.0 and 2428 were genotyped by ILMN 370. The TMMG data set genotyped by AFFY 6.0 (644 413 SNPs passing quality control checks) was imputed using the phased CEU and TSI samples (566 haplotypes) as part of Pilot 3 of the 1000 Genomes Project as a reference by BEAGLE version 3.0 (42,43). Separate imputation was performed on the TMMG data set genotyped on the ILMN 370 (329 315 SNPs passing quality control checks) using the same method. For the meta-analysis of GWAS, we included only imputed genotypes with imputation quality scores >0.6, where the score is defined as the ratio-of-variances (empirical/asymptotic) of each genotype. This score is commonly applied as a quality filter for imputed genotypes and is equivalent to the RSQR_HAT value by MACH and the information content (INFO) measure by PLINK (44). Since the imputation accuracies are relatively low for SNPs with low MAF, we only included imputed genotypes of common variants (MAF >0.01) in the analysis. A consensus set of 6 036 699 high-quality SNPs from each imputed data set was analyzed by PLINK, using a generalized linear model controlling for the genotyping platform and genetic ancestry based on principal component analysis. The imputed genotypes were coded by the genotype probabilities (dosages) for each SNP, which were given less weights in the analysis than individuals with certain genotypes coded by (0, 1, 2). The eigenvector scores with nominal significant ($P < 0.05$) association with case/control status (principal components 1, 2, 3, 4, 5, 6, 7, 11 and 16) and the original genotyping platform were included as covariates in the analysis. The top 40 SNPs were validated using Sequenom genotyping on 1600 samples that were also part of the Tufts/MGH GWAS. The MAFs were compared for these SNPs and showed no significant differences between imputed and genotyped frequencies in cases or controls.

The replication data sets consisted of 5640 cases and 52 174 controls from 10 independent cohorts from JHU, COL, Genentech, Iceland, Wash-U, AUS, RS, FR-CRET, Irish and an independent replication sample from Tufts/MGH. All replication studies applied the same criteria for the diagnosis of cases. Population and shared controls were included in Genentech, Iceland and the RS samples. All participating studies received approval from institutional review boards (IRBs) and conformed to the tenets of the Declaration of Helsinki. All participants signed informed consent as approved by IRBs. Characteristics of each participating cohort are shown in Supplementary Material, Table S2. Samples from FR-CRET, Irish and Tufts/MGH replication data sets were genotyped at the Broad Institute by the Sequenom iPLEX assay. Samples from Wash-U were genotyped at the Sequenom Core Laboratory of Washington University. Samples from AUS were genotyped in-house and at the Murdoch Children's Research Institute Sequenom Platform Facility. Samples from JHU and COL were genotyped by the TaqMan assay, using the ABI PRISM 7900 Sequence Detection System (ABI, Foster City, CA, USA). For the SNPs we intended to replicate, we obtained directly genotyped or imputed results from Genentech, Iceland and RS samples. Genentech samples included 54 non-overlapping cases and 229 controls from the AREDS cohort (genotyped using Illumina Human610-Quad), 347 cases from a Genentech trial (Illumina Human660W-Quad), 3390 controls from the SLE GWAS study (45) (Illumina HumanHap550), 2274 controls from the CGEMS breast cancer study (46) and 2256 controls from the CGEMS prostate cancer study (47). For candidate SNPs not directly genotyped in the Genentech samples, genotype information was imputed using IMPUTE version 2 (48) with combined reference data of CEU and TSI population from the 1000 Genomes Project (June 2010 release) and HapMap3 Project. The Iceland samples were genotyped using Illumina HumanCNV370v1 Bead Array. Candidate SNPs not directly genotyped were imputed using IMPUTE version 2 with the reference data of CEU and TSI population from the 1000 Genomes Project (June 2010 release), HapMap2 Project (release 22) and a reference data set of 500–1000 Icelanders genotyped using the 1 million OmniQuad and CardioMetabo chips from Illumina. Owing to the larger size of the Icelanders data set, the imputation is more reliable based on the Icelanders data set than the imputation based on the HapMap or 1000 Genomes Project. The Rotterdam Study samples were genotyped by Illumina Infinium II HumanHap550 (cases $n = 192$, controls $n =$ 1887) and Illumina Human610-Quad Array (cases $n = 29$, controls $n = 2600$). Candidate SNPs not directly genotyped were imputed using MACH 1.0 (49) with the reference data of CEU and TSI population from the HapMap2 project (release 22). Genotyping and imputation methods used by the Rotterdam Study samples have been described in detail

previously (50). Standard quality control and statistical analysis for these samples were performed by Genentech, Iceland and RS separately. SNPs which met genotype quality control criteria in other replication cohorts were tested for association with advanced AMD, using a generalized linear model in PLINK. We used an additive model for each SNP (0, 1 or 2 minor alleles). The *P*-value for the combined analysis was derived from the effect size estimates and standard errors, using a fixed effects model by METAL (51). Heterogeneity of the association between SNP and disease was evaluated by Cochran's *Q*-test.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

We thank the participants, their families and numerous ophthalmologists throughout the country who participated in this study, the MIGen study group and the Brigham and Women's Hospital PhenoGenetic Project for providing DNA samples that were used in this study, the AREDS Research Group, and Dr J. Barre, Dr. J.C. Danan and P. Ledudal from the Clinical Researches Functional Unit, CHI Creteil, France. The MMAP dataset used for the analyses described in this manuscript was obtained from the NEI Study of Age-Related Macular Degeneration (NEI-AMD) Database found at http://www.ncbi.nlm.nih.gov/gap through dbGaP accession number phs000182.v2.p1. Funding support for NEI-AMD was provided by the National Eye Institute. We would like to thank NEI-AMD participants and the NEI-AMD Research Group for their valuable contribution to this research. Funding support for the Genome-Wide Association of Schizophrenia Study was provided by the National Institute of Mental Health (R01 MH67257, R01 MH59588, R01 MH59571, R01 MH59565, R01 MH59587, R01 MH60870, R01 MH59566, R01 MH59586, R01 MH61675, R01 MH60879, R01 MH81800, U01 MH46276, U01 MH46289 U01 MH46318, U01 MH79469, and U01 MH79470) and the genotyping of samples was provided through the Genetic Association Information Network (GAIN). The datasets used for the analyses described in this manuscript were obtained from the database of Genotypes and Phenotypes (dbGaP) found at http://www.ncbi.nlm.nih.gov/gap through dbGaP accession number phs000021.v3.p2. Samples and associated phenotype data for the Genome-Wide Association of Schizophrenia Study were provided by the Molecular Genetics of Schizophrenia Collaboration (PI: Pablo V. Gejman, Evanston Northwestern Healthcare (ENH) and Northwestern University, Evanston, IL, USA).

Conflict of Interest statement. T.R.B., W.O., T.W.B. and R.R.G. are employees of Genentech, Inc. G.T., O.G., H.S., K.S. and U.T. are employees of and/or own stock or stock options in deCODE genetics. Tufts Medical Center (J.M.S.) and Massachusetts General Hospital (M.J.D.) have filed patent applications related to this research.

FUNDING

We deeply appreciate the support of a generous anonymous donor to the research of J.M.S., without whom the Tufts/MGH genome-wide association study would not have been possible. This research was also supported in part by grants RO1-EY11309, RO1-EY13435, R24-EY017404, P30-EY 001765 and K12-EY16335 from the National Institutes of Health, Bethesda, MD, USA; Massachusetts Lions Eye Research Fund, Inc.; Unrestricted grants and Career Development Award from Research to Prevent Blindness, Inc., New York, NY, USA; Foundation Fighting Blindness, Owing Mills, MD, USA; The Macula Vision Research Foundation; Kaplen Foundation; Widgeon Point Charitable Foundation; the Alcon Research Institute; a Fight for Sight postdoctoral award; the National Health and Medical Research Council of Australia Centre for Clinical Research Excellence No. 529923—Translational Clinical Research in Major Eye Diseases and a Practitioner Fellowship to R.H.G. and Operational Infrastructure Support from the Victorian Government; American Macular Degeneration Foundation; and the Macular Degeneration Research Fund of the Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts Medical Center, Tufts University School of Medicine, Boston, MA, USA. Funding to pay the Open Access publication charges for this article was provided by the Macular Degeneration Research Fund, Tufts Medical Center.

REFERENCES

1. Seddon, J.M. and Sobrin, L. (2008) Epidemiology of age-related macular degeneration. In Albert, D., Miller, J., Azar, D. and Blodi, B. (eds), *Albert & Jakobiec's Principles and Practice of Ophthalmology*. 3rd edn. W.B. Saunders, Philadelphia, Vol. 1, pp. 413–422.
2. Klein, R., Cruickshanks, K.J., Nash, S.D., Krantz, E.M., Javier Nieto, F., Huang, G.H., Pankow, J.S. and Klein, B.E. (2010) The prevalence of age-related macular degeneration and associated risk factors. *Arch. Ophthalmol.*, **128**, 750–758.
3. Ciulla, T.A. and Rosenfeld, P.J. (2009) Antivascular endothelial growth factor therapy for neovascular age-related macular degeneration. *Curr. Opin. Ophthalmol.*, **20**, 158–165.
4. Klein, R.J., Zeiss, C., Chew, E.Y., Tsai, J.Y., Sackler, R.S., Haynes, C., Henning, A.K., SanGiovanni, J.P., Mane, S.M., Mayne, S.T. *et al.* (2005) Complement factor H polymorphism in age-related macular degeneration. *Science*, **308**, 385–389.
5. Hageman, G.S., Anderson, D.H., Johnson, L.V., Hancox, L.S., Taiber, A.J., Hardisty, L.I., Hageman, J.L., Stockman, H.A., Borchardt, J.D., Gehrs, K.M. *et al.* (2005) A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc. Natl Acad. Sci. USA*, **102**, 7227–7232.
6. Haines, J.L., Hauser, M.A., Schmidt, S., Scott, W.K., Olson, L.M., Gallins, P., Spencer, K.L., Kwan, S.Y., Noureddine, M., Gilbert, J.R. *et al.* (2005) Complement factor H variant increases the risk of age-related macular degeneration. *Science*, **308**, 419–421.
7. Edwards, A.O., Ritter, R. III, Abel, K.J., Manning, A., Panhuysen, C. and Farrer, L.A. (2005) Complement factor H polymorphism and age-related macular degeneration. *Science*, **308**, 421–424.
8. Maller, J., George, S., Purcell, S., Fagerness, J., Altshuler, D., Daly, M.J. and Seddon, J.M. (2006) Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. *Nat. Genet.*, **38**, 1055–1059.
9. Li, M., Atmaca-Sonmez, P., Othman, M., Branham, K.E.H., Khanna, R., Wade, M.S., Li, Y., Liang, L., Zareparsi, S., Swaroop, A. *et al.* (2006) CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration. *Nat. Genet.*, **38**, 1049–1054.

10. Gold, B., Merriam, J.E., Zernant, J., Hancox, L.S., Taiber, A.J., Gehrs, K., Cramer, K., Neel, J., Bergeron, J., Barile, G.R. *et al.* (2006) Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat. Genet.*, **38**, 458–462.
11. Maller, J.B., Fagerness, J.A., Reynolds, R.C., Neale, B.M., Daly, M.J. and Seddon, J.M. (2007) Variation in complement factor 3 is associated with risk of age-related macular degeneration. *Nat. Genet.*, **39**, 1200–1201.
12. Fagerness, J.A., Maller, J.B., Neale, B.M., Reynolds, R.C., Daly, M.J. and Seddon, J.M. (2009) Variation near complement factor I is associated with risk of advanced AMD. *Eur. J. Hum. Genet.*, **17**, 100–104.
13. Rivera, A., Fisher, S.A., Fritsche, L.G., Keilhauer, C.N., Lichtner, P., Meitinger, T. and Weber, B.H. (2005) Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum. Mol. Genet.*, **14**, 3227–3236.
14. Jakobsdottir, J., Conley, Y.P., Weeks, D.E., Mah, T.S., Ferrell, R.E. and Gorin, M.B. (2005) Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am. J. Hum. Genet.*, **77**, 389–407.
15. Neale, B.M., Fagerness, J., Reynolds, R., Sobrin, L., Parker, M., Raychaudhuri, S., Tan, P.L., Oh, E.C., Merriam, J.E., Souied, E. *et al.* (2010) Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). *Proc. Natl Acad. Sci. USA*, **107**, 7395–7400.
16. Chen, W., Stambolian, D., Edwards, A.O., Branham, K.E., Othman, M., Jakobsdottir, J., Tosakulwong, N., Pericak-Vance, M.A., Campochiaro, P.A., Klein, M.L. *et al.* (2010) Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. *Proc. Natl Acad. Sci. USA*, **107**, 7401–7406.
17. Gehrs, K.M., Jackson, J.R., Brown, E.N., Allikmets, R. and Hageman, G.S. (2010) Complement, age-related macular degeneration and a vision of the future. *Arch. Ophthalmol.*, **128**, 349–358.
18. Seddon, J.M., Reynolds, R., Yu, Y., Daly, M.J. and Rosner, B. (2011) Risk models for progression to advanced age-related macular degeneration using demographic, environmental, genetic, and ocular factors. *Ophthalmology*, in press.
19. Seddon, J.M., Reynolds, R., Maller, J., Fagerness, J.A., Daly, M.J. and Rosner, B. (2009) Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest. Ophthalmol. Vis. Sci.*, **50**, 2044–2053.
20. Sobrin, L., Maller, J.B., Neale, B.M., Reynolds, R.C., Fagerness, J.A., Daly, M.J. and Seddon, J.M. (2010) Genetic profile for five common variants associated with age-related macular degeneration in densely affected families: a novel analytic approach. *Eur. J. Hum. Genet.*, **18**, 496–501.
21. Kathiresan, S., Voight, B.F., Purcell, S., Musunuru, K., Ardissino, D., Mannucci, P.M., Anand, S., Engert, J.C., Samani, N.J., Schunkert, H. *et al.* (2009) Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat. Genet.*, **41**, 334–341.
22. Manolio, T.A., Rodriguez, L.L., Brooks, L., Abecasis, G., Ballinger, D., Daly, M., Donnelly, P., Faraone, S.V., Frazer, K., Gabriel, S. *et al.* (2007) New models of collaboration in genome-wide association studies: the Genetic Association Information Network. *Nat. Genet.*, **39**, 1045–1051.
23. Sobrin, L., Reynolds, R., Yu, Y., Fagerness, J., Leveziel, N., Bernstein, P.S., Souied, E.H., Daly, M.J. and Seddon, J.M. (2011) ARMS2/HTRA1 locus can confer differential susceptibility to the advanced subtypes of age-related macular degeneration. *Am. J. Ophthalmol.*, **151**, 345–352.
24. Seddon, J.M., Cote, J., Page, W.F., Aggen, S.H. and Neale, M.C. (2005) The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. *Arch. Ophthalmol.*, **123**, 321–327.
25. Barrett, J.C., Hansoul, S., Nicolae, D.L., Cho, J.H., Duerr, R.H., Rioux, J.D., Brant, S.R., Silverberg, M.S., Taylor, K.D., Barmada, M.M. *et al.* (2008) Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat. Genet.*, **40**, 955–962.
26. Haines, J.L., Schnetz-Boutaud, N., Schmidt, S., Scott, W.K., Agarwal, A., Postel, E.A., Olson, L., Kenealy, S.J., Hauser, M., Gilbert, J.R. *et al.* (2006) Functional candidate genes in age-related macular degeneration: significant association with VEGF, VLDLR, and LRP6. *Invest. Ophthalmol. Vis. Sci.*, **47**, 329–335.
27. Corbo, J.C., Lawrence, K.A., Karlstetter, M., Myers, C.A., Abdelaziz, M., Dirkes, W., Weigelt, K., Seifert, M., Benes, V., Fritsche, L.G. *et al.* (2010) CRX ChIP-seq reveals the cis-regulatory architecture of mouse photoreceptors. *Genome Res.*, **20**, 1512–1525.
28. Lamas, J.R., Rodriguez-Rodriguez, L., Vigo, A.G., Alvarez-Lafuente, R., Lopez-Romero, P., Marco, F., Camafeita, E., Dopazo, A., Callejas, S., Villafuertes, E. *et al.* (2010) Large-scale gene expression in bone marrow mesenchymal stem cells: a putative role for COL10A1 in osteoarthritis. *Ann. Rheum. Dis.*, **69**, 1880–1885.
29. O'Reilly, M.S., Boehm, T., Shing, Y., Fukai, N., Vasios, G., Lane, W.S., Flynn, E., Birkhead, J.R., Olsen, B.R. and Folkman, J. (1997) Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell*, **88**, 277–285.
30. Sudhakar, A., Nyberg, P., Keshamouni, V.G., Mannam, A.P., Li, J., Sugimoto, H., Cosgrove, D. and Kalluri, R. (2005) Human alpha1 type IV collagen NC1 domain exhibits distinct antiangiogenic activity mediated by alpha1beta1 integrin. *J. Clin. Invest.*, **115**, 2801–2810.
31. Ribatti, D. (2009) Endogenous inhibitors of angiogenesis: a historical review. *Leuk. Res.*, **33**, 638–644.
32. Sun, J., Hopkins, B.D., Tsujikawa, K., Perruzzi, C., Adini, I., Swerlick, R., Bornstein, P., Lawler, J. and Benjamin, L.E. (2009) Thrombospondin-1 modulates VEGF-A-mediated Akt signaling and capillary survival in the developing retina. *Am. J. Physiol. Heart Circ. Physiol.*, **296**, H1344–H1351.
33. Yu, Y., Reynolds, R., Fagerness, J., Rosner, B., Daly, M.J. and Seddon, J.M. (2011) Association of variants in LIPC and ABCA1 genes with intermediate and large drusen and advanced age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.*, **52**, 4663–4670.
34. Lohmueller, K.E., Pearce, C.L., Pike, M., Lander, E.S. and Hirschhorn, J.N. (2003) Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat. Genet.*, **33**, 177–182.
35. Seddon, J.M., Santangelo, S.L., Book, K., Chong, S. and Cote, J. (2003) A genomewide scan for age-related macular degeneration provides evidence for linkage to several chromosomal regions. *Am. J. Hum. Genet.*, **73**, 780–790.
36. Seddon, J.M., Cote, J., Davis, N. and Rosner, B. (2003) Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist–hip ratio. *Arch. Ophthalmol.*, **121**, 785–792.
37. Age-Related Eye Disease Study Research Group (2001) A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. *Arch. Ophthalmol.*, **119**, 1417–1436.
38. Seddon, J.M., Sharma, S. and Adelman, R.A. (2006) Evaluation of the clinical age-related maculopathy staging system. *Ophthalmology*, **113**, 260–266.
39. Korn, J.M., Kuruvilla, F.G., McCarroll, S.A., Wysoker, A., Nemesh, J., Cawley, S., Hubbell, E., Veitch, J., Collins, P.J., Darvishi, K. *et al.* (2008) Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs. *Nat. Genet.*, **40**, 1253–1260.
40. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J. *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.*, **81**, 559–575.
41. Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. and Reich, D. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.*, **38**, 904–909.
42. Browning, B.L. and Browning, S.R. (2009) A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am. J. Hum. Genet.*, **84**, 210–223.
43. Browning, S.R. and Browning, B.L. (2007) Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. *Am. J. Hum. Genet.*, **81**, 1084–1097.
44. de Bakker, P.I., Ferreira, M.A., Jia, X., Neale, B.M., Raychaudhuri, S. and Voight, B.F. (2008) Practical aspects of imputation-driven meta-analysis of genome-wide association studies. *Hum. Mol. Genet.*, **17**, R122–R128.
45. Hom, G., Graham, R.R., Modrek, B., Taylor, K.E., Ortmann, W., Garnier, S., Lee, A.T., Chung, S.A., Ferreira, R.C., Pant, P.V. *et al.* (2008) Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N. Engl. J. Med.*, **358**, 900–909.
46. Hunter, D.J., Kraft, P., Jacobs, K.B., Cox, D.G., Yeager, M., Hankinson, S.E., Wacholder, S., Wang, Z., Welch, R., Hutchinson, A. *et al.* (2007) A

genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat. Genet.*, **39**, 870–874.
47. Thomas, G., Jacobs, K.B., Yeager, M., Kraft, P., Wacholder, S., Orr, N., Yu, K., Chatterjee, N., Welch, R., Hutchinson, A. *et al.* (2008) Multiple loci identified in a genome-wide association study of prostate cancer. *Nat. Genet.*, **40**, 310–315.
48. Howie, B.N., Donnelly, P. and Marchini, J. (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.*, **5**, e1000529.
49. Li, Y., Willer, C.J., Ding, J., Scheet, P. and Abecasis, G.R. (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet. Epidemiol.*, **34**, 816–834.
50. Estrada, K., Abuseiris, A., Grosveld, F.G., Uitterlinden, A.G., Knoch, T.A. and Rivadeneira, F. (2009) GRIMP: a web- and grid-based tool for high-speed analysis of large-scale genome-wide association using imputed data. *Bioinformatics*, **25**, 2750–2752.
51. Willer, C.J., Li, Y. and Abecasis, G.R. (2010) METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*, **26**, 2190–2191.