Adult Onset Foveomacular Vitelliform Dystrophy Shows Genetic Overlap With Age-Related Macular Degeneration

Shlomit Jaskoll,[1,2] Adi Kramer,[1] Sarah Elbaz-Hayoun,[1] Batya Rinsky,[1] Chiara M. Eandi,[3,4] Michelle Grunin,[1,5] Yahel Shwartz,[1] Liran Tiosano,[1] Iris M. Heid,[6] Thomas Winkler,[6] and Itay Chowers[1]

[1]Department of Ophthalmology, Hadassah-Hebrew University Medical Center, and the Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
[2]Department of Military Medicine and "Tzameret", Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel, and Medical Corps, Israel Defense Forces, Israel
[3]Department of Surgical Sciences, University of Torino, Torino, Italy
[4]Jules-Gonin Eye Hospital, Fondation Asile des Aveugles, Department of Ophthalmology, University of Lausanne, Lausanne, Switzerland
[5]Braun School of Public Health and Community Medicine, Hebrew University of Jerusalem, Jerusalem, Israel
[6]Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany

Correspondence: Itay Chowers, Department of Ophthalmology, Hadassah Medical Center, Jerusalem 91120, Israel; chowers@hadassah.org.il.

Received: August 5, 2024
Accepted: November 4, 2024
Published: November 25, 2024

Citation: Jaskoll S, Kramer A, Elbaz-Hayoun S, et al. Adult onset foveomacular vitelliform dystrophy shows genetic overlap with age-related macular degeneration. *Invest Ophthalmol Vis Sci.* 2024;65(13):53. https://doi.org/10.1167/iovs.65.13.53

Purpose. Adult-onset foveomacular vitelliform dystrophy (AFVD) shares phenotypic similarities with age-related macular degeneration (AMD). The genetic factors associated with AFVD are unknown in >80% of cases. This study evaluated the association of known AMD genetic risk variants with AFVD and compared systemic complement activation in these conditions.

Methods. Clinical, imaging, and genetic data were collected from 50 patients with AFVD (men/women = 25/25, mean age ± SD 73 ± 10 years), 917 patients with AMD (men/women = 377/540, mean age ± SD 77 ± 9 years), and 432 unaffected healthy controls (men/women = 202/230, mean age ± SD 71 ± 8 years). Genotyping focused on 52 single nucleotide polymorphisms (SNPs) linked to AMD. Weighted genetic risk scores (GRS) for 19 complement system associated variants, 7 lipid metabolism associated variants, the remaining 26 variants (other pathways GRS), and for all 52 variants (global score) were derived and correlated with phenotype.

Results. Of the 52 SNPs evaluated, CFH (rs570618) and C2/CFB/SKIV2L (rs116503776 and rs114254831) were associated with AFVD compared with healthy controls (odds ratio [OR] = 2.73, 95% confidence interval [CI] = 1.32–5.73, $P = 0.01$; OR = 0.31, 95% CI = 0.14–0.71, $P = 0.0036$; and OR = 0.41, 95% CI = 0.22–0.74, $P = 0.0025$, respectively). MIR6130/RORB (rs10781182) was negatively associated with AFVD compared with the healthy controls (OR = 0.13, CI = 0.06–0.25, $P < 0.0001$) and AMD (OR = 0.19, CI = 0.10–0.34, $P < 0.0001$). Regression analysis showed complement GRS was positively associated with AFVD compared with controls (OR = 1.42, 95% CI = 1.04–1.95, $P = 0.03$), whereas the other pathways' GRS was negatively associated (OR = 0.46, 95% CI = 0.21–0.98, $P = 0.04$). AMD was positively associated with the complement score, global score, and ARMS2/HTRA1 compared with controls.

Conclusions. Non-monogenic AFVD is associated with AMD risk alleles in the complement cascade, but not in other pathways. Further research is needed to explore complement inhibition for AFVD.

Keywords: age-related macular degeneration (AMD), adult onset foveomacular vitelliform dystrophy (AFVD), genetics, complement system

Adult-onset foveomacular vitelliform dystrophy (AFVD) is a prevalent type of macular degeneration. This condition can cause visual impairment and, in more severe cases, can lead to substantial vision loss due to choroidal neovascularization or retinal pigmented epithelium (RPE) atrophy in the macula area.[1] AFVD is the common phenotype of pattern dystrophies (PDs), along with butterfly-shaped pigment dystrophy (BSPD), with an approximated prevalence that ranges from 1:7400 to 1:8200.[2–4] AFVD can be associated with monogenic mutations in genes including PRPH2, BEST1, and IMPG1/2, or rarely others. The prevalence of such mutations among patients with AFVD vary among different cohorts and ranges from 0% to 18%.[1,5–7]

PD phenotypes like AFVD and BSPD can manifest a varying and overlapping phenotype, and they also share characteristics with age-related macular degeneration (AMD). Both AFVD and AMD are associated with age, although AFVD tends to appear earlier. Individuals affected by either disease

may have a positive family history. Both conditions involve RPE alterations that can progress to atrophy, both manifest retinal deposits (drusen and pseudovitelliform lesions), and both might be complicated by the development of macular neovascularization.[6,8] Acquired vitelliform lesions (AVLs) may also be associated with specific types of drusen, including cuticular drusen and subretinal drusenoid deposits (SRDD).[9]

Fifty-two (52) genetic variants in 34 loci were independently associated with the risk for developing AMD in a large genomewide association study (GWAS).[10] Among these 34 loci, several are related to genes involved in the complement cascade and lipid metabolism. Loci related to the complement pathway include Complement factor H (*CFH*), Complement components 3, 2, and 9 (*C3*, *C2*, and *C9*), Complement factor I (CFI), Transmembrane protein 97/Vitronectin (TMEM97/VTN) and the age-related maculopathy susceptibility 2/high-temperature requirement A serine peptidase 1 (ARMS2/HTRA1) variant, which is suspected to be associated with complement function.[11] However, due to the uncertainty of its role in the complement system, we did not include ARMS2/HTRA1 in the complement genetic score but rather in the other pathways score, which will be detailed later in this manuscript. Of the 34 loci, the protein encoded by Apolipoprotein E (APOE), Cholesteryl ester transfer protein (CETP), ATP binding cassette transporter A1 (ABCA1), and Lipase C (LIPC) genes are associated with lipid metabolism. These genetic findings, as well as histological findings suggested that the alternative pathway of complement activation has a major role in the pathogenesis of AMD, ultimately leading to RPE dysfunction, photoreceptor loss, and neovascularization.[12] Accordingly, complement inhibitors were recently approved to slow the progression of geographic atrophy in atrophic AMD (aAMD).[13] Systemic complement activation has been previously evaluated in AMD, to investigate its role in disease pathogenesis. Interestingly, the exploration of systemic complement activation in AMD has yielded inconsistent findings across several studies.[14–16] Systemic complement activation may reflect immune responses that could contribute to retinal damage, providing a broader understanding of how complement dysregulation influences AMD and AFVD pathology. This systemic perspective, if further clarified, may help elucidate the biological relevance of genetic variants shared between the two conditions and their potential impact on disease mechanisms.

Although AFVD presents phenotypic similarities to AMD, the extent to which AMD-associated genetic variants and complement activation are involved in the pathogenesis of AFVD remains unclear. We have previously reported of an association between the common *HTRA1* variant (rs11200638), that is highly associated with AMD, and AFVD. However, the common risk variants in *CFH* (rs1061170) and *C3* (rs2231099) genes were not associated with AFVD in our previous study that included 35 patients who were diagnosed with AFVD or BSPD.[7]

Here, we aim to obtain a more comprehensive understanding of the genetic factors that are associated with AFVD. Understanding the intricate interplay of genetic predisposition and the complement system in AFVD holds a promise for advancing our therapeutic approaches for this condition, especially as complement inhibitors became available for the treatment of aAMD. To that end, we have compared the genetic risk profile among patients with AFVD and patients with AMD, and unaffected healthy controls, and have evaluated for complement activation and its association with AFVD.

Methods

Patients and Controls

A total of 50 patients with AFVD (men/women = 25/25, mean age ± standard deviation [SD] = 73 ± 10 years, range = 51–93 years), 917 patients with AMD (men/women = 377/540, mean age ± SD = 77 ± 9 years, range = 55–96 years), and 432 unaffected healthy controls (men/women = 202/230, mean age ± SD = 71 ± 8 years, range = 50–97 years), were included in this study. An independent group of 17 patients with AFVD (men/women = 11/6, mean age ± SD = 74 ± 9 years, range = 54–88 years), 18 patients with AMD (men/women = 7/11, mean age ± SD = 72 ±6 years, range = 61–85 years), and 24 unaffected healthy controls (men/women = 14/10, mean age ± SD = 68 ± 6 years, range = 57–80 years) were evaluated for systemic complement activation. None of the patients had a positive family history for AFVD.

All patients and controls were enrolled at the Department of Ophthalmology of the Hadassah-Hebrew University Medical Center in Jerusalem, Israel, between January 2009 and April 2023. Ethical approval for the study was granted by the institutional ethics committee, and all patients provided an informed consent before enrollment. Clinical data and retinal imaging studies were collected from the electronic medical records (EMRs). Diagnosis of AFVD was determined by retina specialists following the criteria that we have previously detailed.[6] Briefly, AFVD diagnosis was based on the Gass's original description,[17] in adult patients exhibiting vitelliform lesions in at least one eye. Exclusion criteria included other pathologies associated with vitelliform lesions, such as Best's disease, vitreomacular traction (VMT), epiretinal membrane (ERM), active systemic cancer, and central serous choroidopathy. If drusen were present, AFVD was diagnosed instead of AMD when the foveal vitelliform lesion was the primary clinical feature. Cuticular drusen with vitelliform lesions were excluded based on the fluorescein angiographic appearance of early hyperfluorescence of the drusen that fades in the later phases of the angiogram. Cases with subretinal drusenoid deposits were also excluded. Drusen were permitted in some of the cases, as they have also been described in the Gass' original description of the phenotype, and because they may also present in cases of proven monogenic AFVD. Diagnosis of AMD was determined according to the Age-Related Eye Disease Study (AREDS) criteria.[18] Unaffected healthy individuals were designated as the control group. To qualify for inclusion as controls, participants underwent retinal examinations that revealed no evidence of retinal disease. Those with significant systemic conditions, including cancer, autoimmune disorders, congestive heart failure, active endocrine conditions, or uncontrolled diabetes, were excluded from the study. Multimodal imaging of representative cases of the three diagnostic categories (AMD, AFVD, and control) can be found in Figure 1.

Disease Staging

In patients with AFVD, the stage of vitelliform lesions (vitelliform, vitelliruptive, pseudohypopyon, and atrophy) was classified based on strict criteria from our previous

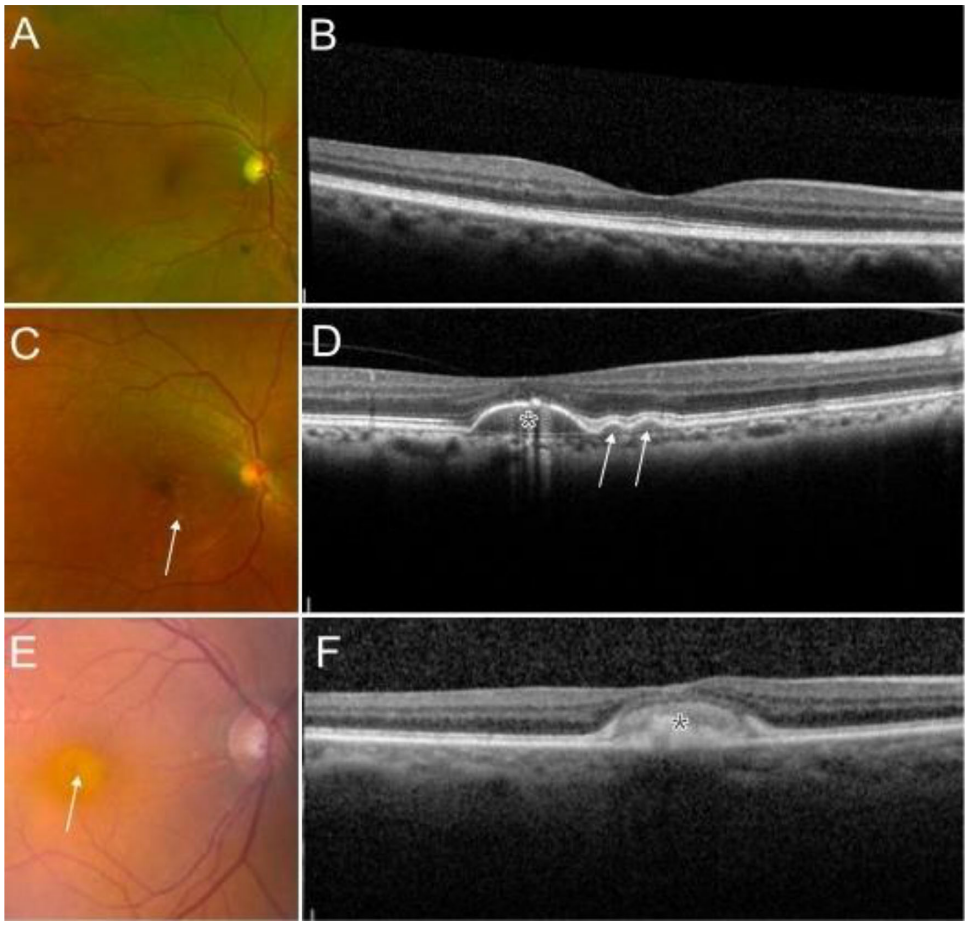

Figure 1. Multimodal imaging of healthy retina, age-related macular degeneration (AMD), and adult-onset foveomacular vitelliform dystrophy (AFVD). (**A, B**) Color fundus photograph (**A**) and spectral-domain optical coherence tomography (SD-OCT) scan (**B**) of the right eye of a 68-year-old woman with no signs of AMD or AFVD (control). Both modalities show unaffected retina. (**C, D**) Color fundus photograph showing typical drusen (**C**) and SD-OCT scan (**D**) of the right eye of an 81-year-old woman with non-neovascular AMD, showing two large drusen (>125 µm; *arrows*) and drusenoid pigment epithelial detachment (PED; *asterisk*). (**E, F**) Color fundus photograph (**E**) and SD-OCT scan (**F**) of the right eye of an 84-year-old man with AFVD, demonstrating a yellow foveal lesion in the color photograph (*arrow*) and a dome-shaped subretinal lesion in the vitelliform stage in the OCT section (*asterisk*).

work.[6] Classification was based on spectral domain optical coherence tomography (SD-OCT; Spectralis, Heidelberg, Germany) imaging from baseline and follow-up examinations over 5 years. Classification of the vitelliform lesions was similar to the one described in the CAM report #5 and according to published histological and multimodal imaging studies.[19–21]

This follow-up span was applicable to a subset of 29 patients (men/women = 15/14, mean age ± SD = 68 ± 9, range = 63–84 years), comprising 55 eyes affected by AFVD. Within this patient subset, a further subdivision was executed to stratify patients based on their progression rate. Consequently, patients were categorized into either the "slow progression" (SP) or "fast progression" (FP) groups. This categorization facilitated the execution of a binary regression analysis aimed at unveiling the potential association between genetic scores and the disease progression rate. Assignment to the SP and FP groups was determined according to the number of stages progressed within the follow-up duration; group A – included eyes exhibiting regression, with disease features improving and manifesting an earlier stage at the second examination, group B – eyes displaying neither progression nor regression, with disease features remaining stable between examinations, group C – included eyes demonstrating progression by one stage, group D – included eyes demonstrating progression by two stages, and group E – included eyes demonstrating progression by three stages. For each patient, the progression rate of the disease was determined as the average between the two eyes when both were analyzed. The SP group included all patients in groups A, B, and C, whereas the FP group included all patients in groups D and E. A correlation analysis was also conducted to ascertain the interplay between genetic scores and progression rates across the five categories.

Genetic Analysis

Six hundred seventy-seven (677) of 1399 samples included in the study were genotyped on a custom Illumina chip platform designed by the International AMD Genomic Consortium (IAMDGC)[10] that contains approximately 250,000 tagging markers for imputation and approximately 250,000 custom markers for AMD. The other samples (n = 722)

Investigative Ophthalmology & Visual Science

underwent sequencing on a parallel platform; the Infinium Global Screening Array version 3.0 (GSA) which has been comprehensively described to ensure robust performance for polygenic scores and genome imputation.[22] The genetic data from both sets were submitted separately to the TOPMed Imputation Server (BioData Catalyst; https://imputation.biodatacatalyst.nhlbi.nih.gov/#!) due to the different chips[23] for TOPMed version 2 imputation.[24] Imputation utilized Eagle version 2.4 for phasing and minimac4 for imputation.[25] Imputation of all autosomal chromosomes and the pseudoautosomal regions of the X chromosome was complete, with no missing data blocks (10 MB chunks). Post-imputation, datasets were combined into a single set with consistent number of markers with identical matches to strand/allele for the reference. Cross-chip comparison revealed uniform variant imputation, confirming successful imputation on both platforms. All patients with AFVD included in this study tested negative for mutations in the genes *PRPH2*, *BEST1*, *IMPG*1 (exons 3, 7, and 13), and *IMPG*2 (exon 3), as we have previously described.[7]

A weighted genetic risk score (GRS) methodology was used to assess the cumulative genetic predisposition of individuals toward a specific outcome. The GRS was calculated for each subject ($i = 1,\ldots, n$) based on a set of k SNPs considered, where the number of risk alleles was encoded as 0, 1, or 2. The weighted GRS (GRS_i) for each patient was computed using a summation of the minor alleles multiplied by external weights ($w_1,\ldots, w_k$) for each variant, which were determined from the marginal genetic effects of the corresponding SNPs estimated in a separate study population. The external weights reflected the approximate influence of each SNP on the risk of developing AMD. Specifically, we extracted the effect sizes (β) from odds ratios ($\exp(\beta)$) based on a fully conditioned analysis conducted in the prior study of IAMDGC.[10] We compared the odds ratios between the Fritsche et al. GWAS and a Leave-One-Out GWAS, where the Israeli samples were completely removed. The odds ratios differed by less than 0.001 decimal points, allowing us to confidently use the Fritsche et al. GWAS as the weights for our analysis. Although newer GWAS-derived PRS models have been published,[26,27] we used the 2016 IAMDGC data for our analysis due to its robust validation and widespread acceptance. These newer studies have shown improved prediction accuracy, particularly in the higher polygenic risk score (PRS) deciles, but without a significant increase in area under the curve (AUC) values. Given the modest incremental gains in accuracy, we chose to continue using the well-validated 2016 PRS for this study. This approach allowed us to account for the varying degrees of contribution of genetic variants related to different molecular pathways to the overall genetic risk, providing a more accurate representation of genetic predisposition in our subsequent genetic analyses.[28] We calculated four GRS highlighting specific pathways associated with AMD. A complement pathway-focused score, which included 19 variants related to the complement pathway, a lipid pathway-focused score, consisting of 7 variants associated with the lipid pathway, a score including the remaining 26 variants out of the 52 variants reported by the IAMDGC, and a global genetic score including all 52 variants. The attribution of variants to their respective molecular pathways was ascertained through a functional enrichment analysis conducted by Fritsche and colleagues, utilizing the INRICH tool.[29] A comprehensive listing of these variants, along with their corresponding molecular pathways, can be found in Supplementary Table S2.

Enzyme-Linked Immunosorbent Assays

Peripheral blood was collected into EDTA-containing tubes. EDTA chelates Ca^{2+} and Mg^{+}, thereby blocking the function of C3 convertases and C1 complex, minimizing any further in vitro activation of the complement system.[30] Samples were placed in 4°C immediately after collection and centrifuged (1400 × g at 4°C for 15 minutes) within 4 hours to isolate plasma. Immediately after centrifugation, samples were placed at −80°C, and the freeze-thaw cycles were avoided altogether. To quantify complement activation in the collected plasma samples, a sandwich ELISA was performed for C3a (550499, BD OptEIA, San Jose, CA, USA), and a competitive ELISA for C3 (ab108822, Abcam Cambridge, England), respectively, according to the manufacturer's instructions. Samples were run in duplicates. Optical density (450 nm) was measured to quantify the ELISA results. A standard curve was generated for every experiment by regression analysis using a four-parameter logistic curve-fit, the standard curves are presented in Supplementary Figure S2. R square of the standard curves was higher than 0.95 in all experiments. To avoid a bias secondary to the initial C3 concentration in different individuals, we calculated a C3a/C3 ratio to estimate complement activation levels.

C-reactive protein (CRP) levels were measured, and three samples, one from each group of patients, were excluded for presenting CRP levels higher than 10 mg/L which indicate an active inflammation that can often lead to an increase in systemic complement activation.[31]

Statistical Analysis

Statistical analysis was performed using SPSS software (version 27, Chicago, IL, USA) and GraphPad Prism Software (version 8, Boston, MA, USA), as we have previously described.[7,32] Briefly, Fisher exact, and χ^2 tests were applied to assess odds ratios, confidence intervals, and significance. When analyzing the 52 SNPs, we corrected for multiple comparisons using the false discovery rate. To explore the relationship between genetic scores and the occurrence of AMD and AFVD, as well as the progression rate of AFVD, we utilized logistic regression, adjusted for age and sex. Additionally, a correlation analysis was performed, yielding Spearman's rank correlation coefficient (Spearman's rho) for each parameter under investigation.

Results

Single Variants Analysis

Distributions of the 52 AMD risk variants across the three distinct groups (AMD, AFVD, and controls) was assessed. A detailed depiction of the minor allele frequency (MAF) can be found in Supplementary Figure S1. For each variant, we conducted a χ^2 analysis in terms of genotype (homozygous for major allele in comparison to heterozygous and homozygous for minor allele combined) and in terms of allele count (number of major alleles in comparison to minor alleles). Additionally, a χ^2 test for trend was conducted to compare the genotypes as separate natural groups (homozygous for major allele, and heterozygous and homozygous for minor allele). Significant results are detailed in Tables 1 and 2, and the full results report can be found in Supplementary Table S1.

Investigative Ophthalmology & Visual Science

Table 1. Significant Results Highlighting Differences in the Genotype of 52 AMD-Associated Genetic Variants Among Patients With AFVD, Patients With AMD, and Unaffected Controls

			AFVD Versus Controls			AFVD Versus AMD			AMD Versus Controls		
Pathway	**Locus**	**Variant**	**OR**	**95% CI**	**Sig.**	**OR**	**95% CI**	**Sig.**	**OR**	**95% CI**	**Sig.**
Complement pathway											
	CFH	rs10922109	0.91	0.49 to 1.62	0.75	1.65	0.89 to 3.04	*0.11*	0.55	0.43 to 0.69	***<0.0001****
		rs570618	2.73	1.32 to 5.73	***0.01***	1.32	0.65 to 2.73	*0.60*	2.07	1.60 to 2.65	***<0.0001****
		rs187328863	0.43	0.04 to 2.48	0.71	0.21	0.02 to 1.17	0.12	2.01	1.21 to 3.29	***0.0041****
	C9	rs62358361	0.00	0 to 77.7	>0.9999	0.00	0 to 5.51	>0.9999	4.30	0.79 to 23.3	*0.05*
	C2/CFB/SKIV2L	rs181705462	1.88	0.73 to 4.29	0.18	2.45	1.00 to 5.53	***0.04***	0.76	0.49 to 1.18	0.25
	C3	rs2230199	0.75	0.37 to 1.45	0.51	0.58	0.29 to 1.14	0.12	1.29	1.01 to 1.62	***0.04***
Lipid pathway											
	APOE	rs429358	1.30	0.63 to 2.60	0.44	1.83	0.91 to 3.70	0.09	0.71	0.51 to 0.96	**0.03**
	CETP	rs17231506	1.10	0.59 to 2.00	0.77	0.81	0.45 to 1.44	0.56	1.35	1.07 to 1.69	***0.01***
	LIPC	rs2043085	2.90	1.14 to 6.91	***0.02***	3.55	1.45 to 8.38	***0.00***	0.82	0.62 to 1.06	0.13
Other pathway											
	TNFRSF10A	rs79037040	2.60	0.85 to 8.23	0.12	1.79	0.60 to 5.60	0.47	1.46	1.04 to 2.01	***0.03***
	MIR6130/RORB	rs10781182	0.13	0.06 to 0.25	***<0.0001****	0.19	0.10 to 0.34	***<0.0001****	0.70	0.48 to 0.99	0.05
	TRPM3	rs71507014	0.64	0.32 to 1.25	0.24	0.94	0.48 to 1.80	0.86	0.69	0.50 to 0.92	***0.01***
	RDH5/CD63	rs3138141	0.60	0.27 to 1.26	0.21	0.42	0.19 to 0.91	***0.03***	1.41	1.07 to 1.84	***0.01***
	RAD51B	rs2842339	6.18	1.04 to 64.2	***0.04***	4.73	0.82 to 48.8	*0.11*	1.31	0.92 to 1.85	*0.15*
	CTRB2/CTRB1	rs72802342	0.00	0 to 0.65	***0.01***	0.00	0 to 0.75	***0.03***	0.73	0.49 to 1.07	0.12
	CNN2	rs67538026	2.58	1.23 to 5.59	**0.02**	1.29	0.62 to 2.80	0.58	1.99	1.60 to 2.47	**<0.0001***
	ARMS2/HTRA1	rs3750846	1.51	0.83 to 2.76	0.18	0.75	0.43 to 1.33	*0.38*	2.01	1.59 to 2.53	***<0.0001****

AMD, age-related macular degeneration; AFVD, adult-onset foveomacular vitelliform dystrophy;; CI, confidence interval; FDR, false discovery rate; OR, odds ratio; Sig., significance.

Bold indicate significance ($P < 0.05$); and *italics* indicate trend ($P < 0.12$).

* Indicates significance after FDR correction.

Table 2. Significant Results Highlighting Differences in Allele Frequencies of 52 AMD-Associated Genetic Variants Among Patients With AFVD, Patients With AMD, and Unaffected Controls

			AFVD Versus Controls			AFVD Versus AMD			AMD Versus Controls		
Pathway	**Locus**	**Variant**	**OR**	**95% CI**	**Sig.**	**OR**	**95% CI**	**Sig.**	**OR**	**95% CI**	**Sig.**
Complement pathway	CFH	rs10922109	0.75	0.48 to 1.13	0.20	1.20	0.79 to 1.80	0.40	0.63	0.52 to 0.74	**<0.0001***
		rs570618	2.04	1.32 to 3.14	**0.0013***	1.40	0.91 to 2.15	0.14	1.47	1.22 to 1.74	**<0.0001***
		rs187328863	0.22	0.02 to 1.24	0.15	0.11	0.01 to 0.61	**0.0046***	1.91	1.16 to 3.14	**0.01**
	C9	rs62358361	0.00	0 to 38.9	>0.9999	0.00	0 to 2.72	0.63	4.25	0.78 to 23.0	0.05
	C2/CFB/SKIV2L	rs116503776	0.31	0.14 to 0.71	**0.0036***	0.41	0.19 to 0.91	**0.04**	0.76	0.56 to 1.01	0.07
		rs114254831	0.41	0.22 to 0.74	**0.0025***	0.41	0.23 to 0.73	**0.0019***	0.99	0.78 to 1.23	0.91
	C3	rs2230199	0.45	0.24 to 0.81	**0.01**	0.38	0.20 to 0.66	**0.0005***	1.18	0.95 to 1.45	0.13
Lipid pathway											
	CETP	rs17231506	0.58	0.36 to 0.93	**0.03**	0.46	0.28 to 0.73	**0.0009***	1.25	1.03 to 1.51	**0.02**
	LIPC	rs2070895	0.56	0.31 to 1.00	0.06	0.56	0.31 to 0.99	0.05	1.00	0.79 to 1.26	>0.9999
Other pathway											
	MIR6130/RORB	rs10781182	0.32	0.19 to 0.51	**<0.0001***	0.33	0.21 to 0.52	**<0.0001***	0.95	0.79 to 1.14	0.61
	RDH5/CD63	rs3138141	0.67	0.33 to 1.35	0.31	0.51	0.24 to 1.03	0.06	1.33	1.04 to 1.68	**0.02**
	RAD51B	rs2842339	2.36	1.27 to 4.60	**0.01**	1.76	0.93 to 3.38	0.08	1.34	1.11 to 1.62	**0.0026***
	ARMS2/HTRA1	rs3750846	0.80	0.50 to 1.28	0.36	0.43	0.27 to 0.66	**0.0002***	1.86	1.52 to 2.26	**<0.0001***

AMD, age-related macular degeneration; AFVD, adult-onset foveomacular vitelliform dystrophy; CI, confidence interval; FDR, false discovery rate; OR, odds ratio; Sig., significance.

Bold indicates significance ($P < 0.05$).

* Indicates significance after FDR correction.

In terms of genotypes, the risk allele for one variant, *C2/CFB/SKIV2L* (rs181705462) was more common in patients with AFVD than in patients with AMD (odds ratio [OR] = 2.44, 95% confidence interval [CI] = 1.00 to 5.53, $P = 0.04$), whereas the other complement variants showed similar distribution in AMD and AFVD. When comparing patients with AMD to healthy controls, 5 out of 19 complement pathway variants, all in the loci of *CFH*, *C9*, and *C3*, were associated with AMD in this cohort. Of the seven variants in genes associated with lipid metabolism, the minor allele of *LIPC* (rs2043085) was more common in AFVD in comparison to controls (OR = 2.90, 95% CI = 1.14 to 6.91, $P = 0.02$) and AMD (OR = 3.55, CI = 1.45 to 8.38, $P = 0.00$). In the comparison between the AMD and control groups, the minor allele of APOE (rs429358) was observed less frequently in the AMD group (OR = 0.71, 95% CI = 0.51 to 0.96). Conversely, the minor allele of CETP (rs17231506) was more prevalent in the AMD group (OR = 1.35, 95% CI = 1.071 to 1.693), whereas the other variants in the lipid pathway showed similar distribution between the groups. A

Investigative Ophthalmology & Visual Science

variant in *MIR6130/RORB* (rs10781182) was less common in patients with AFVD compared to controls (OR = 0.13, 95% CI = 0.06 to 0.25, $P < 0.0001$) and in patients with AFVD compared to patients with AMD (OR = 0.19, 95% CI = 0.10 to 0.34, $P < 0.0001$). When evaluating allelic frequency, *MIR6130/RORB* (rs10781182) remained negatively associated with AFVD compared to controls (OR = 0.32, 95% CI = 0.19 to 0.51, $P < 0.0001$), and AFVD compared to AMD (OR = 0.33, 95% CI = 0.21 to 0.52, $P < 0.0001$).

ARMS2/HTRA1 genotypes (rs3750846; OR = 2.01, 95% CI = 1.59 to 2.53, $P < 0.0001$), and alleles (OR = 1.86, 95% CI = 1.52 to 2.26, $P < 0.0001$) were associated with AMD compared with controls. *ARMS2/HTRA1* (rs3750846) was negatively associated with AFVD compared with AMD in terms of allele count (OR = 0.43, 95% CI = 0.27 to 0.66, $P = 0.0002$), but not in terms of the genotypes.

Genetic Scores-Based Analysis

Four custom genetic scores were generated to obtain an overall assessment of the contribution of specific biologic pathways (complement and lipid) to AFVD compared with AMD and unaffected controls. The global genetic score, including all 52 variants associated with AMD in previous studies, was higher in patients with AMD (mean ± SD = 1.45 ± 0.11) compared with controls (mean ± SD = 0.65 ± 0.07, $P < 0.0001$) and patients with AFVD (mean ± SD = 0.95 ± 0.12, $P = 0.00$). This score showed a borderline significant difference between patients with AFVD (mean ± SD = 0.95

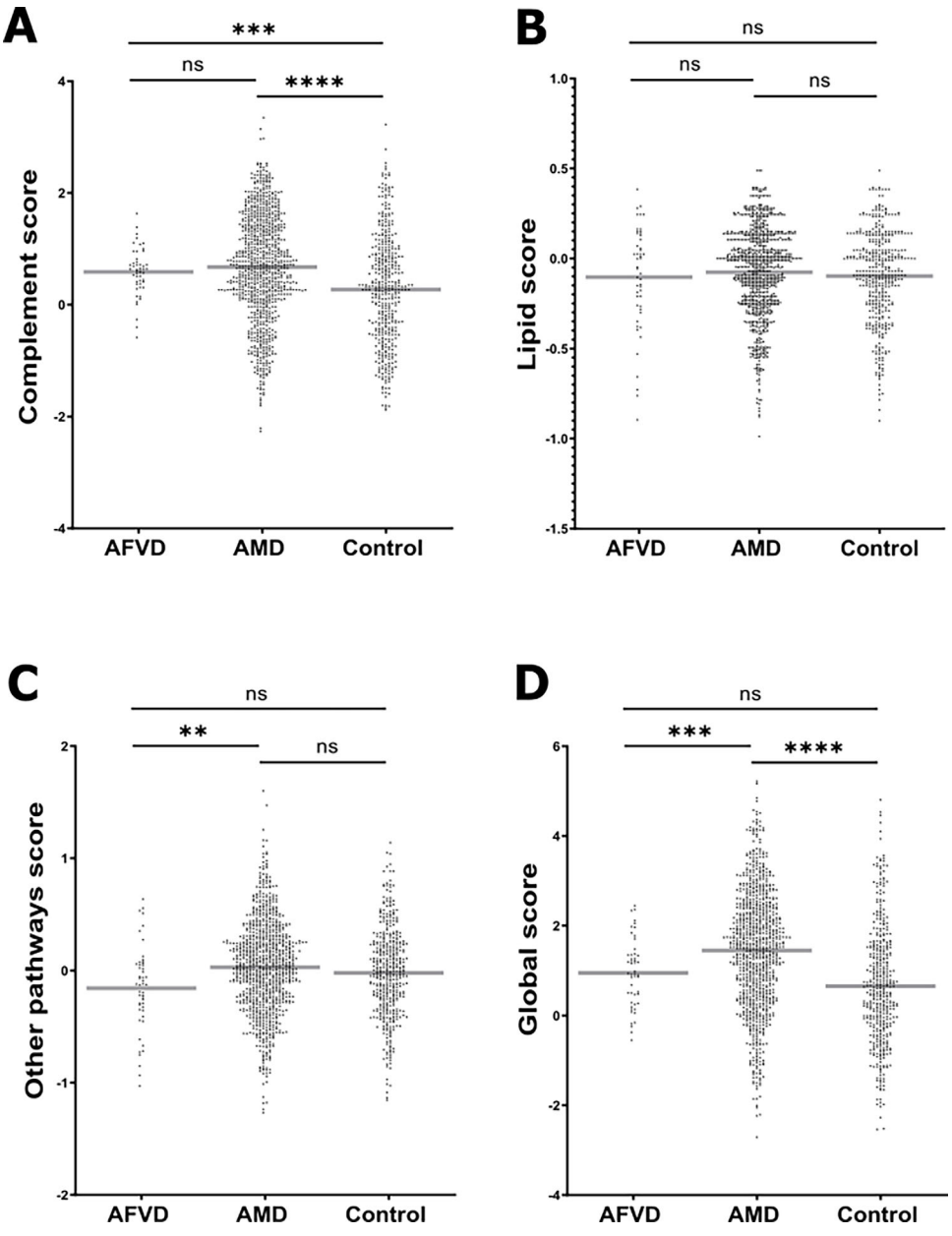

Figure 2. Scatter plots showing the distributions of the genetic risk scores for the (**A**) complement score, (**B**) lipid score, (**C**) other molecular pathways score, and (**D**) global score in control participants, patients with adult-onset foveomacular vitelliform degeneration (AFVD), and patients with age-related macular degeneration (AMD).

Investigative Ophthalmology & Visual Science

Table 3. Logistic Regression Results for AFVD Prevalence and AMD Prevalence in Relation to Genetic Markers

			Regression Coefficients			ROC Curve			
Independent Variables			**OR**	**95% CI**	**Sig.**	**Area**	**Asymptotic 95% CI**	**Std. Error***	**Asymptotic Sig. †**
AMD	ARMS2/HTRA1	Complement score	1.57	1.38 to 1.79	<0.0001	0.72	0.69 to 0.75	0.01	0.00
		Lipid score	1.64	1.01 to 2.68	0.04	0.69	0.66 to 0.72	0.02	0.00
		Other pathways score	1.32	0.98 to 1.79	0.07	0.69	0.66 to 0.72	0.02	0.00
		Global score	1.63	1.47 to 1.80	<0.0001	0.75	0.72 to 0.78	0.01	0.00
		Heterozygous	1.74	1.33 to 2.26	<0.0001	0.72	0.69 to 0.75	0.01	0.00
		Homozygous	4.76	2.95 to 7.70	<0.0001				
AFVD	ARMS2/HTRA1	Complement	1.42	1.04 to 1.95	0.03	0.66	0.60 to 0.72	0.03	0.00
		Lipid	0.96	0.30 to 3.12	0.95	0.60	0.53 to 0.67	0.04	0.02
		Others	0.46	0.21 to 0.98	0.04	0.65	0.57 to 0.72	0.04	0.00
		Global	1.19	0.95 to 1.50	0.12	0.63	0.57 to 0.70	0.03	0.00
		Heterozygous	1.49	0.81 to 2.74	0.20	0.61	0.54 to 0.69	0.04	0.01
		Homozygous	1.05	0.29 to 3.78	0.94				

AMD, age-related macular degeneration phenotype; AFVD, adult-onset foveomacular vitelliform dystrophy; CI, confidence interval; OR, odds ratio; ROC, receiving operating characteristic; Sig., significance; Std., standard.

* Under the nonparametric assumption.

† Null hypothesis: true area = 0.5.

± 0.12) and controls ($P = 0.05$). The complement genetic score was lower in controls (mean ± SD = 0.27 ± 0.06) compared with patients with AFVD (mean ± SD = 0.58 ± 0.07, $P = 0.00$) and compared with patients with AMD (mean ± SD = 0.67 ± 0.05, $P<0.0001$). This score was similar in patients with AFVD and patients with AMD ($P = 0.40$). The lipid genetic score was similar in patients with AFVD (mean ± SD = −0.10 ± 0.04) compared to patients with AMD (mean ± SD = −0.07 ± 0.01, $P = 0.78$), and in patients with AFVD compared to healthy controls (mean ± SD = −0.09 ± 0.04, $P = 0.98$). Similar score values were also detected between patients with AMD and healthy controls ($P = 0.30$). The score summarizing variants unrelated to complement or lipid metabolism was higher in AMD (mean ± SD = 0.03 ± 0.02) compared with AFVD (mean ± SD = −0.15 ± 0.05, $P = 0.004$), and showed a borderline significance level for being lower in AFVD compared with controls (mean ± SD = −0.02 ± 0.05, $P = 0.05$; Fig. 2).

Logistic regression analysis, adjusted for age and sex, was performed to explore the associations among the genetic scores and AFVD or AMD compared with controls. Results are summarized in Table 3 and receiver operating characteristic (ROC) curves indicating meaningful predictive accuracy (AUC > 0.5, asymptotic sig. < 0.05) are presented in Figure 3. The *ARMS2/HTRA1* variant (rs3750846) was analyzed separately as it is a major risk allele for AMD. It was tested in a logistic regression model as an ordinal variable with three categories: 0 = homozygous to the major allele (reference level), 1 = heterozygous, and 2 = homozygous to the minor allele. Carrying the minor allele as a heterozygous (OR = 1.74, 95% CI = 1.33 to 2.26, $P < 0.0001$) or a homozygous (OR = 4.77, 95% CI = 2.95 to 7.70, $P < 0.0001$) was associated with AMD compared to controls. The *ARMS2/HTRA1* variant was also analyzed separately and did not present an association to AFVD compared with controls. Interestingly, when comparing AFVD to controls, the complement score presented a positive association to AFVD and the other molecular pathways' score was found to be negatively associated to AFVD. The complement score demonstrated an OR of 1.42 (95% CI = 1.04 to 1.95, $P = 0.03$) and an AUC of 0.66 (95% CI = 0.60 to 0.72, asymptotic sig. = 0.00). The other molecular pathways' score demonstrated an OR of 0.46 (95% CI = 0.21 to 0.98, $P = 0.04$) and an AUC of 0.60 (95% CI = 0.57 to 0.72, asymptotic sig. = 0.00).

Genetics and Disease Progression

The association between genetic scores and AFVD progression as assessed by vitelliform lesion morphology was evaluated in a subgroup of 29 patients with AFVD ($n = 55$ eyes). Results are presented in Table 4. At baseline, 28 of these 55 eyes (50.9%) manifested the vitelliform stage, whereas only 7 eyes (12.7%) remained at this stage at 5 years. AFVD progression rate was not correlated with genetic scores or *ARMS2/HTRA1*. Similarly, in a regression analysis, none of the genetic scores demonstrated an association to disease progression rate. Regression analysis showed an AUC of 0.81 (95% CI = 0.63 to 0.99, asymptotic sig. = 0.01) for the *ARMS2/HTRA1* variant when used to predict higher progression rate of AFVD. However, it was also not found to be a robustly predictive coefficient ($P > 0.07$).

Systemic Complement Activation

The C3a/C3 ratio was measured in plasma samples from patients with AFVD ($n = 17$), patients with AMD ($n = 18$), and healthy controls ($n = 24$). In the AFVD group, 6 of 17 patients presented an optical density values above the standard curve for C3a ELISA. The C3a values for these samples was determined using a Robust regression and outlier removal (ROUT) methodology to account for outliers with a Q of 10%. Results showed lower C3a levels in patients with AMD (mean ± SD = 3235 ± 532.7 ng/mL) compared to healthy controls (mean ± SD = 3929 ± 476.2 ng/mL, $P = 0.00$), whereas C3a levels in patients with AFVD were not found to be different from patients with AMD or healthy controls (mean ± SD = 3915 ± 726.4 ng/mL, $P = 0.43$ and $P = 0.31$ respectively). The C3a/C3 ratio was similar among patients with AFVD, patients with AMD, and healthy controls. The C3a/C3 ratio at the vitelliform stage (9.17, $n = 1$), pseudohypopyon stage (7.68 ± 1.04, $n = 2$), vitelliruptive stage (12.66 ± 7.44, $n = 6$), and atrophic stage (11.84 ± 4.68, $n = 7$) were similar, and there was no correlation between advanced stage and C3a/C3 ratio (Spearman's rho = 0.228, $P = 0$ .52).

Investigative Ophthalmology & Visual Science

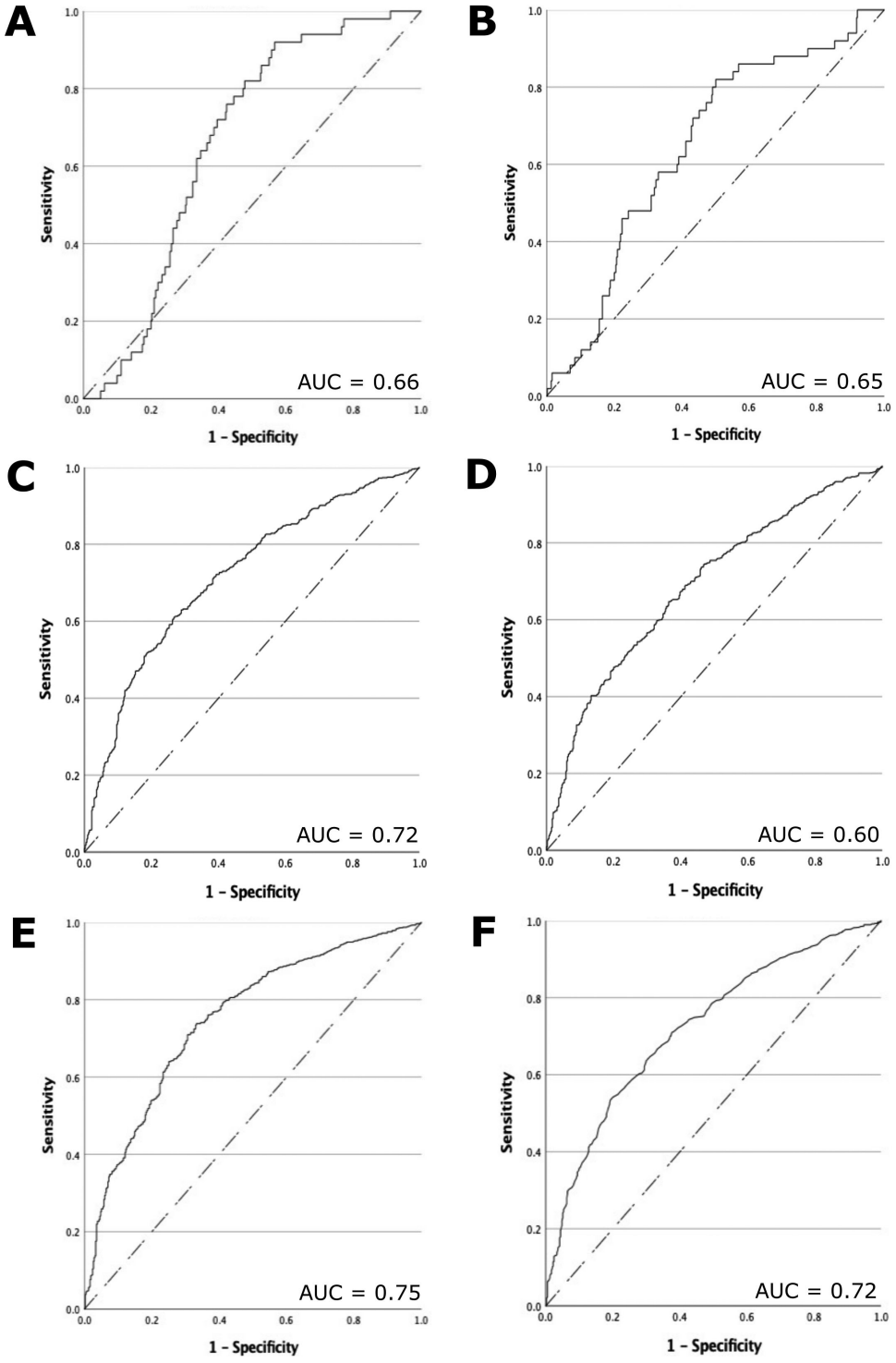

Figure 3. Receiver operating characteristic (ROC) curves for genetic scores with significant association to phenotype. For adult-onset foveomacular vitelliform degeneration (AFVD) phenotype, (**A**) complement score, and (**B**) other molecular pathways score. For age-related macular degeneration (AMD) phenotype, (**C**) complement score, (**D**) lipid score, (**E**) global score, and (**F**) ARMS2/HTRA1. All logistic regression models included age and sex.

Discussion

In this study, we characterized the genetic architecture of AFVD as compared with AMD and unaffected controls. Several individual genetic risk variants that were previously reported for AMD were also associated with AFVD. A variant in *MIR6130/RORB* (microRNA 6130; rs10781182) was negatively associated with AFVD and AMD. MIR6130 is estimated to play a role in regulating the complement C1q C chain and various other proteins. The protective effect of the variant from AFVD may be due to the potential role of MIR6130 in modulating the complement system.[33]

Investigative Ophthalmology & Visual Science

Table 4. Logistic Regression Results for AFVD Progression Rate in Relation to Genetic Markers

	Regression Coefficients			ROC Curve			
Independent Variables	**OR**	**95% CI**	**Sig.**	**Area**	**Asymptotic 95% CI**	**Std. Error***	**Asymptotic Sig.†**
AFVD progression rate							
Complement score	2.02	0.30 to 13.59	0.47	0.75	0.55 to 0.95	0.10	0.04
Lipid score	25.67	0.51 to 1284.55	0.10	0.80	0.61 to 0.99	0.10	0.01
Other pathways score	0.64	0.05 to 8.80	0.74	0.74	0.53 to 0.94	0.11	0.05
Global score	2.07	0.57 to 7.49	0.27	0.74	0.54 to 0.95	0.11	0.05
ARMS2/HTRA1							
Heterozygous	2.21	0.23 to 21.32	0.49	0.81	0.63 to 0.99	0.09	0.01
Homozygous	41.76	0.73 to 2385.69	0.07				

AFVD, adult-onset foveomacular vitelliform dystrophy; CI, confidence interval; OR, odds ratio; ROC, receiving operating characteristic; Sig., significance; Std., standard.

* Under the nonparametric assumption.

† Null hypothesis: true area = 0.5.

To obtain insights into the biological pathways that are involved in AFVD, we calculated a global genetic risk score for all 52 variants, as well as a complement score for 19 variants associated with the complement pathway, and a lipid score for 7 variants associated with the lipid metabolism pathway. Finally, a score for the remaining 26 variants associated from other biological pathways was calculated. This analysis showed that patients with AFVD and patients with AMD had a higher complement genetic score compared with unaffected healthy controls. This was validated in our regression analysis. On the other hand, the score summarizing variants not linked to complement or lipid pathways was lower in patients with AFVD compared with patients with AMD and healthy controls. Patients with AMD also displayed a higher global score compared to patients with AFVD and healthy controls.

These data implicate the complement cascade in AFVD, whereas affects from other pathways including the lipid pathway may be less important in AFVD as compared with AMD. In our attempt to better understand the pathogenesis of AFVD and its similarity to AMD, we also measured complement activation products in the blood plasma of patients with AFVD and patients with AMD, comparing them to healthy controls. There were no differences in complement activation levels across groups, which may be accounted by the limited sample size and the low statistical power (approximately 0.2). Prior studies probing systemic complement activation in AMD yielded inconsistent results, suggesting potential challenges in estimating systemic complement activation in AMD, or its minimal role in these diseases.[30,34–36] Future studies should focus on larger datasets and possibly explore complement activation in the retina to discern its role in AFVD pathogenesis.

Although the complement genetic variants and score are associated with AFVD, we found no link between genetic scores and AFVD progression rate through correlation and regression models. This can possibly imply that the disease progression might result from more intricate interactions of various genetic factors and other factors. Our drive to examine the relationship between the genetic scores and AFVD disease progression was based on a previous work demonstrating a complex relationship between complement pathway variants and AMD disease progression, as well as evidence for an association of an *ARMS2/HTRA1* variant with AMD disease progression once baseline features of the disease are present.[37] However, the limited sample size and statistical power might also underlie the lack of association among complement genetic variants and AFVD progression in the current study.

We previously evaluated the association of pattern dystrophy phenotypes with a few specific major risk variants for AMD including variants in *HTRA1* (rs11200638), *CFH* (rs1061170), and *C3* (rs2231099) in 35 patients with AFVD and patients with BSPD.[7] The current study evaluated a larger cohort of patients (n = 50) and focused on AFVD. Consistent with the earlier report, our recent study excluded an association of the C3 variant and AFVD, but there was a trend toward higher prevalence of this risk allele in AMD compared with AFVD. The current study focused on assessment of the 52 variants linked to AMD in the comprehensive IAMDGC analysis, therefore, the *CFH* (rs1061170) and the *HTRA1* gene promoter (rs11200638) variant were not included. A different variant in the *ARMS/HTRA1* locus, rs3750846, was assessed. Both variants are part of the linkage disequilibrium block for the *ARMS2* and *HTRA1* genes. In our current study, the *ARMS2/HTRA1* (rs3750846) variant showed a greater prevalence in AMD than in AFVD. Notably, whereas our prior smaller-scale study indicated an association between the *ARMS2/HTRA1* variant and AFVD, the current larger study did not replicate this. There was a nonsignificant trend for association of the HTRA1 risk allele genotypes with AFVD, but this was not identified at the allele count. The discrepancy between our current and previous studies with respect to HTRA1 might be attributed to the significantly larger sample size of the current study and the different phenotypes that were evaluated (40% increase for AFVD, 90% for AMD, and 70% for controls).

As complement-targeted therapies emerge, pinpointing the genetic similarities and differences between AMD and AFVD gained clinical importance. Because the complement score was associated with AFVD in the current research, the role of complement inhibition in AFVD should be carefully evaluated. Unlike in AMD, the development of foveal atrophy in AFVD follows the progression of the vitelliform lesion. Determining the ideal time point for application of complement inhibition in AFVD will require additional studies and potentially, AFVD-specific clinical trials. Such trials will be complicated by the difficulty in recruiting a sufficient number of patients and by the particular progression pattern of the disease.

The current study has certain limitations, including its limited sample size and single center design. Additionally, the focus on specific genetic variants might not capture the entire genetic landscape of AFVD. Yet, GWAS require

Investigative Ophthalmology & Visual Science

a significantly larger sample size that is difficult to obtain with AFVD. Furthermore, known monogenic mutations associated with AFVD were excluded in patients enrolled in the current analysis.

In conclusion, we report that AFVD, a common phenotype in the retinal clinic, shares genetic risk variants with AMD, particularly in genes related to the complement cascade. Additional investigation is required to untangle the intricacies of the resemblances and disparities of those diseases, as well as to elucidate the precise contribution of complement activation in AFVD and its potential as a therapeutic target.

Acknowledgments

Supported by Israel Science Fund (#3484/19; IC), Israeli Ministry of Science (#3-1735; MG), and NIH (R01 EY022310 and RES516564; IMH) for IAMDGC.

Disclosure: **S. Jaskoll**, None; **A. Kramer**, None; **S. Elbaz-Hayoun**, None; **B. Rinsky**, None; **C.M. Eandi**, None; **M. Grunin**, None; **Y. Shwartz**, None; **L. Tiosano**, None; **I.M. Heid**, None; **T. Winkler**, None; **I. Chowers**, None

References

1. Chowers I, Tiosano L, Audo I, Grunin M, Boon CJF. Adult-onset foveomacular vitelliform dystrophy: a fresh perspective. *Prog Retin Eye Res*. 2015;47:64–85.
2. Burgess DB, Olk RJ, Uniat LM. Macular disease resembling adult foveomacular vitelliform dystrophy in older adults. *Ophthalmology*. 1987;94(4):362–366.
3. Dalvin LA, Pulido JS, Marmorstein AD. Vitelliform dystrophies: prevalence in Olmsted County, Minnesota, United States. *Ophthalmic Genet*. 2017;38(2):143–147.
4. Francis PJ, Schultz DW, Gregory AM, et al. Genetic and phenotypic heterogeneity in pattern dystrophy. *Br J Ophthalmol*. 2005;89(9):1115–1119.
5. Meunier I, Manes G, Bocquet B, et al. Frequency and clinical pattern of vitelliform macular dystrophy caused by mutations of interphotoreceptor matrix IMPG1 and IMPG2 genes. *Ophthalmology*. 2014;121(12):2406–2414.
6. Tiosano L, Grunin M, Hagbi-Levi S, Banin E, Averbukh E, Chowers I. Characterising the phenotype and progression of sporadic adult-onset foveomacular vitelliform dystrophy. *Br J Ophthalmol*. 2016;100(11):1476–1481.
7. Jaouni T, Averbukh E, Burstyn-Cohen T, et al. Association of pattern dystrophy with an HTRA1 single-nucleotide polymorphism. *Arch Ophthalmol*. 2012;130(8):987–991.
8. Johnson LV., Ozaki S, Staples MK, Erickson PA, Anderson DH. A potential role for immune complex pathogenesis in drusen formation. *Exp Eye Res*. 2000;70(4):441–449.
9. Freund KB, Laud K, Lima LH, Spaide RF, Zweifel S, Yannuzzi LA. Acquired vitelliform lesions: correlation of clinical findings and multiple imaging analyses. *Retina*. 2011;31(1):13–25.
10. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet*. 2016;48(2):134–143.
11. Micklisch S, Lin Y, Jacob S, et al. Age-related macular degeneration associated polymorphism rs10490924 in ARMS2 results in deficiency of a complement activator. *J Neuroinflammation*. 2017;14(1):4.
12. Romero-Vazquez S, Llorens V, Soler-Boronat A, Figueras-Roca M, Adan A, Molins B. Interlink between inflammation and oxidative stress in age-related macular degeneration: role of complement factor H. *Biomedicines*. 2021;9(7):763.
13. Dolgin E. Age-related macular degeneration foils drugmakers. *Nat Biotechnol*. 2017;35(11):1000–1001.
14. Smailhodzic D, Klaver CCW, Klevering BJ, et al. Risk alleles in CFH and ARMS2 are independently associated with systemic complement activation in age-related macular degeneration. *Ophthalmology*. 2012;119(2):339–346.
15. Lechner J, Chen M, Hogg RE, et al. Higher plasma levels of complement C3a, C4a and C5a increase the risk of subretinal fibrosis in neovascular age-related macular degeneration. *Immun Ageing*. 2016;13:4.
16. Ristau T, Paun C, Ersoy L, et al. Impact of the common genetic associations of age-related macular degeneration upon systemic complement component C3d levels. *PLoS One*. 2014;9(3):e93459.
17. Gass JD. A clinicopathologic study of a peculiar foveomacular dystrophy. *Trans Am Ophthalmol Soc*. 1974;72:139–156.
18. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular degeneration: AREDS report no. 18. *Arch Ophthalmol*. 2005;123(11):1570–1574.
19. Jaffe GJ, Chakravarthy U, Freund KB, et al. Imaging features associated with progression to geographic atrophy in age-related macular degeneration: *Classification of Atrophy Meeting Report 5*. *Ophthalmol Retina*. 2021;5:855–867.
20. Brinkmann M, Bacci T, Kar D, et al. Histology and clinical lifecycle of acquired vitelliform lesion, a pathway to advanced age-related macular degeneration. *Am J Ophthalmol*. 2022;240:99–114.
21. Arnold JJ, Sarks JP, Killingsworth MC, Kettle EK, Sarks SH. Adult vitelliform macular degeneration: a clinicopathological study. *Eye*. 2003;17(6):717–726.
22. Nguyen DT, Tran TTH, Tran MH, et al. A comprehensive evaluation of polygenic score and genotype imputation performances of human SNP arrays in diverse populations. *Sci Rep*. 2022;12(1):17556.
23. Das S, Forer L, Schönherr S, et al. Next-generation genotype imputation service and methods. *Nat Genet*. 2016;48(10):1284–1287.
24. Taliun D, Harris DN, Kessler MD, et al. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. *Nature*. 2021;590(7845):290–299.
25. Fuchsberger C, Abecasis GR, Hinds DA. Minimac2: faster genotype imputation. *Bioinformatics*. 2015;31(5):782–784.
26. He W, Han X, Ong JS, et al. Genome-wide meta-analysis identifies risk loci and improves disease prediction of age-related macular degeneration. *Ophthalmology*. 2024;131(1):16–29.
27. Han X, Gharahkhani P, Mitchell P, Liew G, Hewitt AW, MacGregor S. Genome-wide meta-analysis identifies novel loci associated with age-related macular degeneration. *J Hum Genet*. 2020;65(8):657–665.
28. Hüls A, Krämer U, Carlsten C, Schikowski T, Ickstadt K, Schwender H. Comparison of weighting approaches for genetic risk scores in gene-environment interaction studies. *BMC Genet*. 2017;18(1):115.
29. Lee PH, O'Dushlaine C, Thomas B, Purcell SM. INRICH: interval-based enrichment analysis for genome-wide association studies. *Bioinformatics*. 2012;28(13):1797–1799.
30. Brandwijk RJMGE, Michels MAHM, van Rossum M, et al. Pitfalls in complement analysis: a systematic literature review of assessing complement activation. *Front Immunol*. 2022;13:1007102.
31. Pathak A, Agrawal A. Evolution of C-reactive protein. *Front Immunol*. 2019;10:943.
32. Chowers I, Meir T, Lederman M, et al. Sequence variants in HTRA1 and LOC387715/ARMS2 and phenotype and response to photodynamic therapy in neovascular age-related macular degeneration in populations from Israel. *Mol Vis*. 2008;14:2263–2271.

33. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. *Nucleic Acids Res*. 2020;48(D1):D127–D131.
34. Scholl HPN, Issa PC, Walier M, et al. Systemic complement activation in age-related macular degeneration. *PLoS One*. 2008;3(7):e2593.
35. Sivaprasad S, Adewoyin T, Bailey TA, et al. Estimation of systemic complement C3 activity in age-related macular degeneration. *Arch Ophthalmol*. 2007;125(4):515–519.
36. Reynolds R, Hartnett ME, Atkinson JP, Giclas PC, Rosner B, Seddon JM. Plasma complement components and activation fragments: associations with age-related macular degeneration genotypes and phenotypes. *Invest Ophthalmol Vis Sci*. 2009;50(12):5818–5827.
37. Thee EF, Colijn JM, Cougnard-Grégoire A, et al. The phenotypic course of age-related macular degeneration for ARMS2/HTRA1: the EYE-RISK Consortium. *Ophthalmology*. 2022;129(7):752–764.

Investigative Ophthalmology & Visual Science