Supplementary Online Content

Lorés-Motta L, Riaz M, Grunin M, et al. Association of genetic variants with response to anti–vascular endothelial growth factor therapy in age-related macular degeneration. *JAMA Ophthalmol*. Published online May 31, 2018. doi:10.1001/jamaophthalmol.2018.2019

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Inclusion criteria for study participants

Inclusion criteria for all study center patients were defined as neovascularization secondary to AMD confirmed by fluorescein angiogram or optical coherence tomography, age greater or equal to 50 years, and a loading dose of three consecutive injections of bevacizumab (Hoffmann-La Roche, Basel, Switzerland) or ranizibizumab (Hoffmann-La Roche, Basel, Switzerland / Novartis Basel, Switzerland) therapy at monthly intervals (± two weeks). No patients included in the study were treated with aflibercept at the time of study recruitment. We excluded eyes with other retinal morbidities such as myopia greater than 8 diopters, ocular surgery during follow-up or in the two months prior to treatment, previous treatment for neovascular disease, macular hole, staphyloma, or a visual acuity (VA) lower than 2.3 logMAR or 0 Early Treatment Diabetic Retinopathy Study (ETDRS) score letters.

eTable 1. Association of baseline variables with response to anti–vascular endothelial growth factor therapy in neovascular age-related macular degeneration

	Discovery phase (n=678)					Replication phase (n=1,380)						Total (n=2,058)
	Cologne	**Jerusalem**	**Nijmegen**	**Melbourne**	**Sydney**	**Jerusalem**	**Melbourne**	**Leeds**	**BRAMD**	**IVAN**	**EUGENDA**	
N	155	113	121	119	170	146	88	220	215	542	169	
Sex, male (β (SE) in ETDRS letters)	1.3 (2.2)	-9.3 (4.3)	-0.04 (2.5)	-0.9 (2)	1 (2.4)	-1.4 (2.7)	-2.5 (2.7)	-2.5 (1.3)	-2.7 (1.2)	0.6 (1)	-1.7 (1.6)	-1 (0.6)
Sex (*P*-value)	0.558	0.035	0.989	0.656	0.686	0.616	0.364	0.057	0.021	0.556	0.316	0.087
Baseline VA in ETDRS leters (β (SE) in ETDRS letters)	0.2 (0.06)	0.4 (0.07)	0.2 (0.07)	0.3 (0.06)	0.3 (0.06)	0.4 (0.05)	0.3 (0.06)	0.3 (0.04)	0.2 (0.04)	0.3 (0.03)	0.2 (0.04)	0.3 (0.01)
Baseline VA (*P*-value)	0.001	2.03×10^{-8}	0.004	7.17×10^{-7}	7.00×10^{-6}	7.33×10^{-12}	4.04×10^{-7}	3.36×10^{-8}	1.22×10^{-4}	5.53×10^{-18}	1.38×10^{-4}	3.41×10^{-71}
Age , years (β (SE) in ETDRS letters)	-0.4 (0.1)	-0.3 (0.3)	-0.4 (0.2)	-0.2 (0.1)	-0.3 (0.1)	-0.5 (0.2)	-0.2 (0.2)	-0.2 (0.1)	-0.04 (0.1)	-0.04 (0.1)	-0.2 (0.1)	-0.2 (0.04)
Age (*P*-value)	0.016	0.275	0.041	0.140	0.077	0.002	0.286	0.138	0.610	0.579	0.061	8.55×10^{-7}

Influence of the variables age and baseline VA on the change in VA after 3 months was assessed including these variables in a general linear model.

ETDRS = Early Treatment Diabetic Retinopathy Study Cologne = University Hospital of Cologne, Cologne; Jerusalem = Hadassah-Hebrew University Medical Center, Jerusalem; Nijmegen = Radboud university medical center, Nijmegen; Melbourne = Centre for Eye Research Australia, Melbourne; Sydney = Centre for Vision Research, Sydney; Leeds = St. James's University Hospital, Leeds; BRAMD = BRAMD trial cohort, IVAN = alternative treatments to Inhibit VEGF in Age-related choroidal Neovascularisation (IVAN)' trial cohort, EUGENDA = European Genetic Database study cohort; N = number; VA = visual acuity.

eTable 2. Single-variant association analyses of lead variants in the discovery and replication phases by cohort

	Cohort	chr1 rs12138564			chr2 rs13002976			chr3 rs241692			chr7 rs2237435			chr17 rs242939		
		MAF	β (SE)	*P*-value	MAF	β (SE)	*P*-value	MAF	β (SE)	*P*-value	MAF	β (SE)	*P*-value	MAF	β (SE)	*P*-value
Discovery	Cologne	0.312	0.052 (0.031)	0.093	0.409	-0.025 (0.031)	0.436	0.067	0.188 (0.069)	0.007	0.287	-0.084 (0.035)	0.018	0.074	0.041 (0.058)	0.485
	Jerusalem	0.275	0.006 (0.072)	0.935	0.384	-0.132 (0.073)	0.075	0.092	0.331 (0.114)	0.004	0.276	-0.054 (0.086)	0.528	0.088	0.288 (0.120)	0.018
	Nijmegen	0.309	0.088 (0.040)	0.031	0.410	-0.123 (0.036)	0.001	0.051	0.233 (0.098)	0.019	0.275	-0.13 (0.041)	0.002	0.094	0.178 (0.064)	0.007
	Melbourne	0.251	0.113 (0.028)	1.04×10^{-4}	0.387	-0.081 (0.030)	0.009	NA*	NA*	NA*	0.291	-0.072 (0.033)	0.032	0.079	0.151 (0.051)	0.004
	Sydney	0.315	0.073 (0.035)	0.036	0.358	-0.096 (0.038)	0.012	0.068	0.178 (0.077)	0.022	0.304	-0.048 (0.039)	0.217	0.104	0.111 (0.051)	0.030
Replication	Jerusalem	0.459	0.427 (1.141)	0.709	0.398	1.69 (1.331)	0.207	NA^	NA^	NA^	0.337	0.443 (1.183)	0.709	0.092	0.503 (1.654)	0.761
	Melbourne	0.268	0.025 (0.045)	0.583	NA^	NA^	NA^	0.080	-0.113 (0.068)	0.099	0.288	-0.024 (0.04)	0.554	0.080	-0.007 (0.075)	0.921
	Leeds	0.306	0.044 (0.020)	0.028	0.420	0.014 (0.018)	0.460	0.053	-0.025 (0.042)	0.561	0.276	0.029 (0.02)	0.162	0.063	0.047 (0.038)	0.214
	BRAMD	0.285	0.016 (0.019)	0.379	0.404	0.007 (0.017)	0.704	0.051	-0.041 (0.038)	0.287	0.273	0.028 (0.019)	0.138	0.079	-0.052 (0.031)	0.094
	IVAN	0.311	0.009 (0.014)	0.515	0.428	0.002 (0.014)	0.892	0.067	0.021 (0.027)	0.442	0.282	0.009 (0.015)	0.556	0.070	-0.04 (0.027)	0.131
	EUGENDA	0.270	0.017 (0.025)	0.498	0.363	0.044 (0.023)	0.061	0.067	-0.075 (0.044)	0.089	0.249	0.001 (0.024)	0.968	0.051	0.009 (0.052)	0.855

Cologne = University Hospital of Cologne, Cologne; Jerusalem = Hadassah-Hebrew University Medical Center, Jerusalem; Nijmegen = Radboud university medical center, Nijmegen; Melbourne = Centre for Eye Research Australia, Melbourne; Sydney = Centre for Vision Research, Sydney; Leeds = St. James's University Hospital, Leeds; BRAMD = BRAMD trial cohort, IVAN = alternative treatments to Inhibit VEGF in Age-related choroidal Neovascularisation (IVAN)' trial cohort, EUGENDA = European Genetic Database study cohort; chr = chromosome; MAF = Minor allele frequency; SE = Standard error; NA = Not applicable

*Not included due to MAF <0.05, ^ Not included due to call rate <0.9

eTable 3. Single-variant association analysis of variants previously associated with response to anti–vascular endothelial growth factor therapy in neovascular age-related macular degeneration

Study	Chr.	Position[a]	SNP	Gene[b]	Allele	*P*-value Cologne	*P*-value Nijmegen	*P*-value Jerusalem	*P*-value Melbourne	*P*-value Sydney	β (SE) Meta-analysis	*P*-value Meta-analysis
Wickremasinghe SS et al., 2011; Bakbak B et al., 2015*	19	45,412,079	rs7412	*APOE*	T	0.935	0.715	0.453	0.185	0.178	-0.003 (0.027)	0.926
Wickremasinghe SS et al., 2011; Bakbak B et al., 2015*	19	45411941	rs429358	*APOE*	C	0.111	0.696	0.996	0.203	0.434	0.055 (0.027)	0.043
Brantley MA et al., 2007; Imai D et al., 2010*; Kloeckener-Gruissem B et al., 2011; Nischler C et al., 2011; McKibbin M et al., 2012; Smailhodzic D et al., 2012; Menghini M et al., 2012; Tian J et al., 2012*; Hautamaki A et al., 2014; Matsumiya W et al., 2014*; Veloso CE et al., 2014; Piermarocchi S et al., 2015; Medina F et al., 2015*; Shah AR at ., 2016.	1	196,659,237	rs1061170	*CFH*	T	0.074	0.446	0.984	0.397	0.546	0.028 (0.015)	0.059
Teper SJ et al., 2010; McKibbin M et al., 2012; Kang HK et al., 2012*; Tian J et al., 2012*; Abedi F et al., 2013; Kitchens JW et al., 2013; Valverde-Megías A et al., 2017	10	124,220,544	rs11200638[#]	*HTRA1*	A	0.141	0.729	0.892	0.790	0.864	0.011 (0.014)	0.416
Hautamaki A et al., 2013; Hautamaki A et al., 2014; Lazzeri S et al., 2015	4	74,606,024	rs4073	*CXCL8*	T	0.403	0.814	0.532	0.325	0.921	0.014 (0.016)	0.390
Imai D et al., 2010*	17	1,673,276	rs1136287	*SERPINF1*	T	0.615	0.330	0.152	0.625	0.154	0.019 (0.016)	0.226
Wang VM et al., 2012	4	110,638,810	rs2285714	*PLA2G12A*	T	0.849	0.852	0.708	0.805	0.109	-0.014 (0.015)	0.368
Nakata I et al., 2011*	6	43,732,669	rs699946	*VEGFA*	G	0.377	0.141	0.614	0.819	0.297	-0.007 (0.019)	0.687
Imai D et al., 2010*; Lazzeri S et al., 2013; Cruz-Gonzalez F et al., 2014; Hautamaki A et al., 2014	6	43,736,389	rs699947[^]	*VEGFA*	C	0.479	0.087	0.613	0.838	0.972	-0.008 (0.015)	0.581
Abedi F et al., 2013; Chang W et al, 2013*	6	43,742,579	rs833069[~]	*VEGFA*	C	0.933	0.539	0.784	0.460	0.435	0.009	0.559

											(0.015)	
Zhao L et al., 2013	6	43,826,627	rs943080	*VEGFA*	T	0.452	0.571	0.467	0.558	0.918	-0.010 (0.015)	0.492
Hermann MM et al., 2014	4	55,986,238	rs4576072[+]	*KDR*	C	NA	NA	NA	NA	NA	NA	NA
Hermann MM et al., 2014	4	55,966,801	rs6828477	*KDR*	T	0.337	0.554	0.950	0.696	0.773	0.012 (0.016)	0.433
Lazzeri S et al., 2015	4	55,992,366	rs2071559	*KDR*	G	0.975	0.306	0.435	0.723	0.005	0.009 (0.015)	0.555
Lorés-Motta L et al., 2016	10	33,552,695	rs2070296	*NRP1*	T	0.847	0.204	0.561	0.509	0.018	-0.031 (0.021)	0.136
Riaz M and Lorés-Motta L et al., 2016	11	4,389,639	rs4910623	*OR52B4*	A	0.185	0.161	0.756	0.158	0.126	0.022 (0.015)	0.155

SNP = Single nucleotide polymorphism, Cologne = University Hospital of Cologne, Cologne; Jerusalem = Hadassah-Hebrew University Medical Center, Jerusalem; Nijmegen = Radboud university medical center, Nijmegen; Melbourne = Centre for Eye Research Australia, Melbourne; Sydney = Centre for Vision Research, Sydney; Leeds = St. James's University Hospital, Leeds; BRAMD = BRAMD trial cohort, IVAN = alternative treatments to Inhibit VEGF in Age-related choroidal Neovascularisation (IVAN)' trial cohort; EUGENDA = European Genetic Database study cohort; SE = Standard error; NA = Not applicable

[a]Chromosomal position according to the NCBI RefSeq hg19 human genome reference assembly. [b]Closest gene.* Association identified in a non-European descent population. [+]Variant did not pass imputation quality control. [#]In linkage disequilibrium (r^2>0.9) with rs10490924. [^]In linkage disequilibrium (r^2>0.9) with rs833061 and rs1413711. [~] In linkage disequilibrium (r^2>0.9) with rs3025000.

eTable 4. Single-variant association analysis of variants previously associated with age-related macular degeneration

Locus name	Variant[a]	Allele	*P*-value Cologne	*P*-value Nijmegen	*P*-value Jerusalem	*P*-value Melbourne	*P*-value Sydney	β (SE) Meta-analysis	*P*-value Meta-analysis
CFH	rs10922109	C	0.536	0.575	0.702	0.585	0.355	-0.013 (0.018)	0.454
CFH	rs570618	G	0.074	0.435	1.000	0.414	0.546	0.028 (0.015)	0.060
CFH	rs121913059	NF	NF	NF	NF	NF	NF	NF	NF
CFH	rs148553336	NF	NF	NF	NF	NF	NF	NF	NF
CFH	rs187328863	C	0.017	0.686	0.109	0.186	0.472	0.068 (0.039)	0.077
*CFH (CFHR3/CFHR1)*b	rs61818925	G	0.297	0.549	0.594	0.150	0.737	-0.019 (0.017)	0.275
CFH	rs35292876	C	0.013	0.379	0.736	0.105	0.707	0.029 (0.042)	0.487
CFH	rs191281603	NF	NF	NF	NF	NF	NF	NF	NF
COL4A3	rs11884770	C	0.719	0.329	0.962	0.627	0.477	0.007 (0.018)	0.689
ADAMTS9-AS2	rs62247658	C	0.716	0.552	0.866	0.058	0.452	0.028 (0.015)	0.056
COL8A1	rs140647181	C	0.327	0.061	0.389	0.089	0.774	-0.112 (0.049)	0.022
COL8A1	rs55975637	G	0.951	0.541	0.607	0.088	0.388	-0.022 (0.021)	0.310
CFI	rs10033900	C	0.532	0.955	0.571	0.543	0.390	-0.002 (0.016)	0.925
CFI	rs141853578	NF	NF	NF	NF	NF	NF	NF	NF
C9	rs62358361	G	NF	0.007	0.307	0.812	0.151	-0.115 (0.076)	0.130
PRLR/SPEF2	rs114092250	G	0.372	0.799	NF	0.272	0.614	-0.029 (0.05)	0.565
C2/CFB/SKIV2L	rs116503776	G	0.086	0.806	0.448	0.632	0.146	-0.001 (0.027)	0.958
C2/CFB/SKIV2L	rs144629244	G	0.500	NF	0.280	NF	0.523	-0.05 (0.085)	0.558
C2/CFB/SKIV2L (PBX2) b	rs114254831	G	0.416	0.811	0.148	0.014	0.271	0.036 (0.017)	0.033
C2/CFB/SKIV2L	rs181705462	G	0.402	NF	0.927	0.482	0.209	-0.046 (0.057)	0.422
VEGFA	rs943080	NF	NF	NF	NF	NF	NF	NF	NF
KMT2E/SRPK2	rs1142	C	0.941	0.406	0.955	0.668	0.396	-0.005 (0.016)	0.751
PILRB/PILRA	rs7803454	C	0.461	0.240	0.020	0.956	0.292	0.037 (0.019)	0.050
TNFRSF10A	rs79037040	G	0.094	0.952	0.268	0.280	0.041	0.015 (0.016)	0.325
MIR6130/RORB	rs10781182	G	0.498	0.646	0.220	0.209	0.777	0.005 (0.016)	0.729

TRPM3	rs71507014	GC	0.696	0.074	0.401	0.780	0.753	-0.017 (0.014)	0.236
TGFBR1	rs1626340	G	0.673	0.094	0.260	0.393	0.076	0.013 (0.02)	0.530
ABCA1	rs2740488	C	0.305	0.999	0.019	0.774	0.177	0.009 (0.018)	0.631
ARHGAP21	rs12357257	G	0.308	0.710	0.718	0.077	0.249	-0.016 (0.017)	0.372
ARMS2/HTRA1	rs3750846	C	0.153	0.673	0.892	0.790	0.753	0.012 (0.014)	0.409
RDH5/CD63	rs3138141	C	0.620	0.567	0.630	0.877	0.267	0.004 (0.023)	0.856
ACAD10	rs61941274	G	0.203	0.699	NF	0.367	0.017	-0.014 (0.064)	0.831
B3GALTL	rs9564692	C	0.999	0.544	0.384	0.452	0.907	-0.002 (0.017)	0.913
RAD51B	rs61985136	C	0.142	0.360	0.268	0.628	0.243	-0.007 (0.016)	0.672
RAD51B	rs2842339	G	0.368	0.651	0.255	0.511	0.527	-0.006 (0.024)	0.820
LIPC	rs2043085	C	0.224	0.418	0.956	0.809	0.656	-0.009 (0.015)	0.536
LIPC	rs2070895	G	0.242	0.274	0.158	0.463	0.914	0.004 (0.018)	0.844
CETP	rs5817082	C	0.114	0.052	0.105	0.393	0.425	0.033 (0.018)	0.072
CETP	rs17231506	C	0.384	0.041	0.932	0.492	0.037	-0.017 (0.016)	0.286
CTRB2/CTRB1	rs72802342	C	0.750	0.497	0.522	0.794	0.837	-0.001 (0.035)	0.973
TMEM97/VTN	rs11080055	C	0.108	0.862	0.837	0.349	0.483	0.002 (0.015)	0.891
NPLOC4/TSPAN10	rs6565597	C	0.188	0.977	0.036	0.047	0.885	-0.021 (0.017)	0.215
C3	rs2230199	G	0.554	0.671	0.886	0.725	0.291	0.002 (0.019)	0.897
C3	rs147859257	G	NF	NF	NF	0.062	NF	-0.226 (0.12)	0.062
C3 (NRTN/FUT6) b	rs12019136	G	0.219	0.572	0.435	0.953	0.023	0.02 (0.042)	0.633
CNN2	rs67538026	C	0.983	0.221	0.893	0.903	0.197	0 (0.017)	0.999
APOE	rs429358	C	0.111	0.696	0.996	0.203	0.434	0.055 (0.027)	0.043
*APOE(EXOC3L2/MARK4)*b	rs73036519	G	0.193	0.820	0.430	0.192	0.464	-0.005 (0.018)	0.787
MMP9	rs142450006	TTTTC	0.582	0.146	0.715	0.494	0.153	0.002 (0.024)	0.944
C20orf85	rs201459901	TA	0.565	0.220	0.838	0.048	0.738	-0.012 (0.034)	0.723
SYN3/TIMP3	rs5754227	C	0.390	0.313	0.010	0.689	0.706	0.015 (0.025)	0.553
SLC16A8	rs8135665	C	0.405	0.602	0.354	0.731	0.898	-0.003 (0.018)	0.855

NF = Not found, SE = Standard error.

[a]Chromosomal location of the SNP as in Fritsche et al., 2016

eTable 5. Rare protein-altering variants in *C10orf88* and *UNC93B1* included in the gene-based analysis

Gene	Variant[a]	Protein change	Imputation quality (R^2)	CADD score[b]	RAC	Frequency (%)	Single variant *P*-value	β (SE)
C10orf88	c.412G>A	p.Glu138Lys	1	24.6	3	0.4	$4.96x10^{-4}$	-0.618 (0.177)
	c.827T>C	p.Ile276Thr	1	0.038	3	0.4	$2.33x10^{-5}$	-0.749 (0.176)
	c.1258C>T	p.Gln420*	1	38	1	0.1	0.120	<0
UNC93B1	c.385C>A	p.Leu129Ile	1	24.9	7	1.0	$3.33x10^{-7}$	-0.606 (0.118)
	c.626C>T	p.Pro209Leu	1	25.6	7	1.0	$4.21x10^{-7}$	-0.596 (0.117)

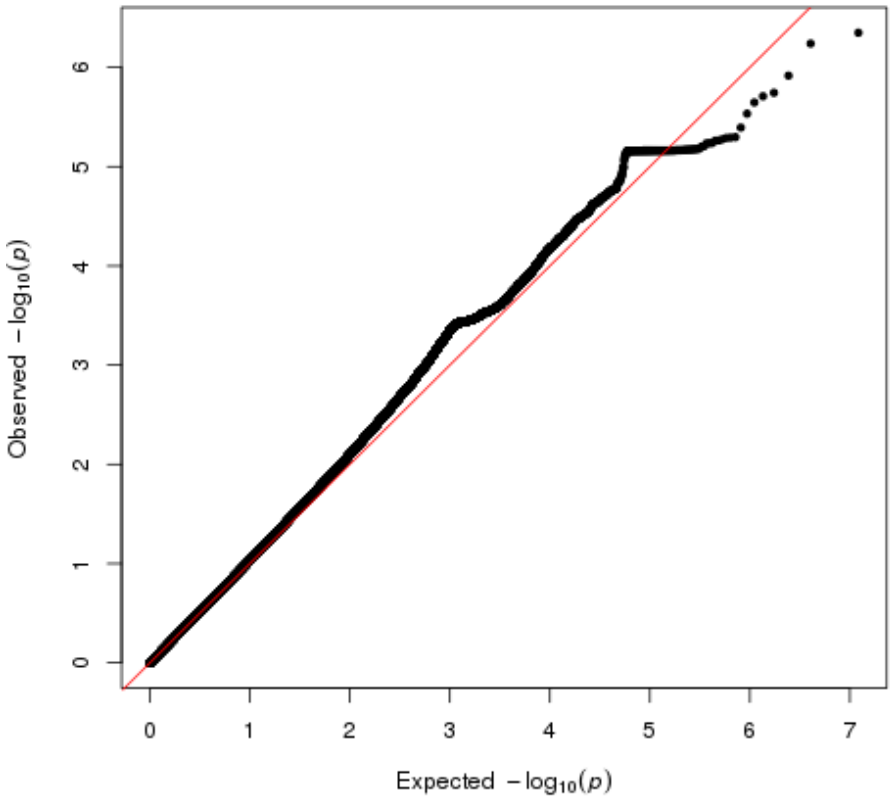
RAC = Rare allele count, SE = Standard error
[a]Positions according to the NCBI RefSeq hg19 human genome reference assembly. [b]The CADD score refers to the PHRED-like scaled C-score for which ≥20 indicates that the variant is predicted to be in the 1% most deleterious substitutions in the human genome.

eTable 6. Gene-based analysis of rare variants in genes previously associated with age-related macular degeneration

Gene	Chromosome	Chromosomal position[a]	N rare variants	RAC	*P*-value
CFH	1	196,621,252-196,716,375	14	40	0.541
CFI	4	110,662,068-110,723,117	10	26	0.026
TIMP3	22	33,198,100-33,255,356	2	4	0.163
SLC16A8	22	38,474,406-38,478,804	5	21	0.007

N = number, RAC = rare allele count
[a]Chromosome and chromosomal position according to the NCBI RefSeq hg19 human

eFigure. Q-Q plot of the single-variant association analysis of response to anti–vascular endothelial growth factor therapy in neovascular age-related macular degeneration



Shown as black dots are the observed *P*-values ($-\log_{10}$(p)) compared to those expected under the null hypothesis.
In the meta-analysis, adjustment for the inflation factor of the different cohorts was conducted when λ>1 (University Hospital of Cologne cohort λ=1.020, Hadassah-Hebrew UMC cohort λ=0.99, Radboud umc cohort λ=1.001, Centre for Eye Research Australia cohort λ=1.005 and Centre for Vision Research cohort λ=1.005).