59: Retina

Transcriptome-wide Association Study Identifies Novel Risk Loci Associated With Age-Related Macular Degeneration

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PURPOSE

Age-related macular degeneration (AMD) is a complex degenerative blindness disorder affecting 200 million individuals yearly, with a global cost of care of $343billion. Previous GWAS implicated 34 main loci associated with AMD (International Age-related Macular Degeneration Genomics Consortium [IAMDGC]) but identifying effective therapeutic targets remains difficult. The innate immune system, particularly monocytes/macrophages, are suspected to play a crucial role in AMD pathogenesis, although underlying mechanisms remain poorly understood.

METHODS

We integrated monocyte eQTL and AMD GWAS data to identify risk genes for AMD. We utilized IAMDGC genomic chip data for 16,108 cases/18,038controls (15,616/16,723 EUR, 207/322 ASN, 50/357 AFR, and 235/636 OTH) and imputed to TOPMed v2 imputation panel, build 38. Principal components were calculated and ancestry-specific GWAS analyses were conducted using a REGENIE Firth test and meta-analyzed with METAL. 51,162,741 variants (MAF>0.1, INFO>0.8) were analyzed across ancestries. We combined genetic data from controls with European ancestry (n=432) with monocyte expression data in several states of stimulation (naive, LPS 2h. LPS 24h, and IFN 24h; Fairfax et al. 2014) to develop elastic-net regression models of genetically-regulated expression. After filtering for models with significant association to gene expression, we performed a transcriptome-wide association study (TWAS) of monocyte expression on AMD in each stimulation state using the MetaXcan software suite. A second complementary modeling strategy using multivariate adaptive shrinkage (MASH),which borrows information across treatments, was also implemented for further TWAS analysis. eQTL genes underwent pathway analysis per treatment condition via enrichR.

RESULTS

16 TWAS significant associations after Bonferroni-correction were identified across the genome, 14 in known AMD loci and 2 novel loci. The top result in LPS stimulated monocytes was the gene PLEKHA1 (P=6.13E-107), and the top result in naive monocytes was the gene HTRA1 (P=6.90E-78), both in the known HTRA1/ARMS2 AMD-associated locus. BUB3 (P=7.24E-10) and NUDT21 (P=4.96E-02) were newly associated with AMD. BUB3 is associated with choroidal thickness in a European population. Our second MASH pipeline identified multiple significant novel loci across the genome.

CONCLUSIONS

These TWAS results represent genes strongly regulated by variants known to be associated with AMD, as well as novel loci. Our results support the role of genetic regulation of monocyte inflammatory processes in AMD, and highlight novel biology underpinning pathogenesis. Validation of these candidate genes using gene expression data may elucidate novel risk genes and therapeutic targets

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TOPMed imputed GWAS

- **16,108 AMD cases**
- **18, 038 controls**
- **51,162,741 variants utilized post QC**

Fairfax Monocytes

- **1.26 million probes of genome data**
- **16,818 harmonized microarray gene expression data**
- **4 treatment conditions: Naïve, LPS, LPS+24, IFN**
- **432 individuals**

TWAS

- **Elastic Net Regression – PrediXcan – detects multiple sets of SNPs predicting gene expression**
- **MashR –multivariate adaptive shrinkage, multi-ancestry, takes weights and adjusts based on correlation patterns in an empirical Bayes algorithm**

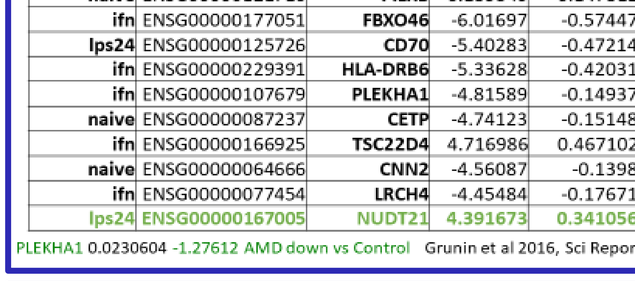
ElasticNet: 2 novel loci and 14 Bonferroni-corrected Associations

treatment	gene	gene_name	zscore	effect_size	pvalue	padj_within	padj_across
lps2	ENSG00000107679	PLEKHA1	-22.3369	-0.53559	1.62E-110	6.13E-107	2.87E-106
naive	ENSG00000166033	HTRA1	19.1275	0.891648	1.49E-81	6.90E-78	2.64E-77
lps2	ENSG00000166033	HTRA1	-10.9911	-0.63066	4.22E-28	1.60E-24	7.48E-24
ifn	ENSG00000237861	NA	-8.60955	-1.06068	7.33E-18	3.59E-14	1.30E-13
naive	ENSG00000154473	BUB3	7.381567	0.484644	1.56E-13	7.24E-10	2.77E-09
lps2	ENSG00000105519	CAPS	6.310727	0.756571	2.78E-10	1.05E-06	4.92E-06
naive	ENSG00000121716	PILRB	6.139549	0.147312	8.28E-10	3.83E-06	1.47E-05
ifn	ENSG00000177051	FBXO46	-6.01697	-0.57447	1.78E-09	8.70E-06	3.15E-05
lps24	ENSG00000125726	CD70	-5.40283	-0.47214	6.56E-08	2.89E-04	1.16E-03
ifn	ENSG00000229391	HLA-DRB6	-5.33628	-0.42031	9.49E-08	4.65E-04	1.68E-03
ifn	ENSG00000107679	PLEKHA1	-4.81589	-0.14937	1.47E-06	7.18E-03	2.60E-02
naive	ENSG00000087237	CETP	-4.74123	-0.15148	2.12E-06	9.83E-03	3.76E-02
ifn	ENSG00000166925	TSC22D4	4.716986	0.467102	2.39E-06	1.17E-02	4.24E-02
naive	ENSG00000064666	CNN2	-4.56087	-0.1398	5.09E-06	2.36E-02	9.03E-02
ifn	ENSG00000077454	LRCH4	-4.45484	-0.17671	8.40E-06	4.11E-02	1.49E-01
lps24	ENSG00000167005	NUDT21	4.391673	0.341056	1.12E-05	4.96E-02	1.99E-01

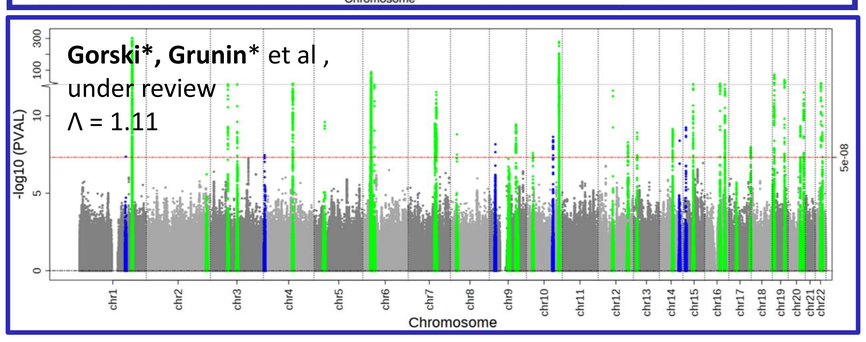
PLEKHA1 0.0230604 -1.27612 AMD down vs Control Grunin et al 2016, Sci Reports Joint Work with Yousef Mustafa

MashR Results (Naïve): 6 novel loci

gene_name	zscore	effect_size	pvalue	var_g	best_gwas_p	largest_weight
PLEKHA1	15.66486	5.447421746	2.63E-55	0.001316	1.10E-198	0.033222968
HTRA1	5.617071	0.769593175	1.94E-08	0.0085	1.63E-15	0.096049347
CKM	5.025168	1.884625915	5.03E-07	0.001253	8.34E-05	0.039163219
PILRB	4.977798	0.492341613	6.43E-07	0.015454	1.29E-05	0.171084557
PDE6G	-4.415	-3.24121075	1.01E-05	0.000347	1.01E-05	0.02534775
TSPAN10	-4.415	-14.29864365	1.01E-05	1.78E-05	1.01E-05	0.005745818
ARHGEF3	-3.84952	-0.456372747	0.000118	0.011759	0.000677063	0.148579311
HIKESHI	3.836907	1.860756635	0.000125	0.000794	3.59E-05	0.028084462
TRIM4	-3.83338	-0.232872794	0.000126	0.041995	1.29E-05	0.234484979
WDR18	3.820099	0.307040602	0.000133	0.028551	0.002386237	0.141798569
SC5D	-3.72639	-0.745462894	0.000194	0.004628	0.00258966	0.061502498
CFLAR	-3.67157	-1.3336287	0.000241	0.001308	0.000241066	0.049946136
TMEM229B	3.608421	1.331365635	0.000308	0.001398	0.000308066	0.051399779
NAALADL1	-3.51119	-0.943516054	0.000446	0.0024	0.000975202	0.047124259
NIN	3.49802	1.079337484	0.000469	0.001638	0.00093812	0.044404133



Fritsche et al 2016, Nature Genetics, GWAS λ=1.13



Pathway Analysis EnrichR

Panther 2016: Phototransduction pathways

Index	Name	P-value	Odds Ratio	Combined score
1	FAS signaling pathway Homo sapiens P00020	0.02301	47.51	179.21
2	Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction Homo sapiens P00028	0.02301	47.51	179.21
3	Apoptosis signaling pathway Homo sapiens P00006	0.07385	14.06	36.64

WikiPathway 2023: Cholesterol Synthesis and Metabolism

Index	Name	P-value	Odds Ratio	Combined score
1	Joubert Syndrome WP4656	0.001449	41.40	270.59
2	Cholesterol Biosynthesis With Skeletal Dysplasias WP4804	0.005239	237.85	1249.08
3	Cholesterol Biosynthesis Pathway WP197	0.01119	101.89	457.73
4	Cholesterol Synthesis Disorders WP5193	0.01342	83.90	361.69
5	Nanomaterial Induced Apoptosis WP2507	0.01490	75.06	315.73
6	Urea Cycle And Metabolism Of Amino Groups WP497	0.01564	71.30	296.47
7	Photodynamic Therapy Induced NF kB Survival Signaling WP3617	0.02594	41.91	153.07
8	Enterocyte Cholesterol Metabolism WP5333	0.02667	40.71	147.56
9	Cholesterol Biosynthesis Pathway In Hepatocytes WP5329	0.03178	33.92	116.98
10	Fas Ligand Pathway And Stress Induction Of Heat Shock Proteins WP314	0.03178	33.92	116.98

KEGG 2021 Human Rankings: Vision and Steroid Synthesis

Index	Name	P-value	Odds Ratio	Combined score
1	Steroid biosynthesis	0.01490	75.06	315.73
2	Phototransduction	0.02080	52.80	204.47
3	Arginine and proline metabolism	0.03686	29.06	95.92
4	Chagas disease	0.07385	14.06	36.64
5	NF-kappa B signaling pathway	0.07525	13.79	35.67
6	TNF signaling pathway	0.08081	12.79	32.17
7	Purine metabolism	0.09253	11.08	26.37
8	Autophagy	0.09800	10.42	24.21
9	Apoptosis	0.1014	10.05	23.01
10	Hepatitis C	0.1115	9.08	19.91

Reactome 2022 Apoptosis pathways

Index	Name	P-value	Odds Ratio	Combined score
1	Death Receptor Signaling R-HSA-73887	0.004745	22.29	119.26
2	TRAIL Signaling R-HSA-75158	0.005985	203.86	1043.43
3	Creatine Metabolism R-HSA-71288	0.007476	158.54	776.21
4	Dimerization Of Procaspase-8 R-HSA-69416	0.008221	142.68	685.01
5	CASP8 Activity Is Inhibited R-HSA-5218900	0.008221	142.68	685.01
6	Activation Of Phototransduction Cascade R-HSA-2485179	0.008221	142.68	685.01
7	NF-kB Activation Thru FADD/RIP-1 Pathway Mediated By Caspase-8 And -10 R-HSA-933543	0.009709	118.89	551.00
8	TRAF3-dependent IRF Activation Pathway R-HSA-918233	0.01119	101.89	457.73
9	Caspase Activation Via Death Receptors In Presence Of Ligand R-HSA-140534	0.01194	95.10	421.09
10	TRAF6 Mediated NF-kB Activation R-HSA-933542	0.01859	59.41	236.74

Table 1: International Age-related Macular Degeneration Genomics Consortium (IAMDGC) Cohort Demographics with Ethnicities and Phenotype Subclassification

	CNV only	GA only	Mixed GA/CNV	late AMD	NoAMD	Intermediate AMD	Large Drusen
AFR	33	8	9	50	357	30	100
ASN	181	23	3	207	322	20	76
EUR	10,361	3,184	2,071	15,616	16,723	2,202	3,795
Other	184	36	15	235	636	67	151