Genetic risk factors for late age-related macular degeneration in India

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Synopsis

Genetic variants in complement pathways, lipid metabolism and angiogenesis increase neovascular age-related macular degeneration risk in the Indian setting. Certain key risk variants are more common in Indian ancestry compared with European.

Abstract

Background/Aims: There are limited data from India on genetic variants influencing late age-related macular degeneration (AMD). We have previously reported associations from a population-based study in India (INDEYE) of early AMD and Single Nucleotide Polymorphisms (SNPs) in ARMS2/HTRA1 and no association with CFH, C2 or CFB. Late AMD cases were too few for meaningful analyses. We aimed to investigate SNPs for late AMD through case enrichment and extend loci for early AMD.

Methods: Fundus images of late AMD hospital cases were independently graded by the modified Wisconsin AMD grading scheme. In total 510 cases with late AMD (14 Geographic Atrophy and 496 neovascular (nvAMD), 1876 with early AMD and 1176 with no signs of AMD underwent genotyping for selected SNPs. We investigated genotype and per-allele additive associations (Odds Ratios (OR) and 95% Confidence Interval (CI) with nvAMD or early AMD. Bonferroni adjusted p-values are presented.

Results: We found associations with nvAMD for CFH *Y402H* variant (rs1061170), OR=1.99, 95% CI 1.67- 2.37, $p=10^{-6}$, *ARMS2* (rs10490924), OR=2.94, 2.45 - 3.52, $p=10^{-9}$, *C2* (rs547154), OR=0.67, 0.53-0.85 p=0.01, *ABCA1* (rs1883025), OR=0.77, 0.65-0.92, p=0.04 and a SNP near *VEGFA* (rs4711751) OR=0.64, 0.54-0.77, $p=10^{-3}$. We found no associations of *TLR3* (rs3775291), *CFD* (rs3826945), *FRK* (rs1999930) or *LIPC* (rs10468017) or *APOE* ε4 alleles with nvAMD or early AMD nor between early AMD and rs1883025 or rs4711751.

Conclusions: The major genetic determinants of nvAMD risk in India are similar to those in other ancestries whilst findings for early AMD suggest potential differences in the pathophysiology of AMD development.

Introduction

Genetic risk variants for late age related macular degeneration (AMD) have been identified, and further confirmed in Genome Wide Association studies (GWAS), the majority in studies of European ancestry.[1] There is less information on late AMD genetic risk in India, with most data coming from one patient/control cohort.[2-4] We have previously reported genetic results from a large population-based study of people aged 60 and over in India (INDEYE) for early AMD with variants in complement factor H (*CFH*), factor B (*CFB*), component 2 (*C2*), *ARMS2/HTRA1*.[5] Late AMD cases were too few for meaningful analyses. In the present paper we present results for late AMD based on an enriched sample, and for other genetic loci with early AMD.

Materials and Methods

INDEYE was conducted between 2005 and 2007 in two locations in south (Tamil Nadu) and north (Haryana) India. The study methods including sampling and recruitment, blood collection, ophthalmological examination and AMD grading along with results on the prevalence of early and late AMD have been published. [6] In the present study, we recruited additional cases of late AMD between 2009 and 2011 from the hospitals that participated in the INDEYE study (All India Institute of Medical Sciences (AIIMS) Delhi, Aravind Eye Hospital Pondicherry, Tamil Nadu) and additionally from Aravind Eye Hospital Madurai, Tamil Nadu. We aimed to achieve 600 late AMD cases plus two population controls per case to detect the two-fold per allele association of Y402H CFH (rs1061170) reported in a meta-analysis of primarily European ancestry[7] at 90% power and alpha <0.001. Initial eligibility criteria were age 60 years and over,

Indian descent and a diagnosis of late AMD by the retinal ophthalmologists. Controls were participants in the INDEYE study with no signs of early or late AMD in either eye.

In both INDEYE and clinic participants, informed written consent was obtained prior to enrolment. If the participant was illiterate the information sheet was read out aloud in the presence of a local witness, and a thumb impression of the participant signified assent. The study complied with the Declaration of Helsinki and ethics approval was received from the Indian Council for Medical Research, the Research Ethics Committees of AIIMS, Aravind Eye Hospital, London School of Hygiene and Tropical Medicine and Queens University Belfast.

Full details of the method of ascertainment of AMD in the population study have previously been published.[6]. In brief two 35degree stereo fundus photographs of each eye were taken and graded at Queens University Belfast (QUB) using the modified Wisconsin Age-Related Maculopathy Grading System.[8] Each eye was classified into 5 mutually exclusive grades, Grade 1-soft distinct drusen (≥63 μm) only *or* pigmentary irregularities only; Grade 2 - soft indistinct (≥125μm) or reticular drusen only *or* soft distinct drusen (≥ 63μm) with pigmentary irregularities; Grade 3 -(soft indistinct (≥ 125μm) or reticular drusen) with pigmentary irregularities; Grade 4 - either Neovascular AMD (nvAMD) (presence of any of the following: serous or haemorrhagic retinal or retinal pigment epithelial detachment, subretinal neovascular membrane, periretinal fibrous scar) or Geographic Atrophy, GA (well-demarcated area of retinal pigment atrophy with visible choroidal vessels). Fundus images of cases recruited from hospital clinics were sent to QUB (color photographs, optical coherence tomograms (OCT)) and graded as above. In all graded images, GA and nvAMD present in the same eye were categorized as nvAMD. Images that showed no signs of any features of early or late AMD were categorized as having no AMD.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using Quiagen kits. SNPs were genotyped using TaqMan assays in an ABI 7900 real-time PCR. We limited our study to genes in biological pathways relevant to AMD pathogenesis including complement activation (*CFH*, *CFB*, *CFD*) and deposition (toll like receptors (*TLR 3*, *4*,*7*), lipid metabolism (*ABCA1*, *APOE*, *CETP*, *LIPC*) or the degradation of the extracellular matrix (*TIMP3*).[9] We investigated two SNPs on chromosome 6, previously reported to be associated with late AMD [10] (*LOC107986598* rs4711751 located near *VEGFA* and *FRK* rs1999930 near *COL10A1)*. We included SNPs in *ARMS2/HTRA1* due to their demonstrated importance in many studies[11] and recent evidence for an ARMS2 role in surface complement regulation.[12] We tested for departures from Hardy Weinberg equilibrium (HWE) in controls and excluded any SNPs with a p value ≤ 0.05. We used logistic regression in Stata14 (StataCorp LP) to examine associations of (i) genotype and (ii) per-allele additive models adjusted for age, sex, and centre. We present additionally Bonferroni adjusted p-values for the number of independent SNPs tested. We created APOE alleles from the SNPs rs429358 (T/C) and rs7412(C/T) resulting in three alleles: ε2 (TT), ε3 (TC) and ε4 (CC). Analyses of APOE alleles used ε3 as the reference group.

Results

The prevalence of early and late AMD in the INDEYE population study has been published.[6] There were 1986 cases of early AMD (1686 Grade1, 289 Grade 2, 11 Grade 3), 53 of late AMD (44 nvAMD 9 GA) and 1228 population controls with no signs of AMD in either eye. Hospital retinal clinics recruited 533 cases based on ophthalmologists' diagnoses. After exclusion of participants without confirmed late AMD or missing blood samples (Figure), 496 nvAMD cases,

1876 early AMD and 1176 controls were available for analysis. We did not investigate GA because of small numbers (n=14). The mean age in years (SD) was 65.3 (5.4) in population controls, early AMD, 67.0 (6.1) and nvAMD, 70.7 (6.9). The number and proportion of women was 600 (51%), 915 (49%) and 179 (36%) respectively. Two SNPs (rs4986790 *TLR4/ TLR7*, rs9621532 *TIMP3*) failed HWE. HWE and MAFs for remaining SNPs are shown in Table 1. We also present MAFs for European and Indian ancestry from the 1000 genome study https://www.ncbi.nlm.nih.gov/**snp** *accessed December 5 2016.* Control frequencies of *APOE* alleles were ε3 (0.73), ε2 (0.09) and ε4 (0.18).

We found additive associations with nvAMD for *Y402H*, (rs1061170), *HTRA1* (rs2672598), *ARMS2* (rs10490924, rs10490923) *CFB* (rs438999, rs547154), *ABCA1* (rs1883025) and SNP rs4711751 close to *VEGFA* (Table 2). We found no associations with *TLR3* (rs3775291), *CFD* (rs3826945), *FRK* (rs1999930) or *LIPC* (rs10468017). There was no association between *APOE* ε4 and nvAMD, OR= 0.72, 95% CI (0.52,1.01).

We combined Grades 2 and 3 of early AMD due to the small numbers of Grade 3. Subsequently we combined all grades of early AMD (1 to 3) because our preliminary analyses revealed no differences in genetic associations for these early stages. There were no associations with early AMD and any of the SNPs (Table 3) or with *APOE* ε4, OR= 0.88, 95% CI (0.73,1.01).

Discussion

CFH and *ARMS2 /HTRA1* have been identified in numerous studies in European [1 11] and East Asian ancestry[13] as the most important genes for late AMD risk with effect sizes around 2.5 and 3 per-allele respectively [1 7 11] and the top two variants at GWAS significance.[1] Our effect sizes of 2 for the C allele of Y402H variant of *CFH* (rs1061170) and 3 for *ARMS2* T allele

(rs10490924) are consistent with these findings and add to the limited evidence for India.[2 3] The MAF of rs1061170 is lower in East Asian (< 0.10) compared to European ancestry (0.3)[7] and higher for rs10490924 (0.4), almost twice that in European ancestry.[9] Our MAFs for rs1061170 (0.32) and rs10490924 (0.32) concur with those for South Asians in the 1000 Genome Study (Table 1) and other sources in India. [2,3,14] It appears that rs1061170 allele frequencies in Indian ancestry are closer to European than East Asian and intermediate between European and East Asian for rs10490924.

We found associations with SNPs in other genes established predominantly in European ancestry including *C2, SKIV2L*, *ABCA1* and in a SNP (rs4711751) in an uncharacterized gene *LOC107986598* close to *VEGFA*.[1] We found a reduced risk with the T allele of *ABCA*1 (rs1883025) but not with *CETP* or *LIPC*. A meta-analysis of European ancestry studies found APOE ε4 haplotype was associated with a 30% lower risk of nvAMD [15]; we observed a similar effect but with wide confidence intervals.

We found no association with early AMD and any of the variants reported in Table 3. We have previously reported results for early AMD and found no association with *Y402H* (rs1061170), *C2* (rs547154), *SKIVL* (rs43899).[5] *ARMS2/HTRA1* variants (rs10490924 and rs2672598) were associated with early AMD, the OR per allele was 1.22, 95% CI (1.13-1.33), p<0.0001 and 1.12 (1.02-1.23), p=0.02 respectively.[5] A GWAS meta-analysis of 4089 early AMD cases, the majority of European ancestry, found associations between SNPs in *CFH* and *ARMS2/HTRA1* but with smaller effect sizes than those reported for late AMD.[16] Analyses by Asian ancestry found no association with any CFH SNP whereas *ARMS2* (rs10490924) was associated with an OR of 1.18 (1.07, 1.13), similar to our study, compared to 1.43 (1.34, 1.54) for European ancestry. The lower prevalence of early AMD in Asia[17] and India[6] may, in part, be explained

by the apparently lesser role of genetic variants compared to studies in European ancestry but caution is warranted due to the paucity of genetic studies of early AMD in Indian and East Asian ancestry.

Limitations

Although we did not attain the 600 planned cases, we confirmed the per-allele two fold risk of rs1061170 and nvAMD hypothesized for the sample size estimates. We had low power to investigate variants with low MAFs (compared to European ancestry) such as FRK, LIPC or to identify smaller effects. The majority of late AMD cases were of nvAMD phenotype similar to studies in East Asia[18] and we could not investigate genetic associations with Geographic Atrophy. It is possible we misclassified population cases of late AMD. We had confirmatory OCTs in 89% of clinic late AMD cases but the population-based study used color images only.

Conclusions

Our findings suggest the major genetic determinants of neovascular AMD risk in India are similar to those in other populations whilst findings for early AMD suggest potential differences in the pathophysiology of AMD development.

Author Contributions

Dr Fletcher had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Fletcher, Ravindran, Chakravarthy, Smeeth, Nitsch.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Fletcher, Rajendran

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Fletcher.

Obtained funding: Fletcher, Chakravarthy Ravindran, Nitsch, Smeeth.

Administrative, technical, or material support: Ravindran, Chakravarthy, Sundaresan, Fletcher

Study supervision: Ravindran, Fletcher

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Table 1. Single Nucleotide Polymorphisms, Minor Allele Frequency (MAF) and test for Hardy Weinberg Equilibrium and corresponding reported MAF in the 1000 genomes project in South Asian and European populations

Chromosome	Gene	SNP[1]	Major/Minor Alleles	HWE[2]	MAF[3]	MAF EUR[4]	MAF SAS[5]
1	*Y402H*	rs1061170	T/C	0.6854	0.323	0.362	0.287
4	TLR3	rs3775291	C/T	0.9347	0.235	0.324	0.263
6	*C2*	rs547154	C/A	0.3813	0.187	0.089	0.156
6	*SKIV2L*	rs438999	A/G	0.6932	0.183	0.089	0.148
6	*LOC107986598*[6]	rs4711751	T/C	1	0.423	0.487	0.330
6	*FRK*	rs1999930	C/T	0.3957	0.075	0.281	0.052
9	*ABCA1*	rs1883025	C/T	0.0797	0.432	0.240	0.413
10	*ARMS2*	rs10490923	G/A	0.7299	0.149	0.130	0.148
10	*ARMS2*	rs10490924	G/T	0.1953	0.319	0.195	0.343
10	*HTRA1*	rs2672598	T/C[7]	0.2969	0.524	0.499	0.464
15	*LIPASE*	rs10468017	C/T	0.9195	0.176	0.283	0.184
16	*CETP*	rs3764261	C/A	0.0737	0.295	0.292	0.321
19	*APOE*	rs429358	T/C	1	0.097	0.155	0.087
19	*APOE*	rs7412	C/T	0.5232	0.050	0.063	0.044
19	*CFD*	rs3826945	T/C	0.8424	0.344	0.313	0.334

[1] Single Nucleotide Polymorphisms (SNP)
[2] p value for tests for departure from Hardy Weinberg Equilibrium (HWE) in controls
[3]Minor Allele Frequency (MAF) in controls
[4] Minor Allele Frequency (MAF) from 1000 genome study for European ancestry available in *https://www.ncbi.nlm.nih.gov/snp*
[5] Minor Allele Frequency (MAF from 1000 genome study for South Asian ancestry available in *https://www.ncbi.nlm.nih.gov/snp*
[6] SNP located near *VEGFA*
[7] Minor allele considered as C for comparison with other studies

Table 2. Association of neovascular age-related macular degeneration with Single Nucleotide Polymorphisms

			1 versus 0 copies of minor allele		2 versus 0 copies of minor allele		Additive per minor allele			
Gene	SNP[1]	Major/Minor Alleles	Odds Ratio[2]	95% CI[3]	Odds Ratio[2]	95% CI[3]	Odds Ratio[2]	95% CI[3]	p	P[4]
Y402H	rs1061170	T/C	1.72	1.31 - 2.28	4.13	2.91 - 5.87	1.99	1.67 – 2.37	10^{-7}	10^{-6}
TLR3	rs3775291	C/T	1.18	0.92 - 1.51	0.88	0.51 - 1.53	1.06	0.87 - 1.30	0.545	
C2	rs547154	C/A	0.62	0.47 - 0 .82	0. 64	0.29 - 1.43	0.67	0.53 - 0 .85	0.001	0.01
SKIV2L	rs438999	A/G	0.63	0.47 - 0.83	0.50	0.21 - 1.22	0.65	0.50 - 0.83	0.001	0.01
LOC107986598	rs4711751	T/C	0.35	0.27 - 0.46	0.65	0.45 - 0 .94	0.64	0.54 - 0 .77	10^{-4}	10^{-3}
FRK	rs1999930	C/T	0.93	0.64 - 1.34	5.94	1.17 - 30.10	1.05	0.74 - 1.49	0.777	
ABCA1	rs1883025	C/T	0.81	0.61 - 1.07	0.58	0.41 - 0.83	0.77	0.65 - 0.92	0.003	0.04
ARMS2	rs10490923	G/A	0.49	0.35 - 0.67	0.85	0.33 - 2.17	0.57	0.43 - 0.75	10^{-3}	0.04
ARMS2	rs10490924	G/T	1.86	1.37 - 2.51	8.73	6.11 - 12.48	2.94	2.45 - 3.52	10^{-10}	10^{-9}
HTRA1	rs2672598	T/C	1.53	1.01 - 2.32	5.42	3.58 – 8.21	2.67	2.19 - 3.25	10^{-9}	10^{-8}
LIPC	rs10468017	C/T	1.13	0.87 - 1.47	1.12	0.56 - 2.23	1.11	0.89 - 1.37	0.370	
CETP	rs3764261	C/A	1.27	0.98 - 1.64	1.26	0.82 - 1.91	1.17	0.98 - 1.41	0.087	
APOE	rs429358	T/C	0.82	0.60 - 1.14	NC[5]		NC[5]			
APOE	rs7412	C/T	0.87	0.58 - 1.32	NC[5]		NC[5]			
CFD	rs3826945	T/C	1.02	0.79 - 1.31	1.05	0.70 - 1.58	1.02	0.85 - 1.23	0.820	

[1]Single Nucleotide Polymorphisms (SNP)
[2] adjusted for age, sex, centre
[3] 95% Confidence interval
[4] Bonferroni adjusted p-value for 13 per -allele tests
[5] Not calculated, no cases with 2 copies of minor allele

Table 3 Association of early age-related macular degeneration with Single Nucleotide Polymorphisms

		1 versus 0 copies of minor allele			2 versus 0 copies of minor allele			Additive per allele		
Gene	SNP[1]	Odds Ratio[1]	95% CI[2]	p	Odds Ratio[1]	95% CI[2]	p	Odds Ratio[1]	95% CI[2]	p
TLR3	rs3775291	1.01	0.86-1.20	0.868	1.13	0.83-1.54	0.425	1.04	0.92-1.16	0.520
LOC107986598	rs4711751	0.95	0.78-1.14	0.558	0.91	0.69-1.20	0.502	0.95	0.84-1.08	0.452
FRK	rs1999930	0.83	0.66-1.05	0.117	1.63	0.39-6.77	0.498	0.88	0.69-1.12	0.285
ABCA1	rs1883025	0.96	0.79-1.17	0.698	0.96	0.77-1.19	0.726	0.98	0.88-1.09	0.699
LIPASE	rs10468017	0.97	0.82-1.14	0.677	1.08	0.67-1.75	0.744	0.99	0.85-1.15	0.913
CETP	rs3764261	1.08	0.91-1.28	0.365	1.10	0.90-1.33	0.339	1.06	0.96-1.17	0.228
APOE	rs429358	0.93	0.77-1.12	0.454	0.80	0.35-1.84	0.594	0.93	0.77-1.10	0.380
APOE	rs7412	0.97	0.74-1.27	0.826	1.38	0.42-4.55	0.594	1.00	0.79-1.27	0.994
CFD	rs3826945	1.02	0.88-1.20	0.758	1.10	0.87-1.39	0.399	1.04	0.94-1.15	0.416

[1] Single Nucleotide Polymorphisms (SNP)
[2] adjusted for age, sex, centre
[3] 95% Confidence interval

Figure Legend

Flowchart of hospital case recruitment and population cases and controls