Analysis of *CFH*, *TLR4*, and *APOE* Polymorphism in India Suggests the Tyr402His Variant of *CFH* to be a Global Marker for Age-Related Macular Degeneration

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Purpose. To screen polymorphisms in complement factor-H (*CFH*), toll-like receptor 4 (*TLR4*), and *APOE* genes as potential risk factors for age-related macular degeneration (AMD) in Indian patients.

Methods. One hundred patients with AMD and 120 normal control subjects were screened for the polymorphisms by restriction digestion and resequencing. Five intragenic SNPs in *CFH* were screened to generate haplotype data in cases and controls. The data were analyzed in conjunction with data from other populations based on genotype and haplotype frequencies, and odds ratios were computed to estimate the risk of AMD in the different genotypes.

Results. Significant association was noted with the *CFH* variant (Tyr402His) among AMD cases ($P = 1.19 \times 10^{-7}$). Individuals homozygous for the mutant genotype CC had a significantly higher risk ($P < 0.0001$) of AMD (OR = 11.52; 95% CI 5.05–26.28) than those carrying a single copy of the C allele (OR = 1.51; 95% CI 0.82–2.80), after adjusting for age, gender, and diabetes. Linkage disequilibrium and haplotype analysis at the *CFH* locus indicated the C-G-T-C-A-G to be a risk haplotype ($P = 0.0003$). No significant differences were observed in the genotype frequencies of *APOE* polymorphisms among patients and control subjects ($P = 0.76$). The carriers of $\varepsilon 4$ allele had a reduced risk ($P = 0.03$) of AMD (OR = 0.42, 95% CI 0.19–0.91). *TLR4* did not exhibit any association with AMD.

Conclusions. The *CFH* polymorphism Tyr402His appears indicative of AMD pathogenesis. Diabetes, age, and gender in the presence of the homozygous "CC" genotype in *CFH* carry an increased risk of AMD. Hence this polymorphism could be used as a potential marker for predictive testing across continents. (*Invest Ophthalmol Vis Sci.* 2006;47:3729–3735) DOI:10.1167/iovs.05-1430

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Age-related macular degeneration (AMD) is a leading cause of irreversible vision loss worldwide and leads to progressive impairment of central vision.[1,2]

It is a late-onset, complex disorder with multifactorial etiology. It is estimated that ~8 million people will have vision loss due to retinal complications including AMD by the year 2020.[3] Epidemiologic surveys have indicated age and smoking as potential risk factors in the pathogenesis of AMD.[4] The estimated prevalence of retinal diseases in India is 10.3%, of which AMD contributes to 1.84% to 2.7%, increasing with age.[5]

Besides senescence and lifestyle, genetic predisposition is recognized to be a risk factor for AMD. Classic genetic studies[6–8] and whole-genome scans have led to the identification of chromosomal loci on 1q, 9q, 10q, 16q, and 22q by linkage analysis, but most underlying genes have yet to be characterized.[9–18] Based on common pathogenic features in AMD, atherosclerosis, and cardiovascular disease, a common biochemical mechanism was proposed for these diseases, and variants in genes involved in inflammation, oxidative stress, and cholesterol metabolism were suggested to be the potential candidates.[19,20] Recent studies have indicated that single-nucleotide polymorphisms (SNPs) in genes regulating innate immunity, such as complement factor-H (*CFH*)[21–26] toll-like receptor-4 (*TLR4*),[27] and *APOE*[28–31], contribute significant susceptibility to AMD. The Tyr402His (T>C) variant in *CFH*, increased the relative risk (RR) of having AMD by four- to fivefold, with an odds ratio (OR) ranging from 2.4 to 4.6 for the carrier C allele and 3.3 to 7.4 for the homozygous CC genotype in several independent studies.[22–25] It was further demonstrated that an early age at diagnosis and family history of AMD was associated with the high-risk allele.[24] A risk haplotype was also identified at the *CFH* locus along with the flanking SNPs.[26] The Asp299Gly SNP in *TLR4* also exhibited a 2.65-fold increased risk of AMD and exhibited an additive risk (OR = 4.13, $P = 0.002$) with allelic variants of *APOE* and ATP-binding cassette transporter-1 (*ABCA1*) involved in cholesterol efflux, suggesting that altered *TLR4* signaling by this variant may influence phagocytic function of RPE, thereby contributing to damage of the RPE.[27] Most of the studies on *APOE* gene polymorphism have indicated an elevated risk of AMD with *APOE-ε2* allele and a reduced risk with the *APOE-ε4* allele.[28–31]

As these studies were performed predominantly on white populations in Western countries, they require replication in well-documented AMD phenotypes from different ethnic groups worldwide to gain a better appreciation of their role in the disease pathogenesis. Moreover, with rapid demographic changes and the increasing number of elderly people in the developing world as well, it is important to analyze these SNPs on a global scale in various ethnic groups so as to anticipate and attempt to manage AMD the world over. Herein, we report on such an analysis of *CFH*, *TLR4*, and *APOE* gene polymorphisms in the background of other clinical and demographic

variables in clinically diagnosed AMD subjects from different states and ethnicities in India.

METHODS

Subjects and Clinical Evaluation

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. One hundred unrelated consecutively diagnosed patients with AMD from seven different states of India along with 120 ethnically matched normal control subjects presenting at the L. V. Prasad Eye institute, Hyderabad, India, were recruited. All the subjects underwent a detailed eye examination that included best corrected visual acuity (using the Snellen chart), slit lamp evaluation of the anterior segment and vitreous, biomicroscopy of the optic nerve head and macula (78 D and 90 D), and examination of the peripheral fundus (through indirect ophthalmoscopy). Fundus photography was performed routinely in all the patients, whereas fluorescein angiography was performed only when necessary.

Diagnosis and classification of AMD was based on the standardized AREDS criteria.[2] The assignment of AMD affection status was independently performed by two retina specialists, based on the evaluation of color fundus photographs and other details of examination from the medical records. In cases of disagreement, re-evaluation was performed by a third observer to confirm the diagnosis. The interobserver agreement was estimated by calculating the κ statistics. The status of the condition was defined by the most severe grade in either eye. Subjects with media opacity and diseases that phenotypically overlap AMD such as a few small drusen, isolated pigmentary disturbances of the RPE, CNV other than AMD, high myopia, retinal detachment, and central serous chorioretinopathy were excluded. The control subjects did not have a family history of AMD or any other ocular or systemic diseases such as diabetes and hypertension. Fundus examination was normal in these subjects, and there was no drusen formation. They were mainly unaffected spouses of the patients or were patients undergoing cataract surgery (whose media was clear enough to allow a fundus examination) at the institute and were matched with the geographical region of habitat of the patients.

Complete information regarding the ethnicity, diet, lifestyle, and systemic conditions were documented on each subject in a predesigned questionnaire. Blood samples from the patients and normal volunteers were collected by venipuncture, with prior informed consent.

Screening of SNPs in the *CFH*, *APOE*, and *TLR4* Genes

Genomic DNA was extracted from the peripheral blood leukocytes according to standard protocols.[32] The specific region of *CFH* containing the T1277C (Tyr240His) polymorphism was PCR amplified by using specific primers[26] followed by restriction digestion analysis with the *Nla*III enzyme at 37°C overnight. The digested products were electrophoresed on 6% nondenaturing polyacrylamide gels, and band patterns were scored from the gel. Genotyping of the *TLR4*-Asp299Gly (rs4986790) and Thr399Ile (rs4986791) SNPs were done by allele-specific PCR and restriction digestion.[33] *APOE* genotyping was performed with a standard technique, as described earlier.[34] Subsets of restriction digests from each gel were further confirmed by resequencing (3100 Genetic Analyzer; Applied Biosystems [ABI], Foster City, CA), with dye termination chemistry (BigDye Terminator; ABI), as per the manufacturer's protocol.

Haplotype Analysis at the *CFH* Locus

Five intragenic SNPs in *CFH* flanking the Tyr402His variant were screened by resequencing on the genetic analyzer (3100 ABI) to generate haplotype data in patients and control subjects. The order of SNPs screened is: promoter −258C>T (rs3753394), exon 2 (I62V; rs800292), intron 6 (IVS6; rs3766304), exon 9 (Y402H; rs1061170), exon 13 (Q672Q; rs3753396), and exon 18 (D936E; rs1065489). The primer sequences and amplification protocols for these SNPs were as published earlier.[26]

Statistical Analysis

Allele and genotype frequencies were estimated by the allele counting method. Hardy-Weinberg estimates for genotypes and estimated haplotype frequencies were calculated using the Haploview software[35] that uses the EM algorithm. Linkage disequilibrium between the intragenic SNPs at the *CFH* locus was also analyzed using the LD plot function of this software. ORs were computed for estimating the risk of AMD with respect to different genotypes. Univariate analysis was performed to check for association of different genotypes and risk factors to AMD followed by multivariate analysis. Logistic regression was used to adjust covariate effects by age, gender, and diabetes. Additive effects between the risk genotypes of different susceptible genes were also assessed. All these calculations were performed in commercial statistical analysis software (SPSS software; SPSS, Chicago, IL).

RESULTS

Among the patients, 13% had an affected relative, whereas the rest were sporadic cases. Consanguinity was observed in 22% of the cases, but neither family history of AMD (P = 0.756) nor consanguinity (P = 0.490) was associated with the disease phenotype. Nearly 75% of the patients had late-stage and proliferative AMD (P = 0.001). The phenotype categories of the patients comprised choroidal neovascular membrane (38%), large drusen (35%), disciform scar (14%), and geographic atrophy (13%). There was good interobserver agreement in assignment of AMD status ($\kappa = 0.91 \pm 0.06$). The mean age for early and late AMD were found to be 62.4 ± 10.2 and 69.2 ± 7.7 years, respectively, among the

TABLE 1. Distribution of Genotype and Allele Frequencies in the *CFH* SNP in AMD Patients across Different Populations

Tyr402His (T>C) SNP	Present Study	Edwards et al.[22]	Haines et al.[23]	Zareparsi et al.[24]	Rivera et al.[25]
Alleles					
T	0.48	0.45	0.06	0.39	0.41
C	0.52	0.55	0.94	0.61	0.59
Significance	$P = 1.19 \times 10^{-7}$	$P = 4.95 \times 10^{-10}$	$P = 0.00006$	$P < 1 \times 10^{-24}$	$P = 6.7 \times 10^{-29}$
Genotypes					
TT	0.27	0.21	NA*	0.14	0.17
TC	0.42	0.47	NA*	0.50	0.45
CC	0.31	0.31	NA*	0.36	0.37
Odds Ratio (95% CI)					
TC	1.51 (0.82–2.80)	NA*	2.45 (1.41–4.25)	4.36 (3.13–6.08)	1.99 (1.61–2.46)
CC	11.52 (5.05–26.28)	NA*	3.33 (1.79–6.20)	5.52 (3.54–8.59)	6.72 (5.14–8.79)

* Not available.

TABLE 2. Distribution of Unadjusted and Adjusted Odds Ratios for the Mutant Genotypes in Different Genes

Gene (Polymorphism)	Genotype	OR (95% CI) [Unadjusted]	*P*	OR (95% CI) [Adjusted]*	*P*
CFH (Y402H)	TC	1.51 (0.82–2.80)	0.375	1.10 (0.55–2.20)	0.783
	CC	11.52 (5.05–26.28)	0.0001	7.81 (3.18–12.44)	0.001
APOE	ε2ε3	1.03 (0.36–2.94)	0.95	0.87 (0.28–2.67)	0.805
	ε3ε4	0.42 (0.19–0.91)	0.03	0.38 (0.16–0.90)	0.028
TLR4 (D299G)	AG	0.75 (0.40–1.39)	0.37	0.76 (0.39–1.48)	0.425
TLR4 (T399I)	CT	0.83 (0.43–1.62)	0.59	0.88 (0.43–1.81)	0.735

* Adjusted for age, gender, and diabetes.

patients, and was 63.9 ± 6.6 years among the control subjects. Significant association was noted between AMD and age ($P = 0.003$) and gender ($P = 0.001$), and women were more prone to the disease (OR = 2.67, 95% CI 1.47–4.84) than were men. When age was incorporated as a continuous variable in the logistic regression analysis, an increase of 10 years in the age of the patients raised the relative risk by 1.79-fold (95% CI 1.22–2.62) and of 5 years by 1.34-fold (95% CI 1.10–1.62). Among the risk factors, only diabetes was significantly ($P = 0.003$) associated with AMD (OR = 3.41, 95% CI 1.51–7.72), whereas there was no association to smoking ($P = 0.317$) and hypertension ($P = 0.446$).

The cohort of cases and control subjects conformed to the Hardy-Weinberg equilibrium. A significantly higher frequency of the C allele of Tyr402His SNP (*CFH*) was noted among AMD cases than in controls (0.52 vs. 0.26; $\chi^2 = 28.02$, 1 *df*; $P = 1.19 \times 10^{-7}$) similar to previous studies (Table 1). Individuals homozygous for the CC genotype had a significantly higher risk ($P < 0.0001$) of AMD (OR = 11.52; 95% CI 5.05–26.28) than those carrying a single copy of the C allele (OR = 1.51, 95% CI 0.82–2.80). The significant association of the CC genotype was consistent ($P < 0.001$), even after adjusting for age, gender, and diabetes (Table 2), providing additional evidence on the functional importance of Tyr402His variant in AMD.

To confirm the association of Tyr402His SNP with AMD, additional intragenic SNPs at the *CFH* locus were typed in patients and control subjects that indicated a significant association of the I62V ($P = 0.0006$) and IVS6 ($P = 0.0026$) variants with AMD, similar to Tyr402His, whereas the remaining SNPs were not informative (Table 3). These findings are exactly similar to those reported in white populations from Iowa and Columbia.[26] The association of the Tyr402His variant with AMD was further confirmed by looking at the pair-wise linkage disequilibrium (LD) among these SNPs (Fig. 1) in our cohort. As is evident from the figure, there was a stronger LD for the Tyr402His and IVS6 SNPs ($D' = 0.84$; 95% CI 0.53–0.94) and a relatively milder LD between the Q672Q and D936E SNPs ($D' = 0.77$; 95% CI 0.58–0.90).

Haplotype analysis using five intragenic SNPs flanking Tyr402His locus revealed 16 different haplotypes among the patients and control subjects. The estimated haplotype frequencies are presented in Table 4. It is clear from the table that the C-G-T-C-A-G haplotype harboring the mutant C allele at the Tyr402 locus was approximately two times higher in the AMD cases than control subjects (31% vs. 15.4%). This was the only risk haplotype ($P = 0.0003$). The association of the risk haplotype was even stronger ($P = 3.06 \times 10^{-8}$) when only the three-locus haplotype comprising the AMD-associated SNPs (I62V, IVS6, and Y402H) were considered (data not shown). Most of the other haplotypes with a C allele at the Tyr402 locus were relatively more frequent among the cases but did not show any significant association (Table 4). A significant association was noted for the C-A-T-T-A-G ($P = 0.024$) and the C-A-C-T-A-G ($P = 0.016$) haplotypes among the control subjects, indicating that these could be protective.

The distributions of genotype frequencies for *APOE* polymorphisms in patients were not significantly different from the control subjects ($P = 0.76$), but the frequency of the ε2 allele was slightly higher in cases than in control subjects (0.04 vs. 0.03), whereas the ε4 allele frequency was slightly higher in control subjects (0.15 vs. 0.07), with no significant differences. The presence of the ε2,ε3 genotype did not indicate any elevated risk of AMD ($P = 0.95$) that was consistent, even after adjustment for age, gender, and diabetes (Table 2); it also showed a decline ($P = 0.83$) in cases of late-stage AMD (OR = 0.87, 95% CI 0.25–3.04). The carriers of the ε4 allele had a marginally reduced risk ($P = 0.03$) of the overall AMD cases, but the OR for the ε3,ε4 genotype with only late-stage AMD was not significant ($P = 0.14$), after adjusting for age, gender, and diabetes (OR = 0.51, 95% CI 0.21–1.25).

There were no significant differences in the genotype distributions for Asp299Gly ($P = 0.36$) and Thr399Ile ($P = 0.59$) SNPs of the *TLR4* gene in cases and controls. Further subanalysis of the dataset after adjusting for such risk factors as age, gender, and diabetes also did not indicate any asso-

TABLE 3. Association of Intragenic *CFH* SNPs to AMD across Different Populations

SNP	dbSNP ID	Iowa Cohort[26] χ^2 (*P*)	Columbia Cohort[26] χ^2 (*P*)	Present Study χ^2 (*P*)
Promoter	rs3753394	NA†	2.84 ($P = 0.089$)	0.65 ($P = 0.417$)
I62V	rs800292	15.0 ($P = 1.1 \times 10^{-4}$)*	26.1 ($P = 3.21 \times 10^{-7}$)*	11.7 ($P = 0.0006$)*
IVS6	rs3766404	NA†	23.0 ($P = 1.59 \times 10^{-6}$)*	9.1 ($P = 0.0026$)*
Q672Q	rs3753396	0.21 ($P = 0.65$)	2.05 ($P = 0.15$)	0.13 ($P = 0.7230$)
D936E	rs1065489	0.64 ($P = 0.8$)	0.53 ($P = 0.46$)	2.98 ($P = 0.0838$)

* Significant.
† NA, not available.

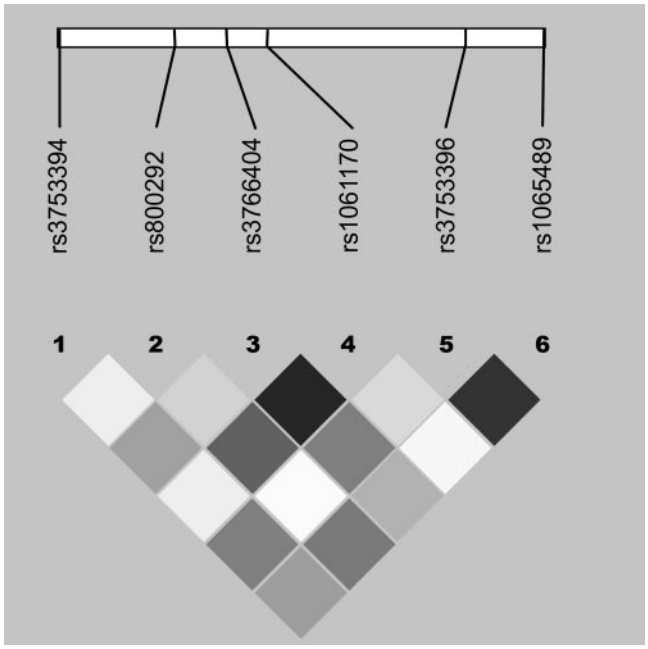

FIGURE 1. Analysis of pair-wise LD across the six *CFH* SNPs in the Indian cohort. *Black*: strong LD (High D′) between rs3766404 (IVS6) and rs1061170 (Tyr402His); *gray* to *light gray* and *white*: moderate to no LD among the SNPs.

ciation with Asp299Gly ($P = 0.42$) and Thr399Ile ($P = 0.73$) SNPs (Table 2). A similar situation was noted with respect to the late-stage AMD phenotypes for these two SNPs ($P = 0.25$ and 0.69 for Asp299Gly and Thr399Ile, respectively). We generated haplotypes for these two SNPs across cases and control subjects but the estimated haplotype frequencies were not significant in the four possible haplotypes (Table 5). Further, we looked into the additive effect due to the interaction of the two genotypes AG (Asp299Gly) and CT (Thr399Ile) in *TLR4*, but the association was not significant ($P = 0.63$) even after adjustment for age, gender, and diabetes (OR = 0.83, 95% CI 0.39–1.77). A similar exercise with the genotypes AG of Asp299Gly (*TLR4*) and $\varepsilon 3,\varepsilon 4$ of *APOE* also did not reveal any significant association ($P = 0.98$) with susceptibility to AMD (OR = 1.03, 95% CI 0.13–7.90). However, the sample size in the present study was far less apt to exhibit sufficient statistical power, than that in the previous study, given the frequency of the minor allele of *TLR4* in their cohort.[27]

TABLE 4. Estimated Haplotype Frequencies of *CFH* in AMD Cases and Controls in the Indian Cohort

Haplotypes	Cases	Controls	*P*
C-G-T-C-A-G	0.308	0.154	0.0003*
C-A-T-T-A-G	0.076	0.148	0.0238*
C-G-T-T-A-G	0.064	0.119	0.0583
C-A-C-T-A-G	0.048	0.113	0.0161*
T-G-T-T-A-G	0.053	0.092	0.136
C-G-C-T-A-G	0.049	0.06	0.6192
T-G-T-T-G-T	0.055	0.052	0.8946
T-G-T-C-A-G	0.075	0.032	0.06
T-A-T-T-A-G	0.035	0.024	0.5313
T-G-T-T-A-T	0.048	0.047	0.956
T-G-C-T-A-G	0.013	0.033	0.1889
T-G-T-C-A-T	0.031	0.014	0.239
C-G-T-C-G-T	0.03	0.005	0.0549
C-A-T-C-A-G	0.019	0.012	0.5852
C-A-C-C-A-G	0.006	0.023	0.1475
T-A-T-C-A-G	0.014	0.01	0.6566

* Significant.

DISCUSSION

Association studies on AMD have provided valuable information on the implication of genetic variants with disease susceptibility.[36] They become even more useful if they are performed across populations of diverse ethnicities.[30,31] The present study enlarges the role of *CFH* polymorphism as an indicator of AMD pathogenesis across continents. Recent reports on white populations showed the Tyr402His SNP of *CFH* to be significantly associated with AMD.[21–26,37–39] Our results support this association and show this SNP to be more universal (Table 1). In addition, we find the increased risk of AMD in the presence of the homozygous CC alleles to be consistent, even after adjustment for age, gender, and diabetes. The relatively higher ORs with the CC genotype in the present study is perhaps due to the smaller sample size compared with previous studies. Despite this, a significant association of the Tyr402His polymorphism in the geographically and ethnically diverse patient cohort from India highlights its global implication in AMD.

The association of the Tyr402His variant was further confirmed by the analysis of SNPs flanking this variant (Table 3), pair-wise LD (Fig. 1) and haplotype analysis (Table 4). The I62V and IVS6 were similarly associated with AMD, as indicated in previous studies, whereas there was no association with the Q672Q and D936E SNPs.[26] Pair-wise LD analysis also indicated a strong LD between IVS6 and Tyr402His.

Haplotype analysis revealed certain similarities among the white (Columbian cohort),[26] Japanese,[40] and Indian patients with AMD (Table 6). The risk haplotype C-G-T-C-A-G observed in the present study was also noted in the Columbian cohort, whereas it was not associated in the Japanese cohort in cases and controls. This could be attributed to the lack of association of the *CFH* variant Tyr402His among the Japanese patients. Of note, the C-A-T-T-A-G haplotype was protective in all the three populations. The other protective haplotype in the Indian and Columbian patients were not shared among each of the two populations. To analyze our data in conjunction with the Japanese data,[40] we reanalyzed the haplotypes based on the four SNP loci (data not shown). It was observed that of the two risk haplotypes G-T-T-A and A-T-T-C in the Japanese cohort, the former haplotype was observed more frequently among the normal control subjects in India, whereas the latter haplotype was completely absent in the present cohort (Table 6). These results strongly suggest the implication of the *CFH* SNPs in the disease pathogenesis and indicate that the risk haplotype could predispose to AMD.

Another interesting observation is the association of diabetes with AMD in our patient cohort. In addition, diabetes in conjunction with the CC genotype of *CFH* appears to confer an elevated risk of AMD (Table 2) that should be

TABLE 5. Estimated Haplotype Frequencies of *TLR4* in AMD Cases and Controls in the Indian Cohort

Haplotypes	Cases	Controls	*P*
A-C	0.861	0.794	0.0893
G-T	0.074	0.113	0.1935
G-C	0.039	0.059	0.373
A-T	0.026	0.034	0.6833

TABLE 6. Distribution of Risk and Protective *CFH* Haplotypes across Different Populations Worldwide

Haplotypes	Columbian Cohort[26]	Japanese Cohort[40]*	Present Study
C-G-T-C-A-G	Risk ($P < 0.00001$)	NS ($P = 0.802$)†	Risk ($P = 0.0003$)
C-A-T-T-A-G	Protective ($P = 0.00003$)	Protective ($P = 0.001$)	Protective ($P = 0.0238$)
C-G-C-T-A-G	Protective ($P = 0.00008$)	—‡	NS† ($P = 0.6192$)
C-A-C-T-A-G	—‡	—‡	Protective ($P = 0.0161$)
--G-T-T-A--	—‡	Risk ($P = 0.028$)	NS† ($P = 0.058$)
--A-T-T-C--	—‡	Risk ($P = 0.004$)	—‡

* The Japanese study was based on four intermediate SNPs only.
† Not significant.
‡ Absent.

investigated further. An earlier study demonstrated that the presence of AMD deteriorates visual acuity earlier in patients with type 2 diabetes than in control subjects.[41] Thus, it would be interesting to see the implications of *CFH* at the molecular level in diabetes and AMD. We also note that unlike a previous study,[23] the age of the patient also provides a potential risk factor in association with the CC genotype (Table 2). It has been postulated that the high-risk allele 402His of *CFH* encodes an isoform with some functional implications in AMD pathogenesis.[24–26] Based on these, we conclude that the Tyr402His polymorphism could be used worldwide as a diagnostic marker in AMD.

In contrast, our results with respect to *APOE* SNPs are consistent with those in other Asian populations,[42,43] which did not exhibit an association of the ε2 allele with AMD; but the ε4 allele that exhibited a significant protective effect in AMD[30,31] is marginally higher among the control subjects in the present study, indicating a similar trend. However *APOE* polymorphisms have indicated marked geographical and ethnic variations with respect to allelic association across patients with AMD worldwide (Table 7).

Turning to the *TLR4* gene, we did not find any significant association of the reported SNPs (Asp299Gly and Thr399Ile) with AMD. The estimated frequencies of the haplotypes A-C, G-T, G-C, and A-T were also not significant (Table 5). The additive effect of the interaction of the high-risk alleles within *TLR4* and between *TLR4* (Asp299Gly) and *APOE* (ε4) also did not reveal any association with susceptibility to AMD, as shown earlier.[27] As has been pointed out, however, a much larger sample size than the present one available to us is needed before associations with the *TLR4* SNPs can be determined.[27] Also, unlike *CFH* and *APOE*, the *TLR4* polymorphism has been relatively less explored across different AMD populations. We may thus have to wait for some time until a global picture with respect to *TLR4* emerges.

To the best of our knowledge, this is perhaps the first study on these three polymorphisms among Indian patients with AMD. The data in the present study underscore the role of *CFH* polymorphisms, particularly Tyr402His as an indicator of AMD pathogenesis across continents. As systemic and demographic variables like diabetes, age, and gender in the presence of the CC genotype in Tyr402His carry an increased risk, these factors should be incorporated in the diagnosis of AMD. Hence, these *CFH* SNPs could be used universally as a potential marker for predictive testing and calls for further studies from different geographical regions. A recent study indicated that variations in the factor B (*BF*) and complement component 2 (*C2*) encoding regulatory proteins of the same pathway, similar to *CFH*, are implicated in AMD.[47] Similar studies in other ethnic groups worldwide would also help in identifying genetic variants in candidate genes that would be of predictive value in AMD.

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TABLE 7. Distribution of *APOE* Alleles and Genotype Frequencies in AMD Patients in Different Studies

APOE Alleles/ Genotypes	Simonelli et al.[44] ($n = 87$)	Klaver et al.[28] ($n = 88$)	Present Study ($n = 100$)	Souied et al.[45] ($n = 116$)	Schmidt et al.[46] ($n = 230$)	Baird et al.[30]† ($n = 252$)	Zareparsi et al.[31] ($n = 632$)
ε2 (%)	9.8	12.5	4.5	9.9	8.7	9.9	9
ε3 (%)	87.3	80.6	88.5	82.8	79.6	78.6	81
ε4 (%)	2.9	6.8	7	7.3	11.7	11.5	10
ε2 ε2 (%)	0	0	0	0.0	1.7	0.8	0.2
ε2 ε3 (%)	18.4	22.7	9	17.2	11.3	14.7	16.5
ε2ε4 (%)	1.2	2.3	0	2.6	2.6	3.6	1.6
ε3 ε3 (%)	75.9	63.6	77	71.6	64.8	62.3	64.6
ε3 ε4 (%)	4.5	11.4	14	6.9	18.3	17.9	16.8
ε4 ε4 (%)	0	0	0	2.6	1.3	0.8	0.3
ε2 carriers* (*P*)	0.031	0.17	0.805	NA‡	0.97	0.18	NA‡
ε2 carriers* OR (95% CI)	5.2 (1.2–23.0)	1.50 (0.80–2.82)	0.87 (0.28–2.67)	NA‡	0.99 (0.59–1.66)	1.69 (0.79–3.61)	NA‡
ε4 carriers* (*P*)	0.168	0.002	0.028	<0.0001	0.56	0.04	0.009
ε4 carriers* OR (95% CI)	0.4 (0.1–1.2)	0.43 (0.21–0.88)	0.38 (0.16–0.91)	0.27 (0.12–0.57)	0.88 (0.58–1.35)	0.58 (0.34–0.98)	0.60 (0.41–0.88)

* With respect to ε3ε3 as the reference genotype.
† Late-stage AMD only.
‡ NA, not available.

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E R R A T U M

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