Variations in *TIMP3* are associated with age-related macular degeneration

Extracellular matrix (ECM) remodeling and degradation have been associated with atrophic changes in the retinal pigment epithelium (RPE) and Bruch's membrane, leading to macular dystrophy. In the paper based on a genome-wide association study (GWAS), Chen et al. (1) discovered a single-nucleotide polymorphism (SNP) located ≈100 kb upstream of *TIMP3* that influenced the susceptibility to age-related macular degeneration (AMD) in very large and diverse cohorts. In an ongoing parallel effort, we undertook screening of 11 candidate genes involved in ECM turnover and degradation, namely fibulin 5 (*FBLN5*), fibulin 6 (*FBLN6*), decorin (*DCN*), lumican (*LUM*), epiphycan (*EPYC*), *MMP1, MMP2, MMP3, MMP9, TIMP2*, and *TIMP3* to understand their involvement in a previously diagnosed cohort of AMD cases (n = 250) and normal controls (n = 250) from India (2).

Initial screening was accomplished by an extensive genotyping of 121 SNPs spanning these genes by using the golden gate assay of Illumina. The selections of these SNPs were based on the knowledge of their prior association with any other age-related conditions and their frequency in the general haplotype map (HapMap) populations. Genotypes were extracted with the Bead Studio software (version 3.0) of Illumina by using a clustering algorithm. Five independent samples in each 96-well plate were provided as replicates for validation of genotypes in the golden gate assay, and only samples with >99% genotype call rates were included for analysis. The association of a SNP was further confirmed by resequencing of the extended region with appropriate primers by using BigDye chemistry (Applied Biosystems) and PCR-based restriction digestion.

There were no deviations from the Hardy–Weinberg equilibrium for these 121 SNPs among the normal controls ($P > 0.05$). Significant differences ($P < 0.05$) were observed in the allele frequencies of some SNPs in *FBLN5*, *FBLN6*, *MMP2*, and *TIMP3* between cases and controls (Table 1), but only two intronic SNPs (rs713685 and rs743751) in *TIMP3* withstood Bonferroni correction for multiple testing ($P = 4.13 \times 10^{-4}$). Haplotypes were generated (by using Haploview software, version 4.1, that uses an EM algorithm) with these two *TIMP3* SNPs that were in complete LD (D' = 1). A risk (C-C) and a protective (T-C) haplotype were observed (Table 2), indicating that this region could have some potential functional implications in AMD. The SNPs in the other genes (*DCN, LUM, EPYC, TIMP2, MMP1, MMP2*, and *MMP3*) did not exhibit any association to AMD (Table 1).

In conclusion, our data demonstrates a strong association of two *TIMP3* SNPs in AMD that need to be explored further. It provides evidence on the involvement of *TIMP3* in a different ethnic population (Indian), thereby supplementing the possible indication of this gene from the GWAS data of Chen et al. (1). Higher levels of TIMP3 in AMD eyes have been suggested to result in the thickening of the Bruch's membrane (3), and recent identification of signature genes in RPE has implicated *TIMP3* as a potential candidate in AMD pathogenesis (4). It would thus be interesting to see the replication of the association of *TIMP3* in AMD in other ethnically diverse populations worldwide.

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Table 1. Distribution of allele frequencies in different candidate genes in AMD in the Indian cohort

Gene	Chromosomal location	Total SNPs screened	Associated SNP(s)	Associated allele	Frequency in AMD cases	Frequency in normal controls	P	Odds ratio (95% confidence interval)
FBLN6	1q24-q25	20	rs721153	C	0.503	0.420	0.0275	1.39 (1.04–1.88)
FBLN5	14q32.1	18	rs929608	A	0.537	0.454	0.0287	1.39 (1.03–1.87)
			rs741198	G	0.825	0.754	0.0216	1.53 (1.06–2.21)
			rs1861085	G	0.709	0.617	0.0099	1.51 (1.10–2.07)
			rs2160079	C	0.740	0.649	0.0084	1.54 (1.18–2.13)
MMP2	16q13	10	rs9302671	A	0.271	0.194	0.0158	1.54 (1.08–2.20)
			rs2241145	C	0.565	0.480	0.0240	1.41 (1.05–1.89)
			rs243836	G	0.525	0.420	0.0051	1.53 (1.13–2.06)
TIMP3	22q12.1-q13.2	24	rs713685*	C	0.907	0.677	5.74×10^{-14}	4.64 (3.04–7.08)
			rs6518799	A	0.119	0.051	0.0014	2.48 (1.40–4.40)
			rs743751*	G	0.136	0.054	2.0×10^{-4}	2.73 (1.57–4.75)
MMP1	11q22-q23	4	—	—	—	—	—	—
MMP3	11q23	4	—	—	—	—	—	—
DCN	12q21.3	10	—	—	—	—	—	—
LUM	12q21.3-q22	6	—	—	—	—	—	—
EPYC	12q21	3	—	—	—	—	—	—
TIMP2	17q25	14	—	—	—	—	—	—
MMP9	20q11.2-q13.1	8	—	—	—	—	—	—

*SNPs that withstood Bonferroni correction.

Table 2. Distribution of estimated haplotype frequencies based on the AMD-associated SNPs in *TIMP3* in the Indian cohort

Haplotypes*	Overall frequency[†]	Frequency in AMD cases	Frequency in normal controls	P[‡]	Odds ratios (95% confidence intervals)
C-C	0.697	0.771	0.623	1.83×10^{-5}	2.04 (1.47–2.84)
T-C	0.207	0.093	0.323	5.73×10^{-14}	0.22 (0.14–0.33)

*The order of the haplotype is rs713685-rs743751.
[†]Only haplotypes with >5% frequency in the general population were considered.
[‡]Permutation P values (n = 10,000 permutations) for both the haplotypes were $P < 10^{-4}$.