Original Communication

Association of apolipoprotein E (RFLP) polymorphism with myopia

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BACKGROUND: Myopia or nearsightedness is a spherical error of refraction, whereby the images are focused in front of retina. Eye, being an organ rich in activated oxygen species, requires a high level of antioxidants to protect the unsaturated fatty acids. Apolipoprotein E (APOE) is one of the proteins that is produced by Muller cells within the retina and is also endowed with antioxidant properties. Genetic polymorphism of APO E is controlled by three common alleles ε3, ε2 and ε4 and rare e1, e4v at the APOE structural gene locus. Different isoforms of APO E differ in their antioxidant properties, and the e4 allele has lesser ability to combat oxidative stress.
AIMS: Myopia being a disease influenced by oxidative stress, the present study was undertaken to find association of myopia with APO E polymorphism.
MATERIALS AND METHODS: A total of 187 myopic cases and 192 controls were genotyped for apolipoprotein E polymorphism.
RESULTS: In both controls and myopic cases, E3/3 genotype was found to be the most frequent one. There was an increase in E3/4 genotype frequency among male probands, high myopia cases and probands with early age at onset, suggesting that the E3/4 genotype might confer risk for myopia development.
CONCLUSION: This association with E3/4 genotype might predispose susceptible individuals to develop high myopia and early onset myopia.

Key words: Apolipoprotein E polymorphism, genotype, Muller cells, myopia, oxidative stress, retinal degeneration

Myopia or nearsightedness is a condition whereby images are focused in front of the retina. Myopia development involves variation in corneal structure or increase in axial length. Refractive error is measured in diopters and is the combined power of the cornea and the lens that is needed to focus distant objects correctly on the retina.[1] There are etiologically distinct forms of myopia. High myopia or pathological myopia causes progressive elongation of the globe and stretching of the scleral wall, leading to a high refractive error of more than 6.0 diopters. The simple or less severe form is known as physiological myopia, which occurs as a result of correlative effect of refractive components of the eye and has refractive error up to 6.0 diopters. Myopia is a disease that is influenced by oxidative stress.[2] The oxygen-rich environment of the eye has phospholipids containing dexahexanoic acid within the photoreceptor cells. Dexahexanoic acid is intensively sensitive to oxidative damage. To protect against oxidative damage, the retina contains antioxidants and antioxidant enzyme systems. APO E is one of the proteins that is produced by Muller cells within the retina and is also endowed with antioxidant properties.[3] APO E gene is mapped on chromosome 19 in a cluster within APO C1 and APO C2. It spans 3.7 Kb including 4 exons. Genetic polymorphism of APO E is controlled by three common alleles ε3, ε2 and ε4 and rare e1, e4v at the APOE structural gene locus.[4,5] Different isoforms of APO E differ in their antioxidant properties, and the e4 allele has lesser ability to combat oxidative stress.[6] Hence the present study has been planned to identify the possible association between APO E polymorphism and myopia progression.

Materials and Methods

Blood samples were collected in EDTA vacuatainer from 187 myopia patients reported at Sarojini Devi Eye Hospital, Kanchan Eye Hospital and Jagadamba Nursing Home. Each of these hospitals was visited twice a week for a period of 20 months. The information regarding age at onset, sex, para, maternal reproductive history, nutritional status, socioeconomic status, familial incidence and parental consanguinity was collected from

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the patients by personally interviewing them on the basis of selected pro forma All the patients under study were clinically examined by ophthalmologists accurately for spherical error of refraction, retinal changes, fundus and macula changes. Age- and sex-matched controls (n = 192) examined by ophthalmologists and found to be without any history of myopia or any other genetic disease were selected randomly from hostels, schools, colleges and various institutions for the purpose of comparison. DNA was isolated using the rapid non-enzymatic method of DNA isolation.[7] The genomic DNA was amplified using specific primers for APOE from Hysel India Ltd. (Forward primer**:** 5' ACA GAA TTC GCC CCG GCC TGG TAC AC-3'; reverse primer: 5' TAA GCT TGG CAC GGC TGT CCA AGG A-3'). Thirty microliters of PCR mix contains 3 ml of Genomic DNA; 3 μl of 10x PCR buffer; 3 μl of 10 mM dNTPs; reverse and forward primers, 3 μl [1 pmol/μl] of each; 0.1 μl [2.5 U/μl] of Taq polymerase; 11.9 μl of DdH_2O; and 3 μl of 10% DMSO. PCR conditions include initial denaturation for 5 min at 95°C and 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension of 70°C for 2 min.[8] The PCR product was subjected to restriction digestion at 37°C overnight by Hha I (Bangalore Genie Pvt. Ltd.). The digested product was run on 14% polyacrylamide gel for 3 h at 200 V under constant 45 mA current. After the electrophoresis, the gel was stained with ethidium bromide (0.2 mg/l) for 10 min, and DNA fragments were visualized under UV transilluminator.

GENOTYPES	FRAGMENT SIZES
E2/2	91, 83 bp
E3/3	91, 48, 35 bp
E4/4	72, 48, 35 bp
E2/3	91, 83, 48, 35 bp
E2/4	91, 83, 72, 48, 35 bp
E3/4	91, 72, 48, 35 bp

Results and Discussion

In our study, we have observed only three genotypes of APO E in the disease group and controls, viz., (ε3/3, ε3/4, ε2/3). Although there are six possible genotypes, several studies have shown variation in number of genotypes, ranging from 3 to 5. The genotype distribution of APO E polymorphism in myopia (ε3/3, 82.4%; ε3/4, 13.9%; ε2/3, 3.7%) does not deviate from that of control (ε3/3; 79.3%,ε3/4; 14.5%,ε2/3; 6.1%), as revealed in Table 1. The allele frequencies also did not show much difference. The relative risk calculated for e3/3 *vs.* e3/4 (χ^2 = 0.811) and ε2/3 *vs.* ε3/4 (χ^2 = 0.451) and ε3/3 *vs.* ε2/3 (χ^2=0.533) did not reveal any significant results.

Table 2 shows the comparison with respect to the sex of the proband, where the distribution of APOE genotypes revealed slightly elevated frequency of ε3/4 (14.6%) genotype in male probands with a corresponding decrease in the ε2/3 genotype (3.1%) as

Table 1: Frequency distribution of apolipoprotein E genotypes in myopia and control groups

Parameter	Genotypes									
	ε 3/3		ε 3/4		ε2/3		Total	Allele frequencies		
	N	%	N	%	N	%		P	Q	R
Myopia	154	82.4	26	13.9	7	3.7	187	0.02	0.90	0.07
Control	192	79.3	26	14.5	11	6.1	179	0.03	0.89	0.08

As compared to controls: Genotype distribution: χ^2 = 1.154

Relative incidence as compared to heterozygotes: ε3/3 versus ε3/4; χ^2 = 0.073; Odds ratio = 0.802, ε3/4 versus ε2/3; χ^2 = 0.661; Odds ratio = 1.571, ε3/3 versus ε2/3; χ^2 = 1.153; Odds ratio = 1.260, **P*<0.05 (Chi-square distribution)

Departure from Hardy - Weinberg equilibrium: Myopia; χ^2 = 0.22146; Control χ^2 = 0.0076

Table 2: Frequency distribution of apolipoprotein E genotypes with respect to sex of the proband

Sex of the	Genotypes									
Proband	ε 3/3		ε 3/4		ε 2/3		Total	Allele frequencies		
	N	%	N	%	N	%		P	Q	R
Male	79	82.3	14	14.6	3	3.1	96	0.017	0.907	0.08
Female	75	82.4	12	13.2	4	4.4	91	0.24	0.907	0.07

χ^2 = 0.267, 2d.f, *P* = 0.875

Table 3: Distribution of apolipoprotein E genotypes with respect to age at onset of the proband

Age of onset	Genotypes									
	ε 3/3		ε 3/4		ε 2/3		Total	Allele frequencies		
	N	%	N	%	N	%		P	Q	R
0-5	19	86.4	3	13.6	-	-	22	0	0.929	0.07
6-10	30	68.2	11	25.0	3	6.8	44	0.041	0.825	0.151
11-15	63	90.0	6	8.6	1	1.4	70	0.007	0.094	0.045
16-20	26	89.7	2	6.9	1	3.4	29	0.018	0.946	0.036
21-25	14	70.0	4	20.0	2	10.0	20	0.059	0.836	0.119
>25	2	100.0	-	-	-	-	2	0	1	0

χ^2 = 14.523, 10d.f, *P* = 0.150

Table 4: Distribution of apolipoprotein E genotypes with respect to types of myopia

Types of myopia	Genotypes									
	ε 3/3		ε 3/4		ε2/3		Total	Allele frequencies		
	N	%	N	%	N	%		P	Q	R
High myopia	47	75.8	14	22.6	1	1.6	62	0.009	0.870	0.129
Low myopia	107	85.6	12	9.6	6	4.8	125	0.025	0.92	0.051

χ^2 = 6.630, 2d.f, *P* = 0.306

compared to corresponding frequencies of female probands [ε3/4 (13.2%), ε2/3 (4.4%) genotypes]. APO E polymorphism studied in different age groups [Table 3] showed elevation of ε3/4 genotype in early onset cases of age 0-10 years (21.2%) as compared to late onset myopia cases of age more than 21 years (12.24%). Not much difference was seen in ε2/3 and ε3/4 genotype distributions with respect to age at onset. The study of APO E polymorphism between types of myopia [Table 4] revealed the elevation of ε3/4 genotype frequency in high myopia (22.6%) as compared to low myopia (9.6%).

The increase in the ε3/4 genotype frequency among male probands, high myopia cases and probands with early age at onset suggest that the ε3/4 genotype confers risk for the development of myopia. Myopia is a disease that is influenced by oxidative stress. In high myopia, the lipid peroxidation results in free radical process, leading to retinal detachments.[9-11] Children, adolescents with progressive myopia and retinal detachments had a reduced ratio between antioxidant activity and radical formation. The progressive myopia was also correlated with the oxidative damage and free radical formation. Eye, being an organ rich in activated oxygen species, requires a high level of antioxidants to protect the unsaturated fatty acids. The ε4 allele is known to have lesser ability to combat oxidative stress as compared to ε2 and ε3 alleles of APO E and thus contributes to the development of high myopia. The association of ε4/4 alleles was also seen with retinitis pigmentosa[12] and glaucoma.[13]

The APO E genotype distribution among familial and nonfamilial cases revealed a decrease in the ε3/4 allele frequency in familial cases (12.3%) as compared to nonfamilial cases (16.9%). Increase in ε3/4 genotype was also observed in consanguineous cases (21.2%) as compared to nonconsanguineous cases (12.3%). The increase of ε3/4 genotype frequency in nonfamilial cases and consanguineous cases suggests that myopia is caused by both environmental triggering factors like oxidative stress near work as well as by the influence of genetic factors. The present study reveals that association of E3/4 genotype might predispose susceptible individuals to have early onset of the condition and high myopia.

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