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## TGFB1 codon 10 polymorphism and its association with the development of myopia: a case-control study

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### Abstract

Excessive axial elongation in progressive high myopia is associated with scleral remodeling events resulting in diminished sclera-fibril architecture of the eye. Transforming Growth Factor beta (TGF- $\beta$ ) is an important pleiotropic growth factor that modulates the levels of specific extracellular matrix (ECM) proteins during scleral remodeling. In the present case-control association study (207 high myopia, 96 low myopia and 250 control cases), we aimed to investigate the genetic association of *TGFB1* codon 10 polymorphism at exon1 (T869C) in myopia patients from South Indian population using PCR-RFLP technique. Genotype distribution in high myopia patients did not reveal significant variation, but there was a slight elevation of heterozygote TC frequency (21.2%) compared to control group (16.8%). However, low myopia cases showed elevated CC genotype frequency (14.6%) as compared to controls (8.0%). Elevated CC genotype frequency was observed in low myopia group among males (21.4% vs 9.3%), and cases with early onset (23.1%), familial incidence (17.2%) and with no parental consanguinity (15.5%). Our results suggested that Individuals with CC genotype might carry sex specific risk to myopia progression especially in early onset myopia cases.

**Keywords:** Axial elongation; scleral remodeling; high myopia; transforming growth factor beta; polymorphism.

### Introduction

Myopia (near-sightedness) is a prevalent multifactorial ocular disorder worldwide, characterized by spherical error of refraction (RE) and retinal defocus that results in decreased visual acuity. The prevalence of myopia has been increasing in recent decades, especially in East Asian countries such as Japan, Singapore, Taiwan and China (Saw, 2003; Xu *et al.*, 2005). High or pathological myopia (RE >6D) is a progressive form with a increasing risk for serious complications such as glaucoma, macular degeneration, retinal detachment, and choroidal neovascularization, which when left untreated appropriately, may eventually lead to permanent vision loss (Young, 2009). It is the fourth most common cause of irreversible blindness. In Asia, the prevalence of high myopia is 1% to 5%, even ranging to 9.1% in some regions (Wong *et al.*, 2000).

It is considered to be a complex, multifactorial condition in which several non-genetic/ environmental components such as near work, excess illumination, nutritional deficiencies, mechanical stress and mental stress, along with the genetic components influence normal emmetropisation mechanisms of the eye contributing to ocular refraction in myopia (Feldkammer and Schaeffel, 2003). Genetic studies have identified 24 gene loci for myopia till date providing an array of potential candidate genes, but failed to identify single causative mutation (Ng *et al.*, 2009).

Myopia is mainly caused due to excessive axial growth of the eye and active remodeling of the ocular sclera has been shown to play a crucial role in axial elongation (McBrien and Gentle, 2003; Rada *et al.*, 2006). Scleral remodeling is an important functional event associated with modulation of quantitative and qualitative levels of extracellular matrix (ECM) proteins such as matrix metalloproteases (MMPs), tissue inhibitors of MMPs collagens, growth factors, basement membranes, small leucine rich repeat proteins (SLRPs), etc. The turnover of ECM is the major mechanism of changing axial length of eye and therefore, altered protein levels of ECM could result in diminished scleral-fibril architecture and defective mechano-transduction pathway of the eye.

Transforming growth factor beta (TGF- $\beta$ ) a pleiotropic cytokine is known to play a role in the proliferation of chondrocytes and fibroblasts as well as in the production of collagens, MMP2 and several other ECM proteins implicated in scleral remodeling of myopia development ((Overall *et al.*, 1989; Seko *et al.*, 1995; Jobling *et al.*, 2004). The three isoforms of TGF- $\beta$  are highly conserved exhibiting distinct isoform specific expression in a time dependent manner (Jobling *et al.*, 2004). The levels of TGF- $\beta$  were found to be reduced significantly in retina, choroid and sclera in correlation with axial elongation of myopia (Honda *et al.*, 1996). It is also one of the target genes related to TGF- $\beta$ -induced

factor gene (*TGIF*), a useful marker for high myopia (Lam *et al.*, 2003). The activation of *TGFB1* expression in ocular tissues mediated by transcription factor *EGR1* (early growth response gene type 1) is thought to influence ocular elongation (Liu *et al.*, 1996).

*TGFB1* is the first myopia susceptibility gene successfully replicated (Zha *et al.*, 2009). *TGF-β1* is encoded by the *TGFB1* gene mapped to chromosome 19q13 (NCBI Entrez Gene 7040 and OMIM 190180), comprising of 7 exons encompassing 23.5 kb of DNA (Patel *et al.*, 2005). There are three association studies from different ethnic groups on single-nucleotide polymorphisms (SNPs) of the *TGFB1* gene and high myopia (Lin *et al.*, 2006; Hayashi *et al.*, 2007; Zha *et al.*, 2009). One study excluded its association but the other two studies reported significant association of the coding SNP rs1800470 with high myopia and suggested *TGFB1* as a myopia susceptibility gene. The codon 10 polymorphism, corresponding to SNP rs1800470 at exon1 (T869C) of the *TGFB1* gene constitutes missense mutation (leucine to proline) in the region encoding signal peptide sequence (Cambien *et al.*, 1996). Our present study is an attempt to understand its association with myopia in South Indian population.

## Materials and Methods

### Source and study design

The present case-control study was conducted during the period of 2005 to 2008 after the approval by Institutional Ethical Committee. A total of 206 high myopia cases (>6 D) and around 98 low myopia (<6 D) cases (to serve as positive control group) were recruited from Maxivision Eye Hospital, Hyderabad after explaining to them the purpose of the study and obtaining their informed consent. All the patients were clinically examined by concerned ophthalmologists. Only patients who had myopia in both the eyes were included. Patients with any known ocular disease, or a history of retinopathy or connective tissue disorders associated with myopia, such as Stickler or Marfan syndromes were not included. Around 250 randomly selected age and sex matched normal healthy individuals without history of myopia and other ocular diseases were used as controls to compare with patient group. Detailed information on clinical, epidemiological and ophthalmic variables was recorded for every patient using a specified proforma. Genomic DNA from the blood samples collected from each in EDTA vacutainers was isolated by

using non-enzymatic salting-out method and used for polymorphic analysis using PCR-RFLP technique (Lahiri and Nurnberg, 1991).

### Genotyping

The amplification of *TGFβ1* exon 1 region (codon 10) was done through PCR (polymerase chain reaction) using gene specific primers (Wu *et al.*, 2004). The forward primer sequence was 5'-ACCACACCAGCCCTGTTCGC-3' and the reverse primer sequence was 5'-AGTAGCCACAGCAGCGGTAGCAGCTGC-3'. PCR was performed with an initial denaturation at 94°C for 3 minutes, followed by 33 cycles of denaturation at 94°C for 50 seconds, annealing at 66°C for 1 minute, elongation at 72°C for 1 minute and by final elongation of 10 minutes at 72°C. The PCR products (size 123 bp) were checked for amplification using 2.5% agarose gel containing ethidium bromide. For RFLP analysis, 5 ml of the PCR product was then digested with 5U of *PstI* restriction endonuclease (New England Biolabs) enzyme at 37°C for 1 hour. Restriction enzyme digested PCR products were subjected to electrophoresis in a 3% agarose gel at 100V for 1 hour. The genotypes were recorded as Leu/Leu (TT), Leu/Pro (TC) and Pro/Pro (CC) based on the band pattern observed (figure-1).

### Statistical analysis

All the statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) 15.0. Allele and genotype frequencies were calculated and Chi square test was done to test the significance of genotype distribution with myopia development. All the P values were two-sided and the level of significance was taken as  $P < 0.05$ .

## Results

The present study recruited 207 high myopia and 96 low myopia cases. The mean age at onset among high myopia cases (14 to 80 years of age) was found to be lower ( $15.1 \pm 0.672$  years) as compared to low myopia cases (19-80 years) who had mean age at onset of  $19.75 \pm 10.78$  years. The genotype distribution of codon 10 polymorphism of *TGFB1* gene in high myopia patients did not reveal significant variation, but there was a slight elevation of heterozygote TC frequency (21.25%) compared to control group (16.8%). However, low myopia cases showed elevated CC genotype frequency (14.6%) as compared to controls (8.0%). When the comparison was made between high and low myopia cases, the frequency of CC

genotype was found to be decreased among high myopia cases (8.7% vs 14.6% low myopia). The  $\chi^2$  values for the distribution were insignificant for high (1.673), low (4.288) and pooled cases (2.844). All the three groups, high myopia, low myopia and control group deviated from Hardy-Weinberg equilibrium ( $\chi^2=20.73$  for high myopia,  $\chi^2=20.97$  for low

myopia and  $\chi^2=42.97$  for control group). The odds ratios were insignificant for all the 3 genotype comparisons and could not indicate any genotype specific risk. The allelic distribution revealed reduction in the C allele frequency among high myopia cases (0.193) as compared to low myopia (0.244) (Table 1).

Figure 1. TGFB1 gene codon 10 (T869C) polymorphism

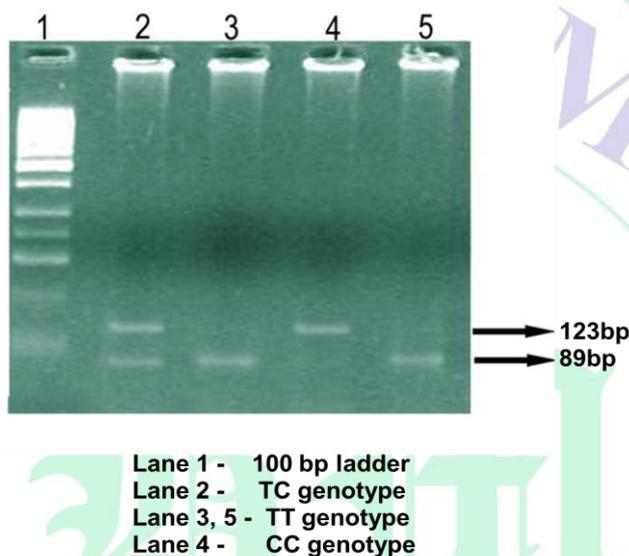


Table 1: Genotype distribution of TGFB1 gene codon 10 polymorphism.

Type of Myopia	Genotype Frequency						Allele Frequency	
	TT		TC		CC		T	C
	N	%	N	%	N	%		
High myopia (207)	145	70	44	21.25	18	8.7	0.806	0.193
Low myopia (96)	63	65.6	19	19.8	14	14.6	0.755	0.244
Total (303)	209	68.64	63	20.8	32	10.56	0.79	0.21
Controls (250)	188	75.2	42	16.8	20	8.0	0.836	0.164
High myopia vs. Controls - $\chi^2$ - 1.673; df-2, p-0.433 Low myopia vs. Controls - $\chi^2$ - 4.288, df-2, p-0.117 Total cases vs. Controls - $\chi^2$ - 2.844, df-2, p-0.241 Odds ratios for High and Low myopia TT vs. TC: 0.736, CI (0.457 to 1.183) and 0.740 CI (0.401 to 1.366) TC vs. CC: 1.164, CI (0.542 to 2.499) and 0.646 CI (0.270 to 1.545) TT vs. CC: 0.857, CI (0.437 to 1.679) and 0.478 CI (0.228 to 1.003)								

The genotype and gene frequency distribution with respect to sex of the proband among high myopia cases did not reveal much variation but low myopia cases showed elevated CC genotype frequency in male probands (21.4%) as compared to female probands (9.3%). The CC genotype frequency

was reduced in high myopia males as compared to low myopia. The C allele frequency was found to be highest in low myopia males (0.29). These observations indicated that C allele might show sex specific risk to develop myopia in males specially low myopia. TGF  $\beta$ 1 polymorphism did not indicate

any association with age at onset in high myopia whereas CC genotype frequency was slightly elevated in early onset cases of low myopia. The comparison of high and low myopia cases with early age at onset indicated significant association of CC genotype with

early onset low myopia. In high myopia, the CC genotype exhibited increasing trend with increase in age at onset whereas the CC genotype frequency declined with advance in age at onset in low myopia group (Table 2).

**Table 2: Genotype distribution with respect to sex of the proband and age at onset.**

Sex of the Proband		Genotype Frequency			Allele Frequency	
		TT N (%)	TC N (%)	CC N (%)	T	C
High Myopia	Male (105)	72(68.6)	24(22.9)	9(8.6)	0.8	0.2
	Female (102)	73(71.6)	20(19.6)	9(8.8)	0.81	0.18
Low Myopia	Male (42)	26(61.9)	7(16.7)	9(21.4)	0.702	0.297
	Female (54)	37(68.5)	12(22.2)	5(9.3)	0.796	0.157
HM: $\chi^2$ -0.327; df-2, (p-0.849); LM: $\chi^2$ -2.925; df-2, (p-0.232)						
Age at Onset						
High Myopia	<10 yrs (66)	49(74.2)	12(18.2)	5(7.6)	0.833	0.166
	10-20yrs (102)	69(67.6)	24(23.5)	9(8.8)	0.794	0.205
	>20 yrs (39)	27(69.2)	8(20.5)	4(10.3)	0.794	0.205
Low Myopia	<10 yrs (13)	9(69.2)	1(7.7)	3(23.1)	0.73	0.26
	10-20 yrs (41)	26(63.4)	8(19.5)	7(17.1)	0.731	0.268
	>20 yrs (42)	28(66.7)	10(23.8)	4(9.5)	0.785	0.214
HM: $\chi^2$ -1.011; df-4, (p-0.9081), Yates $\chi^2$ -0.519, p-0.971 LM: $\chi^2$ -2.924; df-4, (p-0.571), Yates $\chi^2$ -1.311, p-0.859						

High myopia cases could not reveal any association of genotypes with diet but the frequency of C allele carriers (TC and CC) was found to be slightly increased in vegetarian group (38.9%) of low myopia as compared to non-vegetarian group (31.0%). The frequency of C allele was found to be increased in familial cases of high (0.216) and low myopia (0.284) as compared to non-familial cases (0.146 in high myopia and 0.184 in low myopia). Corresponding increase in frequency of CC genotype in familial cases of high myopia and low myopia was also found. The genotype distribution among high and low myopia cases did not show much variation with respect to parental consanguinity but the frequency of C allele carriers was increased in consanguineous cases (41.6%) of low myopia as compared to non-consanguineous cases (33.4%) (Table 3).

Genotype distribution was also studied with respect to contributing ophthalmic variables. The mean RE was found to be increased in both high and low myopia cases with CC genotype. The mean ACD was found to be decreased in high myopia cases but increased in low myopia cases with CC genotype indicates that C allele might contribute to shallower anterior chamber depth during high myopia development. The mean Corneal Thickness showed decline only in low

myopia cases and mean Axial Length did not show much variation in both the myopia groups. However, significant genotype association was not observed with respect to these ophthalmic variables.

### Discussion

The development of high myopia is mainly related to excessive axial elongation of the eye associated with altered scleral-fibril architecture. Excessive axial elongation of the eye facilitated by active scleral remodeling is considered to be one of the most important etiologies in the progression of myopia. Transforming growth factor- $\beta$  is one of the potential candidate genes involved in scleral remodeling that modulates extra cellular matrix (ECM) composition, thereby influencing ocular size axial elongation.

The codon 10 polymorphism at exon1 (T869C) of the TGFB1 gene under study was found to be associated with chronic pancreatitis (Bendicho *et al.*, 2007), myocardial infarction and stroke, renal failure, blood pressure (Rivera, 2001), bone mineral density (Zhou *et al.*, 2000), breast cancer (Ziv *et al.*, 2001), heart failure due to cardiomyopathy (Holweg *et al.*, 2001) and asthma (Holgate *et al.*, 2001) indicating its functional significance in pathogenesis. This gene polymorphism also contributed to the

genetic predisposition to nephropathy in Type 1 diabetes (Patel *et al.*, 2005). This T/C polymorphism results in missense mutation

(leucine to proline) in the signal peptide domain and is known to influence the synthesis of TGFB1 gene.

**Table 3: Genotype distribution with respect to diet, familial incidence and parental consanguinity.**

Diet		Genotype Frequency			Allele Frequency	
		TT N (%)	TC N (%)	CC N (%)	T	C
High Myopia	Vegetarian(62)	42(67.7)	14(22.6)	6(9.7)	0.79	0.209
	Non-Vegetarian(143)	101(70.6)	30(21.0)	12(8.4)	0.811	0.188
Low Myopia	Vegetarian(36)	22(61.1)	9(25)	5(13.9)	0.736	0.263
	Non-Vegetarian(58)	40(69)	9(15.5)	9(15.5)	0.767	0.233
HM: $\chi^2$ -0.185; df-2, (p-0.9116) LM: $\chi^2$ -1.29; df-2, (p-0.524)						
<b>Familial Incidence</b>						
High Myopia	Familial (141)	95(67.4)	31(22)	15(10.6)	0.783	0.216
	Non-Familial (65)	49(75.4)	13(20)	3(4.6)	0.85	0.146
Low Myopia	Familial (58)	35(60.3)	13(22.4)	10(17.2)	0.715	0.284
	Non-Familial (38)	28(73.7)	6(15.8)	4(10.5)	0.815	0.184
HM: $\chi^2$ -2.337; df-2, (p-0.3108), Yates $\chi^2$ -1.539, p-0.463 LM: $\chi^2$ -1.841; df-2, (p-0.398), Yates $\chi^2$ -0.989, p-0.609						
<b>Parental Consanguinity</b>						
High Myopia	Consanguineous (30)	21(70)	7(23.3)	2(6.7)	0.816	0.183
	Non-Consanguineous (176)	123(69.9)	37(21)	16(9.1)	0.803	0.196
Low Myopia	Consanguineous (12)	7(58.3)	4(33.3)	1(8.3)	0.75	0.25
	Non-Consanguineous (84)	56(66.7)	15(17.9)	13(15.5)	0.755	0.244
HM: $\chi^2$ -0.237; df-2, (p-0.888), Yates $\chi^2$ -0.021, p-0.989 LM: $\chi^2$ -1.749; df-2, (p-0.417), Yates $\chi^2$ -0.67, p-0.715						

Three studies investigated the genetic association between single-nucleotide polymorphisms (SNPs) of the *TGFB1* gene and high myopia. A study from Taiwanese Chinese population on *TGF-β1* codon 10 polymorphism (rs1800470) revealed strong association of CC genotype with high myopia (Lin *et al.*, 2006). Later, study by Hayashi *et al.* (2007) on 10 SNPs of the gene revealed no significant association with high myopia and excluded transforming growth factor-beta1 as a candidate gene for myopia in the Japanese. A recent study on 8 SNPs (including rs1800470) in Chinese subjects of Hong Kong revealed the association of 4 SNPs in the 5' half of the *TGFB1* locus with high myopia. This study could successfully replicate the positive finding of Lin *et al.* (2006), supporting the association of *TGFB1* gene with myopia susceptibility (Zha *et al.*, 2009).

In the present study, the genotype distribution of codon 10 polymorphism revealed reduction in the C (proline) allele frequency among high myopia cases as

compared to that of low myopia cases and control group. These findings were in contrast to the earlier study by Lin *et al.* (2006), which reported higher C allele frequency (0.566). Association signals for common complex traits such as myopia appear to be different in different ethnic groups. Our observations also suggest that individuals with C allele may carry sex specific risk and possibly increasing risk for progression in cases with lower age at onset. Elevated C allele frequency in cases with familial incidence also indicates the role of this genetic component in myopia susceptibility. We failed to find any significant genotypic association with the ophthalmic variables that contribute to refraction. Overall, the results suggested that CC genotype might confer risk to develop low myopia in male probands, lower age at onset cases (<10 yrs) and cases on vegetarian diet since these cases are more likely to progress to high myopia. The presence of proline rather than leucine in the hydrophobic region of the signal sequence is thought to alter export of newly

synthesized proteins across membranes of endoplasmic reticulum (Crilly *et al.*, 2002). Proline, due to its cyclic structure alters the  $\alpha$  helical portions of the signal peptide backbone, whereas leucine owing to its aliphatic side chain favours the formation of  $\alpha$  helices. This structural alteration due to nucleotide substitution is thought to affect the protein export through endoplasmic reticulum (Crilly *et al.*, 2002). Signal transduction of the TGF $\beta$ 1 pathway could be thus affected in the eye. However, TGF $\beta$ 1 signaling and its role in ocular refraction is yet to be understood.

Environmental factors can also trigger myopia progression in individuals with genotype specific risk. Hence, there is a need to focus on gene-environment interactions in myopia development. Identification of potential causative variants in the candidate genes including TGF $\beta$ 1 gene are yet to be discovered. Studies to understand the role of TGF $\beta$ 1 in control of axial elongation can greatly help the prophylaxis and treatment of myopia.

### Conclusion

The case control association study of TGFB1 codon 10 polymorphism could not reveal strong association with high myopia in South Indian patients but suggested that CC genotype might confer risk to develop low myopia in male probands, lower age onset cases and cases on vegetarian diet since these cases are more likely to progress to high myopia. The study suggests the role of TGFB1 in low myopia. Further, large scale population based association studies and functional analysis can help to understand its role in the etiopathogenesis of myopia.

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