

# Intronic variants of TGIF1 (variant-008) have a potential to associate with high myopia in ethnic kashmiri population

**KHURSHID I. ANDRABI<sup>1</sup>, S. RASOOL<sup>2</sup> AND T. JAN<sup>2</sup>**

<sup>1</sup>Department of Biotechnology, Science Block, University Of Kashmir, HAZRATBAL SRINAGAR (J & K) INDIA

<sup>2</sup>Department of Statistics, University of Kashmir, HAZRATBAL SRINAGAR (J & K) INDIA

Email : andrabik@uok.edu.in; andrabik@kashmiruniversity.ac.in

**Key words :** Myopia, Ethnicity, Polymorphism, CSGE, TGIF1

**How to cite this paper :** Andrabi, Khurshid I., Rasool, S. and Jan, T. (2012). Intronic variants of TGIF1 (variant-008) have a potential to associate with high myopia in ethnic kashmiri population. *Asian J. Bio. Sci.*, 7 (2) : 241-243.

Myopia is the most common eye disorder which remains a significant ocular health problem associated with increased risk of visual loss around the world (Fredrick, 2002). High myopia considered as more advanced type of myopia may lead to degenerative changes in the eye (degenerative myopia) leading to blindness and often afflicts, people earlier in life when they may still be active professionally (Jacobia *et al.*, 2005). The wide spectrum of myopia-associated disorders strongly argues for an etiologically heterogeneous nature of myopic refractive errors, where multiple factors with genetic and epigenetic effects contribute at different stages during development (Feldkammer and Schaeffel, 2003). The concept that environmental factors influence ocular development has been well established in epidemiological and experimental animal studies (Saw *et al.*, 2002). Despite recognized importance of visual experience in the development of myopia there is abundant evidence for genetic factors determining refractive development (Francois, 1961). First, higher myopia prevalence in developed Asian countries compared to the western world suggests a genetic susceptibility to myopia development. Further, myopic parents are more likely to give rise to offsprings with myopia than non-myopic parents (Goldschmidt, 1981). Linkage studies have mapped at least eight loci (MYP1, MYP2, MYP3, MYP4, MYP5, MYP11, MYP12 and MYP13) responsible for high myopia with Mendelian inheritance (Young, 2004). TGIF1 is expressed in sclera, retina, cornea, and optic nerve and competitively inhibits binding of the retinoic acid receptor to a retinoid-responsive promoter (Young *et al.*, 1998a).

It is possible that mutations in TGIF1 gene may alter its

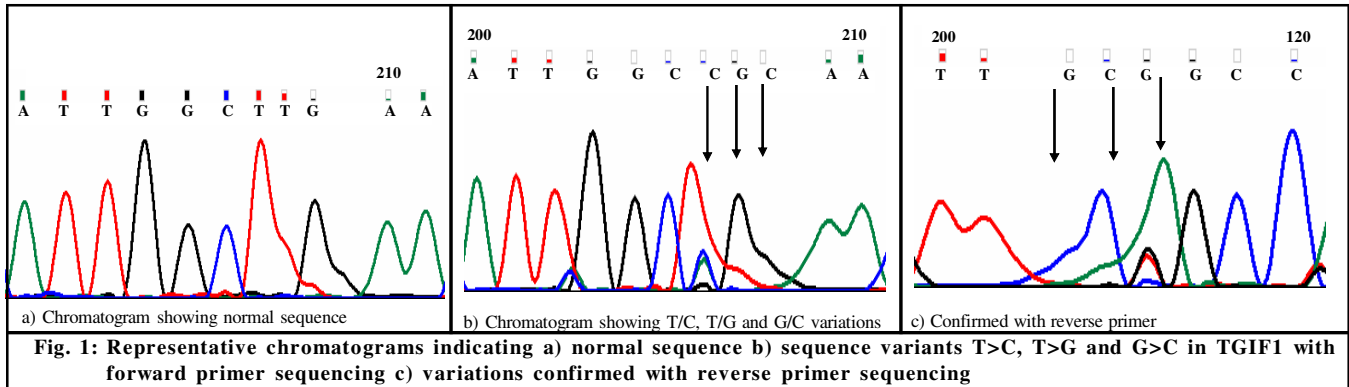
function and hence the phase of eye development, thus making it a potential candidate gene to study high myopia. One study has disregarded TGIF1 as potential contributor to the disease (Scavello *et al.*, 2004) although it has been associated with high myopia wherein six single nucleotide polymorphisms (SNPs) appear to associate with the disease in a Chinese cohort (Lam *et al.*, 2003). However, association could not be replicated in a second Chinese case control study of high myopia individuals (Li *et al.*, 2003). Another study with Japanese subjects failed to identify association of this gene with high myopia (Hasumi *et al.*, 2006). This discrepancy has largely been attributed to ethnic variations in the genetics of this disease.

Kashmiri population being a pure ethnic group provides an ideal scenario to substantiate the contribution of TGIF1 (if any) in the development of high myopia. 52 high myopic and 18 normal controls of Kashmiri ethnicity were recruited for TGIF1 polymorphism studies. DNA was isolated from venous blood samples and amplified by polymerase chain reaction. PCR products were purified and screened for mutations by heteroduplex assay employing CSGE (conformation sensitive gel electrophoresis). Samples showing differential mobility on CSGE were sent out for commercial sequencing. Sequences were analysed using different softwares like Chromas pro and Cluatal X2. After analyzing the data, we observed three novel and adjacent intronic SNPs with potential to have a bearing on the etiology of the disease.

Mutational screening of TGIF1 (variant-008, Transcript ID-ENST00000552383) in 52 high myopia affected and 18

**Table 1: Polymorphism found in intronic region of TGIF1 in ethnic high myopia affected Kashmiri population**

Sr. No.	Wild nucleotide	SNP	SNP type	Codon position	Location
1.	T	T/C or A	Novel	Intronic	Chr.18-3418281
2.	T	T/G	Novel	Intronic	Chr.18-3418281
3.	G	G/C	Novel	Intronic	Chr.18-3418281



normal controls from Kashmir revealed a total of three novel and adjacent sequence variations; T>C/A, T>G and G>C (Table 1) in the intronic region immediately after exon 2 boundary of this variant. All the three variations found in heterozygous state (Fig. 1). First variation T>C/A (T changed to either C or A depicted by dual peaks) was observed at a frequency of 4/18 in normal controls and 26/52 affected samples. Second variation T>G was also present in both control and affected samples at a frequency of 8/18 in controls and 38/52 in affected samples. Sequence variation G>C was not observed in normal controls but was found in affected samples only at a frequency of 14/52. All the three adjacent variations were found to be present together in 10 samples, which were all affected.

The relative frequency of occurrence of variants for SNP T>C/A ( $p$  allele = 0.08,  $p$  genotype = 0.04;  $\chi^2$  allele = 3.06,  $\chi^2$  genotype = 4.07; OR = 2.66; CI (95%) = 0.86-8.23) shows statistical significance of genotype (TC). Likewise the frequency of occurrence for T>G variant ( $p$  allele = 0.11,  $p$  genotype = 0.02;  $\chi^2$  allele = 2.48,  $\chi^2$  genotype = 4.80; OR = 0.49; CI (95%) = 0.20-1.19) also shows significance of genotype (TG) and for G>C variant only  $p$  value could be calculated ( $p$

allele = 0.02;  $p$  genotype = 0.01) which is statistically significant (Chisquare is calculated only if all the expected cell frequencies are equal to or greater than 5).

TGIF has been implicated to be the candidate gene for high myopia by Single Nucleotide Polymorphism (SNP) studies. We examined the hypothesis that polymorphisms within TGIF1 may influence the susceptibility of ethnic Kashmiri subjects to high myopia. The polymorphisms in TGIF1 studied here reveal significant association with an increased risk of having high myopia in Kashmiri patients when compared with control group. These results suggest that there exists high complexity of genetic background for our high myopic population and TGIF has a considerable effect on myopia onset and severity. Future work is needed to investigate other variants of TGIF and the recently reported candidate genes like TGF $\beta$ 1 and HGF.

### Conclusion:

Although  $p$  values for these intronic variations come out to be significant sample size needs to be increased further to establish the potential of these variants in the etiology of high myopia.

## LITERATURE CITED

- Feldkammer, M. and Schaeffel, F. (2003). Interactions of genes and environment in myopia. *Dev. Ophthalmol.*, **37**: 34-49.
- Francois J. (1961). *Heredity in ophthalmology*, Mosby, St. Louis.
- Fredrick, D.R. and Myopia, B.M.J. (2002). **324**: 1195-99.
- Goldschmidt, E. (1981). The importance of heredity and environment in the aetiology of low myopia. *Acta Ophthalmol.*, **59**: 759-762.
- Hasumi, Y., Inoko, H. and Mano, S. (2006). Analysis of single nucleotide polymorphisms at 13 loci within the transforming growth factor-induced factor gene shows no association with high myopia in Japanese subjects. *Immunogenetics*, **58**(12): 947-953.

- Jacobia, F.K., Zrenner, E., Broghammer, M. and Pusch, C.M. (2005).** A genetic perspective on myopia. *Cell Mol. Life Sci.*, **62**: 800-808.
- Lam, D.S.C., Lee, W.S. and Leung, Y.F. (2003).** TGF  $\beta$ - induced factor: A candidate gene for high myopia. *Invest. Ophthalmol. Vis. Sci.*, **44**:1012–1015.
- Li, J., Zhang, Q.J. and Xiao, X.S. (2003).** The SNPs analysis of encoding sequence of interacting factor gene in Chinese population in Chinese. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.*, **20**(5): 454–456.
- Saw, S.M., Chua, W.H., Hong, C.Y., Wu, H.M., Chan, W.Y., Chia, K.S., Stone, R.A. and Tan, D. (2002).** Nearwork in early-onset myopia. *Invest Ophthalmol Vis Sci.*, **43**: 332–339.
- Scavello, G.S., Paluru, P.C. and Ganter, W.R. (2004).** Sequence variants in the transforming growth factor  $\beta$ -induced factor (TGIF) gene are not associated with high myopia. *Invest. Ophthalmol. Vis. Sci.*, **45**: 2091-2097.
- Young, T.L., Ronan, S.M., Drahozal, L.A., Wildenberg, S.C., Alvear, A.B., Oetting, W.S., Atwood, L.D., Wilkin, D.J. and King, R.A. (1998).** Evidence that a locus for familial high myopia maps to chromosome 18 pp. *American J. Hum. Genet.*, **63**: 109-119.
- Young, T.L. (2004).** Dissecting the Genetics of human high myopia: a molecular biologic approach. *Trans. American Ophthalmol. Soc.*, **102**: 423-446.

