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# A novel G26A variation in 5' half of TGIF1 gene associates with high myopia in ethnic Kashmiri population from India

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## Abstract:

This study aims to look at novel variations in TGIF1 gene and explores their potential association with high myopia in an ethnic population from Kashmir (India). Genomic DNA was genotyped for polymorphic variations, and allele frequencies were tested for the Hardy–Weinberg disequilibrium in 240 ethnic Kashmiri cases with high myopia with a spherical equivalent of  $>-6$  diopters (D) and compared with emmetropic controls with spherical equivalent within  $-0.5$ D in one or both eyes represented by a sample size of 228. In this study, we found a novel sequence variation G26A (GAT to AAT) in 5' half of TGIF1 gene (p. aspartic acid  $\rightarrow$  asparagine) at a frequency of 62% (148/240,  $P \leq 0.0001$ ). Variation appears to associate with high myopia significantly ( $P \leq 0.001$ ) as it happens to be present only in high myopia affected individuals. Further, it shows statistical significance for its association with gender and the degree of myopia ( $P \leq 0.05$ ). In addition, *in silico* predictions show that variation likely has an impact on the structure and functional properties of the protein. The assessment of the I-TASSER protein structure showed higher energy for a wild-type protein ( $-5820.186$  kJ/mol) as compared to mutant protein ( $-6595.593$  kJ/mol).

## Keywords:

Ethnic, gene, myopia, novel, TGIF1, variation

## Introduction

Myopia, known as nearsightedness, is the most common cause of visual impairment worldwide. It is a condition of the eye where the light that comes in does not directly focus on the retina but in front of it. It is said to be a consequence of mismatch between the power of optical components and the axial length of the eye.<sup>[1]</sup> This common ocular disorder was earlier thought as a benign refractive error. Nowadays even at low levels, it is associated with high risk for a number of ocular diseases.<sup>[2]</sup> The high and severe grades of myopia worsen to the stage of "high or pathological myopia." This condition

is often associated with complications, leading to reduced vision or blindness.<sup>[3]</sup> The ocular refractive components precisely undergo coordinated physical alterations during ocular growth, to attain and maintain normal emmetropic visual acuity, and hence that image focuses directly on the retinal plane.<sup>[4]</sup> Any discordance between the axial length and other optical refractive components, such as corneal and lenticular curvatures, would result in ametropia and blurred visual acuity.<sup>[5]</sup>

Myopia is being reported as an epidemic occurring worldwide.<sup>[6]</sup> Both genetic and environmental components have been associated with the etiology of this potentially blinding ocular condition, as

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such, making prevention and treatment challenging.<sup>[7]</sup> Furthermore, studies conducted on humans and animals over the past four decades also provide strong evidence for the involvement of both environmental and genetic factors in the progression of myopia.<sup>[8-12]</sup>

Ethnic diversity plays a great role in the development of myopia reaching as high as 70%–90% in some parts of Asia, 30%–40% in Americans and Europeans, and up to 20% in Africans.<sup>[13]</sup> It is a highly prevalent and complex disorder involving both genetic and environmental factors or it may be interplay of both genetic and environmental factors.<sup>[14]</sup> Recent mapping studies have mapped 14 genomic loci to be associated with myopia. MYP2 is a candidate locus of the nonsyndromic autosomal dominant high myopia first identified by Young *et al.*<sup>[15]</sup> There are nine known and six hypothetical genes considered to be candidates based on mapped position within the MYP2 interval. Among other genes present in the MYP2 locus is transforming growth factor beta induced factor 1 (TGIF1).<sup>[16]</sup> TGIF1 is expressed in the sclera, retina, cornea, and optic nerve and competitively inhibits binding of the retinoic acid receptor to a retinoid-responsive promoter.<sup>[15]</sup> 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.

### Experimental procedure

Two hundred and forty Kashmiri adult participants (from India, above 20 years) with high myopia and ethnically matched 228 healthy adult controls (above 20 years) were enrolled for the study from Government Medical College Hospital (Ophthalmology unit) as well as from our ophthalmologist's clinic. This study was performed with informed consent and following all the guidelines for experimental investigations required by the Institutional Board of Research Studies (BORS), of which all authors are affiliated (No: BORS-BT-20109A-2010). Participants were encouraged to narrate all the details relevant to this study such as age, history of onset of myopia, information regarding close work, and any associated ocular complication. Individuals were excluded if there was a known ocular disease such as retinopathy, cataract or if they had a known genetic disease associated with myopia such as Stickler or Marfan syndrome. The ophthalmic evaluation was done which included measuring visual acuity, keratometry, retinoscopy, slit lamp examination of the anterior segment, fundus examination, and measurement of the axial length.

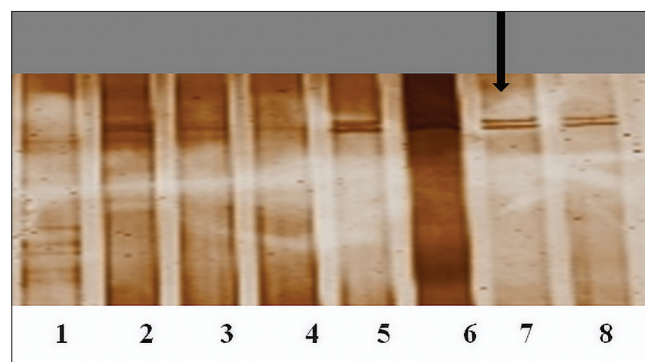
### Methodology

Genomic DNA was prepared from peripheral blood and subjected to amplification by polymerase chain reaction using following primer sequences:

5'-GGGAATAAGTGAGGGGCTCT-3' (sense) and 5'-CCTGAACCAGTCGCAAAGTT-3' (antisense) generating a 472 bp fragment. Purified PCR products were subjected to denaturation and renaturation procedures for the generation of potential heteroduplexes [Figure 1] and analyzed using conformation-sensitive gel electrophoresis strictly as described by Ganguly *et al.*<sup>[17]</sup> Samples that showed unusual mobility during these assays were finally sequenced to confirm the presence of sequence variations along with controls (Scigenom, Cochlin). Sequence results obtained in fasta and pdf formats were analyzed using ClustalX version 2 software<sup>[18]</sup> and by Chromas Pro version 1.49 beta 2 software for the detailed inspection of individual chromatograms allele frequencies were tested for the Hardy–Weinberg disequilibrium. The genotype and allele frequencies were evaluated using the Chi-square test or Fisher's exact test. Odds ratio and confidence interval were also calculated. The amino acid sequence of the protein in fasta format obtained from (NCBI) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) was submitted to an automated server (I-TASSER) ([zhang.bioinformatics.ku.edu/I-TASSER](http://zhang.bioinformatics.ku.edu/I-TASSER)) for three-dimensional (3D) structure prediction.<sup>[19]</sup> The server furnishes predicted 3D structure in pdb format. Swiss-PdbViewer was used for viewing pdb files and computing the free energy of the predicted 3D structures.<sup>[20]</sup> Swiss-PdbViewer includes a version of the GROMOS43B1 force field to allow the energy evaluation of a protein structure (macromolecular) as well as repair the distorted geometry through energy minimization. In this implementation, all computations are done in vacuo without reaction field.<sup>[21]</sup>

### Results

In this study, finally, DNA sequencing was used to confirm the results of heteroduplex assays. In addition to previously reported variations that we have already published the data claiming the association of gene with the disease,<sup>[22]</sup> a novel missense sequence variation G > A [Figure 2] at codons 26 was identified in 5' half



**Figure 1:** Representative conformation-sensitive gel electrophoresis gel showing heteroduplex bands (as indicated by arrow) in all lanes suggestive of the presence of variation

of TGIF1 gene (variant-003, ensemble). An interesting finding of this study was novelty of the variation. The sequence variation G26A (p. aspartic acid > asparagine) which has not been reported till date was present at a frequency of 62% (148/240).

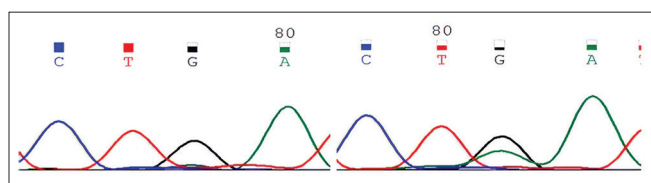
A subtle and statistically significant [ $P \leq 0.001$ ; Table 1] difference in the allelic frequency for this sequence variation was indicative of its possible association with high myopia. Furthermore, it could be associated with gender and the degree of myopia [ $P = 0.01$  and  $P \leq 0.0001$ , Table 2], with the frequency of GA genotype significantly higher in females with degree of myopia  $> -6$  D.

In silico prediction results show that calculated energy for the wild-type protein is more (-5820.186 kJ/mol) as compared to the mutant protein (-6595.593 kJ/mol). This change in energy of mutant protein is suggestive of affecting the protein tertiary structure which may, in turn, have some impact on protein function. Therefore, further studies are needed to elucidate the actual role of this mutation on protein structure and function.

## Discussion

Diverse populations have presented inconsistent profile of association data owing largely to heterogeneous nature of the subject populations. Genetic polymorphisms have widely been in use to test the association of a gene with a commonly seen and multifactorial disease instead of single gene disease. Since ethnic differences do exist, it is imperative to substantiate or dispute the relevance of such polymorphism in genetically purer cohorts.<sup>[13]</sup>

Scavello *et al.*<sup>[16]</sup> reported negative association of TGIF1 gene with high myopia, although six polymorphic variations were reported to be associated with high myopia in a Chinese population.<sup>[23]</sup> However, a second



**Figure 2:** Representative partial chromatogram of (a) normal sample (b) affected sample showing G > A variation at codon 26 in TGIF1 as indicated by arrow

Chinese study excludes the association of these variations with high myopia.<sup>[24]</sup> A Japanese study group also failed to associate TGIF1 gene with high myopia.<sup>[25]</sup> Here, it is evident that discrepancy in the association of TGIF1 gene with high myopia might be due to population heterogeneity. In light of these discrepancies, our results can be of immense value as they substantiate the claims of association of TGIF1 gene with high myopia.

Investigating the genetics of common and complex disorders such as myopia remains one of the great challenges in human genetics. Myopia is considered to be a complex and multigenic condition involving several overlapping signaling pathways, each one mediated by a group of distinct genetic profiles. Therefore, studying the genetic polymorphisms of myopia-related genes can further clarify the relationship between genetics and myopia. The association between myopia and various genetic markers has helped increase our knowledge of prevention and treatment of myopia.<sup>[26]</sup>

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. Two hundred and forty high myopic and 228 normal controls of Kashmiri ethnicity were recruited for TGIF1 polymorphic studies. Two genotypes GG and GA for codon 26 sequence variations occurred at a frequency of 100%: 0.00% in the control group versus 38%:74% in the high myopia group. A subtle and statistically significant [ $P \leq 0.001$ ; Table 1] difference in the allelic frequency was indicative of its possible association with high myopia. Furthermore, variation was significantly associated with gender and the degree of myopia [ $P = 0.01$  and  $P \leq 0.0001$ , Table 2] with the frequency of GA genotype significantly higher in females with degree of myopia  $> -6$  D. The calculated energy for the wild-type protein is more (-5820.186 kJ/mol) as compared to the mutant protein (-6595.593 kJ/mol). This change in energy of mutant protein is suggestive of affecting the protein tertiary structure which may, in turn, have some impact on protein function. Therefore, further studies are needed to elucidate the actual role of this mutation on protein structure and function.

**Table 1: Genotype and allele frequencies of transforming growth factor beta-induced factor 1 gene variation in cases and controls**

Variation	Genotype	Cases (n=240), n (%)	Controls (n=228), n (%)	P	$\chi^2$
429 G>A	GG	92 (38)	228 (100)	<0.001	83.5*
	GA	148 (62)	0		
	AA	0	0		
	G	332 (69)	456 (100)		
	A	148 (31)	0		

\*Pearson's  $\chi^2$

**Table 2: Association of transforming growth factor beta-induced factor 1 gene variations with clinical variables in high myopia affected patients**

Parameters	TGIF1 26 G >A		P ( $\chi^2$ )	OR (95% CI)
	GG	GA + AA		
Age (years)				
≤30	170	78	1.0	0.9832 (0.5661-1.7075)
>30	150	70		
Sex				
Male	188	60	<0.01	0.4787 (0.2732-0.8387)
Female	132	88	(6.74)	
Degree of myopia				
<-6	228	0	<0.0001	NA
-6--12	44	76	(102)	
>-12	48	72		

OR=Odds ratio, CI=Confidence interval, TGIF1=Transforming growth factor beta-induced factor 1, NA=Not available

In addition, the sequence variation is present in coding sequence of the gene affecting the physicochemical properties of the protein causing change from polar negatively charged aspartic acid to polar and neutral amino acid asparagine.

## Conclusion

Focused investigation is needed to establish the precise role played by TGIF1 in the high myopia development, especially in the context of the above-observed results. Therefore, further studies are needed to rule out the actual role of this novel variation on the protein structure and function.

## Ethical approval

This study was approved by Board of Research Studies, Departments of Biotechnology, University of Kashmir. Approval No. BORS-BT-20109A-2010.

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Nil.

## Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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