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ASSOCIATION OF *TGFB1* rs1800470 AND *TGIF* rs2229333 VARIANTS WITH MYOPIA IN MALAYSIAN ADOLESCENTS

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Abstract

Myopia is a global disease with high prevalence in school children. This study aimed to investigate the prevalence of myopia and its association with variants *TGFB1* rs1800470 and *TGIF* rs2229333 among Malaysia adolescents. A total of 565 Malaysian adolescents (206 Chinese, 220 Malay, and 139 Indian) were recruited and refractive error measurements were used for grouping subjects to normal or myopes (≤-0.50D). Concurrently, genomic DNA from buccal cells was genotyped using conventional PCR-RFLP. Allelic and genotype frequency in association with myopia prevalence were statistically analysed. High myopia prevalence (83.0%) was detected in the study population, with females (85.8%) being more myopic than males (78.8%). Highest prevalence of myopia was observed in Chinese (87.4%) followed by Malay (82.3%) and Indian (77.7%) (p<0.001) adolescents. In *TGFB1*, T allele was the minor allele in the Chinese and Malays but not Indians, whereas in *TGIF*, T was the minor allele in all ethnic groups. Interestingly, myopes compared to normal showed higher frequency for CT or combined (CT+CC) genotype for *TGFB1*, but only Malay males showed significantly higher CT (p=0.033, relative risk=1.23) and (CT+CC) (p=0.009, OR (95% CI) = 4.23) respectively. Despite higher frequency of combined (CT+TT) genotype for T risk allele of *TGIF* observed for myopes, no significant association with myopia was detected. The genotype and allele frequency of both variants differed based on sex and ethnicity. This is the first study demonstrating significant association of the *TGFB1* variant with myopic Malay male adolescents, and no association between *TGIF* variant and myopia in this study population.

INTRODUCTION

Myopia which affects almost 2.2 billion people worldwide, is showing an increasing trend with prevalence estimated to reach 49.8% of the world population by 2050 [1,2]. Myopia, also known as near-sightedness, is a refractive error due to light rays entering parallel to the optic axis of the eyes are focused ahead of retina during relaxation of ocular accommodation. Aetiology of myopia includes longer eyeball from anterior to posterior, over curved cornea or increased lens optical power [3]. Different levels of myopia include simple or mild myopia (-0.50D to -2.99D), moderate

myopia (-3.00D to -5.99D), and pathological or high myopia (≥-6.00D). High myopia is significantly associated with ocular pathologies such as glaucoma, cataract, myopic macular degeneration and retinal detachment and blindness [4].

Myopia prevalence in school children has increased over the decades [5-6], with higher myopia prevalence (60%) in Asia compared to the Western countries [7]. In Malaysia, the multi-ethnic population offers a relatively unique comparative study of ethnic and environmental influences on myopia development [8]. Higher myopia prevalence was observed among Chinese and Indians compared to Malays [9] schoolchildren in Malaysia. Interestingly, urban schoolchildren were more myopic than suburban children [10-11]. Some predisposing factors that could increase the risk for development of myopia include age, female sex [12], higher education level, longer near work duration, lesser outdoor time spent [13], and prolonged exposure time to electronic devices [5,14].

An interplay of genetic and environmental factors affecting myopia development and lifestyle can increase the risk of myopia in genetically predisposed individuals under homogenous environments [13]. Hereditary factors, such as children having paternal myopia have contributed to greater myopia risk among adolescents, compared to children with maternal myopia or with myopia in both parents [15].

Numerous polymorphisms in myopia candidate genes and myopia loci have been reported [16]. *TGFB1*, was the first identified myopia susceptibility candidate gene [17-18]. *TGFB1* increases expression and processing of cellular matrix protein receptors, regulates sclera chondrocytes and sclera fibroblasts affecting the sclera shape, such as axial eye length. The rs1800470 (also reported as rs1982073) variant in *TGFB1* involves a change of T to C nucleotide with the corresponding to leucine to proline change at position 10 amino acid of the signal sequence, resulting in a decrease in TGF-β1 secretion [19] and possibly a higher risk of myopia development. Lin et al. [17] reported the C risk allele predisposes to high myopia in the Taiwanese population, however Wang et al. [20] found no association in a Southern China Chinese population study ($N_{high\ myopia}$=288, $N_{control}$=208; p=0.365). Subsequent study by Rasool et al. [21] reported the association of C allele with high myopia in ethnic Kashmiri subjects in India ($N_{high\ myopia}$=247, $N_{control}$=176; p=0.001).

Transforming growth β-induced factor homeobox 1 (*TGIF1* or *TGIF*) is a transcription repressor for retinoid X receptor-dependent transcription factor and *TGF-β* [22]. It is located within 18p11.31, MYP2 region and was reported to show 6 altered sequences (including the rs2229333 variant) with significant differences in genotype frequency between myopic and control subjects in Hong Kong [22]. The variant rs2229333 at position 245 in exon 10, is a nonsynonymous missense mutation of C to T resulting in a change of proline to leucine. This leads to disruption of the coiled motif of *TGIF*, affecting its ability to regulate *TGF-β* signalling pathway [23-24]. Furthermore, *TGIF* repression of retinoic acid receptor will decrease, causing increased in retinoic acid (RA) level leading to higher risk for myopia. Ahmed et al. [23] reported that the T allele of rs2229333 was associated with increased risk for high myopia among the ethnic Kashmiri population ($N_{high\ myopia}$=212, $N_{control}$=239; p=0.015), despite contrary result was found in a Southern China Chinese population ($N_{high\ myopia}$=288, $N_{controls}$=208; p=1) [20].

Considering the ethnic variability in three major Asian populations of Malays, Chinese, and Indian, it will be interesting to study the genetic association of *TGFB1* and *TGIF* variants with myopia in these different ethnic groups in Malaysia. Therefore, we aimed to investigate the genotype of variants rs1800470 and rs2229333, and their association with myopia among 13 to 17 years old Malaysian school children from the Malay, Chinese, and Indian ethnic groups.

MATERIALS AND METHODS

Materials

Genomic DNA extraction was performed using the Wizard® SV Genomic DNA purification system from Promega (Madison, WIs, USA), chemicals for buffer preparation (Na_2HPO_4, $NaH_2PO_4.2H_2O$, NaCl) and DNA agarose were purchased from Sigma-Aldrich Inc., USA. The *Taq* DNA polymerase, deoxynucleotide triphosphates (dNTPs) and restriction enzymes *MspA1I* and *XmaI* were purchased from New England BioLabs (UK), forward and reverse primer pairs were synthesized by First Base Laboratories Sdn. Bhd. (Selangor, Malaysia). Veriti 96 well Thermal Cycler was purchased from Applied Biosystems (Foster City, CA, USA).

Subjects and Refractive Error Measurements

A total of 565 subjects consisting of male and female adolescents (aged 13 to 17) of self-declared Malay, Indian, and Chinese ethnicities were enrolled by convenience ascertainment from secondary schools in Klang Valley and Selangor Darul Ehsan (Malaysia). Informed consent from parent or guardian was obtained prior to recruitment. Ethnicity of all subjects was determined based on self-declaration and on the same parental ethnicity as inclusion criteria, whereas non-Malaysians, Malaysians who are not of the three major ethnicities and who had parents of differing ethnic origin from each other, and twins were excluded in the study. Detailed demographic information of all subjects was obtained by means of questionnaires and anthropometric measurements at the point of ascertainment. The refractive error was measured in diopters (D) using auto-refractor keratometer (PRK-500, Potec, Korea), where a subject was considered myopic when refractive error of -0.50D or worse was measured [3]. This research was approved by UCSI University's research ethics committee, Federal Territory of

Kuala Lumpur State Education Department, and the Ministry of Education, Malaysia.

Genomic DNA Extraction

Oral buccal mucosal cells were obtained by swishing with 10mL of mineral water and used to extract genomic DNA using the Wizard® SV Genomic DNA purification system as previously described [25]. Briefly, buccal cells were washed once in 500 µL of phosphate buffer solution (pH 7.4; 80 mmol/L Na_2HPO_4, 20 mmol/L $NaH_2PO_4.2H_2O$, 100 mmol/L NaCl) and pelleted by centrifugation at 13000 × *g* for 5 min at 4ºC. Cells were lysed in 200 µL of lysis buffer in a microcentrifuge tube, incubated at room temperature for 5 mins and centrifuged at 13000 × *g* for 5 min, after which cell lysate was transferred to a Wizard® SV minicolumn assembly. After centrifugation, the flow through was discarded and the column was washed twice in 650 µL wash solution and a final wash in 250 µL wash solution. The extracted genomic DNA was then eluted in 100 µL of nuclease-free water and was kept at −20°C until use. Extracted genomic DNA was quantified on spectrophotometry at OD_{260nm} and analysed using DNA agarose gel electrophoresis.

Variant Genotyping

Genotyping was performed using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) assays, as previously described [25]. Primer pairs used for PCR amplification (Table 1) generated amplicon size of 273bp and 435bp for rs1800470 *TGFB1* and rs2229333 *TGIF*, respectively. PCR amplification was carried out using a Veriti 96 well Thermal Cycler in a final reaction volume of 25 µL each, containing 0.5–1.0 µg of extracted genomic DNA sample, 1 × PCR reaction buffer, 0.1mM of each dNTPs, 5 pmol of primer pair and 1 unit of *Taq* DNA polymerase. The PCR program consisted of an initial denaturation at 95°C (5 mins), followed by 30 cycles of denaturation at 95°C (45 sec), annealing at 55°C (45 sec), extension at 72°C (45 sec) and a final extension at 72°C for 5 mins. For genotyping, a total of 10 µL PCR amplicons was digested with *MspA1I* and *XmaI* for analysis of the variant rs1800470 and rs2229333 respectively. Briefly, reaction volume comprised of 10 µL PCR amplicon, 6 units of restriction enzyme (*MspA1I* or *XmaI*), 1.5 µL 10X NEB buffer and distilled water to a total volume of 15 µL were incubated at 37ºC for 3 hours followed by agarose gel electrophoresis. RFLP banding profile was determined for rs1800470 and rs2229333 based on the expected banding as shown in Table 2.

Statistical Analysis

The study was carried out using counting method to determine the respective allelic and genotype frequency. The demographic statistics and mean refractive error were analysed using the Statistical for the Social Sciences (SPSS) software version 18. Chi-squared (χ^2) test was used to evaluate the association of genotypic frequencies with myopia, stratified for sex and ethnicity (Malay, Chinese and Indian). Significant difference in statistic was determined when p-value was less than 0.05. In addition, Goodness of fit χ^2 test with one degree of freedom was used to determine whether the observed genotypic frequencies obtained in the study population obey the Hardy –Weinberg equilibrium principles.

Table 1. PCR primers sequence

Variant	Primers	Nucleotide Sequence	Tm (ºC)
rs1800470	*TGFB1-f*	5’ CCTCAGCTTTCCCTCGAGGCCCTCCTACC 3’	63.5
	TGFB1-r	5’ ATGGCCTCGATGCGCTTCCGCTTCACCAGC 3’	63.4
rs2229333	*TGIF-f*	5’ ATTCTCAGAACCCGTTGGCTGAGT 3’	60.1
	TGIF-r	5’ TGGCCFCTATCTGCTGTATATCTGTG 3’	59.8

Table 2. Enzymes used and RFLP profile

Variant	Enzyme	Genotype	Resulting DNA Fragments (bp)
rs1800470	*MspA1I*	Wildtype T/T	169, 64 and 40
		Homozygous C/C	157, 64, 40 and 12
		Heterozygous C/T	169, 157, 64, 40 and 12
rs2229333	*XmaI*	Wildtype C/C	307 and 128
		Homozygous T/T	435
		Heterozygous C/T	435, 307 and 128

RESULTS AND DISCUSSION

Demographics of the 565 adolescents (mean age: 14.85±1.26 years old) recruited for this study via convenient sampling comprised of Malay (38.9%), Chinese (36.5%) and Indian (24.6%) ethnicity, as shown in Table 3.

Refractive Error and Myopia Prevalence

The overall prevalence of myopia was 83%, females (85.8%) were more prevalent for myopia compared to males (78.8%). The mean refractive error of the entire population was -2.11±2.19D, with females (-2.26±2.15D) showing higher mean refractive error than males (-1.90±2.25D). By ethnicity, Chinese adolescents had the highest myopia prevalence (87.4%), followed by Malay (82.3%) and Indians (77.7%), irrespective of sex ($p<0.05$). In accordance, Chinese showed significantly poorer mean refractive error (-2.83±2.63D) than Malays (-1.78±1.91D), and Indians (-1.56±1.52D) ($p<0.001$). No significant difference in refractive index was detected between sex for the different ethnic groups (Table 3).

Table 3. Demographics of population and prevalence of myopia

Number of students (n)		Chinese	Malay	Indian
Male	226	93	76	57
Female	339	113	144	82
Total	565	206	220	139
Myopia prevalence (*n, %*)				
Male	178 (78.8)	78 (83.9)	57 (75.0)	43 (75.4)
Female	291 (85.8)	102 (90.3)	124 (86.1)	65 (79.3)
Total	469 (83.0)	180 (87.4)	181 (82.3)	108 (77.7)
Mean refractive error				
Total population	2.11 ± 2.19	2.83 ± 2.63[a]	1.78 ± 1.91[a]	1.56 ± 1.52[a]
Male	1.90 ± 2.25	2.61 ± 2.82	1.48 ± 1.75	1.29 ± 1.31
Female	2.26 ± 2.15	3.02 ± 2.48	1.94 ± 1.98	1.76 ± 1.63
	(p=0.058)	(p=0.271)	(p=0.091)	(p=0.122)

Note: Mean refractive error (-Mean ± SD Diopter), a $p \leq 0.001$, significant difference between different ethnic group for mean refractive error, irrespective for genders

The high myopia prevalence (83.0%) observed among the Malaysian adolescents coincides with observed prevalence in Asia countries, for example 63.0% (5-20 years old) in Eastern China [26] and 94.9% (12-14 years old) in Japan [27]. The difference in prevalence of myopia between countries may be due to urbanization levels or academic achievement in children. For example, a study of adolescents (7-13 years old) in Chongqing, China reported significantly higher myopia prevalence in urban areas (n=569, 36.7%) compared to rural areas (n=428, 30.1%) [15].

In consideration of multi-ethnic population as reference, Singapore population with Chinese, Malays and Indians is a good proxy for looking at Malaysian population. A total of 69.1% adolescents in Singapore (aged 11-18) were myopic, with highest myopia prevalence observed in Chinese (71.25%) followed by Malays (20.7%), and Indians (8.1%) ($p<0.001$) [28]. A study of Chinese school children (aged 10-12) in Malaysia with myopia is significantly associated with self-declared academic excellence in school ($p=0.041$) and exposure towards electronic devices ($p<0.05$), which may explain for the high prevalence of early onset of myopia (58.4%) in the school-going Chinese children [29].

A separate study on Indian schoolchildren (n=1462, aged 7-11) in Kuala Lumpur showed myopia prevalence of 28.9% [11], which was lower than observed in our Indian adolescent subjects. As a proxy for Malaysian-Indians, a separate study on the South Indian children (n=9616, aged 5-15) suggested a number of risk factors which are significantly associated with the development of myopia including longer time of reading, writing (28-35 hours/>42 hours), computers and video games (>7 hours), and lower exposure to outdoor activities (>14 hours, $p=0.002$) [6]. Furthermore, a South Indian population (n=6984) study reported that myopia progression was significantly associated with age: adolescents less than 15 years old showed drastic annual myopia progression rate (-0.45±0.01D) when compared to those greater than 15 years old (-0.14±0.01D) ($p<0.001$) [30].

The prevalence of myopia was found to be higher in Malaysian female (85.8%) compared to male (78.8%) adolescents (Table 3). Likewise, Goh et al. [31] (aged 7-15) and Awodele [29] (aged 11-18) also observed higher myopia prevalence in female (22.5%) than male (19.0%) in Malaysia and Singapore adolescents (71.3% *v* 66.8%). Despite no significant difference, females (-2.26±2.15D) has higher

mean refractive index compared to males (-1.90±2.25D). Our findings are found to be concordant with a study in eastern China which reported refractive error in females (5-20 years old) (n=2154, -2.66±2.36D) was significantly higher than in males (n=2647, -2.46±2.47D) (p=0.005) [26]. This may be due to the differences in maturational development and the age onset of puberty between males and females; early puberty onset in females may lead to rapid myopia development. An animal study demonstrated that growth hormone injected rats showed significant greater axial length (0.6245±0.0079cm) compared to saline-injected rats (0.5932±0.0149cm) (p=0.0031) after 36 days of treatment [32].

Genotype and Allele Frequency

Genotypes of rs1800470 (*TGFB1*) and rs2229333 (*TGIF*) variants determined for each subject using PCR-RFLP are as shown in Figure 1 and 2. The minor allele frequency (MAF) for rs1800470 (*TGFB1*) and rs2229333 (*TGIF*) is T (0.470) and T (0.188) respectively (Table 4). Despite, C being previously reported as risk allele, in our study the T allele frequency of rs1800470 variant was lower than C allele in Chinese and Malays, corresponding to lower TT genotype frequency compared to CT and CC genotypes. In contrast, lower C allele frequency was observed in Indians corresponding to lower CC genotype frequency compared to CT and TT genotypes. As for variant rs2229333 (*TGIF*), all three ethnic groups showed a lower T (risk) allele frequency compared to C allele corresponding to lower TT genotype compared to CT and CC genotypes (Table 4). Based on the allelic frequency obtained, the $\chi 2$ value was less than 5% of significance level for 1 degree of freedom, inferring that this study population was at Hardy-Weinberg equilibrium.

The allelic frequency for the *TGFB1* rs1800470 variant varies according to population ethnicities. Our study population showed T allele frequency in accordance with T variant of 0.5887 reported in gnomAD database [33], and similarly to East Asian populations (0.4434). In contrast, Malaysian-Indians (0.5827) had allele frequency similar to South Asian (0.5437), Middle Eastern (0.5380) and African or African American population (0.5747). Whereas our studied Chinese (0.3981) and Malays (0.4659) showed frequency closer to the Taiwanese (0.4826) [17] population which showed T as the minor allele. As for *TGIF* rs2229333, the T allele frequency of 0.188 for the whole studied population, is similar to East Asian (0.1612) as reported in gnomAD database. In contrary, lower variant allele frequency is observed in Middle Eastern (0.1203), South Asian (0.1123) and in African or African American (0.0108) [33].

Correlation of Variant and Myopia

Overall, the whole Malaysian adolescent study population showed no significant difference between normal and myopia subjects for genotypes of both variant rs1800470 (*TGFB1*) and rs2229333 (*TGIF*). However, myopes Malay males showing significantly higher frequency of rs1800470 (*TGFB1*) CT and CC genotypes compared to TT (p=0.033) (Table 5). Generally, the CT genotype of rs1800470 (*TGFB1*) showed higher frequency in myopic subjects irrespective of sex, except for Chinese female with higher

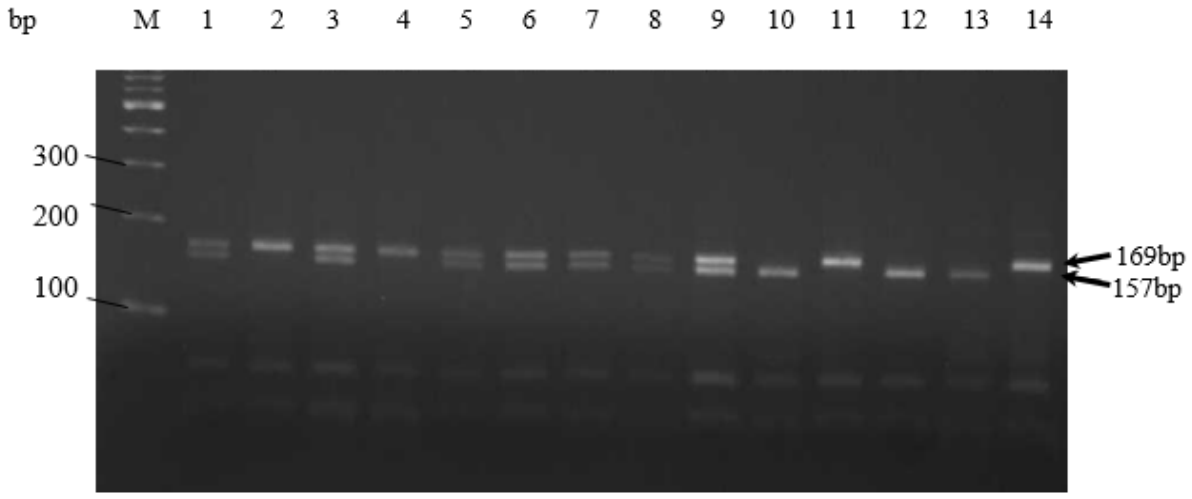

Figure 1. Genotyping of *TGFB1* rs1800470 variant using RFLP for 14 genomic DNA samples. PCR product from the respective DNA samples were digested with *MspA1I* enzyme and separated on a 1% agarose gel electrophoresis. Lane M contained 100bp DNA ladder; Lanes 2, 4, 11 and 14 showed a single 169bp band indicating TT genotype; lanes 1, 3, 5, 6, 7, 8 and 9 showed two bands (169bp and 157bp) indicating heterozygous CT genotype; and lanes 10, 12 and 13 showed a 157bp band, indicating homozygous CC genotype. Only DNA fragments with bigger size (169bp and 157bp) were used for genotype determination because the smaller sized (64bp, 40bp and 12bp) fragments were not clearly visualized on gel after electrophoresis

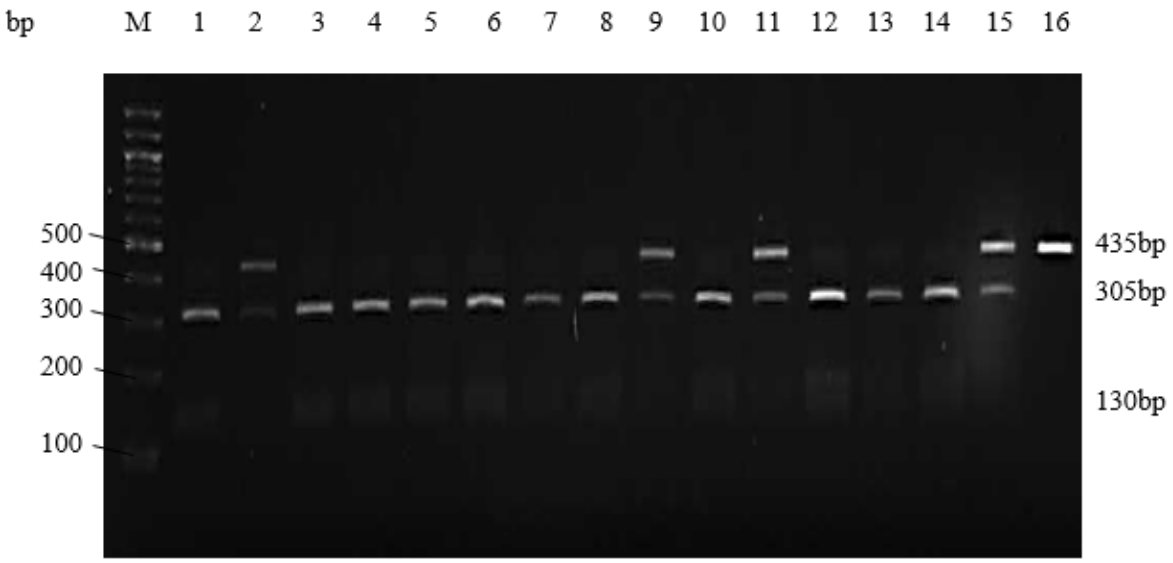

Figure 2. Genotyping of *TGIF* rs2229333 variant using RFLP for 16 genomic DNA samples. PCR product from the respective DNA samples were digested with *XmaI* enzyme and separated on a 1% agarose gel electrophoresis. Lane M contained 100bp DNA ladder; Lanes 1, 3, 4, 5, 6, 7, 8, 10, 12, 13 and 14 showed two bands (305bp and 130bp) indicating CC genotype; lanes 2, 9, 11 and 15 showed three bands (435bp, 305bp and 130bp) indicating heterozygous CT genotype; and lane 16 showed a single 435bp band, indicating homozygous TT genotype

Table 4. Genotype and allelic frequencies of variants

	***TGFB1* (rs1800470)**			***TGIF* (rs2229333)**		
	CC	**CT**	**TT**	**CC**	**CT**	**TT**
Chinese	81 (39.3)	86 (41.7)	39 (18.9)	130 (63.1)	68 (33.0)	8 (3.9)
Malay	67 (30.5)	101 (45.9)	52 (23.6)	149 (67.7)	65 (29.5)	6 (2.7)
Indian	25 (18.0)	66 (47.5)	48 (34.5)	95 (68.3)	37 (26.6)	7 (5.0)
	C		T	C		T
Chinese	248 (60.2)		164 (39.8)	328 (79.6)		84 (20.4)
Malay	235 (53.4)		205 (46.6)	363 (82.5)		77 (17.5)
Indian	116 (41.7)		162 (58.3)	227 (81.7)		51 (18.3)

Note: Frequency (Percentage)

frequency of CC genotype. Pairwise genotype combinations of these variants according to ethnicity and sex are as shown in Table 6. There was no significant difference in genotype frequency of rs2229333 (*TGIF*) between normal and myopia subjects. In Chinese subjects irrespective of sex, CC genotype was more frequent in normal subjects while the CT and TT genotype (T being the risk allele) was more frequent in myopia subjects. This was likewise observed in Malay males and Indian females.

To further investigate prevalence of these variants with myopia, we performed an association test assuming recessive genetic inheritance model. The recessive model of rs1800470 (*TGFB1*), combined genotypes of C risk-allele (CT+CC) compared to the TT genotype showed higher frequency in myopic compared to normal subjects. Furthermore, combined CT+CC was significantly higher in Malay males (p=0.009) with myopia than normal. Despite no significant association, the Chinese irrespective of sex, Malay females and Indian males showed higher frequency for risk genotypes (CT+CC) in myopic subjects. In the case of rs2229333 (*TGIF*), recessive model of combined genotype (CT+TT), with risk T allele, showed no significant association with myopia despite higher frequency observed in Chinese, Malay male and Indian female with myopia (Table 6). The dominant model association test on the other hand, demonstrated no significant association between genotype frequencies with myopia status in the study population (results not shown).

Table 5. Genotype frequency distribution and association with myopia

			***TGFB1* rs1800470**					***TGIF* rs2229333**				
			CC	**CT**	**TT**	**Total**	**P-value**	**CC**	**CT**	**TT**	**Total**	**P-value**
Whole population		Normal	30 (31.3)	35 (36.5)	31 (32.3)	96 (100.0)	0.102	67 (69.8)	27 (28.1)	2 (2.1)	96 (100.0)	0.549
		Myopia	143 (30.5)	218 (46.5)	108 (23.0)	469 (100.0)		307 (65.5)	143 (30.5)	19 (4.1)	469 (100.0)	
Male	Whole population	Normal	13 (27.1)	17 (35.4)	18 (37.5)	48 (100.0)	0.109	33 (68.8)	14 (29.2)	1 (2.1)	48 (100.0)	0.676
		Myopia	51 (28.7)	86 (48.3)	41 (23.0)	178 (100.0)		118 (66.3)	51 (28.7)	9 (5.1)	178 (100.0)	
	Chinese	Normal	5 (33.3)	6 (40.0)	4 (26.7)	15 (100.0)	0.905	11 (73.3)	4 (26.7)	0 (0.0)	15 (100.0)	0.606
		Myopia	26 (33.3)	35 (44.9)	17 (21.8)	78 (100.0)		50 (64.1)	24 (30.8)	4 (5.1)	78 (100.0)	
	Malay	Normal	4 (21.1)	6 (31.6)	9 (47.4)	19 (100.0)	**0.033**[a]	15 (78.9)	4 (21.1)	0 (0.0)	19 (100.0)	0.407
		Myopia	17 (29.8)	30 (52.6)	10 (17.5)	57 (100.0)		38 (66.7)	15 (26.3)	4 (7.0)	57 (100.0)	
	Indian	Normal	4 (28.6)	5 (35.7)	5 (35.7)	14 (100.0)	0.629	7 (50.0)	6 (42.9)	1 (7.1)	14 (100.0)	0.353
		Myopia	8 (18.6)	21 (48.8)	14 (32.6)	43 (100.0)		30 (69.8)	12 (27.9)	1 (2.3)	43 (100.0)	
Female	Whole population	Normal	17 (35.4)	18 (37.5)	13 (27.1)	48 (100.0)	0.594	34 (70.8)	13 (27.1)	1 (2.1)	48 (100.0)	0.699
		Myopia	92 (31.6)	132 (45.4)	67 (23.0)	291 (100.0)		189 (64.9)	92 (31.6)	10 (3.4)	291 (100.0)	
	Chinese	Normal	5 (45.5)	4 (36.4)	2 (18.2)	11 (100.0)	0.961	9 (81.8)	2 (18.2)	0 (0.0)	11 (100.0)	0.315
		Myopia	45 (44.1)	41 (40.2)	16 (15.7)	102 (100.0)		60 (58.8)	38 (37.3)	4 (3.9)	102 (100.0)	
	Malay	Normal	9 (45.0)	6 (30.0)	5 (25.0)	20 (100.0)	0.292	12 (60.0)	8 (40.0)	0 (0.0)	20 (100.0)	0.622
		Myopia	37 (29.8)	59 (47.6)	28 (22.6)	124 (100.0)		84 (67.7)	38 (30.6)	2 (1.6)	124 (100.0)	
	Indian	Normal	3 (17.6)	8 (47.1)	6 (35.3)	17 (100.0)	0.972	13 (76.5)	3 (17.6)	1 (5.9)	17 (100.0)	0.825
		Myopia	10 (15.4)	32 (49.2)	23 (35.4)	65 (100.0)		45 (69.2)	16 (24.6)	4 (6.2)	65 (100.0)	
Malay		Normal	13 (33.3)	12 (30.8)	14 (35.9)	39 (100.0)	0.064	27 (69.2)	12 (30.8)	0 (0.0)	39 (100.0)	0.515
		Myopia	54 (29.8)	89 (49.2)	38 (21.0)	181 (100.0)		122 (67.4)	53 (29.3)	6 (3.3)	181 (100.0)	

Note: Genotype frequency (Percentage), [a] $p<0.05$, significant difference in genotype frequency between normal and myopia group in Malays male for genotype *TGFB1*.

Table 6. Recessive model for genotype frequency of variants

Sex	Ethnic	Genotypes *TGFB1*					Genotypes *TGIF*			
		Group	TT	CT+CC	Total	p-value	CC	CT+TT	Total	p-value
Male	Chinese	Normal	4 (26.7)	11 (73.3)	15 (100.0)	0.679	11 (73.3)	4 (26.7)	15 (100.0)	0.491
		Myopic	17 (21.8)	61 (78.2)	78 (100.0)		50 (64.1)	28 (35.9)	78 (100.0)	
	Malay	Normal	9 (47.4)	10 (52.6)	19 (100.0)	**0.009**[a]	15 (78.9)	4 (21.1)	19 (100.0)	0.313
		Myopic	10 (17.5)	47 (82.5)	57 (100.0)		38 (66.7)	19 (33.3)	57 (100.0)	
	Indian	Normal	5 (35.7)	9 (64.3)	14 (100.0)	0.828	7 (50.0)	7 (50.0)	14 (100.0)	0.178
		Myopic	14 (32.6)	29 (67.4)	43 (100.0)		30 (69.8)	13 (30.2)	43 (100.0)	
Female	Chinese	Normal	2 (18.2)	9 (81.8)	11 (100.0)	0.830	9 (81.8)	2 (18.2)	11 (100.0)	0.137
		Myopic	16 (15.7)	86 (84.3)	102 (100.0)		60 (58.8)	42 (41.2)	102 (100.0)	
	Malay	Normal	5 (25.0)	15 (75.0)	20 (100.0)	0.811	12 (60.0)	8 (40.0)	20 (100.0)	0.496
		Myopic	28 (22.6)	96 (77.4)	124 (100.0)		84 (67.7)	40 (32.3)	124 (100.0)	
	Indian	Normal	6 (35.3)	11 (64.7)	17 (100.0)	0.994	13 (76.5)	4 (23.5)	17 (100.0)	0.559
		Myopic	23 (35.4)	42 (64.6)	65 (100.0)		45 (69.2)	20 (30.8)	65 (100.0)	

Note: Genotype frequency (Percentage), [a] $p<0.05$, significant difference in genotype frequency between normal and myopia Malays male group based on recessive model for genotypes *TGFB1*.

TGF-β regulates fibroblast proliferation and type I collagen, MMP-2 and proteoglycan production, and plays an important role in sclera remodelling by controlling extracellular matrix production [34]. The rs1800470 risk C allele is possibly associated with a higher risk of myopia development due to changes in *TGFB1* mRNA and active form of TGF-β1 in retina, choroid and sclera correlated with axial elongation of myopia [35]. Lin et al. [17] study on Taiwanese Chinese (n=3000) showed that *TGFB1* rs1800470 with CC and CT instead of TT genotype was higher in frequency among high myopes individuals compared to control (p<0.001). In a separate study among Southern Chinese (300 controls, 300 high myopia), the CT and TT genotypes for rs1800470 showed significant association with myopia compared to those with CC genotype (p=0.03) [18]. A study in a Kashmiri Indian population (257 highly myopic, 176 non-myopic) showed that the variant *TGFB1* rs1800470/rs1982073 had significantly higher C risk allele in both CC and CT genotypes in high myopes compared to control subjects (p allele=0.001, p genotype=0.003) [22]. Likewise, our study inferred the potential role of C allele for rs1800470 in myopia development in Malay male adolescents as significant association was observed in CT genotype (p=0.033, relative risk=1.23) and combined genotype CT+CC (p=0.009, OR (95% CI)=4.23).

TGIF is a transcription repressor for retinoid X receptor-dependent transcription factor and TGF-β as mentioned previously, and is expressed in the sclera, retina, cornea and optic nerve [36]. *TGIF* null murine showed upregulation in retinoic acid (RA) target gene expression and increased RA levels can lead to rapid ocular elongation and myopic retina in experimental animals [37-38]. Previous studies observed the T and not C allele for rs2229333 variant was associated with high myopia in Kashmiri subjects (OR (95% CI) =1.46(1.07-1.96), p=0.015) [23]. Likewise, our study showed higher frequency of TT and CT+TT genotypes in myopia compared to normal Chinese, Indians and Malays, except for Indian male and Malay female adolescents. On the contrary other studies on Japanese population involving high resolution screening of 35 microsatellite markers on MYP2 [39] and 13 variants on *TGIF*, (including rs2229333), showed no significant difference was observed between myopia and control groups [40]. Other variants such as the *TGIF* rs2229336 also showed no association with myopia in southeast China subjects [20]. However, two other variants, the *TGIF* rs8082866 was associated with axial length (p=0.013) and corneal curvature (p=0.007), while the *TGIF* rs2020436 was associated with corneal curvature (p=0.022) [41].

Previous and present studies showed inconclusive results for the role of *TGFB1* and *TGIF* variants with myopia development. This is likely attributed to a limitation of this study whereby only two variants from two candidate genes were studied. Expanding study to include reported variants from other myopia candidate genes or from Genome Wide Association Study (GWAS) may help to minimize biases and improve the association study. Another limitation of this study was due to low sample size due to stratification by sex and ethnicity which can be circumvented by increasing the size of overall study population.

In addition, factors influencing the development of myopia such as variable ethnicity of subjects, difference in lifestyle and environmental factors, such as reading, outdoor activities, LED lamps usage, geographical factor and socioeconomic status should be considered when studying myopia development [7,31]. Some of these environmental variables may be quantified using questionnaires or instruments, can potentially improve the quality of future studies to interrogate the potential role of these variants to the aetiology of myopia.

CONCLUSION

The present study reported myopia demographic and high myopia prevalence among Malaysian adolescents, especially among the Chinese ethnic group. Of the two variants studied, only the CT or (CT+CC) *TGFB1* rs1800470 was significantly higher in frequency among the myopic compared to normal Malay male adolescents. Further investigation with a larger sample size and correlation with lifestyle can be performed to verify our findings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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