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Original Article

The Brain-Derived Neurotrophic Factor *(BDNF)* gene Val66Met (rs6265) polymorphism and stress among preclinical medical students in Malaysia

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الملخص

أهداف البحث: تهدف هذه الدراسة لتحديد الرابطة الأليلية والوراثية للجين Val66Met (rs62659) المتعدد الأشكال في جين عامل التغذية العصبية المشتق من الدماغ مع مستوى التوتر لطلاب الطب في المرحلة قبل السريرية في جامعة السلطان زين العابدين، تيرينجانو، ماليزيا.

طرق البحث: في هذه الدراسة المستعرضة، تم ضم ١٢٢ طالبا من جميع طلاب المرحلة قبل السريرية. كما تم توزيع استبانة مقياس الاكتئاب والقلق والتوتر المحققة ٢١، وتم أخذ عينات الدم من كل طالب لاستخراج الحمض النووي. ثم عمل تحليل التنميط الجيني لجين عامل التغذية العصبية المشتق من الدماغ المتعدد الأشكال باستخدام تفاعل البلمرة المتسلسل المحسن بطريقة تقييد طول الجزء المتعدد الأشكال.

النتائج: وافق ١٠٥ طالبا على المشاركة في هذه الدراسة. وكان لدى الطلاب الهنود النمط الوراثي Val/Val أكثر بكثير، بينما كان لدى طلاب الملايو النمط الوراثي Met/Met. واختلفت الأنماط الوراثية الثلاثة لعامل التغذية العصبية المشتق من الدماغ (Val/Val ,Met Val/ وMet/Met) اختلافا كبيرا من حيث مستوى التوتر الملحوظ؛ والطلبة الذين يحملونالنمط الوراثي Val/Val (م= ١٠.٦) كما لوحظ أنهم أقل توترا بشكل ملحوظ بالمقارنة بالطلبة الذين يحملون النمط الوراثي Val/ Met (م = ١٤) والنمط الوراثي Met/Met (م =١٥.١).

الاستنتاجات: في دراستنا، كان الموروث Met مرتبطا بمستوى عالي من التوتر. وعلى حد علمنا، هذه أول دراسة بحثت في هذا المجال عالميا. يجب أن تحفز نتائج هذه الدراسة الباحثين للتركيز على تأثير التوتر على طلاب الطب تحت التأثيرات المحتملة للجينات.

الكلمات المفتاحية: عامل التغذية العصبية المشتق من الدماغ؛ التوتر؛ الأكسدة؛ طلاب الطب

Abstract

Objective: This study aimed to determine the allelic and genotypic association of the Val66Met (rs6265) polymorphism in the *BDNF* gene with stress levels in preclinical medical students of Universiti Sultan Zainal Abidin (UniSZA), Terengganu, Malaysia.

Methods: In this cross-sectional study, we recruited all 122 preclinical medical students. The validated depression anxiety stress scales-21 (DASS-21) questionnaire was distributed and blood samples were collected from each subject for DNA extraction. Genotyping analysis of the *BDNF* gene (Val66Met) polymorphism was performed via an optimised polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: A total of 105 subjects agreed to participate in this study. Indian students were found to more likely have the Val/Val genotype, whereas Malay students were more likely to have the Met/Met genotype ($p = 0.027$). Individuals carrying any one of the three *BDNF* genotypes (Val/Val, Val/Met and Met/Met) differed significantly from each other in terms of their perception of stress ($p = 0.010$); students carrying the Val/Val genotype ($M = 10.6$) perceived significantly lower stress than students carrying the Val/Met ($M = 14$) and Met/Met ($M = 15.1$) genotypes.

Conclusion: In our study, the Met-allele was associated with higher stress levels. To the best of our knowledge, this is the first study investigating this stress-related gene in medical students. The findings from this study should

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trigger more investigators to focus on the impact of stress on genetically predisposed medical students.

Keywords: BDNF; Medical students; Oxidative stress; Stress; Val66Met

Introduction

The Brain-Derived Neurotrophic Factor (BDNF) is one of the most important proteins in the neurotrophin family of neurotrophic factors, and it plays a vital role in promoting the survival of neurons by controlling cell growth and preservation.[1] The most important roles of the BDNF protein include neurogenesis, mood changes, learning, and memory, and it is an essential protein in the brain that regulates eating, drinking, and body weight.[2] The BDNF protein regulates both LTP (long-term potentiation) and LTD (long-term depression), axonal sprouting, synaptic plasticity, dendritic arbour proliferation, and neuronal differentiation.[3] the BDNF protein also plays a significant role in nerve cell differentiation and central nervous system (CNS) control by activating key intracellular signalling series, where cell-to-cell connections occur at the synapses.[4] The BDNF protein is considered an initial regulator of the cognition process on the cellular level; because it is responsible for synergistic connections between synaptic plasticity and neuronal activity.[5]

Interestingly, many stressful and harmful conditions (such as hypoxia and oxidative stress) were reported to be associated with a lack of BDNF protein expression in the CNS and increased free radicals.[6,7] Consequently, the BDNF protein exerts its role in numerous psychiatric disorders including anxiety and depression, and some of the neurodegenerative diseases including Alzheimer disease, epilepsy, and Parkinson's disease. These diseases have a mutual aetiology in which they are triggered by an increased level of stress.[7,8]

This study focused on the analysis of the Val66Met (valine substitution to methionine at codon 66) polymorphism (also known as rs6265) in the *BDNF* gene. Previous studies on human subjects have shown that BDNF protein secretion was significantly lower in Met-allele compared to Val-allele individuals, which led to the hypothesis that individuals who carry two Met-alleles are associated with a higher level of stress compared to those who carry one Met-allele, and those without a Met-allele, respectively.[9,10] Therefore, the *BDNF* Val66Met polymorphism plays a significant role in genetic predisposition to stress disorders.[10]

Although there is still a lack of studies on stress among Malaysian medical students, previous studies have described the Malaysian medical schools as an environment characterised with extra stressful circumstances,[11–13] which often has an adverse effect on students' mental, and consequently, physiological health. The estimated prevalence of emotional disorders related to high stress levels in medical students was found in several studies in Malaysia to be higher compared to the general population.[14–16]

In particular, the early phase of medical studies (preclinical stage) is more stressful for students than the later phases.[17] In addition to general stress factors among medical students, preclinical medical students are required to follow a fixed schedule, and attend early classes daily. Hence, their lifestyle is considered stressful and aggravating, as they are also inundated with self-study, lecture, and laboratory sessions.[18] However, the daily routine of preclinical medical students is characterised by a more intense study schedule than that of clinical medical students, alongside a lack of clarity concerning the aim of their studies, confusion about their role(s) as students, and little time for other activities.[19] Therefore, the lack of studies investigating the problem of high-stress levels among medical students in Malaysia has necessitated our study on stress among preclinical medical students in relation to their genes (*BDNF* gene Val66Met polymorphism). The study subjects were recruited from Universiti Sultan Zainal Abidin (UniSZA), Terengganu, Malaysia. This study, to our knowledge, is the first of its kind conducted globally.

Materials and Methods

Study design and participants

A cross-sectional study was conducted involving all the undergraduate preclinical medical students at Universiti Sultan Zainal Abidin (UniSZA), Kuala Terengganu, Terengganu, Malaysia. A total of 122 preclinical students (66 first year and 56 s year, 39 males and 83 females), were invited to participate in the study on November 21st, 2017.

Inclusion criteria

Healthy, preclinical medical students, non-smokers, not using supplements or any form of medications affecting haematological parameters or stress levels during the study period and in the last three months before participating, and without any family history of hereditary anaemia, such as sickle cell anaemia and thalassemia, were included in this study.

Questionnaire distribution

In the present study, depression anxiety stress scales-21 (DASS-21) was used to assess the severity of stress in clinical and non-clinical samples. The validated DASS-21 questionnaire is based on the three self-report scales designed to measure levels of the negative states of stress, depression, and anxiety.[20]

Blood sample collection and whole blood DNA extraction

A total of 5 ml EDTA blood was collected from each subject. All blood samples were stored at − 80 °C until further analysis. DNA was isolated from blood by using the

GF-1 blood DNA extraction kit (Vivantis, San Jose, CA, USA) according to the manufacturer's instructions.

Restriction fragment length polymorphism (RFLP)

Polymerase chain reaction (PCR) preparation: A PCR master mix was prepared according to a previously described protocol by using a pair of primers (forward primer 5′-ATC CGA GGA CAA GGT GGC-3′ and reverse primer 5′-CCT CAT GGA CAT GTT TGC AG-3′).[21] A total of 11.04 µl dH_2O, 2.5 µl 10 × ViBuffer A [containing 500 mM KCl, 100 mM Tris−HCl (pH 9.1 at 20 °C), and 0.1% TritonTMX-100], 0.16 µl dNTPs, 1.5 µl Chrome Max Taq DNA polymerase [containing Taq DNA Polymerase, Pfu DNA Polymerase, enhancing factors, and mixed with loading dye] (Vivantis, USA), 1.0 µl of each primer, 0.8 µl $MgCl_2$, and 2.0 µl DNA sample was prepared to a final volume of 20 µl for each reaction.

PCR amplification

PCR was carried out in a Veriti® 96-Well thermal cycler (Applied Biosystems, Foster City, CA, USA). After activation of Chrome Max Taq DNA polymerase for 2 min at 95 °C, the reaction mixture was subjected to 35 amplification cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 9 s, and extension at 72 °C for 30 s, followed by a final extension stage at 72 °C for 10 min.

Restriction analysis

The 300 bp amplified product was digested with 10 units (1 µl) of *Eco72I* (*PmlI*) restriction enzyme (recognition site; 5′-CAC↓GTG-3′) (Thermo Scientific, Waltham, MA, USA). A total of 10 µl of the PCR amplified product, 18 µl dH_2O, 2 µl 10 × Buffer Tango (for 100% *Eco72I* digestion) [containing 33 mM Tris-acetate (pH 7.9), 66 mM potassium acetate, 0.1 mg/mL BSA, and 10 mM magnesium acetate], and 1.5 µl *Eco72I* enzyme in a final volume of 31.5 µl for each reaction were mixed gently and subsequently incubated overnight at 37 °C. The *Eco72I* enzyme was inactivated by incubation at 65 °C for 20 min before loading the PCR products.

Gel electrophoresis and visualisation

PCR products were detected after digestion with the restriction enzyme via electrophoresis on 2.5% agarose gel stained with Ethidium Bromide (Vivantis, USA). The DNA was visualised by placing on a UV light source, where the image was captured by using FluorChem (FC2) gel reading system (Cell Biosciences, Santa Clara, CA, USA).

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corporation, Armonk, NY, USA). The relative importance index (RII) was used as an indicator of stress, anxiety, and depression, and the Cronbach's alpha test of internal consistency was used to assess the reliability of the DASS-21 inventory. Frequencies and percentages were used to describe the binary and categorical variables. The chi-squared (χ^2) test of independence was employed to explore bivariate associations between categorical variables. Moreover, one-way ANOVA was used to explore the subjects' demographics and *BDNF* genotypes for statistical differences on metric continuous measured outcome variables (i.e. DASS-21 subscale scores).

Results

Demographic data

Only 109 of 122 students submitted the administered questionnaire. Of the 109 participants, four students were excluded from this study: two of them based on exclusion criteria and the other two because they were from a different ethnic group. Their data may form outliers and raise issues of small sample numbers during statistical analysis. Therefore, the final number of enrolled students was 105 as summarised in Table 1.

Based on ethnicity, 80 (76.2%) students were Malay, while 25 (23.8%) were Indian.

Reliability analysis

The Cronbach's alpha test of internal consistency was used to assess the reliability of DASS-21 and it is subconcepts. It is the most common test used for questionnaire-based Likert scale, to determine if the scale is reliable. In this study, the test suggested that the scale was reliable overall and it measured the students' responses to the stress, anxiety, and depression items consistently. Cronbach's alpha was equal to 0.87, which is above 0.70 as a general cut-off limit (Table 2).

Results of DASS-21

The results of DASS-21 reflected that students were categorised under five groups as summarised in Table 3, and the overall prevalence of stress, anxiety, and depression was calculated in Table 4.

Table 1: The total number of preclinical medical students who enrolled in the present study.

Year of Study	Gender		Total
	Male	Female	
First year	20 (19.04%)	37 (35.23%)	57 (54.3%)
Second year	15 (14.3%)	33 (31.43%)	48 (45.7%)
Total	35 (33.34%)	70 (66.66%)	105 (100%)

Table 2: Reliability analysis of DASS-21 (n = 105).

DASS-21 subscales	Number of items	Cronbach's alpha	Decision
Stress scale	7	0.72	Good
Depression scale	7	0.78	Good
Anxiety scale	7	0.67	Acceptable
DASS-21 overall	21	0.87	Very Good

Table 3: The Frequency of scale categories for DASS-21 subscales (n = 105).

DASS-21 Subscales	Scale Categories				
	Normal	Mild	Moderate	Severe	Extremely Severe
Stress	72 (68.6%)	14 (13.3%)	15 (14.3%)	4 (3.8%)	0 (0.0%)
Anxiety	24 (22.9%)	11 (10.5%)	35 (33.3%)	17 (16.2%)	18 (17.1%)
Depression	62 (59.0%)	19 (18.1%)	18 (17.1%)	1 (0.95)	5 (4.8%)

Table 4: The overall prevalence of DASS-21 subscales (n = 105).

DASS-21 Subscales	Category	*N*	Prevalence
Stress	Yes	33	31.4%
	No	72	68.6%
Anxiety	Yes	81	77.1%
	No	24	22.9%
Depression	Yes	43	41.0%
	No	62	59.0%

Determining the genotypic and allelic frequencies

The Met-allele of the *BDNF* gene (Val66Met) polymorphism was not digested by *Eco72I*, indicated by PCR fragment observed at 300 bp, while the Val-allele was indicated by two fragments at 180 and 120 bp (Figure 1).

The analysis of genotypic and allelic frequencies for the *BDNF* gene (Val66Met) polymorphism is shown in Table 5 and Table 6, respectively.

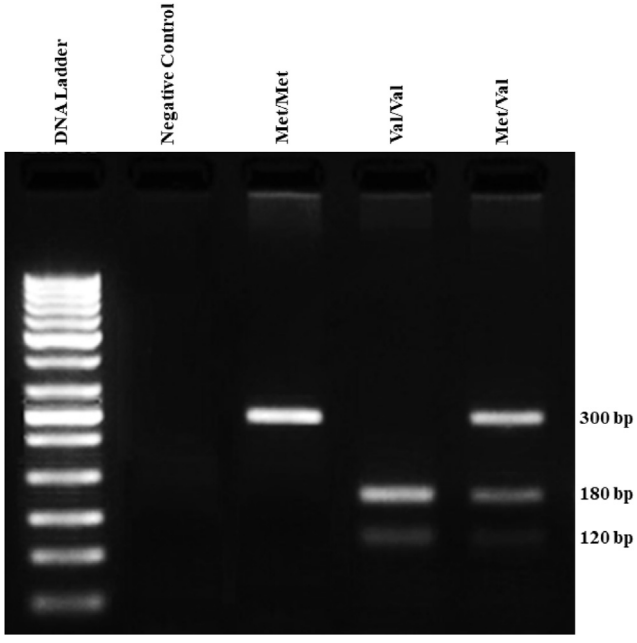

Figure 1:: Restriction analysis of the *BDNF* gene (Val66Met) polymorphism on 2.5% agarose gel.

Table 5: The frequency of *BDNF* genotypes among students.

Genotype	Frequency	Percentage
Val/Val	44	41.9%
Val/Met	39	37.1%
Met/Met	22	21.0%
Total	105	100%

Table 6: Allelic frequency of the Val66Met in students' *BDNF* genes.

Allele	Frequency	Percentage
Val-allele	127	60.5%
Met-allele	83	39.5%
Total	210 (for 105 subjects)	100%

Comparison of the genotypes

The results showed that there was no significant association between the *BDNF* genotype and the gender of the students (p = 0.229). However, there was a significant relationship between the students' ethnicity and the *BDNF* genotype (p = 0.027). Moreover, the *BDNF* genotypes

Table 7: Comparison of students' *BDNF* genotypes to their demographic data and DASS-21 outcomes (n = 105).

Variables	*BDNF* Genotypes			Statistical test	*p* value
	Val/Val n = 44	Val/Met n = 39	Met/Met n = 22		
Sex					
Male	17 (38.6%)	14 (35.9%)	4 (18.2%)	$X^2 = 2.95$	0.229
Female	27 (61.4%)	25 (64.1%)	18 (81.8%)		
Ethnicity					
Indian	16 (36.4%)	7 (17.9%)	2 (9.1%)	$\chi^2 = 7.19$	0.027*
Malay	28 (63.6%)	32 (82.1%)	20 (90.9%)		
Year of Study					
First year	22 (50%)	21 (53.8%)	14 (63.6%)	$\chi^2 = 1.104$	0.576
Second year	22 (50%)	18 (46.2%)	8 (36.4%)		
DASS-21					
Stress score	10.68 (6.87)	14 (5.2)	15.1 (6.28)	F (2.102) = 4.84	0.010*
Anxiety score	11.55 (7)	13.6 (6.5)	12.55 (5.73)	F (2.102) = 1.50	0.354
Depression score	8.80 (7.1)	8.70 (6.8)	8.55 (7)	F (2.102) = 0.008	0.992

Note. The F-ratio is the ratio of the between group variance to the within group variance, if it is accompanied by a p value < 0.05, the ANOVA test is statistically significant.

* Statistically significant difference (p value < 0.05).

Table 8: List of stress studies on preclinical medical students.

Country	Prevalence of stress	Reference
The United States	–	McMurray et al., 1980[24]
The United States	–	Reed et al., 2011[25]
Malaysia	78.3%	Rahman et al., 2013[18]
India	42.5%	Brahmbhatt et al., 2013[26]
Hungary	–	Piko, 2014[27]
KSA	71.7%	Al Sunni and Latif, 2014[28]
Malaysia	16.9%	Fuad et al., 2015[16]
Lebanon	62%	Fares et al., 2016[17]
Thailand	5.6%	Nimkuntod et al., 2016[23]
Malaysia	–	Bhuiyan et al., 2017[29]
India	–	Umadevi et al., 2017[30]
South Africa	29.5%	van Zyl et al., 2017[31]

Table 9: Heterozygosity index and allelic frequencies of the *BDNF* Gene (Val66Met) polymorphism in global populations. Performed in the dbSNP-NCBI up to October 30, 2018.

Population	Individual Group	Heterozygosity index	Alleles	
			Met	Val
African America	–	13%	6.5%	93.5%
Asian	Han Chinese	40%	62%	38%
	Han Chinese	49%	41%	59%
	Han Chinese	46%	60%	40%
	Han Chinese	38%	63%	37%
	Japanese	37%	37%	63%
	Japanese	0.0%	49%	51%
	Japanese	33%	34%	66%
European	Caucasians	25%	17%	83%
	Northern and Western European	34%	19%	81%
	Western and Northern European	29%	18%	82%
	Northern and Western European	28%	18%	82%
Sub-Saharan African	Yoruba Nigerian	0.9%	0.4%	99.6%
	Yoruba Nigerian	0.0%	0.0%	100%

Table 10: The most frequent genotype of the *BDNF* gene Val66Met Polymorphism among different Malaysian ethnic groups.

Malaysian Ethnicity	Most Frequent Genotype	Heterozygosity index	References
Bajau	Val/Met	49%	Sim et al., 2010[38]
Chinese	Val/Val	28%	Mohammed et al., 2014[39]
Chinese	Val/Val	57%	Sim et al., 2010[38]
Indian	Val/Val	27%	Mohammed et al., 2014[39]
Indian	Val/Val	28%	The present study
Kadazan-Dusun	Val/Met	58%	Sim et al., 2010[38]
Malay	Val/Met	40%	The present study
Malay	Val/Met	53%	Sim et al., 2010[38]
Malay	Val/Val	37%	Mohammed et al., 2014[39]

(Met/Met, Val/Met, and Val/Val) differed significantly in their perceived stress levels, F (2,102) = 4.84 (p = 0.010) (Table 7).

Discussion

The paucity of stress studies conducted on preclinical medical students has been proven with the majority of studies conducted within the last decade (Table 8). In this study, the prevalence of stress among preclinical medical students was 31.4%, which can be considered to be moderate or within acceptable levels compared to the results of previous studies on preclinical medical students (Table 8). Also, more than three quarters (77.1%) of the students in this study suffered from increased levels of anxiety and a substantial proportion of them (41.0%) from increased levels of depression. The students' perceived stress correlated with depression and anxiety, depicting that as students perceived greater anxiety and depression their stress tended to rise significantly.[16,22,23]

In 2013, Rahman and her colleagues had conducted a cross-sectional study on preclinical medical students at UniSZA. The study revealed a very high prevalence of stress; a total of 78.3% of students might be having stress related/associated problems. Several stressful causes have been measured, and the primary cause of stress was their academics.[18] Therefore, the results of the present study on the prevalence of stress (31.4%) can be used as an indicator for the improvement of the medical education system and facilities in UniSZA. It can be used to solve the possible academic difficulties that increase stress among preclinical medical students.

In the current study, the DASS-21 questionnaire was used because it is a well-validated and reliable instrument, which requires less time to administer. Moreover, a previous study showed its superiority and improved consonance compared to the full-scale version (DASS-42).[32] A study conducted among preclinical medical students of Universiti Putra Malaysia (UPM) using a similar DASS-21 questionnaire, reported that the prevalence of stress, anxiety, and depression was 16.9%, 52%, and 24.4%, respectively.[22] The prevalence of stress, anxiety, and depression (31.4%, 77.1%, and 41.0%, respectively) in the present study was higher than in the UPM study findings. Another similar study was conducted on preclinical medical students at Suranaree University of Technology, Thailand. The prevalence of stress, anxiety, and depression was 5.6%, 25.7%, and 10.3%, respectively.[23] These findings were lower than the findings in the current study, which prompted the researchers to carefully contemplate the seriousness of the issue as it can later reflect in the students' performance, and their mental and physical health.

Human studies have encountered difficulty in exploring the association between stress and the *BDNF* gene polymorphism, as well as the structural and molecular mechanisms implicating this association due to the complicated genetic background of subjects and dependence on self-report questionnaires to estimate emotional status. Globally, this study is the first to establish the association between the *BDNF* gene (Val66Met) polymorphism and stress levels among medical students. In this cross-sectional, comparative study, the genotypic and allelic frequencies of the *BDNF* gene

(Val66Met) polymorphism were successfully determined and associated with stress levels in preclinical medical students at UniSZA. Furthermore, to date, we have not found any report on the association between genes and stress levels among the Malaysian population.

Our results showed that perceived stress levels among individuals with any one of the three *BDNF* genotypes (Met/Met, Val/Met, and Val/Val) differed significantly, $F(2,102) = 4.84$ ($p = 0.010$). A post-hoc Bonferroni-adjusted pairwise comparison suggested that students with the Val/Val genotype perceived significantly lower stress ($M = 10.6$, $SD = 6.87$) than students who carried the Val/Met ($M = 14$, $SD = 5.2$, $p = 0.049$) and Met/Met ($M = 15.1$, $SD = 6.28$ and $p = 0.022$) genotypes. This showed that those with the Val/Val genotype generally perceived significantly lower stress than those with the Met/Met genotype, but average stress perception between those with the Val/Met and Met/Met genotypes did not differ significantly (Table 7).

In this study, increased stress levels were significantly associated with the Met-allele in the *BDNF* gene Val66Met polymorphism (Table 7), and our findings were consistent with those from eight studies, based on a meta-analysis of 22 studies involving a total of 14,233 participants.[33] The analysed studies provided evidence of a significant association between the Met-allele and increased stress levels.[33] Furthermore, other studies showed that subjects with the Val-allele showed lower levels of stress, which was also in line with this study.[34,35]

In general, *BDNF* gene (Val66Met) polymorphism is a potential risk variant. Several associations with the Val66-Met polymorphism might be due to the various haplotypic backgrounds, in addition to the different interactions between the *BDNF* gene (Val66Met) polymorphism and other environmental or genetic features that might differ among ethnic groups.[36,37]

The reported associations among different ethnic groups may be due to various reasons. A large *BDNF* allele and haplotype diversity was reported among populations globally, and the Met-allele frequencies ranged from 0 to 72% in the different populations,[37] but studies on differences in the *BDNF* gene (Val66Met) polymorphism among different Malaysian ethnicities are still rare and require more attention. To date, there is no data reported for Malaysian population in the most common or global databases, such as dbSNP-NCBI (Table 9).

Till date, there is insufficient and unclear evidence on the *BDNF* gene (Val66Met) polymorphism among Malaysian ethnic populations. This study, through the chi-squared test of independence and path model, showed a significant association between the students' ethnicity and the *BDNF* genotype ($p = 0.027$); Indian students were significantly associated with the Val/Val genotype ($p = 0.007$), whereas Malay students were less likely to have the Val/Val genotype, but more likely to have the Met/Met genotype compared to their Indian counterparts (Table 7).

Although published studies on the *BDNF* gene (Val66-Met) polymorphism in the Malaysian population are lacking, two previous studies were conducted on Malaysian subjects. The most frequent genotype and heterozygosity indices of the *BDNF* gene (Val66Met) polymorphism among different Malaysian ethnic groups, as described by the two studies, are listed in Table 10.

Sim and his colleagues conducted a study with the aim of relating the *BDNF* gene (Val66Met) polymorphism with methamphetamine dependence in the Malaysian population.[38] The study found out that the most frequent genotype in Malay subjects was Val/Met which is in line with our findings, and their heterozygosity index was higher than the heterozygosity index obtained in this study (Table 10). Another study aimed to associate the *BDNF* gene (Val66Met) polymorphism with overweight or obesity in Malaysian adolescents.[39] The study found out that the most frequent genotype in Malay subjects was Val/Val, which is not consistent with our findings, but the heterozygosity index derived is similar with that derived in this study. Moreover, Indian subjects were more likely to carry the Val/Val genotype with 27% heterozygosity index, which is in line with our current findings.

However, both studies failed to prove a significant difference between the genotypes or allele frequency of the *BDNF* gene (Val66Met) polymorphism in different Malaysian ethnicities. This study has shown that Malay subjects are more likely to carry the Met-allele compared to Indian subjects, but this insight requires extensive research with a larger sample size to represent the Malay ethnic group.

Limitations of study

Only a self-administered questionnaire (DASS-21) was used to determine stress levels, and there were no objective measurements (clinically) in this study, which could lead to potential error; if one or more questions was misread or improperly answered, the results could be skewed. Furthermore, it would have been better to measure both BDNF mRNA and protein expression levels than only analysing the *BDNF* gene (Val66Met) polymorphism.

Conclusion

The current study showed that the prevalence of stress among preclinical medical students at UniSZA was within acceptable levels compared to the stress levels reported in previous studies. Val/Val was the most common genotype and the Val-allele was the most common allele in the *BDNF* gene (Val66Met) polymorphism of the enrolled students. However, the Met-allele was associated with a higher stress level, and the Val-allele, with a lower stress level. The findings of this study are essential for building a causation model for the different stress levels among a group of people facing similar stressful events (preclinical medical students), based on the Val66Met polymorphism in the *BDNF* gene. To the best of our knowledge, this is the first study investigating these variables simultaneously in medical students. The data generated from this study will help draw the attention of investigators to focus more on the role of the putative gene associated with stress responses. Considering the important role of the BDNF protein in the brain and the functional effect of the common *BDNF* gene Val66Met polymorphism, this polymorphism is one of the most studied polymorphisms in neuropsychiatric disorders. However, genetic studies have been unable to replicate data consistently. Neuropsychiatric disorders are complex

disorders that depend on several genetic and environmental factors, therefore they cannot be analysed by a conventional genetic association study.

Recommendations

Future studies should analyse the *BDNF* gene (Val66Met) polymorphism together with potential exogenous factors, which could be related with these disorders, via computational methods, such as machine learning techniques/algorithms, to unravel the potential effect of the *BDNF* gene on these disorders.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

This study was approved by the UniSZA Human Research Ethics Committee (UHREC), reference number: UHREC/2017/3/003. Complete useful information about the purpose of the study was provided to the participants, and informed consent was obtained to use their data for research purposes.

Authors contributions

MAIA was responsible for conceptualisation, methodology, validation, formal analysis, investigation, and writing the original manuscript draft. TMARH was responsible for conceptualisation, supervision, and resources. WRWT was responsible for data interpretation and writing (reviewing and editing). II was responsible for conceptualisation, supervision, and writing (reviewing and editing). All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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