BDNF val66met genotype is not associated with psychological distress: A cross-sectional study in Indonesian young adults

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Research article

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Abstract

Background

The number of mental disorders has been increasing but has yet to receive sufficient attention. Healthcare students and professionals tend to have high stress burden. Finding the root cause of psychological distress is important to formulate a method for early detection. The association of BDNF val66met polymorphism to neuropsychiatric disorders has been widely studied. The aim of this study was to interplay between BDNF val66met polymorphism and sociodemographic factors in the pathogenesis of psychological distress among Indonesian students.

Methods

Level of psychological distress and sociodemographic profiling was assessed using the Kessler Psychological Distress Scale (K10) and sociodemographic questionnaires, respectively. Genotyping was performed using polymerase chain reaction-amplified refractory mutation system. Pearson's chi square and binomial logistic tests were used to evaluate the correlation.

Results

This study recruited 148 participants. The psychological distress levels of the participants were well (27.03%), mild (37.16%), moderate (25.00%), and severe (10.81%). Genotypic distributions were AA (25.67%), GA (50.68%), and GG (23.65%). No statistical significance was found in the study ($p > 0.05$).

Conclusion

Psychological distress is not affected by genotypic and environmental factors. Further confirmatory research with larger and broader populations is required.

Background

Steel et al. reported a lifetime prevalence (nearly 30%) of mental disorders (substance use, anxiety, and mood) among adults in 59 countries [1]. The severity of mental disorders has prompted public awareness regarding the importance of mental health; in fact, mental health is highlighted in Sustainable Development Goals. The distinct long-term manifestations of mental disorders are depression and anxiety. Therefore, finding an approach to measure mental health condition predictively is crucial [2]. Psychological distress is a common indicator of mental health in epidemiological and clinical studies [3]. Self-administered or clinician-administered standardized scales, such as the general health questionnaires (GHQ-12,-20,-28,-30), the Kessler scales (K-6,-10), and the symptom checklists (SCL-5,-25; BSI-18), are regularly used to assess psychological distress [4–7]. The various tools and scales of measurement render the prevalence of psychological distress hard to determine, with an approximate estimation of around 5–27%. Factors affecting psychological distress are categorized as inborn and external. Typical inborn psychological distress factors include age, gender, ethnicity, and other sociodemographic factors. External factors, such as experiences, social behavior, income, and occupations, are widely varied among individuals [3]. In particular, work-related burden or occupational stress is associated with mental and medical disorders [8].

Psychological distress affects the neuronal plasticity on brain regions, such as the prefrontal cortex, the hippocampus, and the amygdala, thus altering cognitive processes, such as mood, emotion, learning, and memory [9, 10]. Brain-derived neurotrophic factor (BDNF) is an abundant growth factor in the central nervous system is. It is highly influential in mental

disorders because of its critical roles in neuronal development and plasticity [11]. Reduced mRNA and protein expression levels of hippocampal BDNF have been found in depressive animal and human postmortem studies [12]. Genetic polymorphisms of BDNF (G196A, Val66Met, dbSNP: rs6265) result in the substitution of valine (val) to methionine (met), which modifies the secretion of BDNF and consequently affects mental health [13, 14]. Despite its importance, the interaction between BDNF val66met polymorphism and sociodemographic profile in psychological distress has yet to be studied in developing countries, such as Indonesia. In the present study, we aim to (1) determine the allele and genotype distribution, (2) analyze the association between BDNF val66met polymorphism and psychological distress, and (3) analyze the correlation between sociodemographic factors and psychological distress to evaluate the psychological distress gene environment interaction in an Indonesian student population.

Methods

Study design and ethical considerations

This cross-sectional study was approved by the Board of Ethics, Universitas Padjadjaran (727/UN6.C2.1.2/KE PK/PN.2014) and conducted in accordance with the Declaration of Helsinki. All participants were informed about the study and signed informed consent prior to their participation into the study.

Participants

Recruitment was done by using public notice boards in Universitas Padjadjaran, Sumedang, Indonesia. Healthy young adults aged 18–35 years were eligible to participate in this study. Subjects with a history of mental disorders were excluded.

Phenotype

Subjects were asked to fill in the Kessler Psychological Distress Scale (K10) and sociodemographic questionnaires. The K10 questionnaire was translated, validated, and categorized into well (< 20), mild (20–24), moderate (25–29), and severe (> 29) stress. The sociodemographic questionnaire comprised of questions regarding gender, GPA, housing, and sources of funding for living and tuition fee.

Genotype

DNA was extracted from blood samples using the Purelink Genomic DNA mini kit (Invitrogen®). The quality of DNA was checked using WPA Lightwave II (Biochrom®). Polymorphism genotyping was performed through polymerase chain reaction-amplified refractory mutation system (PCR-ARMS) analysis as described by Sheikh et al. [15]. This genotyping method utilized tetra-primer (Sigma Aldrich, Singapore) consisting of two forward and two reverse primers (Table 1). PCR was conducted using PCR Supermix from Invitrogen® comprising 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM Magnesium Chloride, 220 μM dGTP, 220 μM dATP, 220 μM dTTP, 220 μM dCTP, 22 U/mL recombinant Taq DNA Polymerase, and dan stabilizers. Amplifications were performed using thermocycler (Biometra®) under the following conditions: pre-incubation at 94 ℃ for 5 min, followed by 40 cycles of denaturation at 94 ℃ for 45 s, annealing at 59.8 ℃ for 1 min, extension at 72 ℃ for 1 min, and a final extension at 72 ℃ for 5 min. The products were visualized using 2% agarose gel electrophoresis under 312 nm wavelength (Biometra®). The amplicons were 203 bp (A allele/Met), 253 bp (G allele/Val), and 401 bp (internal control).

Table 1
Tetra primers in BDNF val66met polymorphism analysis

Primers	5′ -> 3′ Sequence
P1 (forward)	CCTACAGTTCCACCAGGTGAGAAGAGTG
P2 (reverse)	TCATGGACATGTTTGCAGCATCTAGGTA
P3 (forward - A allele specific)	ATCATTGGCTGACACTTTCGAACCC**A**
P4 (reverse - G allele specific)	CTGGTC CTCATCCAACAGCTCTTCTATAA**C**

Statistical analysis

Psychological distress was divided into two categories, well and stress. The association between BDNF val66met genotype and psychological distress was analyzed using Pearson's chi-square test, whereas the correlation between sociodemographic factors and psychological distress was evaluated using the generalized linear model with binomial logistic as the model. Deviation of allele frequencies was computed using Hardy–Weinberg equilibrium (HWE). All analyses were conducted using IBM-SPSS version 24.0 (IBM SPSS Statistics, USA, 2016). Statistical significance was set at $p < 0.05$.

Results

Variation of Kessler Psychological Distress Scale (K10) questionnaire result

A total of 148 participants were recruited. The translated version of the K10 questionnaire was tested for its validity and reliability. Results indicated that the translated K10 questionnaire was valid ($r > 0.338$) and reliable (Cronbach's alpha coefficient > 0.80). Forty participants (27.03%) were categorized as well, 55 participants (37.16%) as mild stress, 37 participants (25%) as moderate stress, and 16 participants (10.81%) as severe stress (Table 2).

Genotyping of BDNF val66met and its correlation with the K10 questionnaire

Tetra-primer ARMS genotyping generated three bands for the control amplicon (401 bp) and G and A allele-specific bands (253 and 201 bp, respectively) (Fig. 1). Homozygous samples showed only the control and the G or A allele-specific bands, whereas heterozygous samples showed all three bands. Thirty-eight participants (25.67%) were homozygous AA, 75 participants (50.68%) were heterozygous AG, and 35 participants (23.65%) were homozygous GG. The genotype frequencies were consistent with HWE as shown by $p > 0.05$ (Table 3).

Table 2
Psychological distress and sociodemographic characteristics of participants

Characteristics		
		mean ± SEM
Age		21 ± 0.046
		n (%)
Psychological distress level	Well	40 (27.03)
	Mild	55 (37.16)
	Moderate	37 (25.00)
	Severe	16 (10.81)
Gender	Male	35 (23.6)
	Female	113 (76.4)
Accommodation	Boarding house	99 (66.9)
	Home with parents	44 (29.7)
	Both	5 (3.4)
Source of living allowance	Parents	114 (77.0)
	Scholarship	24 (16.2)
	Both	10 (6.8)
Source of tuition fee	Parents	130 (87.8)
	Scholarship	10 (6.8)
	Both	8 (5.4)
GPA	< 3.0	15 (10.1)
	3 0 ≤ GPA < 3.5	106 (71.6)
	≥ 3.5	27 (18.2)

Table 3
Allele and genotype distribution of BDNF val66met polymorphism

	n (%)
Allele	
A (met)	151 (51.01)
G (val)	145 (48.99)
Genotype	
AA (met/met)	38 (25.67)
GA (val/met)	75 (50.68)
GG (val/val)	35 (23.65)
Hardy-Weinberg equilibrium fulfilled with $p = 0.865$ (inconsistent with HWE if $p < 0.05$)	

BDNF val66met genotype and environmental factor interaction in psychological distress

No statistically significant association was found between BDNF val66met genotype and psychological distress. The correlation between sociodemographic factors was also not significant, except for the source of tuition fee in the female participants with $p = 0.049$ (Table 4).

Table 4
Association between genotype, sociodemographic factors and psychological distress.

	p	
Genotype and psychological distress		
Pearson's chi square test	0.076	
Sociodemographic factors and psychological distress		
	Male (n = 35)	Female (n = 113)
Accommodation	0.671	0.720
Source of living allowance	0.583	0.589
Source of tuition fee	1.000	0.049*
GPA	0.794	0.098

Discussions

The number of suicidal healthcare professionals, such as physicians, pharmacists, nurses, dentists, and veterinarians, is increasing [16]. Pharmacy ranked top three among other healthcare professional degree students in terms of psychological distress and depression as assessed by using GHQ-12 and Beck Depression Inventory-II [17]. The psychological distress level in our study supports pharmacy as one of the top stressful healthcare degrees and professions, with nearly 75% of the students found psychologically distressed. Current results also aligned with previous findings showing no significant correlation between psychological distress and sociodemographic factors, such as accommodation, GPA, source of living allowance, and tuition fee [18].

Our genotyping substantiated the variation of BDNF val66met genotype in different ethnicities, such as Asians and Caucasians [19]. Several neurodegenerative diseases studies also showed similar genotypic distribution of BDNF val66met polymorphism with Asian populations having higher heterogenous Val/Met genotype than homogenous genotypes [19–22]. Our finding suggests that BDNF val66met polymorphism and sociodemographic factors do not influence the pathogenesis of psychological distress.

This insignificant correlation is possibly due to the limitations of our study. First, our study is limited by the number of participants (also imbalanced between gender) and the population of participants, which are primarily highly stress-burdened pharmacy students [16, 17]. Second, the psychological distress questionnaire only focused on the short-term period (the last 4 weeks). Thus, it does not measure the psychological distress over time. Last, the students are subject to response bias, particularly the reluctance to express their true level of psychological distress due to negative image and public judgement toward mental disorders. This finding is supported by a study about depression in medical students where nearly 10% of the participants admitted giving false responses in the questionnaire due to the abovementioned reason [23].

Despite the non-associative findings, our study is the first gene-environment interaction (GxE) study in Indonesia that focused on psychological distress. GxE studies are useful in the development of personalized medicine in preventive (diagnostic) and therapeutic approaches [24]. Up till now, Indonesia is one of the developing countries that has yet to create its own genomic database. The BDNF val66met genotype profile we obtained contributes not only to the genomic database of the Indonesian population but also to that of the Asian ethnicity. According to Indonesia's National Baseline Health Research report, in merely 5 years, the prevalence of emotional mental disorders increased nearly 4% from 6.0–9.8% among Indonesian citizens [25, 26]. Our study also highlighted the importance of increasing public awareness and early screening of mental issues, specifically among healthcare professions. We laid a basis for further research in GxE and provided suggestions that need to be considered in conducting mental health research.

Conclusions

In conclusion, we found no association between BDNF val66met polymorphism, sociodemographic factors, and psychological distress among Indonesian students. Further research with a larger gender-balanced and broader population of participants must be conducted to ascertain these findings. A tool to measure long-term psychological distress and the environmental factors affecting it must also be devised. The clinical significance of BDNF val66met polymorphism has been widely studied for more than a decade in neurodegenerative and neuropsychiatric disorders. To establish rs6265 as a marker for mental health and neuro-disorders, studies should consider BDNF protein level, brain MRI and fMRI on brain structures in association with the val66met polymorphism [27, 28].

Abbreviations

BDNF
Brain-derived neurotrophic factor
K10
Kessler Psychological Distress Scale
PCR-ARMS
Polymerase chain reaction-amplified refractory mutation system
HWE
Hardy–Weinberg equilibrium
GPA
Grade point average
GxE

Gene-environment interaction
Val
Valine
Met
Methionine

Declarations

Availability Of Data And Materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

This cross-sectional study was approved by the Board of Ethics, Universitas Padjadjaran (727/UN6.C2.1.2/KE PK/PN.2014) and conducted in accordance with the Declaration of Helsinki. All participants were informed about the study and signed informed consent prior to their participation into the study.

Consent for publication

Not Applicable

Competing Interests

The authors declare that they have no competing interests.

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Contributions

HN performed investigation and writing original draft. SDA performed statistical analysis, RA performed supervision of writing original draft, MIB designed the methodology, performed supervision and reviewed the writing original draft. All authors read and approved the final manuscript.

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Figures

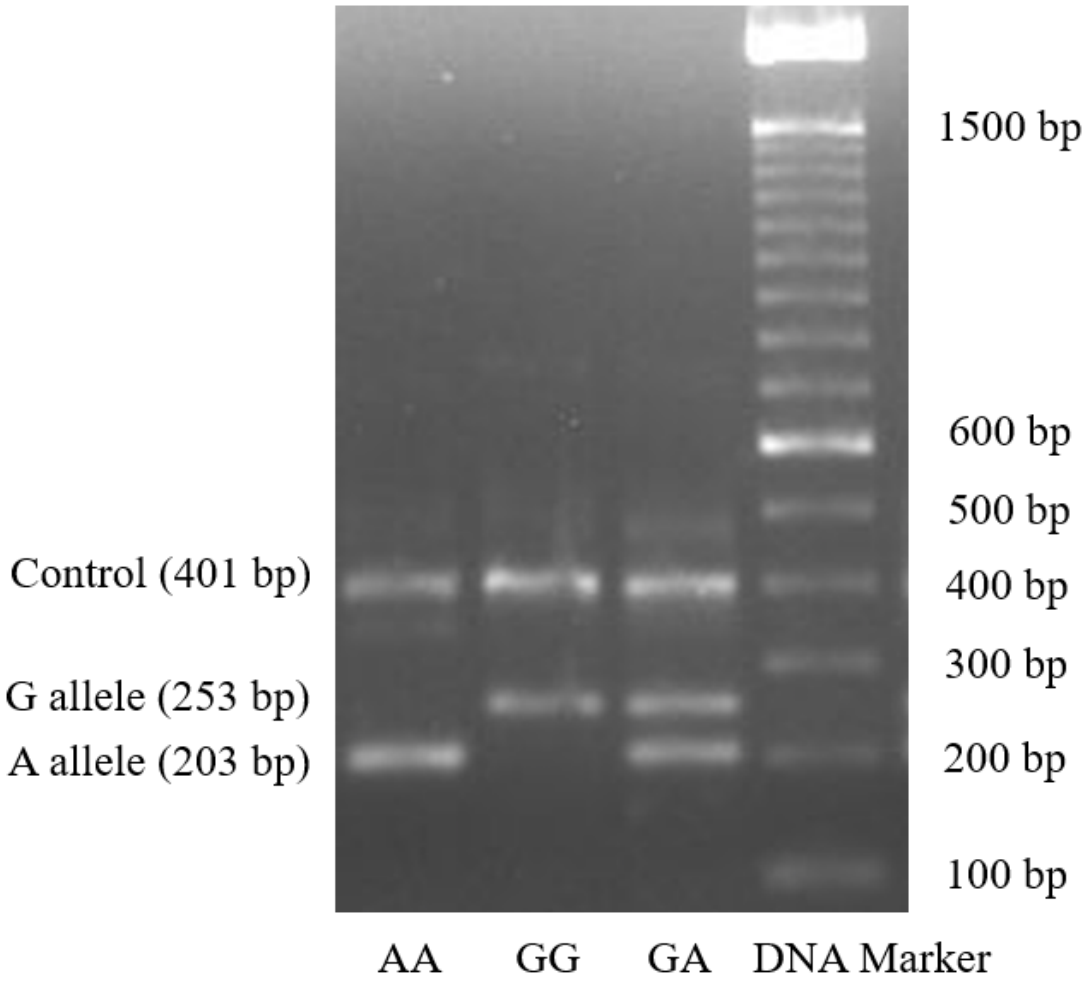

Figure 1

UV visualization of BDNF val66met polymorphism. The PCR-ARMS method resulted in three band namely the control (401 bp), G and A allele specific amplicons (253 and 201 bp).