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

Henry Ng, MMSc[a], Sofa Dewi Alfian, PhD[b,c], Rizky Abdulah, PhD[b,c], Melisa I. Barliana, PhD[a,c,*] iD

Abstract

The number of mental disorders has been increasing but has yet to receive sufficient attention. In particular, 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 and prevention. The association of brain-derived neurotrophic factor val66met polymorphism to neuropsychiatric disorders has been widely studied. To study the interplay between brain-derived neurotrophic factor val66met polymorphism and sociodemographic factors in the pathogenesis of psychological distress among Indonesian Pharmacy students. Level of psychological distress and sociodemographic profiling was collected by using the Kessler Psychological Distress Scale 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. 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 between genotype and psychological distress was found in the study (P = .076). The sociodemographic factors also showed non significance, except for the source of tuition fee among women students (P = .049). Psychological distress is not affected by genotypic and sociodemographic factors. Further confirmatory research with larger and broader populations is required.

Abbreviations: BDNF = brain-derived neurotrophic factor, GPA = grade point average, GxE = gene–environment interaction, HWE = Hardy–Weinberg equilibrium, K10 = Kessler Psychological Distress Scale, Met = methionine, Val = valine.

Keywords: amplified refractory mutation system, BDNF, K10, psychological distress, rs6265, val66met

1. Introduction

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% to 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 (GxE) in an Indonesian Pharmacy student population.

2. Methods

2.1. 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.

2.2. Participants

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

2.3. 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 translated K10 questionnaire was validated twice with different subjects and an interval of 40 days (first validation on October 13; second validation on November 24). The sociodemographic questionnaire comprised of questions regarding gender, grade point average (GPA), housing, and sources of funding for living and tuition fee.

2.4. 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 analysis as described by Sheikh et al.[15] This genotyping method utilized tetra-primer (Sigma Aldrich, Singapore) consisting of 2 forward and 2 reverse primers (Table 1). PCR was conducted using PCR Supermix from Invitrogen (Cat No. 10572-014) with the final concentrations of the agents in one reaction (45 μL Supermix, 0.8 μL primers and 4.2 μL DNA template) are as follows: 19.8 mM Tris-HCl (pH 8.4), 49.5 mM KCl, 1.485 mM magnesium chloride, 198 μM dGTP, 198 μM dATP, 198 μM dTTP, 198 μM dCTP, 0.99 U recombinant Taq DNA Polymerase, and stabilizers. Amplifications were performed using thermocycler (Biometra) under the following conditions: pre-incubation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 45 s, annealing at 59.8°C for 1 minutes, extension at 72°C for 1 minutes, and a final extension at 72 °C for 5 minutes. 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).

2.5. Statistical analyses

Psychological distress was divided into 2 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 adjusting for gender, GPA, accommodation, source of living allowance, source of tuition funding, genotype, and age. Deviation of allele frequencies was computed using Hardy–Weinberg equilibrium (HWE). All analyses were conducted using IBM-SPSS version 24.0 (IBM SPSS Statistics, 2016). Statistical significance was set at $P < .05$.

3. Results

3.1. Validation of Kessler Psychological Distress Scale (K10) questionnaire

A total of 148 participants were recruited (35 men and 113 women). The translated version of the K10 questionnaire was tested for its validity and reliability. Results indicated that the translated K10 questionnaire was valid (2-tailed Pearson correlation $r > 0.338$) 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).

3.2. Genotyping of BDNF val66met and its correlation with the K10 questionnaire

Tetra-primer amplified refractory mutation system genotyping generated 3 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 3 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 $P = .865$ (Table 3).

3.3. BDNF val66met genotype and sociodemographic factors interaction in psychological distress

No association was found between BDNF val66met genotype and psychological distress ($P = .076$). Sociodemographic factors were also not associated with psychological distress in men participants. Meanwhile, for the women participants, the source of tuition fee was correlated with the psychological distress with $P = .049$ (Table 4). Women participants with the source of tuition

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**

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	Men	35 (23.6)
	Women	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		15 (10.1)
	3 0 = GPA < 3.5	106 (71.6)
	=3.5	27 (18.2)
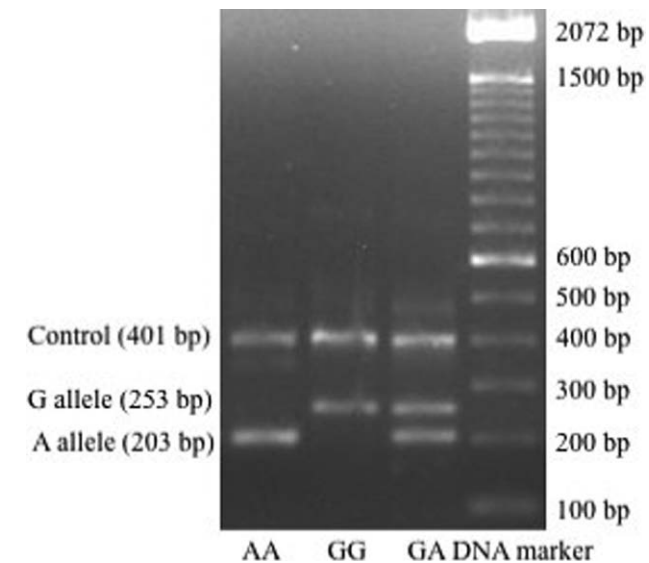
Figure 1. UV visualization of BDNF val66met polymorphism. The polymerase chain reaction-amplified refractory mutation system method resulted in 3 band namely the control (401 bp), G and A allele specific amplicons (253 and 201 bp).

Table 3

Allele and genotype distribution stratified by genders of BDNF val66met polymorphism.

Allele	n (%)		
A (Met)	151 (51.01)		
G (Val)	145 (48.99)		
Genotype	n (%)	Men, n (%)	Women, n (%)
AA (Met/Met)	38 (25.67)	12 (34.40)	26 (23.00)
GA (Val/Met)	75 (50.68)	17 (48.60)	58 (51.30)
GG (Val/Val)	35 (23.65)	6 (17.10)	29 (25.70)

Hardy–Weinberg equilibrium fulfilled with P value of .865 (inconsistent with HWE if P value <.05)

fee from scholarships and parents are more stressful compared to those fully funded by parents with $P = .035$ (Supplementary Table 1, http://links.lww.com/MD/G761). Noteworthy, low GPA was also a factor that potentially affect the psychological distress in women participants with $P = .098$ (Table 4). Specifically, GPA lower than 3.0 was associated with higher psychological distress with $P = .040$ (' Table 1).

4. Discussion

The number of suicidal healthcare professionals, such as physicians, pharmacists, nurses, dentists, and veterinarians, is increasing.[16] Pharmacy ranked top 3 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] Sociodemographic factors have been suggested to contribute to the level of psychological distress as shown by other studies focusing on medical students.[18–20] Therefore, we focus on studying the interplay between genetic polymorphism, sociodemographic factors, and the level of psychological distress in Indonesian Pharmacy students. By comparing the psychological distress level of students from the same year, our study supports pharmacy as one of the top stressful healthcare degrees and professions, with nearly 75% of the students found psychologically distressed. On the other hand, our findings suggest that BDNF val66met polymorphism and sociodemographic factors do not influence the pathogenesis of psychological distress.

Despite the non-significance, our genotyping effort substantiated the variation of BDNF val66met genotype in different ethnicities, where Asians are dominated by heterozygous GA genotype compared to and homozygous GG genotype in Caucasians.[21] 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.[21–24]

Table 4

Association between genotype, sociodemographic factors, and psychological distress.

	P value	
Genotype and psychological distress		
Pearson's chi square test	.076	
Sociodemographic factors and psychological distress		
	Men (n = 35)	Women (n = 113)
Accommodation	.671	.720
Source of living allowance	.583	.589
Source of tuition fee	1.000	.049*
GPA	.794	.098
Genotype	.810	.924
Age	.595	.204

5. Strengths

Despite the non-associative findings, our study is the first 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.[25] 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% to 9.8% among Indonesian citizens.[26,27] 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.

6. Limitations

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.[28]

7. Conclusions

We found no association between BDNF val66met polymorphism, sociodemographic factors, and psychological distress among Indonesian Pharmacy 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.[29,30]

Author contributions

RA and MIB were designed and developed the study; HN responsible for data collection; HN and SDA were responsible for data analysis and curation; HN was responsible for drafting the original manuscript; RA, MIB and HN were responsible for writing, review and editing; RA and MIB were supervised and did final approval of the submitted manuscript.

Conceptualization: Melisa Barliana, Rizky Abdulah.
Data curation: Henry Ng.
Formal analysis: Henry Ng, Sofa Alfian.
Investigation: Henry Ng.
Methodology: Melisa Barliana.
Supervision: Melisa Barliana, Rizky Abdulah.
Writing – original draft: Henry Ng.
Writing – review & editing: Henry Ng, Melisa Barliana, Rizky Abdulah.

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