Genotyping of Opioid Receptor Mu 1 (*OPRM1*) A118G Polymorphism in Indonesian Drug Addicts

Alvina Arum Puspitasari[1], Zullies Ikawati[2]*, Sri Swasthikawati[3], Anindya Rahmawati[1]

[1]Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.

[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia.

[3]Research Centre for Biotechnology, Indonesian Institute of Science, Bogor, Indonesia.

*Corresponding Author E-mail: **zullies_ikawati@ugm.ac.id, ikawati@yahoo.com**

ABSTRACT:

Opioid receptor mu-1 (*OPRM1*) A118G gene polymorphism has been widely studied as a strong candidate gene for increasing risk of drug addiction. However, the association is still controversial concerning the effect of A118G polymorphisms on drug addiction seems to be different inter-ethnic. The prevalence of *OPRM1* A118G polymorphism is found to be low in the European American population but high in the Asian population. To date, there are very limited reports on this polymorphism in the Indonesian population and its association with drug addiction. Here, we carry out the genotyping of *OPRM1* A118G polymorphism in Indonesian population using amplification refractory mutation system polymerase chain reaction (ARMS-PCR) method. The result shows that the *OPRM1* A118G polymorphism is relatively higher compared with other Asian countries. When comparing the frequency of the genotype and allele between the normal (n = 83) and the drug-addicted (n = 83) subjects, no significant difference in prevalence was found in neither genotype (p = 0.066, 95% CI [confidence interval]) nor allele (p = 0.432; 95% CI) between the groups. Our results suggest that there is no direct effect of *OPRM1* A118G gene polymorphism on the risk of drug addiction (including stimulants, marijuana, and benzodiazepine) among Indonesian; instead, social factors might play a key role.

KEYWORDS: *OPRM1*, Opioid Receptor, Drug Addiction, Gene Polymorphism, Nested-ARMS PCR.

INTRODUCTION:

Drug addiction is known as a chronic, relapsing disorder that mainly characterized by compulsive drug seeking and use despite adverse consequences[1]. It also can be marked by the expressions of dysfunctions within specific circuits and regions[2]. Drug addiction can lead to the same effect as other mental disorder on the mental wellbeing that they become deprived of mental and physical control or assessment of their own actions[3].

Various etiologies of drug addiction have been studied, that said, it is occurred from complex combination of environmental and genetic factors[4]. Studies showed that the risk of developing drug addiction is significantly influenced by genetic factors with heritability estimates of around 30%–70%[5,6].

Gene polymorphisms have been extensively studied in relation with various diseases, such as cysteine leukotriene receptor 2 (*CyLTR2* M01 V) and *IL-10* C-597A gene in asthma[7,8], glutathione-s-transferase M1 and T1 gene[9,10] and tumor necrosis factor-308 (*TNF-308*) gene[11] with various types of cancer, and *IL-10*-1082A\G gene in thalassemia[12]. Not only physical health disorder, studies has also revealed the association of gene polymorphisms with various mental disorders. Study by Maddhuri et al. has revealed that *PPP3R1* (CnB 5I/5D) gene polymorphism is associated with mental retardation (intellectual disability)[13], while for drug addiction, opioid receptor mu-1 (*OPRM1*) gene, which encodes the mu-opioid receptor, has said to be one of the most studies gene for the case[14].

A study by Hancock et al. (2015) concludes that the *OPRM1* gene has been associated with heroin addiction in European Americans[15]. Another study also suggests that polymorphism in *OPRM1* variant, A118G, contributes to the mechanism of addiction liability that is shared across different addictive substances, including alcohol, opioid, cannabis, cocaine, and nicotine[16] and amphetamine[17]. Molecularly, the minor allele (118G) of *OPRM1* is known to be less physiologically functional. This might be related to the diminished mRNA and protein levels in some brain regions of individuals who are 118G carriers[18]. The G allele is the minor allele across multiple human populations, with frequencies ranging from 4% in African American samples to ~16% in European-ancestry samples and to over 40% in some Asian samples[16]. Therefore, it can be seen that the prevalence of *OPRM1* A118G polymorphism is found to be low in the European American population but high in the Asian population.

The individuals having G (glycine) instead of A (adenine) show increased susceptibility to addiction, as shown by a study in the Asian population[19]. On the contrary, European ancestors who are carriers of 118G have a modest protective effect on general substance dependency[16]. Meanwhile, in a large Bulgarian case-control study conducted by Nikolov et al. (2011), it has been stated that A118G allele failed to show evidence of an association between the polymorphism and addiction[20]. Hence, the effect of A118G polymorphisms on drug addiction remains unclear and seems to be ethnic specific.

In Indonesia, drug addiction cases in 2019 have increase 0.03 % from the previous year, reaching more than 3.4 million cases[21]. The most drugs abused are stimulant drugs like amphetamine derivatives, cannabis, and depressants. Genetic variability in the *OPRM1* μ-opioid receptor gene is also reported to influences the subjective effects of amphetamine[17]. To date, there are very limited reports on this polymorphism in the Indonesian population and its association with drug addiction. Despite the variation of drugs abused in Indonesia, it is of interest to study the prevalence of *OPRM1* A118G gene polymorphism in the Indonesian population and to investigate its relation to drug addiction in young adults, since drug abuse in Indonesia mostly occurs in young adults.

MATERIAL AND METHODS:

Participants:

Eighty-three drug-addicted male patients and 83 non-addicted control subjects (male) participated in this research. All participants were in the range of 18-48 years of age. The addicted subjects were undergoing rehabilitation treatment in the National Narcotic Board Rehabilitation Center (NNBRC). The control subjects (non-addicted) of this study were selected among indigenous Indonesian from around Yogyakarta area, who had been declared healthy by clinician, fulfilled the inclusion criteria, and were willing to be involved in research. They were recruited by posting notices. Participants who had any history of cancer and nuclear or mitochondrial DNA-related diseases that may affect the DNA, were excluded from this study. Both of drug addict patients and control subjects participated in this study were provided written informed consent prior the enrolment. All participants were interviewed extensively for drug abuse (e.g., marijuana, methamphetamine, and 3,4-methylenedioxymethamphetamine [MDMA], etc), addictive diseases, medical profile, as well as family origin and ethnic background of study subjects and their ancestors. Blood sample collection was then performed for genotyping analysis of *OPRM1* polymorphism. The study was conducted after obtaining ethical clearance from the Ethics Commission of Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Instrumentation:

The proposed work was carried out with Applied Biosystems Veriti™ 96-well Thermal Cycler (Foster City, California, USA) to perform PCR. Other laboratory instruments including EDTA tubes, one set micropipette (Eppendorf®) (1-10 µl, 2-20 µl, 20-200 µl, 200-1000 µl), microcentrifuge tube and rack (Eppendorf®), QIAamp® DNA blood mini purification kit (Qiagen, Hilden, Germany), biochrom spectrophotometers (NanoVue Plus™), gel electrophoresis system (Mupid-2plus®), personal microcentrifuge (Cole-Parmer), vortex (Thermo Scientific), centrifuge (Eppendorf® 5424 R), and gel documentation system (G Box Chemi XRQ, Syngene). As for non-laboratory instruments including notifications (calls for participants), participant inform consent forms, and personal data forms.

ARMS-PCR Genotyping of *OPRM1* A118G:

From all participants, 3 mL of venous blood was drawn by a vacutainer system directly into ethylene diamine tetraacetic acid (EDTA) tubes for DNA isolation and genotyping examination. Genomic DNA was isolated and purified using the QIAamp® DNA blood mini purification kit. Genotyping of the *OPRM1* gene is targeted at the A118G allele using a nested amplification refractory mutation system polymerase chain reaction (ARMS-PCR) technique based on a method performed once by Zahari et al. (2016)[22]. The first PCR was performed using a specific primer (Table 1) that amplifies the region of interest in the *OPRM1* gene, with the concentration of extracted genomic

Table 1: Allele-specific Polymerase Chain Reaction (PCR) Primers for *OPRM1* A118G Genotyping by ARMS-PCR

PCR	Primer	Sequence (5′-3′)	Fragment size (bp)	Primer concentration (µM)
First PCR	µEX1 FW	AAA GTC TCG GTG CTC CTG GCT	420	0.40
	µEX1 RV	TGG GAG TTA GGT GTC TCT TTG TA		0.40
Second PCR				
Wildtype primer	µ118A	CAA CTT GTC CCA CTT AGA TGG C**A**	267	0.25
Mutant-type primer	µ118G	CAA CTT GTC CCA CTT AGA TGG C**G**		0.25
Common primer	µEX1 RV	TGG GAG TTA GGT GTC TCT TTG TA		0.25

DNA used as a template is 10–20 ng. Then, it was followed by second PCR using allele-specific primers to differentiate single nucleotide polymorphism (SNP) in the allele of interest. The mixture processed under cycling conditions as shown in Table 2. This two-step PCR method for *OPRM1* genotyping had been validated for reproducibility and specificity through direct sequencing. PCR was performed using ready-to-use PCR mix (My Taq™ HS Red Mix, Bioline). The obtained products were then electrophoresed in a 1.5% agarose in 1X Tris-borate-EDTA buffer.

Table 2: Cycling condition for *OPRM1* A118G Genotyping by ARMS-PCR

Cycle condition	Time	First PCR	Second PCR
Pre-denaturation	02:00 minute	94°C	94°C
Denaturation	00:30 minute	94°C	94°C
Annealing	00:30 minute	52°C	67°C
Extention	-	72°C – 01:00 minute	72°C – 00:45 minute
Final extention	07:00 minute	72°C	72°C
Total cycle	-	25	20

Statistical Analysis:

Prevalence analysis of *OPRM1* A118G gene polymorphism is conducted by calculating allele frequency proportions and checking for deviation with Hardy–Weinberg equilibrium (χ^2HW) as a second measure of quality control[23]. Comparison of genotype and allele distribution between the two groups in association with drug addiction was analyzed by chi-square test, and the risk of *OPRM1* A118G gene polymorphism toward drug addiction was examined by calculating the odds ratio (OR). The chi-square test and power calculation was set at 5% statistical significance. All statistical data were analyzed using the SPSS statistical package (IBM SPSS Statistics for Windows, Version 23.0).

RESULTS:

Participants Characterization:

The participants from both drug-addicted patients and non-addicted control subjects did not differ significantly in term of age (p = 0.058), which averaged between 26 and 28 years. Characteristics of the research subjects obtained in this study could be seen in Table 3.

Table 3: Demographic Characteristics of Participants

Characteristics	Drug-addicted N (%)	Control N (%)	*p value*
Age (mean ± SD)	28 ± 5	26 ± 5	0.058
Drug Abuse			<0.001
Stimulant	80 (68.4)	0 (0.0)	
Depressant	11 (9.4)	0 (0.0)	
Cannabis	26 (22.2)	0 (0.0)	
Family history of drug addiction			0.001
Yes	10 (12.0)	0 (0.0)	
No	73 (88.0)	83 (100.0)	
Smoking status			<0.001
Yes	82 (98.8)	2 (2.4)	
No	1 (1.2)	81 (97.6)	
Alcohol consumption			<0.001
Often (± once per day)	35 (42.2)	0 (0.0)	
Sometimes (± once per week)	9 (10.8)	0 (0.0)	
Seldom (less than once per month)	9 (10.8)	2 (2.4)	
Never	30 (36.2)	81 (97.6)	
Education level			<0.001
Non-college*	76 (91.6)	3 (3.6)	
College	7 (8.4)	80 (96.4)	
Employment status			<0.001
Unemployed	49 (59.0)	2 (2.4)	
Employed	30 (36.1)	57 (68.7)	
Student	4 (4.8)	24 (28.9)	

*Non-collage defined by subjects who have either elementary, junior high or senior high school as their latest level of education

The family history of drug addiction (p = 0.001), the smoking status, and the characteristics of alcohol consumption were significantly different (p<0.001) between the two groups, wherein the drug-addicted groups were found to be much higher. As for the education level, there is a significant association between drug addiction and education level (p<0.001) with only 8.4% of subjects in the drug-addicted group were in college level, whereas the others were in a non-college level. On the contrary, almost all non-addicted participants have a college level of education. Similar results were also found between employment status and drug addiction (p<0.001).

Distribution of *OPRM1* A118G Gene Polymorphism:

Genotype and allele distributions of *OPRM1* A118G in Indonesian population both in the drug-addicted patients and non-addicted control subjects are shown in Table 4. The results show a high frequency of A118G gene polymorphism in Indonesian population, with the total frequencies of A/G and G/G at 47.6% and 36.7%, respectively, whereas the wild type (A/A) was only found in 15.7% of the total population. Genotypes distribution of *OPRM1* A118G gene polymorphism examined in this study was in Hardy–Weinberg equilibrium with chi-square test result showed that the value of genotypes distribution of A118G observed in this study was equal with the expected values in the population (χ^2HW = 0.00).

Table 4: Genotype and Allele Distributions of the *OPRM1* A118G Polymorphism

Genotype/ Allele	Drug-addicted N (%)	Controls N (%)	Total N (%)	*p* value*
Genotype				
Wildtype (A/A)	11 (13.3)	15 (18.1)	26 (15.7)	0.066
Heterozygous (A/G)	47 (56.6)	32 (38.5)	79 (47.6)	
Homozygous	25 (30.1)	36 (43.4)	61 (36.7)	

mutant (G/G)				
Total	83 (100.0)	83 (100.0)	166 (100.0)	
Allele				
A	69 (41.6)	62 (37.3)	131 (39.5)	0.432
G	97 (58.4)	104 (62.7)	201 (60.5)	
HWE value**	χ^2_{HW} = 2.28	χ^2_{HW} = 2.58	χ^2_{HW} = 0.00	
Odds ratio	0.838 (95% CI 0.539 to 1.302)			

*p-value was analyzed by chi-square test 95%

**The χ^2_{HW} (chi-square test) value indicates the difference between expected and observed values for genotype based on Hardy–Weinberg equilibrium (HWE).

A slightly higher frequency of wild type (A/A) was found in non-addicted controls (18.1%) compared with that of in drug-addicted group (13.3%). However, no significant difference was found in neither genotype (p=0.066) nor allele (p=0.432) in comparison between groups. The study also showed that A118G allele polymorphism provides no significant risk to the development of drug addiction in this population (OR = 0.838; 95% CI 0.539–1.302; Table 4). The dominant and recessive models were also determined for the association, with an assumption that G allele is the high-risk allele (Table 5). However, neither dominant nor recessive model gave substantial association between A118G polymorphism and drug addiction (dominant model: OR=0.693, 95% CI 0.297–1.613, P=0.393; recessive model: OR=0.563, 95% CI 0.297–1.066, P=0.077). Both ORs showed no significant values as they both less than one.

Table 5: Genotype and Allele Distributions of the *OPRM1* A118G Polymorphism

Polymorphism	Genotype	Drug-addicted N (%)	Controls N (%)	Odds ratio	*p* value*
Dominant model	A/A	11 (13.3)	15 (18.1)	0.693 (95% CI 0.297	0.393
	G carriers (AG+GG)	72 (86.7)	68 (81.9)	to 1.613)	
Recessive model	G/G	25 (30.1)	36 (43.4)	0.563 (95% CI 0.297	0.077
	A carriers (AA+AG)	58 (69.9)	47 (56.6)	to 1.066)	

*p-value was analyzed by chi-square test 95%

When looking in more detail concerning the substance abused, there were 3 groups of substances which were commonly used; they were stimulants (amphetamine derivatives), cannabis, and depressants, as displayed in Table 5. When we analyzed the association of individual group of substance with the *OPRM1* A118G gene polymorphism in dominant model, we found no association in all type of substance. The same was found in recessive and codominant model analysis (table is not displayed).

DISCUSSION:

Previous research revealed that nicotine, alcohol, and marijuana use during two periods of adolescence is associated with a smaller gray matter volume in two brain areas at age 25 years. As the brain matures during adolescence (10–19 years old), it passes through stages in which it is particularly sensitive to substance exposure[24]. Therefore, young adults were chosen as the subject criteria in this study. All participants in this study were men, considering that gender differences could affect the results, as previous research reported that women have fewer addiction disorders and often show symptom at a later age than men[25].

The group's mean age (± SD) at first use was 19.8±4.8 years; therefore, this could mean that most of them used drugs above high school age (≥18 years old). Although this may also be influenced by bias in the process of recruiting control subjects since it was done around the campus area, the results were still similar to the results of a general social survey in the United States, which found a significant association between drug addiction and education level[26]. A longitudinal study in an African American population followed for more than 35 years by Fothergill et al. (2008) also showed that in adulthood, drug addiction was significantly more likely to develop in those who dropped out of high school or only had a high school degree compared with those who obtained a college degree[27]. The similar is true for Indonesian population, where the highest prevalence of drug abuser was those who only had elementary or junior high school degree and those who seek attention, as reported by the National Narcotic Board (Indonesia)[21].

An individual who moves from working to unemployment is known to experience an increase in odds of taking illegal drugs. This may be related to an urge to ease the pressure of financial difficulties posed by unemployment that affects families, and an increase in spare time accompanied by loss of employment or an increased personal contact with unemployed people[28]. Those might underlie the significant differences found in our study concerning the employment status between groups.

From various studies of *OPRM1* A118G gene polymorphism distribution worldwide, the frequency of 118G allele in Indonesian population tended to be the highest among that observed in other Asian population studies, reaching 60.5% of the population, followed by Malaysia with around 53.1% of the population[29]. Our earlier study in healthy non-addicted individuals involving female subjects showed similar results, which found that the 118G allele frequency was 60.4%[30]. This result is in accordance with the previous study reporting that this gene polymorphism was found to be low in African American and European populations but was high in the Asian population, which was over 40%[16]. Other Asian population showing relatively high frequency of 118G allele is Japanese, which reaches 45.3 %[31]. Tan et al (2003) have also studied the genotype and allele distribution for this gene between different ethnic groups and significantly high allele frequencies were found in Chinese (35.1%) and Indians (44.2%) groups[32]. Even though most Asian population showed high G allele frequency, Pakistani and Turkish populations were reported to have relatively lower G allele frequency than other Asians, which are 20%[33], and 12.6%[34], respectively. A study by Hofer et al. (2009) concluded that this strikingly different allele frequency between continents could result from demography rather than from positive selection or local adaptation[35]. Another study also stated that each ancestral population might contribute to unequal allele frequencies[36]. This could explain the prevalence similarities of this gene polymorphism found between Indonesian and Malaysian populations, since the two countries have similar demographics and ancestral characters.

This study compared the *OPRM1* A118G gene polymorphism in drug-addicted male young adults and non-addicted control subjects. However, we did not find any association between the *OPRM1* A118G gene polymorphism with drug addiction in our study under dominant and recessive models. Our finding is on the contrary with that studied by Turkan et al. (2019), confirming that there is association between the rs1799971 (G) allele frequency and opioid and other substance addiction[34].These dissimilar findings occurred might be due to the different frequency of G118 allele between the Turkish and Indonesian population, which was much lower in both addicted subjects (16.1 % vs 58.4%) and control subjects (8.4% vs 62.7%). The high frequency of G118 allele in Indonesian population may contribute to the lack of association of this polymorphism to the drug addiction. Another study in Pakistan also reported the significant association of SNP A118G with drug addiction, however, the substance abused in the study was specifically opioid, which was the main drug acting on μ opioid receptor[33]. In addition, the G118 allele frequency in healthy Pakistani population was relatively lower than in Indonesian population (14 % vs 62.7%).

Despite no association between the *OPRM1* A118G gene polymorphism with drug addiction under dominant and recessive models, our study showed a significant association between the *OPRM1* A118G gene polymorphism with the substance addiction under co-dominant model. In this model of inheritance, the disease risk associated with AG genotype (heterozygote) individuals lies between that of AA (wildtype homozygote) and GG (minor allele homozygote) individuals[37]. Therefore based on this model, our results implied that the presence of single minor allele (G) in heterozygote genotypes (AG) significantly increased the risk of addiction about two times than both homozygotes (AA and GG), which do not statistically differ between them, instead of the presence of single allele G (dominant model) or two copies of allele G (recessive model) in the association of increasing risk of addiction.

These findings were not in line with the previous researches which reported that there was a significant association between A allele with opioid dependence in Hispanic subjects[38] and heroin dependence in Indians[32]. Our results were also different with Tan et al. (2003) findings about G allele that has been indicated to be protective against heroin dependence in Indians[32]. These inconsistent results might suggest that the implication of *OPRM1* A118G gene polymorphism is varies among different populations. Previously, Schwantes-An et al. (2016) stated that *OPRM1* A118G gene polymorphism is associated with addiction susceptibility of different addictive substances[16]. Their study examined five specific substances, including alcohol, opioid, cannabis, cocaine, and nicotine and concluded that A118G contributed to the mechanisms of addiction liability that were shared across those different addictive substances. Aside from the difference in sample size, more than half of drug-addicted patients in our study (60.2%), had disorders due to the use of other drugs, including stimulants i.e. methamphetamine and ecstasy (MDMA), so it might contribute to the different from those examined by Schwantes-An et al. (2016)[16].

Although there is also a study supporting the important role of the μ-opioid receptor (MOR) in modulating the development of methamphetamine-induced behavioral sensitization via dopaminergic neurotransmission[39], molecularly, MORs do not directly affect the action of stimulant such as methamphetamine. Unlike opioid that directly binds to MOR as its primary sites of action[40], methamphetamine rather acts by increasing neuronal release of dopamine into the nucleus accumbens via alterations in both the dopamine transporter (DAT) and the vesicular monoamine transporter-2. Concurrent with reuptake inhibition, methamphetamine also induces dopamine efflux into the synapse. While marijuana, as the second most abused drugs in this study, acts via the endocannabinoid type 1 receptor, it inhibits gamma-aminobutyric acid inputs into ventral tegmental area dopamine cells[41]. As can be seen, MOR has no direct role in this mechanism of action of both methamphetamine and marijuana. Therefore, the types of drugs abused might also underlie why there is no significant relationship between *OPRM1* A118G gene polymorphism and drug addiction in this study. Besides, in the case of methamphetamine as the most used drug in our study, Stafford et al. (2019) found that there is a candidate gene i.e. trace amine-associated receptor 1 gene, *Taar1*, which can interact with *OPRM1* variant to influence methamphetamine consumption and response[42]. It means that the *OPRM1* gene is not the solely gene associated with drug addiction,

especially in the case of methamphetamine addiction.

The result of this study was still similar to previous ones stating that there is no association between *OPRM1* A118G gene and drug addiction[20,43]. It is interesting because Nikolov et al. (2011) studied on heroin users, where heroin is a drug acting primarily on μ opioid receptor, so one can expect the association[20]. Previously, in vitro experiments showed that G allele was associated with higher binding affinity to the endogenous ligand, β-endorphin, but lower potency of the exogenous opioid ligands, such as morphine[44]. However, the study by Ahmed et al. (2018) show that computational analysis predicts the A118G mutation does not damage the extracellular domain of encoded proteins, which suggests that it may not affect the binding affinity of ligands for μ-opioid receptors[33]. However, a reduced expression of mutated receptors is showed. While some studies stated that G allele increase the risk of addiction[45,19], in contrary, another studies stated that G allele have a modest protective effect or it was A allele which is associated with liability of heroin addiction[32,15,16]. Hancock et al. (2015) suggested that nearby SNPs may underlie the inconsistent associations between A118G and heroin addiction, or in other words, differences in relevant genetic background might be a possible cause of the different results across various populations in response to A118G polymorphism[15].

Above all, some factors other than genetics might be a key role in substance addiction in Indonesian young adults. Given the lower prevalence of sobriety-conducive, and sobriety-supportive, social contexts in the general population, young adulthood represents the life stage wherein the highest rates of alcohol and other drug use occur[46]. According to a meta-analysis by Aghaii, Kamaly, and Esfahani. (2012), the tendency to become addicted was affected as moderate to high for the environmental factors and low to moderate for the individual factors[47]. Therefore, the environmental factors, such as education level, employment status, and family condition, might have a major implication on drug addiction in young adults. As well as the other type of addiction, such as nomophobia which is also mainly caused by the environment factor, it is important to re-establish the human-human interactions, giving importance to social interaction to reduce the risk of addiction[48].

Regarding this high prevalence of G carriers in Indonesia, although there is no association found in drug addiction cases, it may still be related to other cases such as altered side effects in opioid therapy and the response to analgesia since some studies showed those relations[49]. There was a limitation that might influence the results of this research; that was the moderate sample size. Actually, we calculated the sample size based on Nagaya et al. (2012), which resulted minimum 67 subjects per group with 80% study power[45]. However, from this present study, we found that our power of study is 65,69%. Therefore, an additional study with larger sample size would be needed to confirm our results and address this issue. Nevertheless, despite its limitation, our study is the first report concerning the prevalence of *OPRM1* A118G polymorphism in drug addicted individual in Indonesia, and contribute to the database of this genetic study. In the future toward personalized medicine, research may be carried out regarding these gene polymorphisms in relation to addiction therapy and analgesic response in the Indonesian population.

ACKNOWLEDGEMENT:

The authors gratefully acknowledge financial support from Research Directorate of Universitas Gadjah Mada under Grant [number 3041/UN1/DITLIT/DIT-LIT/LT/2019].

CONFLICT OF INTEREST:

The authors of the current study have no conflicts of interest to disclose.

REFERENCES:

1. American Psychiatric Association. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Substance-Related and Addictive Disorders. Arlington, VA, The American Psychiatric Association (APA). 2013.
2. Sharma M, Nagori K, Agrawal S, Verma VS, Swarnakar H, Vaishnav S, Nazir M, Khan N, Singh A. Drug Abuse: A Curse for Human Society. Asian J. Res. Pharm. Sci. 2014; 4(2): 99-109
3. Dogra SK, Bagga R. Drug Addiction vis-a-vis Defense of Insanity in India: A Comparative Study. Res. J. Humanities and Social Sciences. 2019; 10(2): 559-567
4. Duaux E, Krebs MO, Loo H, Poirier MF. Genetic Vulnerability to Drug Abuse. European Psychiatry. 2020; 15(2): 109-114
5. Hart A, de-Wit H, Palmer A. Genetic Factors Modulating the Response to Stimulant Drugs in Humans. Curr Top Behav Neurosci. 2012; 12: 537–577
6. Clarke T, Crist R, Kampman K, Dackis C, Pettinati H, O'Brien C, Oslinb D, Ferraroa TN, Lohoff FW, Berrettini WH. Low frequency genetic variants in the μ-opioid receptor (OPRM1) affect risk for addiction to heroin and cocaine. Neuroscience Letters. 2013; 542: 71– 75
7. Hasan A, Azawi IH, Al-Hilali HA. Cysteine leukotriene receptors type 2 (CyLTR2 M01 V) gene polymorphism and its role in Iraqi asthmatic patients. Research J. Pharm. and Tech. 2019; 12(9): 4125-4128
8. Gaidan AM, Abbas AA, Hassan MA, Hashim HM, Al-Mayah QS. The Impact of IL-10 C-597A Gene Polymorphisms on Asthma Susceptibility. Research J. Pharm. and Tech. 2017; 10(9): 2867-2870
9. Patel SK, Sinha M, Mitra M. Glutathione-S-transferase M1 and T1 gene Polymorphism as Risk Factors of Oral Squamous Cell Carcinoma: A Preliminary Investigation. Research J. Pharm. and Tech. 2012; 5(7): 918-920
10. Thakre TR, Singh A, Mitra M. Investigation on Glutathione-S-transferase M1 and T1 gene polymorphisms as risk factor in Cervical Cancer. Research J. Pharm. and Tech. 2016; 9(12): 2295-2300
11. Thakre TR, Singh A, Mitra M. Association of TNF-308 gene polymorphism with Cervix Cancer susceptibility among women of Chhattisgarh. Research J. Pharm. and Tech. 2019; 12(5): 2339-2342
12. Surhan RK, Darweesh M, Al-Obiadi AB. IL-10-1082A\G gene polymorphism and production in β-thalassemia major and association with HCV infection. Research J. Pharm. and Tech. 2018; 11(6): 2603-2608
13. Maddhuri S, Vemuri PK, Tatineni J, Puppala J. Association of Calcineurin B (PPP3R1) Functional Gene Polymorphism in South Indian Children with Idiopathic Mental Retardation. Research J. Pharm. and Tech 2020; 13(2): 599-603
14. Crist R, Reiner B, and Berrettini WH. A Review of Opioid Addiction Genetics. Current Opinion in Psychology. 2019; 27: 31–35
15. Hancock DB, Levy J, Gaddis N, Glasheen C, Saccone N, Page G, Hulse GK, Wildenauer D, Kelty EA, Schwal SG, et al. Cis-Expression Quantitative Trait Loci Mapping Reveals Replicable Associations with Heroin Addiction in OPRM1. Biological Psychiatry. 2015; 78: 474–484
16. Schwantes-An T, Zhang J, Chen L, Hartz S, Culverhouse R, Chen X, Coon H, Frank J, Kamens HM, Konte B, et al. Association of the OPRM1 variant rs1799971 (A118G) with non-specific liability to substance dependence in a collaborative de novo meta-analysis of European-ancestry cohorts. Behavior genetis. 2016; 46: 151–169