International Journal of Scientific Advances
ISSN: 2708-7972
Volume: 2 | Issue: 5 | Sep - Oct 2021 Available Online: www.ijscia.com
DOI: 10.51542/ijscia.v2i5.14

Analysis of Single Nucleotide Polymorphism A118g Gene Opioid Receptor Mu-1 (OPRM1) In Opioid Analgesic Patients in Indonesia

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ABSTRACT

Introduction: Opioid Receptor Mu-1 or Opioid Receptor Mu-1 (OPRM1) is a prime candidate for pharmacogenetic studies related to the variability of patient response to opioids. This variant is high frequency in Asian populations that is associated with differences in sensitivity and increased need for opioid analgesics in the treatment of pain. Therefore, this study was conducted with the aim of analyzing OPRM1 in patients receiving opioid analgesics in Indonesia, especially in the city of Surabaya.

Method: The method used is the Polymerase Chain Reaction–Restriction Fragment Length Polymorphism (PCR-RFLP), where the data collected is recorded and tabulated and then analyzed using SPSS version 20.0.

Result: The results showed that the frequency of SNP A118G of the OPRM1 gene was found to be quite high, namely 58 (72.5%) out of a total of 80 subjects. In 40 male subjects, the GG genotype frequency was found to be the highest at 19 (47.5%). While the genotype frequencies of AA, AG and GG were found to be almost comparable in women.

Conclusion: Therefore, it can be concluded that the frequency of SNP A118G of the OPRM1 gene was found to be high in the Indonesian population and had a high tendency in the male population, although it was not statistically significant.

Keywords: OPRMI gene; SNP A118G; opioid receptors; Male and Female; PCR-RFLP

INTRODUCTION

Opioids are the main analgesia used for the treatment of moderate to severe pain in patients. The analgesic efficacy of opioids such as fentanyl and morphine are known to vary widely. Therefore, the incidence of pain experienced is very different. This makes it difficult to predict optimal pain management in each patient (López Soto et al., 2013) (Boules et al., 2015) (Taqi et al., 2019). For this reason, it is important to analyze the mechanisms responsible for this inter-individual variation. Various non-genetic factors have an impact on the patient's pain sensitivity such as: age, gender, anxiety, liver and kidney function, type of surgery and preoperative pain. Advances in genetic research indicate that genetic polymorphisms contribute to patient variability in response to opioid treatment (Taqi et al., 2019) (Puspitasari et al., 2020).

Genetic and psychological variations are increasingly being recognized as contributing to pain relief, analgesic efficacy and safety. Differences in analgesic efficacy related to body composition, metabolism, and hormonal profile have been demonstrated. Gender psychological and social elements have also been associated with altered pain experience and analgesic use profiles, although with significant individual variation. Experimentally, in men with neuropathic pain, macrophages are the most active immune cells. In women, neuropeptides, which are protein-like substances, are more prominently released by neurons.

The mu-1 opioid receptor, encoded by the Opioid Receptor Mu-1 (OPRM1) gene, is a prime candidate for pharmacogenetic studies of patient response variability to opioids because it is the primary site of action for many endogenous opioid peptides including -endorphins and enkephalins, as well as their target The main drugs for opioid analgesics are fentanyl and morphine. A number of single nucleotide polymorphisms (SNPs), otherwise known as single nucleotide polymorphisms, have been found in the OPRM1 gene (Kasai & Ikeda, 2011) (López Soto et al., 2013) (Hwang et al., 2016) (Boules et al., 2015) (Taqi et al., 2019) (Puspitasari et al., 2020).

This substitution is thought to lead to the loss of the N-glycosylation site in the extracellular region of the receptor. Several studies evaluating the effect of SNP 118A>G on the exogenous opioid-induced intracellular signaling cascade (DAMGO and morphine) at the mu-1 opioid receptor demonstrated a two-fold stronger inhibition of Ca2+ channel current in neurons with the A allele than in those expressing the receptor with the A allele. G. This suggests reduced function of the G allele variant receptor function which may result in reduced effects of opioid drugs (Ikeda et al., 2005) (Pasternak & Pan, 2013).

The polymerase chain reaction (PCR) discovered by Kary Mullis in 1985 is known to be an effective and sophisticated procedure for amplification of DNA.

This process is similar to the process of DNA replication in cells (Chou & Hsu, 2021). The result of this DNA segment multiplication causes the duplicated DNA segment to be easily detected because of its high concentration (Boules et al., 2015) (Taqi et al., 2019). Electrophoresis visualization of the PCR amplification of the exon 1 gene fragment OPRM1 with forward and reverse primers will appear in the 193 base pairs (bp) band (Udayakumar & Udayakumar, 2021).

Through this, it can be seen that the A118G SNP exon 1 of the OPRM1 gene has an impact on the variability of the amount of opioid analgesic consumption and is useful in predicting individual selectivity to opioids to optimize patient pain control and to avoid side effects(Kasai & Ikeda, 2011) (López Soto et al., 2013) (Hwang et al., 2016) (Boules et al., 2015) (Taqi et al., 2019) (Puspitasari et al., 2020). Since the Asian population is the largest for the incidence of SNP A118G gene OPRM1, this study was structured with the aim of analyzing OPRM1 in patients receiving opioid analgesics in Indonesia, especially in the city of Surabaya.

METHOD

This study is an unpaired categorical comparative analytical study with a cross sectional design (risk factors and effects viewed at the same time / snapshot of the population) to analyze differences in genotype and allele frequencies in the A118G SNP of the Opioid Receptor Mu-1 gene (OPRM1) with the method PCR-RFLP in patients with opioid analgesics in one hospital in Indonesia, namely RSUD Dr. Soetomo Surabaya.

This research was conducted from October 2020 to March 2021, where sampling was carried out at RSUD Dr. Soetomo Surabaya and laboratory examinations were carried out at the Integrated Laboratory of the Faculty of Medicine, University of North Sumatra (FK-USU).

The subjects in this study were all male and female patients with surgery who received opioid analgesics at RSUD Dr. Soetomo Surabaya Indonesia. Subjects were taken by consecutive random sampling, that is, all subjects who met the inclusion criteria and were not included in the exclusion criteria were sampled in this study.

RESULT

Demographic Characteristics of Research Subjects

Subjects with male gender in this study amounted to 40 (50%) patients and 40 (50%) female patients. The mean age of men was 43.6 ± 15.9, women were 42.0 ± 14.1 and the total subject was 42.8 ± 14.1. The mean weight of men was 64.2 ± 9.7, women was 65.6 ± 11.4 and the total subject was 64.9 ± 10.5. The mean height of men was 166.6 ± 4.8, women were 156.8 ± 5.5 and the total subject was 161.7 ± 7.1.

The mean body mass index (BMI) for men was 23.0 ± 3.0, women were 26.8 ± 4.1 and the total subject was 24.91 ± 4.02. The majority of the subjects in this study (91.25%) were Javanese. Based on the Physical Status of the American Society of Anesthesiologist (PS ASA) score, 65.0% of the subjects were obtained with a PS ASA score of 2, consisting of 27.5% men and 37.5% women, the rest of the subjects were obtained with a PS ASA score of 1 and 3.

As many as 50% of patients underwent orthopedic surgery which consisted of surgery for long bones of the extremities and vertebrae. Subjects who underwent neurosurgery surgery were 6.25% who were all men with vertebral spine surgery. Subjects who underwent digestive surgery were 3.75% of patients (all men), consisting of open cholecystectomy and gastrectomy operations. Subjects who underwent plastic surgery were 6.25% (5.0% men and 1.25% women), consisting of burn surgery and facial bone reconstruction. Subjects who underwent general surgery were 6.25% (3.75% men and 2.5%), consisting of debridement, necrotomies, skin grafts and amputation of diabetic feet. Subjects who underwent urological surgery, namely TUR-P were 1.25% with male gender. Subjects who underwent Ear Nose Throat (ENT) surgery, namely FESS, were also 1.25% male. The last subject was 25% of female patients who underwent obstetric surgery, namely TAH.

Electrophoresis Results of PCR Products and PCR-RFLP Products Research Subjects

PCR, RFLP and electrophoresis analysis have been carried out on a total of 80 blood samples of the subjects of this study. From the results of electrophoresis of PCR products, 193 bp fragments were obtained in the visualization process. Furthermore, RFLP and electrophoresis were performed on all subjects' PCR products and 193 bp fragments were obtained in 22 (27.50%) research subjects, namely lane numbers 1, 5, 7, 13, 23, 24, 31, 37, 40, 41, 44, 46, 48, 50, 51, 52, 53, 61, 68, 75, 79 and 80 which indicate the subject has an AA genotype. In 27 (33.75%) research subjects, namely lane numbers 2, 3, 4, 16, 18, 28, 29, 30, 33, 34, 35, 36, 47, 54, 55, 56, 58, 59, 60, 63, 64, 65, 66, 67, 72, 73 and 74 fragments of 193 and 169 bp were obtained, indicating that the subject had the AG genotype. Meanwhile, 31 (38.75%) research subjects, namely lane numbers 6, 8, 9, 10, 11, 12, 14, 15, 17,19, 20, 21, 22, 25, 26, 27, 32, 38, 40, 42, 43, 45, 49, 57, 62, 69, 70, 71, 76, 77 and 78 fragments of 169 bp were obtained which indicated that the subject had a GG genotype.

Allele frequency distribution on SNP A118G gene OPRM1 all research subjects

The distribution of allele frequencies in the A118G SNP of the OPRM1 gene for all research subjects can be seen in table 1.

TABLE 1: The distribution of allele frequencies in the A118G SNP of the OPRM1 gene for all research subjects

Allele Distribution	Homozygote AA N (%)	Heterozygote AG N (%)	Homozygote GG n (%)	Total N (%)
A	44 (27,5)	27 (16,9)	0 (0,0)	71 (44,4)
G	0 (0,0)	27 (16,9)	62 (38,75)	89 (55,6)
Total	44 (27,5)	54 (33,75)	62 (38,75)	160 (100)

The table describes the frequency of the G allele, which was found to be 89 (55.6%) more than the A allele 71 (44.4%) of the 160 alleles in the subjects of this study. The majority of G alleles are present in GG homozygous subjects, and the majority of A alleles are present in AA homozygous subjects.

Based on the total genotype frequency of the research subjects, the frequency of the AA genotype was 44 (27.5%), the AG genotype 54 (33.75%) and the GG genotype 62 (38.75%), it can be concluded that the frequency of the A118G SNP polymorphism of the OPRM1 gene was found in 72.5% of subjects, this figure was obtained from the addition of the AG genotype (33.75%) and the GG genotype (38.75%).

Distribution of allele frequencies in SNP A118G gene OPRM1 based on gender of research subjects

The frequency distribution of the A and G alleles in the A118G SNP of the OPRM1 gene based on the sex of the research subjects is shown in table 2.

TABLE 2: Distribution of allele frequencies in SNP A118G gene OPRM1 based on gender of research subjects

Allele	Sex		
	Men	Women	Total
	N (%)	N (%)	N (%)
A	30 (37,5)	41 (51,2)	71 (44,4)
G	50 (62,5)	39 (48,8)	89 (55,6)
Total	80 (100,0)	80 (100,0)	160 00,0)

Table 2 shows that in male subjects, the G allele frequency was greater than the A allele, which was 62.5%. In contrast to male subjects, female subjects found that the frequency of the A allele was greater than that of the G allele, which was 51.2%.

Differences in Genotype Frequency Distribution in SNP A118G Gene OPRM1 based on Gender

The distribution of genotype frequencies in the A118G SNP of the OPRM1 gene based on the sex of the research subjects is shown in table 3.

TABLE 3: Differences in the genotype frequency distribution of SNP A118G gene OPRM1 based on the sex of the research subjects.

Genotype	Men	Women	Total	P-Value
	N (%)	N (%)	N (%)	
Wild type (AA)	9 (22,5)	13 (32,5)	22 (27,5)	0,267*
Mutan Heterozigot (AG)	12 (30,0)	15 (37,5)	27 (33,75)	
Mutan homozigot (GG)	19 (47,5)	12 (30,0)	31 (38,75)	
Total	40 (100,0)	40 (100,0)	80 (100,0)	
HWE (χ2 Value)	0,139 *(P>0,05)*	0,839 *(P>0,05)*	0,466 *(P>0,05)*	

** Difference is not significant, p > 0.05*

Based on the Chi-square test, it can be concluded that there is no significant difference in the frequency distribution of the SNP A118G genotype of the OPRM1 gene based on the sex of the subject in this study with p = 0.267 (p > 0.05). The frequency distribution of the A118G SNP genotype of the OPRM1 gene in this study, both in men, women and in total subjects, did not have a significant deviation based on the Hardy-Weinberg Equilibrium/HWE (value 2).

Differences in Allele Frequency Distribution in SNP A118G Gene OPRM1 Based on Gender of Research Subjects

The difference in allele frequency distribution in SNP A118G gene OPRM1 based on the sex of the research subjects can be seen in table 4.

TABLE 4: Differences in allele frequency distribution in SNP A118G gene OPRM1 based on gender of research subjects.

Allele	Men	Women	Total	P-Value (Chi-Square)	OR[1]
	N (%)	N (%)	N (%)		
A	30 (37.5)	41 (51.2)	71 (44.4)	0.08*	0,571
G	50 (62.5)	39 (48.8)	89 (55.6)		
Total	80 (100)	80 (100)	160 (100)		

** Difference is not significant, p > 0.05*

Based on the Chi-square test conducted, it can be concluded that there is no significant difference in the distribution of the SNP A118G allele frequency of the OPRM1 gene based on the sex of the research subjects, with p value = 0.08 (p > 0.05). Given the p value > 0.05, the OR value in this study cannot be used as a recommendation to conclude which sex is more at risk of having the A or G allele in the A118G SNP of the OPRM1 gene.

DISCUSSION

Through the results of the study, it was found that the frequency of the wild type (AA) genotype was at 27.5%. Then genotype heterozygous mutant (AG) in 33.75% and homozygous mutant genotype (GG) in 38.75% of the total 80 subjects.

In this study, 72.5% of subjects had at least 1 G allele in their genotype, namely the mutant heterozygous (AG) and homozygous mutant (GG) genotypes]. This condition is in accordance with the research of Puspitasari et al. (2020) who got 82.2% or have at least 1 G allele in their genotype. These two studies illustrate the high frequency of the G allele in the Indonesian population, which reaches more than 70% of the population.

Another study conducted by Zahari et al. (2018) in Malaysia, found the frequency of wild type (AA) genotypes in 23.0% of subjects. Then there is a heterozygous mutant genotype (AG) in 47.8% and a homozygous mutant genotype (GG) in 29.2% of a total of 161 subjects. This study is in line with the study of Zahari et al. (2018) who also found a high number of subjects with at least 1 G allele in their genotype, namely 77.0%.

Allele Frequency Distribution of SNP A118G Gene OPRM1 in Research Subjects

This study found that the SNP frequency of 118A allele was 44.4% and 118G allele was 55.6% from 160 alleles in a total of 80 subjects. This certainly further confirms the high frequency of the G SNP A118G allele of the OPRM1 gene in the population in Indonesia. One of them is in the city of Surabaya.

The systematic review and meta-analysis of Hwang et al. In 2014, the 118G allele frequency in Asia included Korea, Singapore, and China which accounted for 38.8%, 39.2% and 37.1% of the population, respectively. Meanwhile, the European population, namely Italy and Pennsylvania, were 18.4% and 14.0%, respectively. It is seen that the prevalence of SNPs for this gene is generally found to be high in Asian populations.

In contrast to South Asian countries, including Pakistan and India, the prevalence of the G allele in the A118G SNP of the OPRM1 gene tends to be lower, 14.5% and 17.8%, respectively (Ahmed et al., 2018) (Kumar et al., 2012). These frequencies are more similar to the allele frequency distributions in African American and European populations, which range from 4% to 16% of the population (Schwantes-An et al., 2016). Thus, this study and the research of Puspitasari et al. (2020) shows that Indonesia, with the majority of subjects being Javanese, tends to have the highest 118G allele frequency (55.6-60.4%) among Asian countries, followed by ethnic Malays (53.1%). This also supports the theory that demographics may be a contributing factor, given that Malaysia may have the closest demographic and ethnic characteristics to Indonesia.

Differences in Genotype Frequency Distribution in SNP A118G Gene OPRM1 with Gender

Genotype distribution based on sex in this study was found in male genotypes, namely homozygous mutants (GG) of 47.5%, followed by AG 30% and the lowest was AA, which was 22.5%. Meanwhile, for female gender, this study found that the highest genotype frequency was GG, which was 38.75%, followed by AG at 33.75% and the lowest was AA, which was 27.5%.

Then it was found that there was no deviation in the prevalence of SNP A118G gene OPRM1 of this study subject with all values of HWE ($\chi 2$) > 0.05 both male or female and total subjects. In this study, there was also no significant difference in the frequency distribution of the A118G SNP gene OPRM1 genotype based on the sex of the subject with p value > 0.05 (p = 0.46).

Differences in Allele Frequency Distribution in SNP A118G Gene OPRM1 Based on Gender

The distribution of allele frequencies by gender, in this study, 37.5% of the 118A allele was found in males and 62.5% of the 118G alleles of a total of 80 alleles, while in females the 118A allele was 51.2% and the 118G allele was 48.8%. of a total of 80 alleles. Puspitasari et al. (2020) found out that 33.5% of 118A alleles were obtained for men and 66.5% of 118G alleles from a total of 158 alleles, while in women the 118A allele was 45.6% and the 118G allele was 54.4% of the total 158 alleles.

Based on this description, it can be concluded that this research is in line with the research of Puspitasari et al. (2020) which also found that the frequency of the G allele in men was higher than in women. Although in this study, there was no significant difference in the frequency distribution of the SNP A118G allele of the OPRM1 gene based on the sex of the subject with a p value > 0.05 (p = 0.08), while Puspitasari et al. getting male sex has a higher G allele frequency distribution with a P value <0.05 (p = 0.029).

Through this, it can be seen that the Indonesian population requires high doses of opioid analgesics. In addition, the average male gender has a tendency to require higher doses than women.

CONCLUSION

Therefore, it can be concluded that the frequency of SNP A118G of the OPRM1 gene was found to be high in the Indonesian population and had a high tendency in the male population, although it was not statistically significant.

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