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Association between opioid receptor gene polymorphism and postoperative pain response in Koreans

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Background: The objective of this study was to investigate the association between A118G single nucleotide polymorphism (SNP) of human μ-opioid receptor (OPRM1) gene and the postoperative pain response in Korean patients undergoing thyroidectomy.
Methods: Fifty two adult patients undergoing thyroidectomy were enrolled in this study. Their blood samples were genotyped for the A118G polymorphism. Pain intensity was assessed by a verbal numerical rating scale (VNRS) at postanesthesia care unit, postoperative 6, 24, and 48 hours. Mechanical pain threshold was assessed using electronic von Frey preoperatively and repeated at postoperative 24 and 48 hours on the forearm and periincisional regions.
Results: Of the 50 patients, 23 patients were A118 homozygous (AA), 19 patients were heterozygous (AG), and 8 patients were 118G homozygous (GG). The VNRS score was higher in patients with GG genotype than other genotypes at PACU ($P < 0.05$). Mechanical pain thresholds on the forearm and periincisional area were decreased at postoperative 24 and 48 hours from the preoperative values in all genotypes ($P < 0.05$). However, the changes in pain thresholds were similar among the genotypes.
Conclusions: A118G SNP of OPRM1 gene is associated with inter-individual difference in immediate postoperative pain score in Korean population. **(Anesth Pain Med 2012; 7: 343～347)**

Key Words: Mechanical pain threshold, Opioid receptor, Pain, Polymorphism.

INTRODUCTION

Postoperative pain is a common and distressing complication in patients undergoing surgery. Various analgesics such as opioids have been used to reduce moderate to severe pain during perioperative periods. However, the pain perception after surgery is well known to vary among different individuals [1], therefore, it is difficult to predict the pain response after surgery in some patients. Although the mechanism of this inter-individual difference is not clear, genetic factors may play an important role.

The human μ-opioid receptor (OPRM1) gene regulates the analgesic effect of endogenous and exogenous opioids, and the function of OPRM1 gene has been found to be associated with the single nucleotide polymorphism (SNP) of this gene [2-4]. Among various SNPs, the most common SNP in the OPRM1 is the A118G SNP occurring at nucleotide 118 consisting of adenine (A) to guanine (G) transition. Several studies investigated the clinical implication of A118G SNP of OPRM1 for pain response. However, the effect of this polymorphism on pain perception and analgesic requirement for postoperative pain is controversial [5,6]. Moreover, ethnicity is regarded as an independent predictor of postoperative pain perception [7].

To our knowledge, the relationship between A118G SNP of OPRM1 and the pain response in Korean populations has not been investigated. Therefore, in this study, we investigated the association between A118G SNP of OPRM1 and the postoperative pain response in Korean patients undergoing thyroidectomy.

MATERIALS AND METHODS

After obtaining approval from Institutional Review Board, written informed consent was obtained from each patient. Fifty two adult patients (American Society of Anesthesiologists physical status classification 1－2) undergoing thyroidectomy were enrolled in this study. We excluded patients with a drug abuse, chronic pain or psychiatric history, known hypersensitivity to opioids, and use of opioids within 24 hours before the study. Patients with hepatic or renal disease were also

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excluded. Before the surgery, all patients were instructed about the use of the verbal numerical rating scale (VNRS) and given a quantitative sensory test (QST) of mechanical threshold using electronic von Frey (EVF3™, Bioseb, Vitrolles Cedex, France).

No premedication was administered to the patients. Electrocardiography, noninvasive blood pressure and pulse oximetry were monitored continuously after the patient's arrival at the operating room. Anesthesia was induced with thiopental 4−5 mg/kg, remifentanil 0.5 μg/kg and rocuronium 0.6 mg/kg intravenously. The trachea was intubated and the patient's lungs were ventilated with 50% oxygen in air. Sevoflurane 1.5−2.0 vol% and remifentanil 0.1−0.2 μg/kg/min were used for maintenance of anesthesia. A bispectral index score (BIS) monitor (A-2000 BIS Monitor™, Aspect Medical System Inc., Newton, USA) was monitored continuously to maintain between 45 and 55 during the procedure. Patients were kept warm using a forced-air warming system (Bair-Hugger™, Augustine-Medical, Eden Prairie, MN, USA) to maintain body temperature at 36.0−37.0°C. Thirty minutes before the end of surgery, ondansetron 0.1 mg/kg was administered intravenously for prevention of postoperative nausea and vomiting (PONV). At the end of surgery, neuromuscular block was reversed with neostigmine 0.03 mg/kg and glycopyrrolate 0.004 mg/kg. After adequate spontaneous respiration, endotracheal extubation was performed. Patients who reported a VNRS score > 4 or requested analgesics were treated with tramadol 50 mg IM. The total dose of remifentanil consumption during anesthesia, and awakening time were recorded.

Pain intensity was assessed by an 11-point VNRS for postoperative 48 hours: 15 min after postanesthesia care unit (PACU) admission, postoperative 6, 24, and 48 hours. Possible complications such as PONV, headache, and drowsiness were also recorded at the same time. Mechanical pain threshold was assessed using electronic von Frey preoperatively and repeated at postoperative 24 and 48 hours on the forearm and 3 periincisional regions (both ends and middle of the horizontal skin incision), and a mean value was calculated.

All patients underwent peripheral blood sampling for isolation of genomic DNA. Samples were stored at −20°C until DNA extraction. Genomic DNA was prepared from peripheral blood samples using a nucleic acid isolation device, QuickGene-mini80 (FUJIFILM, Tokyo, Japan). The genotyping of A118G opioid receptor gene variants (rs1799971) was screened using single base primer extension assay using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer's recommendation. Briefly, the genomic DNA was amplified with polymerase chain reaction (PCR) with forward (F) and reverse (R) primer pairs and standard PCR reagents in 10 μl reaction volume, containing 10 ng of genomic DNA, 0.5 pM of each oligonucleotide primer, 1 μl of 10X PCR buffer, 250 μM dNTP and 0.25 U i-StarTaq DNA polymerase (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). Primers were designed on the basis of target gene sequences: 5′-GTGATGAGCCTCTGTGAACT-3′ (F), 5′-TCACATACATGACCAGGAAG-3′ (R). The PCR reactions were carried out as follows: one cycle at 95°C for 10 min, and 35 cycles at 95°C for 30 s, 55°C for 1 min, 72°C for 1 min followed by one cycle of 72°C for 10 min. After amplification, the PCR products were treated with 1 U each of shrimp alkaline phosphatase (SAP) (USB Corporation, Cleveland, OH, USA) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37°C for 75 min and 72°C for 15 min to purify the amplified products. One μl of the purified amplification products were added to the SNaPshot Multiplex Ready reaction mixture containing 0.15 pM of genotyping primer (GGTCAACTTGTCCCACTTAGATGGC) for primer extension reaction. The primer extension reaction was carried out for 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. The reaction products were treated with 1 U of SAP at 37°C for 1 hour and 72°C for 15 min to remove excess fluorescent dye terminators. One μl of the final reaction samples containing the extension products were added to 9 μl of Hi-Di formamide (ABI, Foster City, CA, USA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by electrophoresis in ABI Prism 3730xl DNA analyzer (Applied Biosystems, USA). Analysis was carried out using Genemapper software (version 4.0; Applied Biosystems, USA).

Data were analyzed using SPSS version 13.5 (SPSS Inc., Chicago, IL, USA). All values were expressed as mean ± SD or number of patients. The frequency of the SNP was assessed for deviation from Hardy-Weinberg equilibrium using Fisher's exact test. Frequency differences in genotype, demographic data, VNRS, and mechanical pain threshold were compared by chi-square test, Fisher's exact test, ANOVA with Bonferroni correction and Kruskal-Wallis test as appropriate. A $P < 0.05$ was considered significant.

RESULTS

A total of 52 patients were enrolled on this study. However, two cases were excluded due to technical errors in gene

Table 1. Genotypes Frequency of Human μ-Opioid Receptor A118G Polymorphism

A118G genotypes			
AA	AG	GG	Total
23 (46)	19 (38)	8 (16)	50

Values are number of patients (%). AA: wild homozygous, AG: mutant heterozygous, GG: mutant homozygous.

Table 2. Demographic and Clinical Data

	AA	AG	GG
Sex (M/F)	5/18	5/14	2/6
Age (yr)	44.9 ± 11.4	42.1 ± 9.9	42.3 ± 10.9
Weight (kg)	59.6 ± 9.0	66.3 ± 13.5	63.4 ± 9.2
Height (cm)	161.8 ± 6.3	161.8 ± 8.3	164.6 ± 8.5
Duration of anesthesia (min)	111.3 ± 33.2	131.9 ± 47.1	122.9 ± 42.9
Remifentanil dose (μg)	739.6 ± 320.6	862.0 ± 392.6	828.3 ± 200.1
Awakening time (min)	8.1 ± 4.2	8.2 ± 4.3	7.9 ± 2.2

Values are mean ± SD or number of patients. AA: wild homozygous, AG: mutant heterozygous, GG: mutant homozygous.

analysis. Hence, data from 50 patients were analyzed in this study. Genotype frequencies are summarized in Table 1. Of the 50 patients, 23 patients were A118 homozygous (AA), 19 patients were heterozygous (AG), and 8 patients were 118G homozygous (GG). The genotype frequencies of OPRM1 SNPs were in Hardy-Weinberg equilibrium. There were no significant differences in demographic and clinical data according to genotypes (Table 2).

Pain scores were similar among the genotypes except at PACU. The VNRS score was higher in patients with GG genotype than other genotypes at PACU ($P < 0.05$). There were no significant differences in the number of patients who requested rescue analgesics among the 3 genotypes (Table 3). Mechanical pain thresholds on the forearm and periincisional area were decreased at postoperative 24 and 48 hours from the preoperative values in all genotypes ($P < 0.05$). However, the changes in pain thresholds were similar among the genotypes (Table 4).

There were no significant differences in the incidence of PONV, headache, drowsiness, and dizziness among the 3 genotypes (Table 5).

Table 3. Pain Scores and Analgesic Consumption

	AA (n = 23)	AG (n = 19)	GG (n = 8)
VNRS			
PACU	5 (3－7)	6 (4－8)	7 (5－9)*
Postoperative 6 h	4 (2－6)	3 (1－5)	3 (1－5)
Postoperative 24 h	3 (1－5)	3 (2－4)	3 (2－4)
Postoperative 48 h	2 (1－3)	2 (1－3)	2 (1－3)
Rescue analgesics	15 (65.2)	12 (63.2)	6 (75.0)

Values are median (range) or number of patients (%). AA: wild homozygous, AG: mutant heterozygous, GG: mutant homozygous, VNRS: verbal numerical rating scale, PACU: postanesthesia care unit. *$P < 0.05$ compared with AA and AG.

Table 4. Mechanical Pain Thresholds

Mechanical pain thresholds (g)	AA (n =23)	AG (n =19)	GG (n =8)
Forearm			
Preoperative	125.5 ± 54.8	122.6 ± 58.0	116.7 ± 47.8
Postoperative 24 h	92.4 ± 40.6*	94.0 ± 32.5*	83.0 ± 41.8*
Postoperative 48 h	89.1 ± 36.9*	98.4 ± 51.1*	93.1 ± 48.9*
Periincisional area			
Preoperative	99.3 ± 66.7	97.2 ± 40.8	93.1 ± 51.8
Postoperative 24 h	79.0 ± 43.6*	82.0 ± 42.1*	78.7 ± 42.2*
Postoperative 48 h	80.0 ± 30.3*	83.1 ± 25.3*	80.1 ± 54.9*

Values are mean ± SD. AA: wild homozygous, AG: mutant heterozygous, GG: mutant homozygous. *$P < 0.05$ compared with preoperative values.

Table 5. Incidence of Adverse Effects for Postoperative 48 Hours

	AA (n =23)	AG (n =19)	GG (n =8)
PONV	8	7	3
Headache	1	2	1
Drowsiness	3	3	1
Dizziness	1	1	1

Values are number of patients. AA: wild homozygous, AG: mutant heterozygous, GG: mutant homozygous. PONV: postoperative nausea and vomiting.

DISCUSSION

Our study demonstrates that there was an association between A118G SNP of OPRM1 gene and the immediate postoperative pain score in Korean patients. The VNRS score was higher in patients with GG genotype than other genotypes at PACU.

There are some evidences that genetic polymorphism of OPRM1 gene may influence the pain response and analgesic requirement [8-12]. In a recent study, A118G SNP was associated with pain perception and patient-controlled intravenous morphine consumption for postcesarean analgesia [1]. In this study, we also found an association between A118G SNP of OPRM1 gene and the postoperative pain score at PACU in patient undergoing thyroidectomy. It is likely that patients with 118GG genotype have a greater pain perception due to increased pain sensitivity in the immediate postoperative periods. Postoperative pain is a very serious distress after surgery. Therefore, if we are able to expect the pain response after surgery, personalized pain treatment can be provided with less complication. However, further study should be performed to evaluate the effect of genetic polymorphism in OPRM1 gene on the opioid receptor expression in pain process.

We found that the pain response during 6−48 hours after surgery did not differ according to genotypes. In this study, almost 90% or more of all patients undergoing thyroidectomy did not suffer pain between 6 and 48 hours. Therefore, this may explain the lack of association at this time period. The number of patients who requested rescue analgesics was slightly higher in patients with 118GG genotype; however, it was statistically insignificant due to small sample size of patients with GG genotype.

In this study, we could find that the allele frequency for the 118G allele in Korean population was 0.350. This frequency is similar to the other Asian populations whose reported range is 0.321−0.485 [4,13,14], and significantly higher than the range of 0.074−0.200 reported for Caucasian population [10,14,15]. Tan, et al. demonstrated that there was an ethnic difference in pain perception and patient-controlled analgesia usage for postoperative pain [16]. They also reported that ethnicity would be the most significant factor to opioid usage after surgery. In this study, our study sample is the most homogenous in that it involved only Korean patients undergoing the same surgery; therefore it represents the association between A118G SNP of OPRM1 gene and the postoperative pain score in Korean patients.

Previous studies evaluated the association between a quantitative sensory test (QST) and opioid receptor gene polymorphism with contradictory results. Fillingim et al. [17] demonstrated that the A118G SNP of OPRM1 gene was associated with pressure pain sensitivity in healthy adult. However, Huang et al. [5] reported that there was no significant association between the A118G SNP of OPRM1 gene and pressure pain threshold. Although the mechanisms that represent the inter-individual variability in pain sensitivity is unclear, we found that the changes in mechanical pain thresholds were similar among the A118G OPRM1 genotypes in Korean population.

There are several contributing factors affecting postoperative pain. It is well known that gender and a type of surgery are risk factors for postoperative pain [18]. In this study, there were no significant differences in gender ratios according to genotypes. Moreover, other factors that affect postoperative pain such as anesthetic agent and type of surgery, were also controlled in this study, therefore, they would have minimal effect on our results.

There are several limitations to our study. First, the sample size is relatively small for a genetic association study. Sample size calculation was performed based on a preliminary study. The sample size required to detect a mean difference of 2 in VAS with a SD of 1.7 and a power of 80% at an alpha level of 0.05 was 24 patients in AA group. On the basis of an estimated prevalence of A118G SNP of OPRM1 gene in the preliminary study, we calculated that we needed at least 52 patients to be enrolled. It may not be appropriate to apply our result to clinical practice, as a larger scale study should be required to demonstrate the clinical implication of genetic variation of OPRM1 gene. Although a larger scale study will be required, this results may be helpful to present the basic information of personalized pain management in Korean population. Second, we evaluated only one polymorphism of the opioid receptor gene. Further studies about other polymorphisms that can affect the activity of receptor, drug transporter, and drug metabolizing enzyme will be required.

In conclusion, A118G SNP of OPRM1 gene is associated with inter-individual difference in immediate postoperative pain score in Korean population. Therefore, OPRM1 gene genotypes may be a clinical predictor for immediate postoperative pain.

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