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Original Article

118A>G AND IVS2+691G>C POLYMORPHISMS OF OPRM1 GENE HAVE NO INFLUENCE ON COLD-PAIN SENSITIVITY AMONG HEALTHY OPIOID-NAIVE MALAY MALES

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ABSTRACT

Objective: Common polymorphisms of the mu-type opioid receptor (OPRM1) including 118A>G and IVS2+691G>C may affect experimental pain responses in healthy subjects, and the effect could be ethnic-dependent. The aim of this study was to investigate the influence of these *OPRM1* polymorphisms on cold-pressor pain responses among healthy opioid-naive Malay males.

Methods: Pain-threshold, pain-tolerance, and pain-intensity in response to the cold pressor test (CPT) were measured in healthy opioid-naive Malay males. DNA was extracted from the collected venous blood before PCR-genotyping. Repeated measure analysis of variance (RM-ANOVA) was used to compare CPT responses and *OPRM1* polymorphisms (118A>G and IVS2+691G>C) according to their genotypes and allelic additive models, genotype dominant and recessive models, haplotypes, and diplotypes.

Results: A total of 152 participants were recruited. Both 118A>G and IVS2+691G>C polymorphisms were not associated with cold-pressor pain-threshold, pain-tolerance and pain-intensity despite using genotypes and allelic additive models and genotype dominant and recessive models (all $p>0.05$). Likewise, there were no significant associations between haplotypes and diplotypes for the 118A>G and IVS2+691G>C polymorphisms and the three cold-pain responses (all $p>0.05$).

Conclusion: The common *OPRM1* polymorphisms (i.e., 118A>G and IVS2+691G>C), are not associated with cold-pressor pain responses in healthy opioid-naive Malay males. However, this may be unique for this particular ethnicity. Other polymorphisms may be more relevant for this population, and this should be further investigated.

Keywords: Cold pressor test (CPT), Mu-type opioid receptor (OPRM1), Opioid receptor, mu 1 gene (*OPRM1*), Pain-threshold, Pain-tolerance, Pain-intensity, Opioid-naive, Male, Malays

INTRODUCTION

The mu-type opioid receptor (OPRM1) is a major opioid receptor in human. Together with the δ-opioid receptor (DOR) and κ-opioid receptor (KOR), they are the binding sites for endogenous opioid peptides [1, 2] and exogenous opioids, including methadone [3]. Studies have shown that activation of the OPRM1 system was associated with reductions in the sensory and affective ratings of pain experience [4]. Variability in pain modulation and inter-individual differences in treatment outcomes with opioid-based analgesic therapy may be a result of variations in the OPRM1 gene [5-12]. A previous study in healthy males found that a low OPRM1 binding potential in the striatum was associated with a low cold pain-threshold [13]. Thus, it is hypothesized that individuals with low OPRM1 binding potential have low receptor density, and consequently, low level of OPRM1-mediated suppression of pain pathways, leading to increased experimental pain sensitivity [13].

The 118A>G polymorphism being the most common variation of the OPRM1 gene is found to exert influences on experimental pain responses in healthy subjects [14, 15] but the effect may be ethnic-dependent [16]. Individuals with *118G* allele but not the wild-type allele exhibited lower sensitivity to pressure pain (or higher pressure pain-threshold) [14]. Other less common but important polymorphisms of the OPRM1 gene have included IVS2+31G>A (dbSNP rs9479757) and IVS2+691G>C (dbSNP rs2075572). While IVS2+31G>A polymorphism was associated with a higher pressure pain-threshold in healthy adult females [17] but IVS2+691G>C polymorphism has not been previously studied.

Southeast Asia is a highly populated and culturally diverse region with ethnic Malays consisted the largest population group, mainly populating countries including Malaysia, Indonesia and southern part of the Philippines. Males of ethnic Malays consisted the majority of opioid-dependent patients on methadone treatment in Malaysia, but it is not known whether *OPRM1* polymorphisms influence the inter-individual variations in pain responses. The current study aimed to investigate the influence of common *OPRM1* polymorphisms (i.e., 118A>G and IVS2+691G>C) on cold-pressor pain responses among healthy opioid-naive Malay males. Results of this study would be helpful to determine whether these polymorphisms are suitable for further studies in opioid-dependent patients.

MATERIALS AND METHODS

Study participants

Participants comprised 152 opioid-naive Malay males between 18 and 63 y of age (mean = 27.46 y). They were randomly sampled from within the hospital compound. The participants consisted of staffs and students. Written informed consents were obtained from each participant prior to enrolment. This study was part of a larger study to investigate the genetic factors that may influence cold-pressor pain responses in opioid-dependent patients on methadone treatment (National Medical Research Register (NMRR) number: NMRR-13-524-16614). It was approved by the Human Research Ethics Committee (HREC), Universiti Sains Malaysia (USM) in Kelantan, Malaysia (Reference number: USMKK/PPP/JEPeM (253.3) [14].

Assessment of study participants

Urine drug screens for morphine, tetra-hydrocannabinol, amphetamines and benzodiazepines using drugs of abuse rapid test, F. A. C. T. S ™ 4 in 1 Combo Dipcard Rapid Test (MOR/ THC/ AMP/BZO) (Scientifacts Sdn. Bhd., Malaysia) were performed for each participant twice in one week prior to CPT. Only subjects with two consecutive negative urine tests were allowed to continue with the study. A history of analgesics consumption within 72 h prior to study entry and a positive history of any painful conditions were exclusion criteria. Subjects with any known acute or chronic medical, surgical and psychiatric illnesses that required concurrent medical, surgical or psychiatric therapy and severe cognitive impairment which might interfere with pain assessments and/or communication were also excluded from the study.

Cold pressor test (CPT)

The CPT method utilized in the current study was adapted from Chen *et al.* (1989) and Compton *et al.* (2001) and had been described else where [20]. Briefly, the CPT apparatus consisted of a 48-quart cool box filled with a mixture of two-thirds crushed ice and one-third tap water. A constant temperature of 0–2 °C was maintained by adding ice intermittently. The non-dominant hand and forearm of the participant would be placed in the ice bath with their palm flat at the bottom of the box, with ice water covered the hand and approximately 10 cm of the forearm. The test was truncated at 300 s, since after this time, the numbness would set in and the pain diminished [19, 21, 22]. Pain-threshold was defined as the first experience of pain that can be identified, pain-tolerance as the time elapsed when the participant had to withdrew his hand (i.e., the most severe pain that a subject was willing to tolerate) and pain-intensity as the maximal pain experienced during test on a visual analogue scale (VAS; 0–100). We examined the cold-pressor responses six times over a 24 h period [i.e., at 0 h (at about 8.00 am), and at 2, 4, 8, 12, and 24 h after the first CPT], in order to minimise the possible diurnal variations in cold-pressor pain response [23].

PCR genotyping for 118A>G, IVS2+31G>A and IVS2+691G>C polymorphisms of *OPRM1*

Venous blood (2.5 ml) samples for genotyping were collected in tubes containing sodium citrate and the blood samples were stored at–20 °C until further processing. Genomic DNA was extracted from the unclotted venous blood using QIAamp® DNA Blood Mini Kit (Qiagen Gmbh, Hilden, Germany) according to the manufacturer's instructions. The quantity and quality of the extracted genomic DNA were determined on the NanoDrop®ND-1000 Spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA) with measurements performed at 260 and 280 nm.

A two-step PCR method for simultaneous *OPRM1* and *CYP2B6* genotyping were developed by the Institute for Research in Molecular Medicine (INFORMM) and this had been validated for reproducibility and specificity through direct sequencing [24]. All PCR reactions were performed in standard 0.2 ml Eppendorf PCR tubes and carried out in a volume of 25 µl comprising buffer [10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM EDTA, 0.1% Triton X-100, 50.0% glycerol (v/v)]. The reactions were performed on the Applied Biosystems® Veriti® 96-Well Thermal Cycler (Applied Biosystems, Carlsbad, CA, USA).

Briefly, the first step PCR ('Set A') was performed using specifically designed primers (table 1) to isolate out regions of interest that contain the relevant *OPRM1* polymorphisms (118A>G, IVS2+31G>A and IVS2+691G>C) that were later used for the second allele-specific PCR to avoid amplifications of similar sequences in the human genome that may be located outside the gene. PCR reaction mixture for Set A contained 1.0 U of Biotool® DNA *Taq* Polymerase (Biotools, Biotechnological & Medical Laboratories, SA, Madrid, Spain), 2.0 mM $MgCl_2$, 0.2 mM dNTPs (Biotools, Biotechnological & Medical Laboratories, SA, Madrid, Spain) and 0.10–0.25 µM of the primers (Invitrogen, Waltham, MA, USA). The cycling conditions were optimized for Set A. Ten microliters of the first PCR products of Set A were analyzed using 2.0% agarose gel (Promega Corporation, Madison, WI, USA) and 1 x TBE (Tris, Borate, EDTA) at 100 V for 60 min. Two microliters of the diluted first step PCR products of Set A were used as a template for detection of wild-type or mutant-type alleles in second step PCR. The second step PCR reaction was carried out using identical reaction mixture described for the first step PCR, with the exceptions of primer concentrations shown in table 1. The cycling conditions were again optimized and ten microliters of the second PCR products were again analyzed using 2.0% agarose gel (Promega Corporation, Madison, WI, USA) and 1 x TBE at 100 V for 60 min.

Data and statistical analysis

The sample size was calculated prior to recruitment based on the Cohen sample size table [25], using medium population effect size (ES) at the power of 0.80 for an α value of 0.05. Samples of 64 alleles or subjects per group were required for comparisons of means of two groups (under the allelic additive model, genotype dominant and recessive model).

Genotyping data were analyzed using the population genetic data analytical program, Golden Helix SNP and Variation Suite 7 (SVS 7, version 7.3.1; Golden Helix Inc., Bozeman, MT, USA) based on an expectation-maximization (EM) algorithm for the following procedures: (a) the calculation of *OPRM1* alleles and genotypes frequencies; (b) the estimation of heterozygosity in each polymorphism in Hardy-Weinberg proportion; (c) the estimation of maximum-likelihood haplotype frequency.

Table 1: *OPRM1* primers used for allele-specific multiplex PCR of OPRM1 118A>G (dbSNP rs1799971), IVS2+31G>A (dbSNP rs9479757) and IVS2+691G>C (dbSNP rs2075572)

PCR	Primer	Sequence (5'–3')	Fragment size (bp)	[Primer] (µM)
First PCR Set A	µ EX1 FW	aaa gtc tcg gtg ctc ctg gct	420	0.10
	µ EX1 RV	tgg gag tta ggt gtc tct ttg ta		0.10
	µ INT2 FW	tag att tcc gta ctc ccc gaa	1020	0.20
	µ INT2 RV	cgc aag atc atc agt cca tag		0.20
Second PCR Set 1				
Common primer	µ EX1 RV	tgg gag tta ggt gtc tct ttg ta		0.25
Wild-type primers	µ 118 A FW	caa ctt gtc cca ctt aga tgg ca	267	0.25
Mutant-type primers	µ 118 G FW	caa ctt gtc cca ctt aga tgg cg		0.25
Second PCR Set 2				
Common primer	µ INT2 RV	cgc aag atc atc agt cca tag		0.15
Wild-type primers	µ 691G FW	gct ctg gtc aag gct aaa aat g	240	0.15
Mutant-type primers	µ 691C FW	gct ctg gtc aag gct aaa aat c		0.15
Second PCR Set 3				
Common primer	µ INT2 FW	tag att tcc gta ctc ccc gaa		0.25
Wild-type primers	µ 31G RV	aac ata tca ggc tgt gaa ccc	162	0.25
Mutant-type primers	µ 31A RV	aac ata tca ggc tgt gaa cct		0.25

RESULTS

Distributions of *OPRM1* polymorphisms

The *118A/G*, *IVS2+31G>A* and *IVS2+691G>C* allele of OPRM1 gene were successfully amplified from all 152 subjects. Genotyping analysis revealed that one subject possessed polymorphism in the IVS2+31 locus of the OPRM1 gene (table 2). The genotype at the locus was heterozygous for *IVS2+31A* allele (IVS2+31G>A). The distribution of OPRM1 118A>G, IVS2+31G>A and IVS2+691G>C genotypes were in Hardy-Weinberg equilibrium (HWE) (p>0.129). Assuming a mutant-type allele was a high-risk allele, genotype frequencies under the dominant and recessive models were determined. The most likely haplotype pair or diplotype in each individual was estimated and the haplotype frequency distributions were obtained with an expectation-maximum (EM) algorithm (table 2).

The lack of associations of 118A>G and IVS2+691G>C polymorphisms with pain sensitivity

Due to low frequency of IVS2+31G>A polymorphism in the current study samples, further, analyses were not performed for this polymorphism, and thus, haplotype patterns were constructed from the two polymorphisms of *OPRM1* (118A>G and IVS2+ 691G>C).

The 118A>G and IVS2+691G>C polymorphisms were not associated with pain-threshold, pain-tolerance and pain-intensity despite using genotypes and allelic additive models and genotype dominant and recessive models (all p>0.05) (table 3, 4 and 5). Likewise, there were no significant associations between haplotypes and diplotypes for the 118A>G and IVS2+691G>C polymorphisms and the three cold-pain responses (all p>0.05) (table 3, 4 and 5

Table 2: Allele, genotype, haplotype and diplotype distributions for the three screened polymorphisms of *OPRM1* in opioid-naive Malay males

Polymorphism		N	Frequency (%)	95% CI of frequency		HWE *p* value
				Lower limit	Upper limit	
118A>G						
Genotype (N = 152)	AA	35	23.0	16.3	29.7	0.748
	AG	74	48.7	40.8	56.6	
	GG	43	28.3	21.1	35.5	
Allele (N = 304)	A	144	47.4	41.8	53.0	
	G	160	52.6	47.0	58.2	
Dominant model	AA	35	23.0	16.3	29.7	
	AG+GG	117	77.0	70.3	83.7	
Recessive model	AA+AG	109	71.7	64.5	78.9	
	GG	43	28.3	21.1	35.5	
IVS2+691G>C						
Genotype (N = 152)	GG	1	0.7	0.0	2.0	0.129
	GC	45	29.6	22.3	36.9	
	CC	106	69.7	62.4	77.0	
Allele (N = 304)	G	47	15.5	11.4	19.6	
	C	257	84.5	80.4	88.6	
Dominant model	GG	1	0.7	0.0	2.0	
	GC+CC	151	99.3	98.0	100.0	
Recessive model	GG+GC	46	30.3	23.0	37.6	
	CC	106	69.7	62.4	77.0	
IVS2+31G>A						
Genotype (N = 152)	GG	151	99.3	98.0	100.0	1.000
	GA	1	0.7	0.0	2.0	
	AA	0	0.0	0.0	0.0	
Allele (N = 304)	G	303	99.7	99.1	100.0	
	A	1	0.3	0.0	0.9	
Dominant model	GG	151	99.3	98.0	100.0	
	GA+AA	1	0.7	0.0	2.0	
Recessive model	GG+GA	152	100.0	100.0	100.0	
	AA	0	0.0	0.0	0.0	
Haplotype (N = 304)[a]						
1.	GCG	158	52.0	46.4	57.6	
2.	ACG	99	32.6	27.3	37.9	
3.	AGG	44	14.5	10.5	18.5	
4.	GGG	2	0.7	0.0	1.6	
5.	AGA	1	0.3	0.0	0.9	
Diplotype (N = 152)						
1.	ACG/GCG	51	33.6	26.1	41.1	
2.	GCG/GCG	41	27.0	19.9	34.1	
3.	GCG/AGG	23	15.1	9.4	20.8	
4.	ACG/AGG	19	12.5	7.2	17.8	
5.	ACG/ACG	14	9.2	4.6	13.8	
6.	GCG/GGG	2	1.3	0.0	3.1	
7.	AGA/ACG	1	0.7	0.0	2.0	
8.	AGG/AGG	1	0.7	0.0	2.0	

N, number of subject/allele/haplotype/diplotype; CI, confidence interval; HWE, Hardy-Weinberg equilibrium, [a]Haplotype patterns were constructed from the three screened polymorphisms of *OPRM1* (118A>G, IVS2+691G>C and IVS2+31G>A)

Table 3: Influences of 118A>G and IVS2+691G>C polymorphisms on pain-threshold in opioid-naive Malay males

Polymorphism	N	Mean#	95% CI		*F*-stat. (*df*)[a]	*p* value*
			Lower limit	Upper limit		
118A>G						
Genotype (N = 152)						
AA	35	62.40	40.83	83.98	0.73 (2)	0.483
AG	74	48.88	34.05	63.72		
GG	43	45.69	26.22	65.15		
Allele (N = 304)						
A	144	55.46	44.91	66.01	1.26 (1)	0.263
G	160	47.16	37.15	57.17		
Dominant model						
AA	35	62.40	40.90	83.91	1.40 (1)	0.238
AG+GG	117	47.71	35.95	59.47		
Recessive model						
AA+AG	109	53.22	41.00	65.45	0.42 (1)	0.518
GG	43	45.69	26.22	65.15		
IVS2+691G>C						
Genotype (N = 152)						
GG	1	20.90	-107.10	148.89	0.29 (2)	0.747
GC	45	46.40	27.32	65.48		
CC	106	53.37	40.93	65.80		
Allele (N = 304)						
G	47	45.32	26.83	63.81	0.45 (1)	0.504
C	257	52.15	44.24	60.06		
Dominant model						
GG	1	20.90	-106.82	148.61	0.22 (1)	0.640
GC+CC	151	51.29	40.90	61.68		
Recessive model						
GG+GC	46	45.85	27.03	64.67	0.43 (1)	0.511
CC	106	53.37	40.97	65.76		
Haplotype (N = 304)[b]						
GC	158	47.40	37.32	57.48	0.94 (3)	0.422
AC	99	59.73	46.99	72.46		
AG	45	46.06	27.18	64.95		
GG	2	28.55	-61.04	118.14		
GC	158	47.40	37.33	57.46	1.34 (2)	0.263
AC	99	59.73	47.01	72.44		
Combined AG and GG	47	45.32	26.86	63.77		
GC	158	47.40	37.32	57.47	1.08 (1)	0.299
Not GC	146	55.09	44.61	65.57		
AC	99	59.73	47.03	72.42	2.66 (1)	0.104
Not AC	205	46.92	38.10	55.74		
AG	45	46.06	27.16	64.96	0.32 (1)	0.571
Not AG	259	51.97	44.09	59.84		
Diplotype (N = 152)						
AC/GC	51	55.81	37.92	73.71	0.76 (4)	0.554
GC/GC	41	46.52	26.56	66.48		
GC/AG	23	33.51	6.86	60.16		
AC/AG	20	63.01	34.43	91.59		
Others[c]	17	57.71	26.71	88.71		
AC/GC	51	55.81	37.94	73.69	0.41 (1)	0.523
Not AC/GC	101	48.71	36.01	61.41		
GC/GC	41	46.52	26.58	66.46	0.28 (1)	0.597
Not GC/GC	111	52.78	40.66	64.90		
GC/AG	23	33.51	7.04	59.98	2.03 (1)	0.156
Not GC/AG	129	54.23	43.05	65.40		
AC/AG	20	63.01	34.51	91.52	0.79 (1)	0.377
Not AC/AG	132	49.29	38.19	60.38		

N, number of subject/allele/haplotype/diplotype; CI, confidence interval, # Means for cold pain-threshold (seconds); * *p*-value is significant at<0.05, [a]Repeated measured ANOVA between-group analysis was applied, [b]Haplotype patterns were constructed from the two polymorphisms of *OPRM1* (118A>G and IVS2+691G>C), [c]Diplotype with frequency less than 10.0% were pooled under 'Others' (included AC/AC, GC/GG, AG/AG)

Table 4: Influences of 118A>G and IVS2+691G>C polymorphisms on pain-tolerance in opioid-naive Malay males

Polymorphism	N	Mean#	95% CI		*F*-stat. (*df*)[a]	*p* value*
			Lower limit	Upper limit		
118A>G						
Genotype (N = 152)						
AA	35	73.92	50.24	97.60	0.72 (2)	0.491
AG	74	57.35	41.06	73.63		

GG	43	58.02	36.66	79.39		
Allele (N = 304)						
A	144	65.40	53.82	76.99	0.90 (1)	0.344
G	160	57.71	46.72	68.70		
Dominant model						
AA	35	73.92	50.32	97.52	1.44 (1)	0.232
AG+GG	117	57.60	44.69	70.51		
Recessive model						
AA+AG	109	62.67	49.24	76.10	0.13 (1)	0.717
GG	43	58.02	36.64	79.41		
IVS2+691G>C						
Genotype (N = 152)						
GG	1	29.11	-111.47	169.68	0.20 (2)	0.817
GC	45	57.62	36.66	78.57		
CC	106	63.25	49.59	76.90		
Allele (N = 304)						
G	47	56.41	36.10	76.71	0.27 (1)	0.602
C	257	62.26	53.58	70.94		
Dominant model						
GG	1	29.11	-111.09	169.30	0.21 (1)	0.649
GC+CC	151	61.57	50.16	72.98		
Recessive model						
GG+GC	46	57.00	36.33	77.67	0.25 (1)	0.619
CC	106	63.25	49.63	76.86		
Haplotype (N = 304)[b]						
GC	158	57.98	46.90	69.06	0.65 (3)	0.586
AC	99	69.09	55.10	83.09		
AG	45	57.28	36.52	78.05		
GG	2	36.63	-61.85	135.11		
GC	158	57.98	46.91	69.04	0.89 (2)	0.412
AC	99	69.09	55.12	83.07		
Combined AG and GG	47	56.40	36.12	76.69		
GC	158	57.98	46.91	69.04	0.75 (1)	0.387
Not GC	146	65.01	53.50	76.52		
AC	99	69.09	55.14	83.05	1.77 (1)	0.185
Not AC	205	57.62	47.92	67.32		
AG	45	57.28	36.53	78.04	0.17 (1)	0.676
Not AG	259	62.06	53.41	70.71		
Diplotype (N = 152)						
AC/GC	51	63.93	44.27	83.60	0.68 (4)	0.609
GC/GC	41	59.07	37.13	81.00		
GC/AG	23	42.75	13.46	72.03		
AC/AG	20	76.82	45.42	108.23		
Others[c]	17	66.12	32.05	100.18		
AC/GC	51	63.93	44.30	83.57	0.10 (1)	0.751
Not AC/GC	101	60.05	46.10	74.01		
GC/GC	41	59.07	37.16	80.97	0.06 (1)	0.810
Not GC/GC	111	62.20	48.89	75.51		
GC/AG	23	42.75	13.67	71.82	1.89 (1)	0.172
Not GC/AG	129	64.67	52.40	76.95		
AC/AG	20	76.82	45.57	108.08	1.10 (1)	0.296
Not AC/AG	132	59.01	46.85	71.18		

N, number of subject/allele/haplotype/diplotype; CI, confidence interval, # Means for cold pain-tolerance (seconds); * *p*-value is significant at<0.05, [a]Repeated measured ANOVA between-group analysis was applied, [b]Haplotype patterns were constructed from the two polymorphisms of *OPRM1* (118A>G and IVS2+691G>C), [c]Diplotype with frequency less than 10.0% were pooled under 'Others' (included AC/AC, GC/GG, AG/AG)

Table 5: Influences of 118A>G and IVS2+691G>C polymorphisms on pain-intensity scores in opioid-naive Malay males

Polymorphism	N	Mean#	95% CI		*F*-stat. (*df*)[a]	*p* value*
			Lower limit	Upper limit		
118A>G						
Genotype (N = 152)						
AA	35	65.24	60.55	69.93	0.79 (2)	0.457
AG	74	65.05	61.82	68.27		
GG	43	61.94	57.71	66.17		
Allele (N = 304)						
A	144	65.14	62.84	67.43	1.20 (1)	0.274
G	160	63.38	61.20	65.55		
Dominant model						
AA	35	65.24	60.54	69.93	0.24 (1)	0.623
AG+GG	117	63.90	61.33	66.47		
Recessive model						

AA+AG	109	65.11	62.46	67.76	1.58 (1)	0.211
GG	43	61.94	57.72	66.16		
IVS2+691G>C						
Genotype (N = 152)						
GG	1	66.67	38.77	94.56	0.02 (2)	0.984
GC	45	64.11	59.95	68.27		
CC	106	64.23	61.52	66.94		
Allele (N = 304)						
G	47	64.22	60.19	68.25	0.00 (1)	0.996
C	257	64.21	62.49	65.93		
Dominant model						
GG	1	66.67	38.86	94.47	0.03 (1)	0.861
GC+CC	151	64.19	61.93	66.46		
Recessive model						
GG+GC	46	64.17	60.07	68.27	0.00 (1)	0.980
CC	106	64.23	61.53	66.93		
Haplotype[b] (N = 304)						
GC	158	63.52	61.33	65.72	0.89 (3)	0.448
AC	99	65.30	62.53	68.07		
AG	45	64.78	60.67	68.89		
GG	2	51.67	32.17	71.16		
GC	158	63.52	61.33	65.72	0.49 (1)	0.613
AC	99	65.30	62.53	68.08		
Combined AG and GG	47	64.22	60.19	68.25		
GC	158	63.52	61.33	65.72	0.79 (1)	0.374
Not GC	146	64.95	62.67	67.24		
AC	99	65.30	62.53	68.07	0.89 (1)	0.345
Not AC	205	63.68	61.76	65.61		
AG	45	64.78	60.66	68.89	0.09 (1)	0.769
Not AG	259	64.11	62.40	65.83		
Diplotype (N = 152)						
AC/GC	51	65.10	61.18	69.02	0.23 (4)	0.922
GC/GC	41	62.44	58.07	66.81		
GC/AG	23	64.93	59.09	70.77		
AC/AG	20	64.42	58.15	70.68		
Others[c]	17	64.61	57.82	71.40		
AC/GC	51	65.10	61.21	68.99	0.31 (1)	0.581
Not AC/GC	101	63.76	61.00	66.53		
GC/GC	41	62.44	58.11	66.77	0.90 (1)	0.346
Not GC/GC	111	64.86	62.23	67.50		
GC/AG	23	64.93	59.13	70.72	0.07 (1)	0.791
Not GC/AG	129	64.08	61.64	66.53		
AC/AG	20	64.42	58.20	70.63	0.00 (1)	0.944
Not AC/AG	132	64.18	61.76	66.60		

N, number of subject/allele/haplotype/diplotype; CI, confidence interval, # Means for cold pain-intensity scores; * *p* value is significant at<0.05, [a]Repeated measured ANOVA between-group analysis was applied, [b]Haplotype patterns were constructed from the two polymorphisms of *OPRM1* (118A>G and IVS2+691G>C), [c]Diplotype with frequency less than 10.0% were pooled under 'Others' (included AC/AC, GC/GG, AG/AG)

DISCUSSION

The allelic frequencies of *OPRM1* polymorphisms in the present study were similar to previous reports from Singapore [*118G* = 45 (95% CI 39.0, 51.1) and *IVS2+691C* = 79.5 (95% CI 74.7, 84.4)] [27] with the exception of *IVS2+31G* allele. The frequency of *IVS2+31G* allele in our study was similar to other Asian populations for example, in the Taiwan population, the reported frequency was 2.8% (95% CI 0.1, 5.5)][17].

Among the identified polymorphisms within the OPRM1 gene, 118A>G polymorphism is the most frequently studied in the literature but the evidence is, unfortunately, inconsistent with regards to its association with pain sensitivity to various experimental stimuli [14, 15, 17, 28, 29]. In the present study, 118A>G and IVS2+691G>C polymorphisms were not associated with cold-pain responses among the Malay males. Only one published study which was similar to ours where the same cold-pain technique was used but in the Japanese subjects [29]. Although the association of cold pain sensitivity with vs. without *118G* allele was significant in this Japanese population but the response difference was only one second (7%) [29]. Tan*, et al.* (2009) found an association between 118A>G genotypes and pain-intensity among the non-laboring Chinese women undergoing caesarean section but again no such association was found among the Malays. This suggests that ethnicity may be a major determinant for the role of 118A>G polymorphism in pain sensitivity [17].

There are other explanations to our negative results. There may be differences in experimental pain models (for example electrical or heat pain rather than cold), gender, study sample size, frequency of mutant-type *118G* allele and whether study participants had been pre-medicated with centrally acting agents. Furthermore, the clinical impact of 118A>G polymorphism in pain sensitivity remains controversial. An earlier *in vitro* functional study has demonstrated that *118G* allele altered the β-endorphin binding affinity [31]. Moreover, a recent study did not find any marked functional differences between variant and wild-type receptors in terms of morphine, morphine-6-glucuronide and β-endorphin binding affinities and potencies [32]. They also found both the variant receptor and wild-type receptors exhibited robust receptor internalization and they showed similar desensitization time courses [32]. But *in vitro* studies showed that there was a lower expression of the receptor protein corresponding to the *118G* allele [32, 33]. In addition, it is likely that variations of other candidate genes may be more relevant in our population including *CYP2D6*, *ABCB1, COMT*, and other opioid receptor genes including OPRD1 and OPRK1 genes [34].

Some study limitations need to be highlighted. Firstly, we only studied Malay males, but this was to control for gender effect on

pain sensitivity [35-41] besides the fact that Malay males were the majority of opioid-dependent patients on methadone treatment in Malaysia. Secondly, our study did not evaluate for psychological distress including anxiety or stress which might influence pain perception [42]. Lastly, the sample size was inadequate for post-hoc studies and it was underpowered because the observed numbers of subjects with AA and GG genotypes were small. Thirdly, only one pain modality (i.e., cold-pain) was studied.

CONCLUSION

The current study indicates that the common *OPRM1* polymorphisms (i.e., 118A>G and IVS2+691G>C) are not associated with cold-pressor pain-threshold, tolerance and intensity in healthy opioid-naive Malay males. However, this may be unique for this particular ethnicity. Other polymorphisms may be more relevant for this population and this should be further investigated.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest

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