Sequence Variations in the Mu-opioid Receptor Gene (OPRM1) Associated with Human Addiction to Heroin

Jinxiu Shi1†, Lijian Hui1†, Yonghai Xu2, Feng Wang2, Wei Huang3*, and Gengxi Hu1*

1Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China; 2Nanjing Drug Abuse Control Bureau, Nanjing, Jiangsu, China; 3National Human Genome Center at Shanghai, Shanghai, China.

† These two authors contributed equally to this work.

*Correspondence to: Gengxi Hu, Shanghai Institute of Cell Biology and the Shanghai Life Science Center, Chinese Academy of Sciences, 320 Yue-yang Road, Shanghai 200031, China; Tel.: 86-21-64378218; Fax: 86-21-64718563; E-mail: xbshu@sunm.shcnc.ac.cn; or Wei Huang, Shanghai Human Genome Center, 351 Guo-shoujing Road, Shanghai 201203, China; Tel.: 86-21-50801919-43; Fax: 86-21-50801922; E-mail: huangwei@chgc.sh.cn

Contract grant sponsor: Special Grant for International Cooperation of the Chinese Academy of Sciences and the National Ministry of Science and Technology; Contract grant number: G-19980510007.

Human mu-opioid receptor (OPRM1) is the major site for the analgesic action of most opioid drugs such as morphine, methadone and heroin. It was previously reported that a single nucleotide polymorphism (SNP) in exon1 (c.118A→G) of OPRM1 might modestly alter the affinity in beta-endorphin-Mu interaction. Using denaturing high performance liquid chromatography (DHPLC) the complete coding region of the OPRM1 gene was screened for SNPs in Han-Chinese heroin addicts and normal control. Three novel SNPs were detected, one in exon3, one in intron3 and one in the 3' untranslated region. The SNP c.118A→G reportedly altered the interaction of Mu receptor with opioid had no statistically significant correlation with heroin addition in Han Chinese. However, addicted subjects with the SNP in intron2 (IVS2 +31G→A) tended to show much higher heroin intake dosages than those without this SNP. We also observed that individuals carrying both SNP c.118A→G and IVS2 +31G→A consumed relatively more drugs compared to other addicts. Thus our study further highlights the importance of studying the various regions of the mu opioid receptor gene, coding as well as non-coding, for genetic markers that may be linked to, or directly contribute to opioid drug-seeking behavior. © 2002 Wiley-Liss, Inc.

KEY WORDS: SNP; OPRM1; heroin; addiction; association

INTRODUCTION

Mu-opioid receptor gene (OPRM1; MIM# 600018; GenBank NM_000914) is an important component of the self-rewarding system, interacting with multiple endogenous opioid peptides including beta-endorphin (Selly et al., 1992), and endomorphins (Zadina et al., 1997). It also mediates the effects of several important opioid analgesic agents and drugs such as morphine, methadone, fentanyl (Basbaum et al., 1984; Pasternak, 1993), and especially
Allergic variants at the OPRM1 locus have been identified in both mouse and human (Uhl et al., 1999; Bond et al., 1998; Hoehe et al., 2000). In mouse, SNPs in OPRM1 significantly contributed to the differences in the modulation by opiates (Uhl et al., 1999). In human, the c.118A→G SNP in exon1 transition that caused an Asn40Asp substitution in the Mu receptor led to increased affinity of the receptor to its endogenous ligand, opioid peptide beta-endorphin, in vitro (Bond et al., 1998). It was suggested that genetic variation in OPRM1 gene might affect human addiction to opioid (Uhl et al., 1999).

We examined the genomic diversity of OPRM1 gene with regards to the susceptibility to heroin addiction using denaturing high performance liquid chromatography (DHPLC), which identifies SNPs based on the variation in melting temperature (Kuklin et al., 1997-98). Five SNPs including three novel and two previously reported (c.118A→G and IVS2+31G→A) were detected. The c.118A→G transition does not independently affect the heroin addiction, although it changed the affinity of mu receptor to beta-endorphin in vitro (Bond et al., 1998). Our results showed, however, the carriers of IVS2+31G→A in intron 2 had higher level of addiction to heroin.

MATERIALS AND METHODS

Study subjects

This study was approved by an institutional review board of the Chinese Academy of Sciences. Blood samples from heroin addicts and normal control were included in this study. All addictive subjects were former heroin-dependent addicts without other drug abuse. Addicts were then undergoing methadone maintenance treatment in Nanjing Drug Abuse Control Bureau. Detailed information including health conditions, heroin-intake routes, drug abuse history and daily intake dose was obtained when the abusers entered the methadone maintenance treatment. The drug dosage, on which the initial dose of methadone was based, was questioned before the methadone treatment. Soon after the beginning of treatment, drug dosage was questioned again in regard to the patients' response to methadone. Only subjects with consistent report on abuse history (median: 12 months, interquartile: 4.5-22 months) and effective dose of methadone concurred with empirical dose were included in this study. The daily intake dose ranged from 0.1g/day to 3g/day, and the median was 0.4 g/day and interquartile range was 0.2-0.8g/day. Participants recruited by advertisement were used as normal control and confirmed to be without drug or alcohol abuse history by a questionnaire. Control samples were selected so that the age (minimum=15, maximum=44, average=27.3±5.7) and sex (male:female=1:0.7) distributions were similar to that of the addicts. All subjects studied were Han Chinese. Detailed information is available at www.cell.ac.cn/sample-info.htm.

Amplification of samples

Genomic DNA was prepared from peripheral blood samples with standard phenol extraction protocol. Primers were designed to amplify the four coding exons of the OPRM1 gene. Exon3 was amplified in two overlapping pieces to optimize the separation of mutants from wild-type sequences in DHPLC. A typical amplification was performed in a 25µl reaction volume, containing 50ng of genomic DNA, 0.2µM of each primers, 100µM dNTPs, 20mM Tris-Cl (pH8.8), 10mM KCl, 1.0-1.5mM MgCl2 and 2.5U Pfu polymerase (Stratagene, La Jolla, CA) for 35 cycles at 94°C for 45 second, 63 oC for 1 minute, and 72 oC for 2 minutes in GeneAmp® PCR system 9700 (PE Applied Biosystem). Primer sequences for exon1 were F1: GCG, CTT, GGA, ACC, CGA, AAA, GTC; R1: CAT, TGA, GCC, TTG, GGA, GTT, A.; for exon2, F2: TGC, AAA, TTT, ATT, ATT, GGA, AGC; R2: CAA, CAT, ATC, AGG, CTG, TGA, ACC; for exon3, F3-1: ATG, TTG, CTG, CTA, ATT, TTT, CC; R3-1: CGT, AAA, TGT, GAA, TGG, GAC, TC; F3-2: TGG, CTC, CAA, AGA, AAA, GGA; R3-2: TGT, CAT, CCC, CAG, TAG, ATA, TAC, C; for exon4, F4: GAA, AAA, CTG, AGG, CTT, GCA, GGT, G; R4: CAC, ATT, CTT, GAA, GCA, ACC, TGC, TT.

DHPLC analysis and sequence determination

DHPLC was carried out on an automated HPLC instrument equipped with a DNA separation column (Transgenomic, San Jose, CA). For exons 1, 2, 3 (2 fragments) and 4, the temperature of the DHPLC column were 65°C, 58°C, 60°C and 59°C, and the acetonitrile gradient was 56-63%, 56-67%, 53-59%, 56-63% and 56-62%, respectively. PCR products displaying altered DHPLC chromatograph were bidirectionally sequenced by Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE, Foster City, CA). Sequencing was performed on an automated DNA sequencer ABI 377 (PE Applied Biosystem).
Statistics Method

To screen for all possible SNPs at OPRM1, 96 samples (48 addicts and 48 control individuals) were first analyzed. Only those SNPs that were located in an exon or observed more than once were studied in an expanded sample. For the rare sequence variations in frequencies lower than 1%, no effort was made to accurately determine their frequencies. The expanded sample size was determined by given allowable sampling error width (95% confidence interval of the population means). The SNP sites were studied in different numbers of samples based on the frequencies in order to assure the statistical significance. One hundred and forty-five cases and 48 controls were analyzed in c.118A>G SNP and 232 cases and 160 controls were analyzed in IVS2 +31G>A. In total 145 heroin addicts were analyzed in both c.118A>G and IVS2 +31G>A sites. Hardy-Weinberg equilibrium was checked using the chi square test.

Of the 193 individuals studied in allele c.118A→G, 145 were heroin addicts and for allele IVS2 +31G→A, 232 out of the 392 subjects were drug dependent (Tables 1 and 2). Assuming the genotype observed by Hoehe et al. (Hoehe, 2000), our case-control sample had a power of 70% at SNP IVS2 +31G→A (set statistical significance level \( \alpha = 0.05 \)). The frequency difference between addicts and control subjects was examined by chi square test. The distribution of the drug dosage did not follow Normal distribution; thus nonparametric Ridit analysis was used to examine the differences of addicts’ heroin intake dose among the genotypes (Table 2). The interaction between SNPs c.118 A→G and IVS2 +31G→A can not be tested by Two-Way ANOVA because the daily intake dose was not normally distributed. Mann-Whitney test was applied for the examination of the co-effect of both SNPs.

For analysis of linkage disequilibrium, Arlequin was applied. Linkage disequilibrium between c.118A→G and IVS2 +31G→A were tested for genotypic data using a likelihood-ratio test, whose empirical distribution is obtained by a permutation procedure (Slatkin and Excoffier, 1996).

RESULTS

We investigated the sequence diversity of the OPRM1 gene using DHPLC analysis. Five SNPs in exon 1, exon 3, intron 2, intron 3, and 3'UTR respectively, were observed (Table 1). Genotype frequencies of these SNPs were in Hardy-Weinberg equilibrium, making selection bias less likely (Chi-square test, \( P > 0.05 \)). Three of them were novel and submitted to HGBASE. However, the c.17C→T in exon1 detected in a frequency of 1.9% in Caucasian population (Bond et al., 1998) and other SNPs locating in OPRM1 gene (Hoehe et al., 2000) were not observed in Han Chinese samples, presumably due to the different ethnic populations, low frequencies, or different region of screening.

Table 1. SNPs in the Human OPRM1 Gene of Han Chinese

<table>
<thead>
<tr>
<th>SNP Name</th>
<th>Nucleotide Position</th>
<th>Amino Acid Change</th>
<th>Tested Samples/Alleles</th>
<th>Minor Alleles</th>
<th>SNP Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.118A→G</td>
<td>118(^1) (exon 1)</td>
<td>Asn→Asp</td>
<td>145 cases and 48 controls/383</td>
<td>99* (65 heterozygotes and 17 homozygotes)</td>
<td>25.8%</td>
</tr>
<tr>
<td>IVS2 +31G→A (SNP0005754322)</td>
<td>31 bp downstream of exon 2</td>
<td>—</td>
<td>232 cases and 160 controls/784</td>
<td>33§ (33 heterozygotes)</td>
<td>4.2%</td>
</tr>
<tr>
<td>c.877G→A (SNP000574323)</td>
<td>877(^7) (exon 3)</td>
<td>Ile→Val</td>
<td>98 cases and 48 controls/292</td>
<td>1 (1 heterozygote)</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>IVS3 -77G→A (SNP000574324)</td>
<td>77 bp upstream of exon 4</td>
<td>—</td>
<td>48 cases and 48 controls/192</td>
<td>1 (1 heterozygote)</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>3'UTR +9C→T (SNP000574325)</td>
<td>9 bp downstream of stop codon</td>
<td>—</td>
<td>48 cases and 48 controls/192</td>
<td>1 (1 heterozygote)</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

\(^1\): Position in the CDS starting from the ATG initial codon.
\(^7\): SNP Frequency = Detected Alleles / (Tested Samples x 2) x 100%.

*: 47 heterozygotes (AG) and 13 homozygotes (GG) in case group and 18 heterozygotes and 4 homozygotes in control.
§: 18 heterozygotes in case and 15 heterozygotes in control.
Of the two SNPs, c.118A→G and IVS2 +31G→A, which presented frequencies higher than 1% in the population studied, there was no significant difference in SNP distribution between heroin addicts and the control population (Chi-square test, P>0.05). In addition, no significant difference was observed in length of heroin addiction history (Kruskal-Wallis test, P>0.05), health conditions, age, and sex (Chi-square test, P>0.05) among the addicts with different OPRM1 allele types. These results were consistent with the epidemiological data which suggested that social factors such as education background, employment status and financial income level were much more important in affecting the drug-administration than genetic factors (Pang et al., 1999).

When the genotypes of the subjects were plotted against the daily drug intake dose, it was observed that the addicts heterozygous at SNP IVS2 +31G→A tended to have a higher daily intake dose of heroin compared to the GG homozygotes (Table 2, Ridit test, P<0.05). However, no significant difference was observed among the addicts with c.118AA, c.118AG, and c.118GG genotypes (Ridit test, P>0.05).

Table 2. Genotype and Heroin Intake Dosage Association

<table>
<thead>
<tr>
<th>Daily Intake Dose(g/day)</th>
<th>c.118A→G</th>
<th>IVS2 +31G→A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>0.1-0.5</td>
<td>70(0.82)</td>
<td>33(0.70)</td>
</tr>
<tr>
<td>0.6-1.0</td>
<td>10(0.12)</td>
<td>11(0.23)</td>
</tr>
<tr>
<td>1.1-2.0</td>
<td>3(0.04)</td>
<td>2(0.04)</td>
</tr>
<tr>
<td>2.1-3.0</td>
<td>2(0.02)</td>
<td>1(0.02)</td>
</tr>
</tbody>
</table>

On the other hand, our observation suggests that the c.118G→A SNP may play a moderate role in opioid physiology. Median daily-heroin-intake in the six addicts who had both a heterozygous allele in SNP IVS2 +31G→A and at least one “G” allele in c.118A→G (median: 1g/day, interquartile: 1-1.5g/day) was 2.5-fold higher than the average (median: 0.4g/day, interquartile: 0.2-0.8g/day) (Table 3). Compared to the 139 addicts with other genotypes, which were analyzed both in c.118A→G and IVS2 +31G→A, individuals with c.118 AG/GG and IVS2 +31 GG showed relatively high daily dose intake (Mann-Whitney Test, P=0.001). However, no linkage disequilibrium between these two SNPs was observed by Arlequin (P>0.05).

Table 3. Heroin Intake Dosage of Individuals with Both c.118 A→G and IVS2 +31G→A SNPs

<table>
<thead>
<tr>
<th>Individual Genotype</th>
<th>c.118A→G</th>
<th>IVS2 +31G→A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GG</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>GA</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>GA</td>
</tr>
<tr>
<td>Daily Intake Dose (g/day)</td>
<td>1 1 2 1.5 1</td>
<td>0.8</td>
</tr>
<tr>
<td>Median (Interquartile Range) (g/day)</td>
<td>1(1-1.5)</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The SNP frequencies we detected on the OPRM1 gene were not identical to that previously reported Berrettini et al.,1997; Bond et al., 1998; Bergen et al., 1997; Hoehe et al., 2000), presumably due to the different ethnic populations involved in our study. For example, we didn’t find the c.17C→T SNP in our tested population, although the frequency of the SNP was once reported 10% in healthy Caucasian and African-American volunteers and 22% in cocaine/opioid addicts (Berrettini et al., 1997). Consistent with this observation, the frequency of the c.17C→T SNP varied significantly in other case reports of different populations (Bond et al., 1998; Bergen et al., 1997).

No significant difference was observed between addicts with c.118AA and c.118AG/GG genotypes, suggesting that the SNP alone did not affect the heroin addiction although it altered the affinity between the mu receptor and beta-endorphin in vitro (Bond et al., 1998). Gelernter and his colleagues studied the c.17C→T and c.118A→G SNPs in exon1 in a number of populations, and did not observe any relationship between these two SNPs and substance dependence status (Gelernter et al., 1999). Another report also indicated no significant association between the particular genotype of OPRM1 at this allele and heroin or alcohol dependence (Franke et al., 2001).
Our data was consistent with their results, suggesting that biological effect of the SNP c.118A→G was rather subtle, if any. On the other hand, the SNP c.118G→A SNP may play a moderate role in opioid physiology as heroin addicts with genotypes of c.118AG/GG and IVS2 +31GA tend to have 2.5-fold higher daily heroin intake than the median of all populations. It is possible that SNPs coordinately contribute to the drug addiction. More samples with the particular genotypes should be examined to confirm the coordinate function of the two SNPs.

The SNP IVS2 +31G→A, which was associated with drug consumption, was located in an intron. The mechanism by which this SNP contributed to heroin addiction has yet to be discovered. Numerous SNPs in introns of Mu receptor gene associated with specific phenotypes were reported (Hu et al., 1998; Horikawa et al., 2000). Possible SNP(s) genetically linked to the IVS2 +31G→A SNP might change the regulation of the expression of OPRM1 gene. Analyses of the OPRM1 5’ flanking sequence might help to find sequence variants related to the functional changes (Uhl et al., 1999). It was also possible that the influence of the SNP IVS2 +31G→A on heroin usage is more direct. For example, it may reside in an intron-based transcription enhancer or silencer, thus different alleles may have different impacts on the transcription of mu receptor gene. The other possibility, suggested by Hohe and colleagues (Hohe et al., 2000), maybe the disruption of (A/T)GGG repeat, which was known to be able to regulate alternative splicing (Strand-Pugnet et al., 1995).

Although our data doesn't give support to the correlation between c.118A→G and heroin addiction, it favors the idea that SNPs located in other regions may influence drug intake. Because we only studied the exon region and very short intron of OPRM1, a genetic screening for SNPs in the promoter region of the OPRM1 gene and the measurement of the OPRM1 expression level in patients with different genotypes should be proposed in the further study of the role of genetic variations in the susceptibility of drug abuse.

ACKNOWLEDGMENTS

The authors thank Drs. Lei Yu, Wolfgang Hennig and Shijie Xu for their suggestion and careful proofreading of this paper. We are appreciated for Dr. Naqing Zhao’s helpful consultation in statistic analyses.

REFERENCES


