Genome-wide association study identifies a potent locus associated with human opioid sensitivity

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Supplementary Materials and Methods

1. Subjects, surgery and clinical data

1.1. Patients who underwent painful cosmetic surgery

1.1.1. Subjects

Enrolled in this multistage genome-wide association study (GWAS) were 355 healthy patients (American Society of Anesthesiologists Physical Status I, age 15-50 years, 125 males and 230 females) who were scheduled to undergo cosmetic orthognathic surgery (mandibular sagittal split ramus osteotomy) for mandibular prognathism at Tokyo Dental College Suidoubashi Hospital. Patients with chronic pain, those taking pain medication, and those who had experienced Raynaud’s phenomenon were excluded. The study protocol was approved by the Institutional Review Board, Tokyo Dental College, Chiba, Japan, and the Institutional Review Board, Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science), Tokyo, Japan. Written informed consent was obtained from all of the patients and also from parents if required. The detailed demographic and clinical data of the subjects are provided in Supplementary Table S1.

1.1.2. Anesthesia and surgery

The surgical protocol and subsequent postoperative pain management were fundamentally the same as a previous study\(^1\). The patients received an intravenous bolus injection of fentanyl, 2 \(\mu\)g/kg, before the orthognathic surgery. Afterward, general anesthesia was induced with target-controlled infusion of propofol using a target-controlled infusion pump (TE-371, Terumo, Tokyo, Japan). Vecuronium, 0.1 mg/kg, was administered to facilitate nasotracheal intubation. After the induction of anesthesia, 10 ml of venous blood was sampled for the preparation of DNA specimens. General anesthesia was maintained with propofol at a target blood concentration of 4-6 \(\mu\)g/ml. Vecuronium was administered at a rate of 0.08 mg/kg/h. The lungs were ventilated with oxygen-enriched air.
Local anesthesia was performed on the right side of the surgical field with 8 ml of 2% lidocaine that contained epinephrine, 12.5 μg/ml, and right mandibular ramus osteotomy was performed. Local anesthesia was then administered on the left side, and left mandibular ramus osteotomy was performed. The bilateral mandibular bone segments were fixed in appropriate positions. Whenever systolic blood pressure or heart rate exceeded +20% of the preinduction value during surgery, intravenous fentanyl, 1 μg/kg, was administered.

1.1.3. Postoperative pain management

At the end of the surgery, rectal diclofenac sodium, 50 mg, and intravenous dexamethasone, 8 mg, were administered at the request of surgeons to prevent postoperative orofacial edema/swelling. After emergence from anesthesia and tracheal extubation, droperidol, 1.25 mg, was administered intravenously to prevent nausea/vomiting, and intravenous patient-controlled analgesia (PCA) with a fentanyl-droperidol combination (2 mg fentanyl and 5 mg droperidol diluted in normal saline in a total volume of 50 ml) commenced using a CADD-Legacy PCA pump (Smiths Medical Japan, Tokyo, Japan). The PCA settings included a bolus dose of 20 μg fentanyl on demand and a lockout time of 10 min. Continuous background infusion was not employed. Droperidol was coadministered with fentanyl to prevent nausea/vomiting because our preliminary study showed a high incidence (up to 30%) of nausea/vomiting with PCA fentanyl in young females. Patient-controlled analgesia was continued for 24-h postoperatively. In cases of treatment-refractory adverse effects or inadequate analgesia, PCA was discontinued, and rectal diclofenac sodium, 50 mg, was prescribed as a rescue analgesic as required. Intraoperative fentanyl use and postoperative PCA fentanyl use during the first 24-h postoperative period were recorded. Doses of fentanyl administered intraoperatively and postoperatively were normalized to body weight.

1.2. Patients who underwent major abdominal surgery
1.2.1. Subjects

The recruitment of the subjects and the basic protocol of postoperative pain management were described in a previous report\textsuperscript{2,3}. The subjects used in the current association study were 112 patients who underwent major open abdominal surgery (age 28-80 years, 60 males and 52 females), mostly gastrectomy for gastric cancer and colectomy for colorectal cancer, under combined general and epidural anesthesia at Research Hospital, Institute of Medical Science, The University of Tokyo, or Toho University Sakura Medical Center. Peripheral blood or oral mucosa samples were collected from these subjects for gene analysis. Patients who underwent surgery without substantially severe pain, such as Laparoscopy-Assisted Distal Gastrectomy (LADG), were excluded from the analyses. The study protocol was approved by the Institutional Review Boards at the Institute of Medical Science, The University of Tokyo, Tokyo, Japan, Toho University Sakura Medical Center, Sakura, Japan, and Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science), Tokyo, Japan. All of the subjects provided informed, written consent for the genetic studies. The detailed demographic and clinical data of the subjects are provided in Supplementary Table S5.

1.2.2. Clinical data

Postoperative pain was managed primarily with continuous epidural analgesia with fentanyl or morphine. Fentanyl or morphine was diluted with 0.25% bupivacaine in a total volume of 100 ml and infused at a constant rate of 2 ml/h through a catheter placed in the lower thoracic or upper lumbar epidural space. Whenever the patient complained of significant postoperative pain despite continuous epidural analgesic, appropriate doses of opioids, including morphine, buprenorphine, pentazocine, and pethidine, or nonsteroidal anti-inflammatory drugs (NSAIDs), including diclofenac and flurbiprofen, were administered as rescue analgesics at the discretion of the surgeons. The clinical data collected in the present study included age, gender, height, body weight, postoperative diagnosis, type of operation, duration of operation, and doses of rescue analgesics (opioids or NSAIDs)
administered during the first 24-h postoperative period, for which analgesic therapy would be required in most patients.

To allow intersubject comparisons of rescue analgesic doses required during the first 24-h postoperative period, the doses of opioids and NSAIDs administered as rescue analgesics during this period were converted to the equivalent dose of systemic fentanyl according to previous reports\textsuperscript{2,3}. The total dose of rescue analgesics administered was calculated as the sum of systemic fentanyl-equivalent doses of all opioids and NSAIDs administered to patients as rescue analgesics during the same period. Doses of rescue analgesics administered postoperatively were normalized to body weight.

1.3. Patients with methamphetamine dependence/psychosis

1.3.1. Subjects

Enrolled in the study were 203 patients with methamphetamine (METH) dependence (age 18-69 years, 165 males and 38 females), mostly having comorbid METH-induced psychosis (age 19-69 years, 155 males and 30 females). Patients with an individual or family history of drug dependence or major psychotic disorders, such as schizophrenia and bipolar disorders, were excluded. Consensus diagnoses of METH dependence were made by at least two trained psychiatrists according to the criteria of the \textit{International Classification of Diseases} (ICD) or \textit{Diagnostic and Statistical Manual of Mental Disorders}, 4th edition (DSM-IV), on the basis of interviews and medical records, and abusers who used METH but displayed no dependence/psychosis were excluded from the analyses. Control subjects were included for comparisons with patient subjects and consisted of 221 age- and gender-matched healthy people (age 19-84 years, 170 males and 51 females). All of the subjects were unrelated Japanese and lived in Japan. This study was approved by the Institutional Review Board at the Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science) and the Ethics Committee of each participating institute of the Japanese Genetics Initiative for Drug Abuse
Written informed consent was obtained from all of the patients and control subjects. The detailed demographic and clinical data of the patient subjects are provided in Supplementary Table S6.

1.3.2. Clinical data

A large number of patients were dependent not only on METH but also other drugs, such as cannabinoids, cocaine, lysergic acid diethylamide, and opioids. To estimate the severity of and liability to dependence, patients with METH dependence/psychosis were divided into subgroups according to their multisubstance abuse status. A total of 142 patients were polydrug abusers, and 54 patients were exclusively dependent on METH. The characteristics of the subjects used in this study were also described in several other reports.

1.4. Patients with alcohol dependence

1.4.1. Subjects

The subjects who participated in the study were a total of 438 male Japanese patients with alcohol dependence (age 31-77 years, 438 males and 0 females) who came to the Kurihama Alcoholism Centre for treatment of alcoholism and were diagnosed with either alcohol dependence or alcohol abuse based on the Structured Clinical Interview for DSM-III-R (SCID). The age- and gender-matched control group consisted of 349 unrelated Japanese subjects (age 20-82 years, 349 males and 0 females). Samples with homozygous (*2/*2) genotypes in the aldehyde dehydrogenase-2 gene (ALDH2), which is known to be a strong negative risk factor for alcohol abuse, were excluded from the study. The study protocol was approved by the Institutional Review Boards at the Kurihama Alcoholism Centre, Yokosuka, Japan, and Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science), Tokyo, Japan. All of the subjects provided informed, written consent for the genetic studies. The detailed demographic and clinical data of the
patient subjects are provided in Supplementary Table S7.

1.4.2. Clinical data

Information about clinical parameters was attached to the genomic DNA samples of the subjects offered by the Kurihama Alcoholism Centre for the treatment of alcoholism. The clinical data collected in the present study included several parameters, such as age at onset, number of drugs used, and genotype of the \textit{ALDH2} gene, along with several psychological scales, such as the Self-rating Depression Scale (SDS)^9, Sensation Seeking Scale (SSS)^10, and MacAndrew Alcoholism (MAC) Scale^11 (Supplementary Table S7). The characteristics of the subjects used in the present study were provided in previous reports^12-14.

1.5. Patients with eating disorders

1.5.1. Subjects

The subjects recruited were 228 Japanese patients with eating disorders (age 13-69 years, 16 males and 211 females) who had been diagnosed, evaluated, and treated at one of three hospitals (Kurihama Alcoholism Center, Akagi Kougen Hospital, and Kobe University Hospital). The subjects were diagnosed with anorexia nervosa, bulimia nervosa, or eating disorders not otherwise specified. An age- and gender-matched control group consisted of 268 unrelated Japanese subjects (age 18-39 years, 10 males and 249 females). The controls were employees and students at the Kurihama Alcoholism Center and Tokyo Metropolitan Institute of Gerontology. The study protocol was approved by the Institutional Review Boards at the Kurihama Alcoholism Centre, Yokosuka, Japan, Akagi Kougen Hospital, Shibukawa, Japan, Kobe University Hospital, Kobe, Japan, and Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science), Tokyo, Japan. All of the subjects provided informed, written consent for the genetic studies. The detailed demographic and clinical data of the patient subjects are provided in Supplementary Table S8.
1.5.2. Clinical data

Information about clinical parameters was obtained by three of the medical doctors in face-to-face interviews using a clinical assessment form developed for this study. DSM-IV criteria were used to diagnose eating disorders. The clinical data collected in the present study included several parameters, such as age at onset, course of the disorder, the presence or absence of other psychiatric disorders such as substance dependence, and changes in weight (Supplementary Table S8). Control subjects were also asked to answer the questions to measure the symptoms and concerns characteristic of eating disorders based on the Eating Attitudes Test (EAT-26)\textsuperscript{15}. No control subject showed any signs of abnormal eating behavior. The characteristics of the subjects used in this study were also described in several other reports\textsuperscript{16,17}.

1.6. Healthy volunteers with personality profile data

1.6.1. Subjects

The subjects enrolled in the current association study were a total of 500 healthy volunteers who lived in the Kanto area of Japan and kindly agreed to participate in the study (age 20-72 years, 253 males, 242 females, and 5 gender-unknown subjects). Oral mucosa samples were collected from these subjects for the gene analysis. Additionally, the Temperament and Character Inventory (TCI)\textsuperscript{18-20}, a self-report measure of temperament and character dimensions, was used to profile the personalities of these subjects. The study protocol was approved by the Institutional Review Board at the Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science), Tokyo, Japan. All of the subjects provided informed, written consent for the genetic studies. The detailed demographic and clinical data of the subjects are provided in Supplementary Table S10.

1.6.2. Personality profile data
The TCI was used to assess the personality profiles of all of the subjects. The TCI is a 2-point self-report scale that consists of 240 questionnaire items in the full version, originally developed by Cloninger et al. as a psychobiological model of the structure and development of personality that accounts for dimensions of both temperament and character. The temperament dimensions are defined in terms of individual differences in associative learning in response to novelty, danger, or punishment and consist of novelty seeking (NS), harm avoidance (HA), reward dependence (RD), and persistence (P), which are independently heritable, manifest early in life, and involve preconceptual biases in perceptual memory and habit formation. Character development was defined in terms of insight learning or reorganization of self-concepts, in which insight involves the conceptual organization of perception and was defined as the apprehension of relationships. Each aspect of the self-concept corresponds to one of three character dimensions, including self-directedness (SD), cooperativeness (C), and self-transcendence (ST).

The TCI used in the present study was based on the shortened 125-item questionnaire of the longer 240-item Japanese version of the TCI, for which internal reliability and construct validity were confirmed among different Japanese populations. Three of the four TCI temperament dimensions, NS, HA, and RD, are further subdivided into four, four, and three subscales (NS1-4, HA1-4, and RD1, RD3, and RD4, respectively), whereas the three TCI character dimensions, SD, C, and ST, are further subdivided into five, five, and three subscales (SD1-5, C1-5, and ST1-3, respectively). These subscales indicate the following: NS1, exploratory excitability; NS2, impulsiveness; NS3, extravagance; NS4, disorderliness; HA1, worry/pessimism; HA2, fear of uncertainty; HA3, shyness with strangers; HA4, fatigability and asthenia; RD1, sentimentality vs. insensitivity; RD2, persistence; RD3, attachment vs. detachment; RD4, dependence vs. independence; S1, responsibility vs. blaming; S2, purposefulness; S3, resourcefulness; S4, self-acceptance vs. self-striving; S5, congruent second nature; C1, social acceptance vs. social intolerance; C2, empathy; C3, helpfulness; C4, compassion vs. revengefulness; C5, pure-hearted.
principles; ST1, self-forgetful vs. self-conscious experience; ST2, transpersonal identification vs. self-differentiation; ST3, spiritual acceptance vs. rational materialism. The 125-item version of the TCI properly incorporates the appropriate number of items within each scale and subscale.

1.7. Postmortem specimens for expression analysis

1.7.1. Subjects

To examine the mRNA expression levels of the gene proximal to the SNP associated with opioid sensitivity, postmortem human brain specimens were obtained from the Stanley Medical Research Institute (Bethesda, MD) as samples independent of those in the association study with opioid sensitivity (SMRI samples). The samples comprised a total of 105 human DNA samples extracted from the human occipital cortex and 100 RNA samples extracted from the human anterior cingulate cortex of the same specimens. The racial backgrounds of the subjects from which the samples for the study were obtained were 103 European American, one African American, and one Native American (age 19-64 years, 69 males and 36 females). Other characteristics of the subjects were detailed in a previous report and the website of the institute (http://www.stanleyresearch.org/dnn/; accessed March 1, 2012). The study protocol was approved by the Institutional Review Board at the Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science), Tokyo, Japan.

2. Genotyping

2.1. Whole-genome genotyping

2.1.1. Genotyping procedure

A total of 355 DNA samples from the patients who underwent painful cosmetic surgery were used for genotyping. Total genomic DNA was extracted from whole-blood samples using standard procedures. The extracted DNA was dissolved in TE buffer (10 mM tris-HCl and 1 mM ethylenediaminetetraacetic acid [EDTA], pH 8.0). The DNA concentration was adjusted to 100 ng/μl.
for whole-genome genotyping using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Briefly, whole-genome genotyping was performed using the Infinium assay II with an iScan system (Illumina, San Diego, CA) according to the manufacturer’s instructions. Five kinds of BeadChips were used for genotyping 40, 67, 6, 119, and 123 samples, respectively: HumanHap300 (total markers: 317,503), HumanHap300-Duo (total markers: 318,237), Human610-Quad v1 (total markers: 620,901), Human1M v1.0 (total markers: 1,072,820), and Human 1M-Duo v3 (total markers: 1,199,187). Some of the BeadChips included a number of probes specific to copy number variation markers, but most of the BeadChips were for SNP markers on the human autosome or sex chromosome. Approximately 300,000 SNP markers were commonly included in all of the BeadChips. For further analyses of polymorphisms other than these SNPs around the candidate region, genotype data that resulted from whole-genome genotyping were used.

2.1.2. Quality control

The data for the whole-genome genotyped samples were analyzed using BeadStudio or GenomeStudio with the Genotyping module v3.3.7 (Illumina) to evaluate the quality of the results. The genotype data from all five of the BeadChips were merged to analyze all of the samples simultaneously (i.e., only the markers common to all of the BeadChips were included in the analysis, and the others were automatically excluded). In the data-cleaning process, the samples with a genotype call rate of less than 0.95 were excluded from further analyses. As a result, one sample was excluded from further analyses. Markers with a genotype call frequency of less than 0.95 or “Cluster sep” (i.e., an index of genotype cluster separation) of less than 0.1 were excluded from the subsequent association study. A total of 295,036 SNP markers survived the filtration process and were used for the GWAS (Supplementary Figure S1). Markers were not excluded based on the results of \( \chi^2 \) tests to compare the distributions of the obtained genotype data with the theoretical
distributions expected from Hardy-Weinberg equilibrium in the initial step, but the tests were conducted for the SNPs that survived after the final stage, confirming that no significant deviation of the observed distributions from the expected distributions was observed, in which markers with $P$ values (df = 1) greater than approximately $2 \times 10^{-7}$ (0.05 / 300,000) were considered significantly deviated (Supplementary Table S12).

2.2. Genotyping for specific SNPs

2.2.1. Genotyping procedure and quality control

To genotype the rs2952768 and rs2254137 SNPs, the TaqMan allelic discrimination assay (Life Technologies, Carlsbad, CA) was adopted. For samples that were not appropriately genotyped by this assay, direct sequencing was alternatively adopted to genotype the rs2952768 SNP. A total of 112, 203, 438, 228, 500, and 105 DNA samples from the patients who underwent major abdominal surgery, patients with METH dependence/psychosis, patients with alcohol dependence, patients with eating disorders, healthy volunteer subjects, and SMRI, respectively, were used for genotyping the rs2952768 SNP. Additionally, a total of 105 DNA samples from the postmortem specimens for the expression analysis were used for genotyping the rs2254137 SNP, although genotyping this SNP for other samples was not conducted because of the strength of the linkage disequilibrium (LD) with the rs2952768 SNP. Distribution of genotype of the rs2952768 SNP in patients with METH dependence/psychosis, patients with alcohol dependence, and patients with eating disorders is provided in Supplementary Table S9.

For the SMRI samples, genomic DNA was extracted and adjusted to 10 ng/µl at the Stanley Medical Research Institute before sending it to Tokyo Institute of Psychiatry (currently Tokyo Metropolitan Institute of Medical Science). For the remainder of the samples, total genomic DNA was extracted from whole-blood or oral mucosa samples using standard procedures or as described in a previous report$^{23}$. The extracted DNA was dissolved in TE buffer (10 mM tris-HCl and 1 mM
EDTA, pH 8.0) before use. The DNA concentration was adjusted to approximately 5-50 ng/μl for genotyping the SNPs using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

To perform the TaqMan allelic discrimination assay with a LightCycler 480 (Roche Diagnostics, Basel, Switzerland), TaqMan SNP Genotyping Assays (Life Technologies) that contained sequence-specific forward and reverse primers to amplify the polymorphic sequence and two probes labeled with VIC and FAM dye to detect both alleles of the rs2952768 and rs2254137 SNPs (Assay ID: C__11510543_20 and C__11514151_20, respectively) were used. Real-time polymerase chain reaction (PCR) was performed in a final volume of 10 ml that contained 2×LightCycler 480 Probes Master (Roche Diagnostics), 40×TaqMan Gene Expression Assays, 5 ng genomic DNA as the template, and up to 10 ml H₂O equipped with 2×LightCycler 480 Probes Master. The thermal conditions were the following: 95°C for 10 min, followed by 45 cycles of 95°C for 10 s and 60°C for 60 s, with a final cooling at 50°C for 30 s. Afterward, endpoint fluorescence was measured for each sample well, and each genotype was determined based on the presence or absence of each type of fluorescence.

To perform direct sequencing, primers were designed to cover the polymorphic site within the 2q33.3-2q34 locus on the basis of the reference genomic contig sequence in the National Center of Biotechnology Information database (GenBank Accession No. NT-005403). PCR was performed with forward and reverse primers suitable for the region. Following the cycle sequencing reaction with a BigDye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies) according to the manufacturer’s instructions and purification of the PCR products, the DNA sequences of the fragments were determined using the automated sequencer ABI PRISM 3100 Genetic Analyzer (Life Technologies). The sequences of the forward and reverse primers used for PCR were 5′-ATAGTGCTTTAGCTGGGCGAGGTG-3′ and 5′-TGGCAGGCAGTAAACAACAGATG-3′, respectively, and the latter sequence was also used for the cycle sequencing reaction.
3. Real-time quantitative PCR (qPCR)

3.1. qPCR procedure

The SMRI RNA samples were treated with DNase I using the RNase-Free DNase Set (Qiagen, Hilden, Germany) at room temperature (20-25°C) for 10 min, and then clean-up was performed using the RNeasy MinElute Cleanup Kit (Qiagen). First-strand cDNA for use in the real-time qPCR was synthesized with the SuperScriptIII First-Strand synthesis system for qRT-PCR (Life Technologies) with 100 ng purified total RNA according to the manufacturer’s protocol and diluted properly with diethylpyrocarbonate (DEPC)-treated H₂O before the experiments.

To perform real-time qPCR with a LightCycler 480 (Roche Diagnostics), TaqMan Gene Expression Assays (Life Technologies) were used as a probe/primer set specified for the FAM119A (METTL21A) gene (Assay ID: Hs01592552_m1) and CREB1 gene (Assay ID: Hs00231713_m1) and a probe/primer set for the ACTB gene, a house-keeping gene that encodes β-actin (Assay ID: Hs99999903_m1). PCR was performed in a final volume of 10 μl that contained 2× LightCycler 480 Probes Master, 0.5 μl TaqMan Gene Expression Assay, 0.5 μl cDNA as the template, and up to 10 μl H₂O equipped with 2× LightCycler 480 Probes Master. The PCR program was the following: 95°C for 10 min, 45 cycles of 95°C for 10 s and 60°C for 30 s, followed by 95°C for 10 s, 50°C for 30 s, and 50-70°C (continuously) at a rate 0.06°C/s, with final cooling at 50°C for 30 s. The expression level of the FAM119A (METTL21A) gene or CREB1 gene was normalized to the expression level of the ACTB gene for each sample, and relative mRNA expression levels were compared between the genotype subgroups for each gene. The experiments were performed in triplicate (separate experiments) for each sample, and average values were calculated for normalized expression levels.

4. Statistical analysis

4.1. Genome-wide association study
A multistage GWAS was conducted for the patients who underwent painful cosmetic surgery to investigate the association between genetic variations and opioid sensitivity. Among 355 subjects, one subject lacked postoperative clinical data and another subject did not pass the criteria for quality control (see section 2.1.2) in our preliminary analysis. Therefore, a total of 353 subjects were used for our multistage GWAS (118, 117, and 118 subjects for the first-, second-, and final-stage analyses, respectively). The subjects recruited within several years were randomly categorized into three independent groups to minimize bias in the clinical data, indicating that the samples and clinical data collected were not used in chronological order for our first-, second-, and final-stage analyses. In our preliminary analysis that used merged markers between different BeadChips with BeadStudio or GenomeStudio, 295,036 SNPs were selected for the analyses.

As an index of opioid sensitivity, postoperative PCA fentanyl use during the first 24-h postoperative period was used because analgesic requirements likely reflect the efficacy of fentanyl in each individual. Prior to the analyses, the quantitative values of postoperative fentanyl requirements (µg/kg) were natural-log-transformed for the approximation to the normal distribution according to the following formula: \( \text{Value for analyses} = \ln (1 + \text{postoperative fentanyl requirement} [\mu g/kg]) \). To explore the association between the SNPs and phenotype, linear regression analyses were conducted in each stage of the analysis, in which postoperative fentanyl use (µg/kg; log-transformed) and the genotype data for each SNP were incorporated as dependent and independent variables, respectively. Additive, dominant, and recessive genetic models were used for the analyses because of the previously insufficient knowledge about the genetic factors associated opioid sensitivity. The association study included both female and male subjects for autosomal markers, although male genotypes were excluded from the analysis of X chromosome markers. All of the statistical analyses were performed using gPLINK v. 2.050, PLINK v. 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/; accessed March 1, 2012)\(^{24}\), and Haploview v. 4.1\(^{25}\).

The GWAS procedure is summarized in Supplementary Figure. S1. In the first-stage analysis of
118 subjects, the SNPs that showed statistical $P$-values less than 0.05 were selected as the candidate SNPs for the second-stage analysis among the 295,036 SNPs. For these SNPs, the second-stage analysis was conducted; again, the SNPs that showed $P < 0.05$ were considered potent candidates and selected for further final-stage analysis. In the final stage, an association study was conducted to examine whether the possible association between the SNPs selected until the second stage and the phenotypic trait would be strictly replicated. In this stage, the $Q$-values of the false discovery rate were calculated to correct for multiple testing in addition to the $P$-values based on previous reports\textsuperscript{26,27}. The SNPs that showed $Q < 0.05$ in the analysis were considered genome-wide significant.

To calculate $Q$-values, SFDR (Stratified False Discovery Rate) Software (http://www.utstat.toronto.edu/sun/Software/SFDR/index.html; accessed March 1, 2012)\textsuperscript{28} was used. A log quantile-quantile (QQ) $P$-value plot as a result of the GWAS for the combined samples was subsequently drawn to check the pattern of the generated $P$-value distribution, in which the observed $P$-values against the values expected from the null hypothesis of uniform distribution, calculated as $-\log_{10}$ ($P$-value), were plotted for each model. All of the plots were mostly concordant with the expected line ($y = x$), especially over the range of $0 < -\log_{10}$ ($P$-value) $< 5$, indicating no apparent population stratification of the samples used in the study (Supplementary Figure S2).

4.2. Association study for candidate SNPs

Additional analyses were conducted to corroborate the possible association between the SNPs and opioid sensitivity in the subjects who underwent painful cosmetic surgery after the multistage GWAS. The samples included in these analyses were from the patients who underwent major abdominal surgery, patients with methamphetamine dependence/psychosis, patients with alcohol dependence, patients with eating disorders, healthy volunteers with personality profile data, and postmortem specimens for the expression analysis. For all of the genotype data used in these analyses, the distributions were checked using the $\chi^2$ test, and the absence of significant deviation
from the theoretical distribution expected from Hardy-Weinberg equilibrium was confirmed (Supplementary Table S13). For all of the statistical analyses described below, SPSS 18.0J for Windows (International Business Machines Corporation, Armonk, NY), gPLINK v. 2.050, PLINK v. 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/; accessed March 1, 2012)\textsuperscript{24}, and Haplovie v. 4.1\textsuperscript{25} were used. The criterion for significance was set at $P < 0.05$.

In the analysis of the patients who underwent major abdominal surgery, the calculated total dose of rescue analgesics administered during the first 24-h postoperative period (see section 1.2.2) was used as an index of opioid sensitivity. Prior to the analysis, the quantitative values of postoperative analgesic requirements ($\mu$g/kg) were natural-log-transformed for the approximation to the normal distribution according to the following formula: 

\[ \text{Value for analysis} = \ln (1 + \text{postoperative analgesic requirement} \ [\mu\text{g/kg}]) \]

To explore the association between the SNPs and phenotype, Student’s $t$-test or analysis of variance (ANOVA) was performed, in which Bonferroni correction for multiple comparisons was used for the post hoc tests. For these analyses, postoperative analgesic use ($\mu$g/kg; log-transformed) and the genotype data of each SNP were incorporated as dependent and independent variables, respectively.

$\chi^2$ tests were performed to investigate the contribution of the SNPs to the vulnerability to presence of serious symptom of substance dependence. These analyses were conducted by comparing the genotype and allelic distributions between the patients and control subjects or between subcategories in patient subjects who presented a presence or absence of or tendency toward serious dependence. For the analyses in which the number of subjects in a cell in $2 \times 2$ contingency tables was less than five, Fisher’s exact tests were conducted instead of $\chi^2$ tests.

In the analysis of healthy volunteers with personality profile data (see section 1.6.2), raw TCI scores were processed. The score on each subscale in each dimension was averaged (specifically, the total score was divided by the number of items in each subscale). The average score on each subscale was averaged to calculate the overall score on each dimension (specifically, the sum of the average
score on each subscale was divided by the number of subscales in each dimension), which was used as the endpoint in the association study. Prior to the analysis, quantitative values of the overall score on each dimension (ranging from 0 to 1) were natural-log-transformed for the approximation to the normal distribution according to the following formula: \( \text{Value for analysis} = \ln(1 + \text{endpoint TCI score}) \). To explore the association between the SNPs and phenotype, linear regression analyses were conducted, in which the endpoint TCI score (log-transformed) and genotype data of each SNP were incorporated as dependent and independent variables, respectively. The correction of multiple tests for the analyses of the seven phenotypes was not performed in the present exploratory study.

In the analysis of the postmortem specimens for the expression analysis, the calculated expression level of the FAM119A (METTL21A) gene or CREB1 gene normalized to the ACTB gene for each sample (see section 3) was used. Prior to the analysis, the quantitative values of the relative mRNA expression level were natural-log-transformed for the approximation to the normal distribution according to the following formula: \( \text{Value for analysis} = \ln(1 + \text{relative expression level}) \). To explore the association between the SNPs and phenotype, Student’s \( t \)-test or ANOVA was performed, in which Bonferroni correction for multiple comparisons was used as the post hoc test. For these analyses, the relative expression level and genotype data of each SNP were incorporated as dependent and independent variables, respectively.

5. Additional in silico analysis

5.1. Reference of databases

To scrutinize the candidate locus, SNPs, and genes possibly associated with human opioid sensitivity, several databases were referenced, including the dbSNP database (http://www.ncbi.nlm.nih.gov/snp/; accessed March 1, 2012), HapMap database (http://hapmap.ncbi.nlm.nih.gov/index.html.ja; accessed March 1, 2012)²⁹, and BioGPS database (http://biogps.org/#goto=welcome; accessed March 1, 2012).
Selection Tool for SNP in Large Scale Association Study) search
(http://fastsnp.ibms.sinica.edu.tw/pages/input_CandidateGeneSearch.jsp; accessed March 1, 2012)\textsuperscript{30},
which prioritizes SNPs based on the predicted functional effects and estimated risk according to the opinion of Tabor et al.\textsuperscript{31}, was utilized to estimate the functional impact of the SNPs within the close region upstream from rs2952768 (Supplementary Table S11). However, the information of the SNPs within the close region downstream from rs2952768 was not available on this database.

5.2. Power analysis

Statistical power analyses were preliminarily performed using G*Power version 3.0.5\textsuperscript{32}. Power analyses for the linear regression analyses revealed that the expected power (1 minus type II error probability) was 98.7\% and 98.6\% for the Cohen’s conventional “medium” effect size of 0.15\textsuperscript{33} when the type I error probability was set at 0.05 and the sample sizes were 118 and 117, respectively, corresponding to the sample sizes of the first- and second-stage analyses in the current study. However, for the same type I error probability and sample sizes of 118 and 117, the expected power decreased to 33.2\% and 32.9\%, respectively, when the Cohen’s conventional “small” effect size was 0.02. Conversely, the estimated effect sizes were 0.0676 and 0.0682 for the same type I error probability and sample sizes of 118 and 117, respectively, to achieve 80\% power. Therefore, a single analysis in the current study was expected to detect true associations with the phenotype with 80\% statistical power for effect sizes from large to moderately small, but not too small, although the exact effect size has been poorly understood in cases of SNPs that greatly contribute to opioid sensitivity.

We adopted a consecutive three-stage analysis with approximately 100 samples for each stage in the present study, because several recent pharmacogenomic studies that analyzed approximately 100 cases detected genome-wide significant associations, suggesting that at least some pharmacogenomic effects tend to be larger and involve fewer genes than those detected in GWASs for complex diseases\textsuperscript{34}.

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5.3. Linkage disequilibrium analysis

To identify the relationships between the SNPs identified in the polymorphism screening, LD analysis was performed for a total of 127 samples from patients who underwent cosmetic orthognathic surgery (see section 1.1.1) for the locus of the 170 kbp region that surrounds the rs2952768 SNP on chromosome 2 using Haploview v. 4.1\textsuperscript{25}. For the estimation of LD strength between the SNPs, the commonly used $D'$ and $r^2$ values were pairwise-calculated using the genotype dataset of each SNP. Linkage disequilibrium blocks were defined among the SNPs that showed “strong LD,” based on the default algorithm of Gabriel et al.\textsuperscript{35}, in which the upper and lower 95% confidence limits on $D'$ for a strong LD were set at 0.98 and 0.7, respectively. For comparison, LD patterns between the SNPs with minor allele frequency $\geq$ 0.05 in the same genomic position were similarly analyzed using Haploview v. 4.1\textsuperscript{25} based on the genotype data for the population of Han Chinese in Beijing, China (CHB), and Japanese in Tokyo, Japan (JPT), downloaded from the HapMap database. Although the SNPs incorporated were not exactly the same in both analyses, the LD pattern generated using our samples was mostly comparable to that derived from the database, in which the LD block that included the rs2952768 SNP ranged from the position of the rs16839387 or nearby rs1862951 SNP to the rs7594560 SNP (Figure 1c and Supplementary Figure S4).

5.4. Imputation analysis

For the fine mapping of SNPs more strongly associated with human opioid sensitivity, imputation analysis was conducted to extrapolate the missing genotype data between our data obtained from whole-genome genotyping. The analysis was performed using the “--proxy-assoc” and several related commands within the PLINK v. 1.07 software\textsuperscript{24}, based on the whole-genome genotyping and further genotyping data and local genotype data of the Japanese and Han Chinese populations extracted from the 2 Mb region that surrounds the rs2952768 SNP, which is located at 208,202,479
on chromosome 2 in the HapMap database\textsuperscript{29}. After the imputation analysis, an additional association study was conducted as mentioned above based on the imputed genotype data.

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Supplementary Table S1.
The demographic and clinical data of the subjects undergoing painful cosmetic surgery.

|                           | n   | Minimum | Maximum | Mean  | SD   | Median |
|---------------------------|-----|---------|---------|-------|------|--------|
| Gender                    |     |         |         |       |      |        |
| male                      | 125 |         |         |       |      |        |
| female                    | 230 |         |         |       |      |        |
| Age                       | 355 | 15      | 52      | 25.92 | 7.63 | 23.00  |
| Height (cm)               | 355 | 143     | 258     | 164.78| 10.07| 163.00 |
| Weight (kg)               | 355 | 38      | 128     | 57.61 | 10.86| 55.00  |
| 24-h postoperative analgesic use (μg/kg) | 354 | 0       | 13.82   | 2.93  | 2.57 | 2.27   |
| Ln-transformed            |     |         |         |       |      |        |
| 24-h postoperative analgesic use (μg/kg) | 354 | 0       | 2.70    | 1.17  | 0.64 | 1.18   |
**Supplementary Table S2.**
Top candidate SNPs selected from 3-stage GWAS (additive model).

| Rank | SNP     | CHR | Position | 1st stage | 2nd stage | Final stage | Combined | Related gene |
|------|---------|-----|----------|-----------|-----------|-------------|----------|--------------|
|      |         |     |          | β         | P         | β          | P        | Q            | β     | P         |               |
| 1    | rs2952768 | 2   | 208202479| 0.281     | 0.0119    | 0.239      | 0.0247   | 0.3465       | 0.0003 | 0.0175*   | METTL21A (FAM119A)† |
| 2    | rs7591784 | 2   | 208209975| 0.2719    | 0.0143    | 0.2268     | 0.0324   | 0.3576       | 0.0002 | 0.0175*   | METTL21A (FAM119A)† |
| 3    | rs2709386 | 2   | 208205279| 0.281     | 0.0119    | 0.2268     | 0.0324   | 0.3465       | 0.0003 | 0.0175*   | METTL21A (FAM119A)† |
| 4    | rs11004819| 10  | 51683646 | 0.1824    | 0.0467    | 0.2819     | 0.0015   | 0.2169       | 0.0173 | 0.4237    | ASAH2          |
| 5    | rs7761742 | 6   | 78477151 | 0.3132    | 0.0022    | 0.2374     | 0.029    | 0.2237       | 0.0442 | 0.4237    | HTR1B          |
| 6    | rs12496846| 3   | 14748271 | 0.201     | 0.0308    | 0.3867     | 0.0011   | 0.218        | 0.0322 | 0.4237    | C3orf20        |
| 7    | rs698705  | 19  | 52629278 | -0.3925   | 0.018     | -0.3644    | 0.0071   | -0.3108      | 0.0354 | 0.4237    | SLC8A2         |
| 8    | rs10052295| 5   | 11030322 | -0.3984   | 0.0425    | -0.4829    | 0.0351   | -0.5218      | 0.0233 | 0.4237    | CTNNB2         |
| 9    | rs10513342| 9   | 120522154| -0.1747   | 0.0343    | -0.1937    | 0.0193   | -0.1718      | 0.0438 | 0.4237    | ASTN2, TRIM32, TLR4† |

CHR, chromosome number; Position, chromosomal position (bp); Q, Q value for FDR correction of multiple comparison; Related gene, the nearest gene from the SNP site; * Significant after FDR correction (Q < 0.05); †, modified from the Illumina annotation file
### Supplementary Table S3.
Top candidate SNPs selected from 3-stage GWAS (dominant model).

| Rank | SNP     | CHR | Position | 1st stage | 2nd stage | Final stage | Combined | Related gene |
|------|---------|-----|----------|-----------|-----------|-------------|----------|--------------|
|      |         |     |          | β         | P         | β          | P        | Q            | β          | P        |
| 1    | rs2429239 | 5   | 81772551 | 0.3248    | 0.0073    | 0.3642     | 0.0019   | 0.3394      | 0.0045     | 0.4234   | 0.344    | 4.9E-07  | FLJ41309 |
| 2    | rs224938  | 5   | 81752932 | 0.3075    | 0.0104    | 0.3393     | 0.0037   | 0.3473      | 0.003      | 0.4234   | 0.3317   | 9.6E-07  | FLJ41309 |
| 3    | rs1465040 | 4   | 123147706 | 0.3682    | 0.002    | 0.3039     | 0.0099   | 0.3054      | 0.0095     | 0.4234   | 0.328    | 1.27E-06 | TRPC3     |
| 4    | rs7479309 | 11  | 27268390 | 0.308     | 0.0151    | 0.3211     | 0.0184   | 0.4         | 0.0021     | 0.4234   | 0.3441   | 4.22E-06 | CCDC34    |
| 5    | rs2195731 | 9   | 23775967 | 0.3371    | 0.0097    | 0.2969     | 0.0246   | 0.3368      | 0.0102     | 0.4234   | 0.3221   | 1.85E-05 | ELAVL2    |
| 6    | rs10223341| 6   | 130537576 | 0.9925   | 0.0091    | 0.994      | 0.0288   | 1.56        | 0.0152     | 0.4529   | 1.085    | 3.72E-05 | SAMD3     |
| 7    | rs13266712| 8   | 41103217 | 0.6278    | 0.0356    | 0.5517     | 0.0263   | 0.5533      | 0.0061     | 0.4234   | 0.5641   | 4.27E-05 | ATP6V1G1P2  |
| 8    | rs1478684 | 11  | 27293921 | 0.2886    | 0.0284    | 0.3211     | 0.0184   | 0.3265      | 0.0171     | 0.4529   | 0.312    | 5.37E-05 | CCDC34    |
| 9    | rs2045935 | 11  | 27294419 | 0.2886    | 0.0284    | 0.3211     | 0.0184   | 0.3265      | 0.0171     | 0.4529   | 0.312    | 5.37E-05 | CCDC34    |
| 10   | rs1474797 | 1   | 185233816 | 0.413     | 0.0097    | 0.2964     | 0.0367   | 0.2818      | 0.0484     | 0.6634   | 0.3264   | 0.000113 | PLA2G4A   |
| 11   | rs6877560 | 5   | 81951057 | -0.2663   | 0.027     | -0.2439    | 0.0453   | -0.2805     | 0.0194     | 0.473    | -0.2657  | 0.000118 | FLJ41309 |
| 12   | rs12517913| 5   | 177438401 | 0.2505    | 0.0491    | 0.2838     | 0.0303   | 0.2379      | 0.0475     | 0.6634   | 0.258    | 0.000321 | RPL19P9   |

CHR, chromosome number; Position, chromosomal position (bp); Q, Q value for FDR correction of multiple comparison; Related gene, the nearest gene from the SNP site; ✝, modified from the Illumina annotation file
# Supplementary Table S4.
## Top candidate SNPs selected from 3-stage GWAS (recessive model).

| Rank | SNP     | CHR | Position | 1st stage | 2nd stage | Final stage | Combined | Related gene |
|------|---------|-----|----------|-----------|-----------|-------------|----------|--------------|
|      |         |     |          | β      | P         | β     | P     | Q        | β    | P      |                                 |
| 1    | rs7591784 | 2   | 208209975 | 0.5039 | 0.0184 | 0.4611 | 0.0223 | 0.672 | 0.0002 | 0.0145* | 0.5527 | 9.38E-07 | METTL21A (FAM119A) † |
| 2    | rs2952768 | 2   | 208202479 | 0.5142 | 0.017  | 0.4611 | 0.0223 | 0.6619 | 0.0002 | 0.0145* | 0.5531 | 1.04E-06 | METTL21A (FAM119A) † |
| 3    | rs2709386 | 2   | 208205279 | 0.5142 | 0.017  | 0.4611 | 0.0223 | 0.6619 | 0.0002 | 0.0145* | 0.5531 | 1.04E-06 | METTL21A (FAM119A) † |
| 4    | rs2254137 | 2   | 208152273 | 0.4197 | 0.0427 | 0.4006 | 0.0336 | 0.607 | 0.0007 | 0.0296* | 0.4819 | 1.12E-05 | CREB1 † |
| 5    | rs1898198 | 10  | 51679654  | 0.3512 | 0.0414 | 0.4286 | 0.0086 | 0.4757 | 0.0045 | 0.1188 | 0.4196 | 1.28E-05 | LOC728532 |
| 6    | rs11004819| 10  | 51683646  | 0.3534 | 0.0393 | 0.424  | 0.0091 | 0.4709 | 0.0048 | 0.1188 | 0.4172 | 1.34E-05 | ASAH2 |
| 7    | rs698705  | 19  | 52629278  | -0.8197| 0.0135 | -0.7457| 0.0054 | -0.6382| 0.0295 | 0.389  | -0.7244| 1.82E-05 | SLC8A2 |
| 8    | rs10052295| 5   | 11030322  | -0.8093| 0.0343 | -0.9614| 0.0346 | -1.042 | 0.0226 | 0.3539 | -0.9219| 0.000162| CTNND2 |
| 9    | rs1577822 | 6   | 63643099  | -0.3692| 0.0365 | -0.3171| 0.0311 | -0.334 | 0.0207 | 0.3539 | -0.3326| 0.000165| KHDRBS2 † |
| 10   | rs2459572 | 6   | 64051334  | -0.6184| 0.0385 | -0.7322| 0.0239 | -0.6462| 0.0481 | 0.431  | -0.6635| 0.000247| GLULD1 |

CHR, chromosome number; Position, chromosomal position (bp); Q, Q value for FDR correction of multiple comparison; Related gene, the nearest gene from the SNP site; *, Significant after FDR correction (Q < 0.05); †, modified from the Illumina annotation file.
Supplementary Table S5.
The demographic and clinical data of the subjects undergoing major abdominal surgery.

|                        | n   | Minimum | Maximum | Mean   | SD    | Median |
|------------------------|-----|---------|---------|--------|-------|--------|
| Gender                 |     |         |         |        |       |        |
| male                   | 60  |         |         |        |       |        |
| female                 | 52  |         |         |        |       |        |
| Age                    | 112 | 28      | 80      | 63.13  | 10.06 | 63.00  |
| Height (cm)            | 112 | 133     | 175     | 157.89 | 8.00  | 158.00 |
| Weight (kg)            | 112 | 38      | 77      | 55.83  | 9.97  | 54.00  |
| 24-h postoperative analgesic use (μg/kg) | 112 | 0       | 6.48    | 0.77   | 1.15  | 0.00   |
| Ln-transformed         |     |         |         |        |       |        |
| 24-h postoperative analgesic use (μg/kg) | 112 | 0       | 2.01    | 0.42   | 0.51  | 0.00   |
**Supplementary Table S6.**
The demographic and clinical data of the subjects with methamphetamine dependence/psychosis.

|                                     | n  | Minimum | Maximum | Mean  | SD    | Median |
|-------------------------------------|----|---------|---------|-------|-------|--------|
| Gender of all patients with dependence |    |         |         |       |       |        |
| male                                | 165|         |         |       |       |        |
| female                              | 38 |         |         |       |       |        |
| Gender of patients comorbid with psychosis |    |         |         |       |       |        |
| male                                | 155|         |         |       |       |        |
| female                              | 30 |         |         |       |       |        |
| Age (years)                         | 202| 18      | 69      | 37.82 | 12.05 | 35.00  |
| First use of methamphetamine (years)| 200| 13      | 46      | 20.78 | 5.49  | 19.00  |
| Latency of psychosis                |    |         |         |       |       |        |
| < 3 years                           | 94 |         |         |       |       |        |
| ≥ 3 years                           | 84 |         |         |       |       |        |
| The number of drugs used            | 186| 1       | 10      | 3.12  | 1.71  | 2.00   |
Supplementary Table S7.
The demographic and clinical data of the subjects with alcohol dependence.

|                             | n   | Minimum | Maximum | Mean  | SD   | Median |
|-----------------------------|-----|---------|---------|-------|------|--------|
| Gender of all patients with dependence |     |         |         |       |      |        |
| male                        | 438 | 438     | 0       |       |      |        |
| female                      | 0   | 0       |         |       |      |        |
| Age (years)                 | 438 | 31      | 77      | 50.59 | 8.76 | 51.00  |
| Age of onset                | 415 | 19      | 72      | 40.49 | 10.71| 40.00  |
| SDS score                   | 383 | 23      | 67      | 42.67 | 7.75 | 42.00  |
| SSS score                   | 367 | 2       | 31      | 14.98 | 5.84 | 15.00  |
| MAC score                   | 368 | 10      | 35      | 21.40 | 4.24 | 21.00  |
| **ALDH2 genotype**          |     |         |         |       |      |        |
| *1/*1                       | 387 |         |         |       |      |        |
| *1/*2                       | 51  |         |         |       |      |        |
| *2/*2                       | 0   |         |         |       |      |        |
| The number of drugs used    |     |         |         |       |      |        |
| 0                           | 393 |         |         |       |      |        |
| 1                           | 36  |         |         |       |      |        |
| ≥ 2                         | 9   |         |         |       |      |        |

SDS: Self-rating Depression Scale, SSS: Sensation Seeking Scale, MAC: MAC Scale
### Supplementary Table S8.
The demographic and clinical data of the subjects with eating disorders.

|                                             | n    | Minimum | Maximum | Mean  | SD    | Median |
|---------------------------------------------|------|---------|---------|-------|-------|--------|
| **All patients with eating disorders**      | 228  |         |         |       |       |        |
| Anorexia nervosa (male/female)              | 87 (6/81) |       |         |       |       |        |
| Bulimia nervosa nervosa (male/female)       | 125 (9/116) |     |         |       |       |        |
| Others (male/female)                        | 15 (1/14) |      |         |       |       |        |
| **Age (years)**                             | 222  | 13      | 69      | 26.01 | 7.07  | 25.00  |
| **Height (cm)**                             | 158  | 145     | 170     | 157.84| 5.33  | 158.00 |
| **Current weight (kg)**                     | 130  | 28      | 106     | 47.64 | 14.30 | 45.00  |
| **Maximum weight (kg)**                     | 152  | 41      | 115     | 58.83 | 13.55 | 55.00  |
| **Minimum weight (kg)**                     | 152  | 19      | 73      | 37.46 | 9.16  | 37.00  |
| **Difference between maximum and minimum weight (kg)** | 149 | 5 | 53 | 21.07 | 9.80 | 19.00 |
| **Onset of eating disorders (years)**       | 190  | 10      | 40      | 18.30 | 4.77  | 17.00  |
### Supplementary Table S9. Distribution of genotypes of the polymorphism rs2952768 and odds ratio between control and patient subjects

| n | Genotype | Allele frequency | $\chi^2$ | OR [95% CI] |
|---|----------|------------------|---------|-------------|
|   | T/T | T/C | C/C | T | C | genotype | allele | dominant | recessive | C allele (VS. T) | C/C+T/C genotype (VS. T/T) | C/C genotype (VS. T/T+T/C) |
| METH dependence/psychosis | control | 221 | 84 | 107 | 30 | 0.622 | 0.378 | 0.199 | 0.046 | 0.142 | 0.011 | 0.97 | 0.93 | 1.03 |
| patients | 201 | 80 | 93 | 28 | 0.629 | 0.371 | 0.905 | 0.830 | 0.706 | 0.916 | [0.73-1.28] | [0.63-1.37] | [0.59-1.79] |
| Alcohol dependence | control | 349 | 166 | 147 | 36 | 0.686 | 0.314 | 1.122 | 0.965 | 0.442 | 1.000 | 1.11 | 1.10 | 1.26 |
| patients | 436 | 197 | 184 | 55 | 0.663 | 0.337 | 0.571 | 0.326 | 0.506 | 0.317 | [0.90-1.38] | [0.83-1.46] | [0.80-1.96] |
| Eating disorders | control | 266 | 131 | 117 | 18 | 0.712 | 0.288 | 2.676 | 2.032 | 1.019 | 2.323 | 1.22 | 1.20 | 1.64 |
| patients | 226 | 101 | 101 | 24 | 0.670 | 0.330 | 2.292 | 1.54 | 0.313 | 0.127 | [0.93-1.60] | [0.84-1.71] | [0.86-3.10] |

$n$: the number of samples, OR: odds ratio, 95% CI: 95% confidence interval.
Supplementary Table S10.
The demographic data of the healthy subjects with personality profile data.

|                          | n  | Minimum | Maximum | Mean  | SD    | Median |
|--------------------------|----|---------|---------|-------|-------|--------|
| All subjects             | 500|         |         |       |       |        |
|                         | male | 253     |         |       |       |        |
|                         | female | 242     |         |       |       |        |
|                         | unknown | 5       |         |       |       |        |
| Age (years)              | 481 | 20      | 72      | 33.07 | 12.55 | 29.00  |
| Mean score of TCI        |     |         |         |       |       |        |
| Novelty seeking          | 499 | 0.05    | 0.95    | 0.50  | 0.19  | 0.49   |
| Harm avoidance           | 499 | 0.00    | 1.00    | 0.57  | 0.23  | 0.60   |
| Reward dependence        | 499 | 0.00    | 1.00    | 0.67  | 0.19  | 0.67   |
| Persistence              | 499 | 0.00    | 1.00    | 0.55  | 0.34  | 0.60   |
| Self directedness        | 499 | 0.00    | 1.00    | 0.66  | 0.21  | 0.68   |
| Cooperativeness          | 499 | 0.04    | 1.00    | 0.73  | 0.16  | 0.76   |
| Self transcendedce       | 499 | 0.00    | 0.93    | 0.32  | 0.20  | 0.27   |
| Mean score of TCI (Ln-transformed) |     |         |         |       |       |        |
| Novelty seeking          | 499 | 0.05    | 0.67    | 0.40  | 0.13  | 0.40   |
| Harm avoidance           | 499 | 0.00    | 0.69    | 0.44  | 0.15  | 0.47   |
| Reward dependence        | 499 | 0.00    | 0.69    | 0.50  | 0.12  | 0.51   |
| Persistence              | 499 | 0.00    | 0.69    | 0.42  | 0.23  | 0.47   |
| Self directedness        | 499 | 0.00    | 0.69    | 0.50  | 0.13  | 0.52   |
| Cooperativeness          | 499 | 0.04    | 0.69    | 0.54  | 0.09  | 0.57   |
| Self transcendedce       | 499 | 0.00    | 0.66    | 0.27  | 0.14  | 0.24   |

TCI: Temperament and Character Inventory.
| SNP ID (rs) | Position | Linked | LD | Linked in the database | LD in the database | Gene Symbol | RefSeq mRNA | Region | Possible Functional Effects | Lower Risk | Upper Risk |
|------------|----------|--------|----|------------------------|-------------------|-------------|-------------|--------|-----------------------------|-------------|------------|
| rs1862951  | 208077017|        | +  |                        |                   | AC007879.1   | ENST00000412387 | intronic | Intronic with no known function | 0           | 0          |
| rs16839837 | 208079256| +      |    |                        |                   | AC007879.1   | ENST00000412387 | intronic | Intronic with no known function | 0           | 0          |
| rs2360969 | 208081241 | +      |    |                        |                   | AC007879.1   | ENST00000412387 | intronic | Intronic enhancer             | 1           | 2          |
| rs16839849 | 208087226 | +      |    |                        |                   | AC007879.1   | ENST00000412387 | intronic | Intronic enhancer             | 1           | 2          |
| rs10932200| 208095930 | +      |    |                        |                   | AC007879.1   | ENST00000412387 | intronic | Intronic enhancer             | 1           | 2          |
| rs2253206  | 208100223 | +      |    |                        |                   | CREB1        | ENST00000432329 | 5upstream | Promoter/regulatory region     | 1           | 3          |
| rs2551640  | 208116138 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic with no known function | 0           | 0          |
| rs10932201| 208134502 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs11904814 | 208135043 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs16839883 | 208137587 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs6740584  | 208137596 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs3770704  | 208147363 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs2551923  | 208150021 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs2541377  | 208152273 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs2551645  | 208159033 | +      |    |                        |                   | CREB1        | ENST00000432329 | intronic | Intronic enhancer             | 1           | 2          |
| rs17520056 | 208186300 | +      |    |                        |                   | FAM119A      | ENST00000448807 | 5upstream | Upstream with no known function | 0           | 0          |
| rs2551947  | 208187820 | +      |    |                        |                   | FAM119A      | ENST00000448807 | intronic | Intronic enhancer             | 1           | 2          |
| rs2551946  | 208188750 | +      |    |                        |                   | FAM119A      | ENST00000448807 | intronic | Intronic enhancer             | 1           | 2          |
| rs4234080  | 208197346 | +      |    |                        |                   | FAM119A      | ENST00000448807 | 5utr    | Promoter/regulatory region     | 1           | 3          |
| rs2551941  | 208200388 | +      |    |                        |                   | FAM119A      | ENST00000448807 | 5upstream | Upstream with no known function | 0           | 0          |
| rs2952768  | 208202479 | +      |    |                        |                   | FAM119A      | ENST00000448807 | 5upstream | Upstream with no known function | 0           | 0          |

Linked, SNP showing strong LD ($r^2 \geq 0.86$) with the rs2952768 based on the current study (Figure 1c); LD, SNP included in the same LD block as the rs2952768 based on the current study (Figure 1c); Linked in the database, SNP showing strong LD ($r^2 \geq 0.67$) with the rs2952768 in the HapMap database; LD in the database, SNP included in the same LD block as the rs2952768 in the HapMap database; RefSeq mRNA, Transcript ID in the Ensemble database; Lower Risk, lower level of predicted functional effects based on the FastSNP database; Upper Risk, Upper level of predicted functional effects based on the FastSNP database; Linked, SNP showing strong LD ($r^2 \geq 0.67$) with the rs2952768 in the database; LD, SNP included in the same LD block as the rs2952768 in the database; Upper Risk, Upper level of predicted functional effects based on the FastSNP database; 5upstream, 5'-upstream region of the gene; 5utr, 5'-untranslated region of the gene.
Supplementary Table S12. Distribution of genotypes of the SNPs selected after 3-stage GWAS and the result of tests for Hardy-Weinberg equilibrium.

| Model   | Rank | SNP       | n  | Genotype | Allele frequency | χ²  | P    |
|---------|------|-----------|----|----------|------------------|-----|------|
|         |      |           |    | A/A      | A    | B    |       |
|         | Additive | 1      | rs2952768 | 353 | 158 | 160 | 35 | 0.674 | 0.326 | 0.357 | 0.550 |
|         | Additive | 2      | rs7591784 | 351 | 158 | 158 | 35 | 0.675 | 0.325 | 0.243 | 0.622 |
|         | Additive | 3      | rs2709386 | 353 | 160 | 158 | 35 | 0.677 | 0.323 | 0.195 | 0.658 |
|         | Additive | 4      | rs11004819 | 353 | 137 | 164 | 52 | 0.620 | 0.380 | 0.066 | 0.798 |
|         | Additive | 5      | rs7761742 | 353 | 149 | 171 | 33 | 0.664 | 0.336 | 2.618 | 0.106 |
|         | Additive | 6      | rs12496846 | 353 | 176 | 141 | 36 | 0.698 | 0.302 | 0.955 | 0.328 |
|         | Additive | 7      | rs698705  | 350 | 265 | 70  | 15 | 0.857 | 0.143 | 11.764 | 0.001 |
|         | Additive | 8      | rs10052295 | 353 | 137 | 164 | 52 | 0.620 | 0.380 | 0.066 | 0.798 |
|         | Additive | 9      | rs10513342 | 353 | 95  | 173 | 85 | 0.514 | 0.486 | 0.128 | 0.721 |
|         | Dominant | 1      | rs2429239 | 353 | 203 | 134 | 16 | 0.765 | 0.235 | 1.083 | 0.298 |
|         | Dominant | 2      | rs224938  | 353 | 173 | 157 | 23 | 0.712 | 0.288 | 2.582 | 0.108 |
|         | Dominant | 3      | rs1465040 | 353 | 174 | 150 | 29 | 0.705 | 0.295 | 0.176 | 0.674 |
|         | Dominant | 4      | rs7479309 | 353 | 102 | 183 | 68 | 0.548 | 0.452 | 0.764 | 0.382 |
|         | Dominant | 5      | rs2195731 | 352 | 251 | 96  | 5  | 0.849 | 0.151 | 1.542 | 0.214 |
|         | Dominant | 6      | rs10223341 | 353 | 347 | 6   | 0  | 0.992 | 0.008 | 0.026 | 0.872 |
|         | Dominant | 7      | rs13266712 | 353 | 330 | 21  | 2  | 0.965 | 0.035 | 5.889 | 0.015 |
|         | Dominant | 8      | rs1478684 | 353 | 93  | 183 | 77 | 0.523 | 0.477 | 0.536 | 0.464 |
|         | Dominant | 9      | rs2045935 | 353 | 93  | 182 | 78 | 0.521 | 0.479 | 0.385 | 0.535 |
|         | Dominant | 10     | rs1474797 | 353 | 281 | 68  | 4  | 0.892 | 0.108 | 0.003 | 0.960 |
|         | Dominant | 11     | rs6877560 | 353 | 205 | 121 | 27 | 0.752 | 0.248 | 2.299 | 0.129 |
|         | Dominant | 12     | rs12517913 | 353 | 122 | 176 | 55 | 0.595 | 0.405 | 0.419 | 0.518 |
|         | Recessive | 1     | rs7591784 | 351 | 158 | 158 | 35 | 0.675 | 0.325 | 0.243 | 0.622 |
|         | Recessive | 2     | rs2952768 | 353 | 158 | 160 | 35 | 0.674 | 0.326 | 0.357 | 0.550 |
|         | Recessive | 3     | rs2709386 | 353 | 160 | 158 | 35 | 0.677 | 0.323 | 0.195 | 0.658 |
|         | Recessive | 4     | rs2254137 | 352 | 161 | 153 | 38 | 0.675 | 0.325 | 0.034 | 0.855 |
|         | Recessive | 5     | rs1898198 | 350 | 134 | 164 | 52 | 0.617 | 0.383 | 0.025 | 0.875 |
|         | Recessive | 6     | rs11004819 | 353 | 137 | 164 | 52 | 0.620 | 0.380 | 0.066 | 0.798 |
|         | Recessive | 7     | rs698705  | 350 | 265 | 70  | 15 | 0.857 | 0.143 | 11.764 | 0.001 |
|         | Recessive | 8     | rs10052295 | 353 | 260 | 86  | 7  | 0.858 | 0.142 | 0.001 | 0.971 |
|         | Recessive | 9     | rs1577822 | 350 | 136 | 150 | 64 | 0.603 | 0.397 | 3.857 | 0.050 |
|         | Recessive | 10    | rs2459572 | 353 | 217 | 123 | 13 | 0.789 | 0.211 | 0.758 | 0.384 |

n: the number of samples, Model: the genetic model in which candidate SNPs were selected by GWAS.
A/A: homozygote for the major allele in each SNP, A/B: heterozygote for the major allele in each SNP, B/B: homozygote for the minor allele in each SNP.
Supplementary Table S13. Distribution of genotypes of the rs2952768 and rs2254137 SNPs and the result of tests for Hardy-Weinberg equilibrium.

| Sample cohort | SNP    | n      | Genotype | Allele frequency | $\chi^2$ | P    |
|---------------|--------|--------|----------|------------------|----------|------|
|               |        |        | A/A | A/B | B/B | A  | B  |            |            |       |
| Abdominal     | rs2952768 | 112    | 44  | 56  | 12  | 0.643 | 0.357 | 0.885 | 0.347 |
| METH          | rs2952768 | 221    | 84  | 107 | 30  | 0.622 | 0.378 | 0.196 | 0.658 |
| patients      | rs2952768 | 201    | 80  | 93  | 28  | 0.629 | 0.371 | 0.014 | 0.907 |
| Alcohol       | rs2952768 | 349    | 166 | 147 | 36  | 0.686 | 0.314 | 0.167 | 0.683 |
| patients      | rs2952768 | 436    | 197 | 184 | 55  | 0.663 | 0.337 | 1.358 | 0.244 |
| Eating        | rs2952768 | 266    | 131 | 117 | 18  | 0.712 | 0.288 | 1.434 | 0.231 |
| patients      | rs2952768 | 226    | 101 | 101 | 24  | 0.670 | 0.330 | 0.028 | 0.866 |
| Healthy       | rs2952768 | 496    | 222 | 205 | 69  | 0.654 | 0.346 | 3.708 | 0.054 |
| SMRI          | rs2952768 | 105    | 49  | 45  | 11  | 0.681 | 0.319 | 0.020 | 0.889 |
|              | rs2254137 | 105    | 49  | 45  | 11  | 0.681 | 0.319 | 0.020 | 0.889 |

$n$: the number of samples, A/A: homozygote for the major allele in each SNP, A/B: heterozygote for the major allele in each SNP, B/B: homozygote for the minor allele in each SNP, Abdominal: Patients undergoing major abdominal surgery, METH: Patients with methamphetamine dependence/psychosis, Alcohol: Patients with alcohol dependence, Eating: Patients with eating disorders, Healthy: Healthy volunteers with personality profile data, SMRI: Postmortem specimens for expression analysis.
**Supplementary Figure S1.** Schematic illustration of the multistage GWAS. Potent candidate SNPs associated with human opioid sensitivity were selected in a three-stage GWAS.
**Supplementary Figure S2.** Log quantile-quantile (QQ) $P$-value plot as a result of the genome-wide association study for the combined samples. (a) Plot of the results from the additive model. (b) Plot of the results from the dominant model. (c) Plot of the results from the recessive model.
**Supplementary Figure S3.** Association study between the rs2952768 SNP and seven dimensions of the Temperament and Character Inventory (TCI) in healthy volunteer subjects. (a) Results for novelty seeking (NS). (b) Results for harm avoidance (HA). (c) Results for reward dependence (RD). (d) Results for persistence (P). (e) Results for self-directedness (SD). (f) Results for cooperativeness (C). (g) Results for self-transcendence (ST). The data are expressed as mean ± s.e.m. *P < 0.05; N.S., P ≥ 0.05.
Supplementary Figure S4. State of linkage disequilibrium (LD) between the SNPs in the genomic position from 208,070,000 to 208,240,000 on chromosome 2, based on the genotype data for the CHB + JPT population downloaded from the Hapmap database. Numbers in squares in which two SNPs face represent the percentage of the $r^2$ values calculated from the genotype data of the SNPs.