Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2008 October 5; 147B(7): 1028–1039. doi:10.1002/ajmg.b.30701.

Multiple *OPR* genes influence personality traits in substance dependent and healthy subjects in two American populations

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Abstract

Background—Personality traits are among the most complex quantitative traits. Certain personality traits are associated with substance dependence (SD); genetic factors may influence both. Associations between opioid receptor (*OPR*) genes and SD have been reported. This study investigated the relationship between *OPR* genes and personality traits in a case-control sample.

Methods—We assessed dimensions of the five-factor model of personality in 556 subjects: 250 with SD [181 European-Americans (EAs) and 69 African-Americans (AAs)] and 306 healthy subjects (266 EAs and 40 AAs). We genotyped 20 *OPRM1* markers, 8 *OPRD1* markers, and 7 *OPRK1* markers, and 38 unlinked ancestry-informative markers in these subjects. The relationships between *OPR* genes and personality traits were examined using MANCOVA, controlling for gene-gene interaction effects and potential confounders. Associations were decomposed by Roy-Bargmann Stepdown ANCOVA.

Results—Personality traits were associated as main or interaction effects with the haplotypes, diplotypes, alleles and genotypes at the three *OPR* genes (0.002 < p < 0.046 from MANCOVA; 0.0004 < p < 0.049 from ANCOVA). Diplotypes TTAGGA/TTCAGA at *OPRM1* had main effects on Extraversion (p = 0.008), and diplotypes *OPRM1*^{TTCAGA/TTCAGA} and *OPRD1*^{CAC/TAC} had interaction effects on Openness (p = 0.010) after conservative correction for multiple testing.

Conclusions—The present study demonstrates that the genes encoding the mu-, delta-, and kappa-opioid receptors may contribute to variation in personality traits. Further, the three *OPR* genes have significant interaction effects on personality traits. This work provides additional evidence that personality traits and SD have a partially overlapping genetic basis.

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Author contributions. XL, HK and JG conceived and designed the experiments. XL, HK, LZ, HZ and JG performed the experiments. XL, LZ and SW analyzed the data. XL and LZ wrote the paper.

Competing interests. No competing interests to declare.

Keywords

Personality; Substance dependence; *OPR* genes

Introduction

Quantitative personality traits are genetically complex. At least 33 genetic studies, using family, twin, linkage and association study designs, have consistently demonstrated genetic factors as contributing to individual differences in major dimensions of personality. These studies identified at least 5 chromosome regions and 15 specific genes contributing to personality traits [reviewed by Luo et al., 2007a]. This evidence supports the hypothesis that personality traits are determined by multiple genes, a hypothesis that was tested in the present study in relation to the opioid receptor (*OPR*) genes – the genes encoding the μ , δ , and κ opioid receptors (*OPRM1*, *OPRD1*, and *OPRK1*, respectively).

Many scales have been developed to measure human personality traits. It is now well accepted that the five-factor model (FFM) [Digman, 1990] provides an excellent representation of personality trait structure. This model can be operationalized via the NEO Five-Factor Inventory (NEO-FFI) [Costa and McCrae, 1997]. According to the FFM, the range of variation in human personality structure, which can be viewed as hundreds of personality traits, can be described in terms of five basic dimensions: Extraversion, Agreeableness, Conscientiousness, Neuroticism and Openness to Experience (i.e., Factors I to V). In the present study, we apply a comprehensive analytic procedure to hundreds of personality traits represented by these five personality factors, and then decompose each personality factor in a stepwise fashion (see Methods and Figure 1). We have applied this methodology previously [Luo et al., 2007a;b].

The personality trait structure reflected by the FFM has been demonstrated to be universal among highly diverse cultures with distinct languages, between men and women, between older and younger adults, and between European-Americans and other population groups [Costa et al., 1991; McCrae and Costa, 1997]. This universality might reflect a common biological basis for personality traits across distinct cultures, populations, ages and sexes. However, sex and age have also been reported to be directly implicated as affecting personality scores [Hernandez-Avila et al., 2004]. Furthermore, associations between personality traits and substance dependence (SD) could be modified by sex and age [reviewed Luo et al., 2007a]. Previous studies suggested that some gene effects on personality (e.g., *ADH4* and *CHRM2* genes) might be modified by age, sex, ethnicity and affection status [reviewed by Luo et al., 2007a;b]. To date, there has not been evidence as to whether *OPR* gene effects on personality are modified by these covariates or confounders (age, sex, ethnicity, and/or diagnosis) or not. Therefore, we comprehensively analyzed the *OPR* gene effects in the overall sample first (including older and younger adults, European-Americans and African-Americans, men and women, and both substance-dependent and healthy individuals, to maximize the power), then decomposed the positive findings in the sub-samples.

Many studies have provided evidence that personality characteristics may play a central role in the development of SD (including alcohol dependence and opioid dependence in these studies) [reviewed by Luo et al., 2007a]. Many other studies suggest that common genetic factors may underlie some portions of the association between personality traits and alcohol dependence [reviewed by Luo et al., 2007a]. The findings from these studies highlight the potential role of personality as a genetically determined risk factor for alcohol dependence. In the context of this theoretical framework, one of the aims of the present study was to

investigate whether variation in the *OPR* genes represents a genetic factor that underlies both personality traits and SD.

Opioid receptors are mainly distributed in the paraventricular gray matter, the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the mesolimbic systems; distributions in other brain areas have also been reported. The three classical opioid receptors are μ (MOR), δ (DOR), and κ (KOR), with the main endogenous ligands for these being β -endorphin (for MOR), enkephalin (for DOR and MOR), and dynorphin A (for KOR). These ligands bind to opioid receptors to activate intracellular signaling cascades that are responsible for various physiological effects. Some exogenous MOR agonists (e.g., opium, morphine, and heroin) induce euphoria and have reinforcing or rewarding effects – this is a key mechanism of the development of opioid addiction [Bond et al., 1998]. Some exogenous MOR and DOR agonists or antagonists (e.g., naltrexone, naloxone, and TIPP[ψ]) antagonize these euphoric effects and may be used to treat SD. Some DOR agonists, and most KOR agonists, have aversive effects.

Altered opioidergic cellular functions modulate several neurotransmission systems, among which the dopaminergic system is the most important. MOR agonists stimulate MORs in the limbic VTA of the midbrain, generating physiological effects that increase dopamine release in the NAc [e.g., Spanagel et al., 1992; Herz, 1997; Kreek, 2000]. Enhanced dopamine levels stimulate D1 dopamine receptors in the VTA or D2 dopamine receptors in the NAc, causing euphoria, which further promotes drug reinforcement and reward [Di Chiara and Imperato, 1988]. DOR agonists stimulate DORs in the NAc, generating dopaminergic actions similar to those of MOR agonists [Herz, 1997; Kreek, 2000]. KOR agonists stimulate presynaptic KORs in the NAc, increasing dopamine re-uptake and reducing dopamine release and reward, effects opposite to those of MOR and DOR agonists [Herz, 1997; Kreek, 2000; Di Chiara and Imperato, 1988; Priszczak et al., 2005].

Alcohol has effects on different points in the opioidergic signaling pathway. Repeated ethanol administration influences the biosynthesis of prodynorphin, pro-opiomelanocortin, and pro-enkephalin in discrete regions of rat brain, and these alterations change the availability of endogenous ligands [Przewlocka et al., 1992; Turchan et al., 1999]. Alcohol can also induce conformational changes in peptide ligands for opioid receptors [Rapaka et al., 1986a;b], or directly stimulate endorphin release [Gianoulakis and Barcomb, 1987]. Enhanced levels of endogenous opioid ligands produce euphoric effects that are thought to contribute to alcohol dependence. In contrast, opioid agonists and antagonists can modify alcohol consumption [Herz, 1997]; opioid antagonists can decrease alcohol drinking, possibly by reducing euphoric effects and craving, and thus can be used to treat alcohol dependence. For example, numerous studies have shown the MOR antagonist naltrexone to be efficacious in the treatment of alcohol dependence [Srisurapanont and Jarusuraisin, 2005].

Alcohol has effects on opioid receptors as well. Certain metabolites of alcohol (tetrahydroisoquinolines) can bind to opioid receptors [Trachtenberg and Blum, 1987]. Alcohol at low concentrations enhances endogenous opioid receptor binding density (B_{max}) and activity [Charness, 1989], thereby increasing the euphoric effects that contribute to alcohol dependence. Cocaine dependence may have some mechanisms similar to those seen in alcohol dependence, particularly dopamine-mediated ones. In sum, the evidence supports a role for opioid receptor-mediated neurotransmission in the reward, tolerance, and withdrawal effects of alcohol and cocaine.

Illegal drug dependence (primarily, cocaine dependence and/or opioid dependence) is one of the most common disorders comorbid with alcohol dependence. Many studies have

demonstrated that drug dependence has a number of features in common with alcohol dependence, including symptomatology, neuropsychological impairments, hypothesized pathogenetic mechanisms, and response to specific treatments [reviewed by Luo et al., 2005a]. Further, drug dependence has been reported to share some susceptibility genes with alcohol dependence, including several reports that *OPRM1* variation moderated susceptibility to alcohol dependence and/or drug dependence [reviewed by Luo et al., 2005a]. In the present study, 57.5% (111/193) of subjects with alcohol dependence had comorbid drug dependence, and 66.1% (111/168) of subjects with drug dependence had comorbid alcohol dependence (see Subjects section). Based on these findings and our sample structure, alcohol dependence and drug dependence were jointly studied as one phenotype, i.e., SD, in the present study.

OPR gene variation influences the transcription levels, mRNA stability, translational efficiency, and density or affinity of opioid receptors. For example, expression of the Asp40 allelic variant in *OPRM1* reportedly results in a three-fold increase in MOR binding affinity for β -endorphin and a three-fold increase in activation of G protein coupled potassium channels [Bond et al., 1998]. Agonists may exert different effects when interacting with different allelic forms of the protein products of *OPR* genes. Genetic variation in *OPR* genes may be expected to result in phenotypic variation. Specifically, some of the individual genetic variability in susceptibility to the development of SD may be due to polymorphism at the *OPR* genes. This has been directly demonstrated by several studies. For example, the variant allele A of *OPRM1*^Δ-2044C/A (SNP1: rs17180961) was associated with “alcohol + opioid” dependence in European-Americans [Luo et al., 2003]; variants (SNPs 9–14, 16, and 18–19) that map to each of the two major *OPRM1* haplotype blocks are associated with both drug and alcohol dependence [Zhang et al., 2006]; the variant allele C and genotype C/C of *OPRD1*^Δ+921C/T (SNP27: rs2234918) were associated with heroin dependence in German subjects [Mayer et al., 1997]; the genotypes of several *OPRD1* markers, including rs569356 (upstream), rs1042114 (SNP21: exon 1), rs2236855 (intron 1), rs2298896 (SNP24: intron 1), rs421300 (SNP25: intron 1), and rs204076 (SNP28: downstream) were associated with alcohol dependence and/or drug dependence in European-Americans [Zhang et al., 2008]; and the common allele C of *OPRK1*^Δ+36A/C (SNP34: rs1051660) was associated with opioid dependence in a mixed American sample [Yuferov et al., 2004]. Thus, personality traits may plausibly also be associated with the *OPRM1*, *OPRD1* and *OPRK1* genes, due to their strong physiologic (and in some cases, genetic) link to SD [reviewed by Luo et al., 2007a], and their potential associations with levels of other neurotransmitters (e.g., dopamine, serotonin, epinephrine, and norepinephrine, GABA, glutamate, adenosine, and NPY) that may be modulated by opioidergic activity. Some studies have reported direct links between personality traits and *OPRM1* polymorphisms [Wand et al., 2002]; there was a non-significant trend for the presence of the variant Asp40 allele to be associated with lower total conscientiousness scores; further examination revealed a significantly lower score on the competence subscale (i.e., a facet of the conscientiousness factor) among individuals with the variant allele [Wand et al., 2002]. In summary, considerable data support the hypothesis that *OPR* genetic variation contributes to the determination of behavioral phenotypes; there are much fewer available data supporting a relationship of this variation with personality traits.

In the present study, we examined a large set of *OPR* markers (that might either be directly functional, or that might potentially be in linkage disequilibrium (LD) with some unknown functional locus or loci) and aimed to fine-map those loci. Because the three kinds of endogenous opioid ligands interact with one another, as do the three types of opioid receptors, the physiological effects mediated by different opioid receptors may be additive or counteractive. Therefore, the interaction effects among these three genes on personality dimensions were also considered in the present study.

Materials and Methods

1. Subjects

We studied a total of 556 subjects, each of whom completed the NEO-FFI instrument; 250 subjects with SD [181 European-Americans (EAs) and 69 African-Americans (AAs)] and 306 healthy subjects (266 EAs and 40 AAs) were included. The cases (144 males; 106 females) met DSM-IV criteria [American Psychiatric Association, 1994] for a lifetime diagnosis of alcohol dependence ($n=193$) and/or drug dependence ($n=146$ with cocaine dependence; $n=97$ with opioid dependence). 57.5% (111/193) of subjects with alcohol dependence had comorbid drug dependence, and 66.1% (111/168) of subjects with drug dependence had comorbid alcohol dependence. Diagnoses were made using the Structured Clinical Interview for DSM-III-R (SCID) [Spitzer et al., 1992], the computerized Diagnostic Interview Schedule for DSM-III-R (C-DIS-R) [Blouin et al., 1988], or a checklist comprised of DSM-III-R symptoms. The healthy subjects (112 males; 194 females) were recruited by advertisement from the general population, with many recruited from local college campuses. They were screened using the SCID or the C-DIS-R to exclude major Axis I disorders, including alcohol or drug dependence, psychotic disorders (including schizophrenia or schizophrenia-like disorders), mood disorders, and major anxiety disorders. The affected and healthy subjects were similar socioeconomically. The average ages were 37.9 ± 9.3 years for cases and 27.7 ± 8.6 years for healthy subjects. All subjects were recruited at the University of Connecticut Health Center (UCHC) and gave informed consent before participating in the study, which was approved by the UCHC Institutional Review Board. This sample is the same as that used in previous studies [Luo et al., 2007a;b].

2. Marker inclusion

Following extraction of genomic DNA from peripheral blood by standard methods, 20 SNPs at *OPRM1*, 8 SNPs at *OPRD1*, 7 SNPs at *OPRK1* (see Table 1), and 38 ancestry-informative markers (AIMs) unlinked to the *OPRM1*, *OPRD1* and *OPRK1* genes, including 37 STRs and one Duffy antigen gene (*FY*) marker (rs2814778) [Luo et al., 2005a; b; Yang et al., 2005] were genotyped. *OPR* markers were selected from the published literature, from the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) of the National Center for Biotechnology Information (NCBI) or from the Applied Biosystems SNP database (<http://www.appliedbiosystems.com/>). SNP selection was based on published data, information content, minor allele frequency, functional potential, LD structure, and validation evidence [Zhang et al., 2006; 2008]. Many of these markers are tagSNPs in the HapMap database (www.hapmap.org). All markers were bi-allelic SNPs with most having minor allele frequencies > 0.1 in a EA population (except SNPs 1–6, for which the minor alleles are rare in EA) and were validated previously. These SNPs covered from 5' to 3' of each *OPR* gene, with distances shown in Table 1.

3. Genotyping

- i. Eight *OPRM1* markers (SNPs 1–8), 3 *OPRD1* markers (SNP21, SNP22 and SNP27) and the *FY* marker were genotyped by a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technique, as described and reported elsewhere [Luo et al., 2003; 2004; Zhang et al., 2008; Gelernter and Kranzler, 2000].
- ii. All of the other SNPs were genotyped with a fluorogenic 5' nuclease assay method, i.e., the TaqMan technique, using the ABI PRISM® 7900 Sequence Detection System (ABI, Foster City, CA, USA) [Luo et al., 2005b]. All genotyping was performed in duplicate and compared to ensure validity of the data. Mismatched

genotypes, which constituted ~0.5% of the total number of duplicate genotypes performed, were discarded.

- iii. The 37 STR markers were genotyped by a Fluorescence Capillary Electrophoresis (FCE) technique using the ABI PRISM® 3100 semiautomated capillary fluorescence sequencer [Yang et al., 2005].

4. Data analysis

Since the sample here is the same as that used in previous studies [Luo et al., 2007a;b], the rationale and procedures of the following methods are detailed elsewhere and will only be described briefly here (especially for the first seven methods): (i) Assessment of Personality: The 60-item NEO-FFI was used to assess personality dimensions in cases and healthy individuals, to yield scores in five major domains of personality (Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness to Experience). (ii) Pairwise correlation analysis: The intercorrelation between any two personality factors was tested by Pearson pairwise correlation analysis. (iii) Linkage disequilibrium (LD) analysis: Pairwise LD between any two markers in *OPRM1*, *OPRD1* or *OPRK1* was analyzed separately by population, i.e., EAs and AAs, via the program Haploview [Barrett et al., 2005]. (iv) Hardy-Weinberg Equilibrium (HWE) test: HWE of the genotype frequency distribution for each marker was tested separately by population and diagnosis. (v) Ancestry proportion estimation: The ancestry proportions of each subject were estimated by integrating the information from 38 AIMs, to detect admixture and measure its magnitude in African-Americans (AAs) and European-Americans (EAs), so that EAs and AAs could be combined in a single analysis in which subjects were characterized by continuous ancestry proportions. (vi) Univariate ANCOVA: A series of univariate ANCOVA tests were employed to prioritize the theoretical importance of the five personality factors in the associations with *OPR* genes. (vii) Individual haplotype and diplotype probability estimation was done by the program PHASE. Only the “tagSNPs” ($r^2 = 0.8$) were included in haplotype reconstruction.

Other methods employed include:

(viii) Stepwise MANCOVA: The analysis procedure throughout the present study is illustrated in Figure 1. MANCOVA, as a core analytic method in this study, implemented in SPSS 14.0, was employed to test associations between genes and personality traits.

In the haplotypewise and diplotypewise MANCOVA models, five personality factors that are correlated served as one composite dependent variable; haplotype or diplotype probabilities served as predictor variables; diagnosis, age, ancestry proportions, and sex served as covariates; and two-way interactions between the predictor variables and covariates and between intra-gene or inter-gene haplotypes or between inter-gene diplotypes were also considered as independent predictor variables. The inter-gene haplotype or diplotype interactions represent the gene-gene interactions in this study.

When positive findings from the above haplotypewise or diplotypewise analysis were obtained, allelewise and genotypewise MANCOVA was performed, using allele or genotype data to fine-map the loci contributing to personality. All of the MANCOVAs were run as backward stepwise analyses, with only those variables that were statistically significant (i.e., $p < 0.05$) retained in the final equations.

(ix) Stepwise Roy-Bargmann Stepdown Analysis [Tabachnick and Fidell, 1996]: When we obtained positive findings using MANCOVA, we sought to identify the factors contributing to the positive findings by the application of Roy-Bargmann Stepdown Analysis, which is a stepdown ANCOVA. First, we tested the highest-priority personality factor with a univariate ANCOVA. Then we tested the next personality factor with ANCOVA, and used all higher-

priority personality factors as covariates. Other covariates and predictor variables were those remaining in the final MANCOVA equations.

Five personality factors need five separate Roy-Bargmann Stepdown Analyses. To control the inflation of type I error due to multiple tests, α is usually set at 0.01 ($=0.05/5$). However, (a) since the five personality factors are intercorrelated, correcting the α for five independent times may be too conservative; (b) five ANCOVAs were combined in tandem into a series of Roy-Bargmann Stepdown analyses following a single priority order (shown in Results), and thus avoided multiple tests. It has been argued that a series of Roy-Bargmann Stepdown analyses in a certain priority order needs no correction for multiple tests [Tabachnick and Fidell, 1996]; and (c) gene effects on multigenic quantitative personality traits are usually modest in magnitude, so that suggestive gene effects ($0.01 < p < 0.05$) might still be important (particularly given the comparatively small sample size). Consequently, to prevent information loss, we set α at 0.05.

(x) Gene-disease association analysis: The allele and genotype frequencies of the *OPRM1*, *OPRD1* and *OPRK1* markers in different populations are shown in Table 2. Associations between the alleles, genotypes, haplotypes, diplotypes and the phenotypes were analyzed by comparing their frequency distributions between affected and healthy subjects (within EAs and AAs) with exact tests. Multiple testing was corrected by the program SNPSpD (a Bonferroni correction that takes marker correlation into account) [Nyholt, 2004].

We have reported association studies on *OPRM1*, *OPRD1* and *OPRK1* markers previously in larger samples [Luo et al., 2003; Zhang et al., 2006; 2008; Gelernter and Kranzler, 2000] that contain all or most of the present sample (as well as additional subjects). Gene-disease association analysis is not the major focus in the present study; rather, the data were re-analyzed here to compare gene-disease and gene-personality associations in the same samples, to ascertain whether the disease and personality traits share genetic risk. To take into account interaction effects on phenotypes between markers or between haplotypes or diplotypes, we also performed backward stepwise logistic regression analysis in which the diagnosis served as the dependent variable; ancestry proportions, sex and age served as covariates; and the markers and the two-way interactions between markers and between covariates and markers served as predictor variables; or the haplotypes, diplotypes, and the two-way interactions between intra-gene or inter-gene haplotypes or between inter-gene diplotypes served as predictor variables. Only those variables with statistical significance were retained in the last step of the regression equation.

Results

As described and discussed elsewhere in detail for this sample [Luo et al., 2007a;b]: (i) Two ancestries i.e., European and African were detected in our sample, and the ancestry proportions for each individual were estimated. (ii) The personality scores for the different factors of the NEO Five-Factor inventory (NEO-FFI) were: Extraversion (4–48, mean (SD) = 28.7 ± 6.9), Agreeableness (10–46, mean (SD) = 31.0 ± 6.6), Conscientiousness (0–48, mean (SD) = 31.8 ± 7.8), Neuroticism (2–48, mean (SD) = 20.4 ± 9.4), and Openness to Experience (9–45, mean (SD) = 28.4 ± 6.5). (iii) Pairwise correlation analysis showed that, in the whole sample, there were significant bivariate correlations between personality factors (all $p < 0.001$). Among these, Neuroticism was negatively correlated with other personality factors ($r < 0$); all other correlations were positive ($r > 0$). Every personality factor and the linear combination of all personality factors were normally distributed. (iv) A series of univariate ANCOVA tests showed the following priority order of the personality factors: Neuroticism (F4) > Agreeableness (F2) > Conscientiousness (F3) > Extraversion (F1) > Openness (F5). Other results include the following:

1. *OPR* markers were located in several haplotype blocks. The pairwise LD was stronger in EAs than in AAs. These results are consistent with what we have reported previously on a superset of this sample [Zhang et al., 2008].

The definitions and locations of the markers at *OPRM1*, *OPRD1* and *OPRK1* are shown in Table 1. The allele and genotype frequencies are shown in Table 2 (only those markers with $p < 0.05$ in association analysis are listed). The common haplotype and diplotype frequencies are shown in Table 3.

Four *OPRM1* markers (i.e., -1793T/A, -1699insT, -1320A/G, and -111C/T) were in complete LD ($D' = 1$) and formed a four-variant sequence pattern both in EAs and AAs [Luo et al., 2003]. In EAs, the minor haplotype frequency of this sequence pattern was close to 0. In AAs, the minor haplotype frequency of this pattern was close to 0.08, and this pattern extended to marker +17C/T. SNP9 to SNP14 and SNP15 to SNP20 were in two haplotype blocks in EAs [Zhang et al., 2006], but in eight haplotype blocks in AAs (Figure 2). LD maps of other markers at *OPRD1* and *OPRK1* are shown in Figure 2. The “tagSNPs” selected by the aforementioned criteria were SNPs 1, 7, 10, 12, 17, and 19 at *OPRM1*, SNPs 22, 26, and 27 at *OPRD1*, and SNPs 30, 34, and 35 at *OPRK1*. These “tagSNPs” were used to construct haplotypes for analysis (see Table 3).

2. MANCOVA indicated that personality traits were related to the multi-locus haplotypes and diplotypes. Single-locus markers at *OPRM1*, *OPRD1*, or *OPRK1* had main or interaction effects on personality traits. Some effects were modified by diagnosis and sex (Table 4).

MANCOVA showed significant haplotypewise, diplotypewise, allelewise, and genotypewise associations between personality traits and *OPR* genes (Table 4). The haplotypes TTCAGA and TTCAAA at *OPRM1*, and the haplotypes TAC at *OPRD1* and ACA at *OPRK1* had significant interaction effects on personality traits ($p = 0.045$ and 0.020 , respectively). The diplotypes TTAGAA/TTAGGA and TTAGGA/TTCAGA at *OPRM1* had main effects on personality traits ($p = 0.027$ and 0.015 , respectively). The diplotypes *OPRM1*^{TTCAAG/TTCAGA} and *OPRD1*^{CAC/TAC}, *OPRM1*^{TTCAAG/TTCAGA} and *OPRD1*^{CAC/TAC}, and *OPRM1*^{TTCAAG/TTCAGA} and *OPRK1*^{ACA/ACG} had significant interaction effects on personality traits ($0.004 \leq p \leq 0.040$). Most of the contributory haplotypes and diplotypes contained the haplotypes *OPRM1*^{TTCAAG} ($f = 0.412$), *OPRD1*^{TAC} ($f = 0.196$), and *OPRK1*^{ACA} ($f = 0.062$). Allelewise MANCOVA showed that SNP9 and SNP10, SNP11 and SNP12, and SNP17 and SNP18 at *OPRM1* had significant interaction effects on personality traits ($0.002 \leq p \leq 0.021$), and SNP27 at *OPRD1* had effects on personality traits, modified by sex ($p = 0.007$). Genotypewise MANCOVA showed that *OPRD1*^{SNP28} and *OPRK1*^{SNP30} had significant effects on personality traits, modified by sex and diagnosis ($p = 0.046$ and 0.043 , respectively).

3. Roy-Bargmann Stepdown Analyses showed that *OPRM1*, *OPRD1*, and *OPRK1* haplotypes, diplotypes and markers had main effects and/or interaction effects on different personality factors (Table 5).

Haplotypewise Roy-Bargmann Stepdown Analyses showed that the haplotypes TTCAGA and TTCAAA (constructed by SNPs 1, 7, 10, 12, 17, and 19) at *OPRM1*, and the haplotypes TAC (constructed by SNPs 22, 26, and 27) at *OPRD1* and ACA (constructed by SNPs 30, 34, and 35) at *OPRK1* had significant interaction effects on Agreeableness and Openness ($0.022 \leq p \leq 0.048$). Diplotypewise Roy-Bargmann Stepdown Analyses showed that the diplotype TTAGAA/TTAGGA had main effects on Agreeableness and Neuroticism. TTAGGA/TTCAGA at *OPRM1* had main effects on Extraversion and Agreeableness ($0.012 \leq p \leq 0.039$). The diplotypes *OPRM1*^{TTCAAG/TTCAGA} and *OPRD1*^{CAC/TAC},

OPRM1^{TTCAGA/TTCAGA} and *OPRD1*^{CAC/TAC}, and *OPRM1*^{TTCAGA/TTCAGA} and *OPRK1*^{ACA/ACG} had significant interaction effects on Agreeableness, Conscientiousness, Neuroticism and/or Openness ($0.010 \leq p \leq 0.049$). The signs and magnitudes of the standardized regression coefficients (β s) for these effects can be seen in Table 5.

Allelewise Roy-Bargmann Stepdown analyses showed that the alleles SNP9^T and SNP10^A at *OPRM1* had interaction effects on Agreeableness ($\beta < 0$; $p = 0.001$). The alleles SNP17^G and SNP18^A at *OPRD1* had interaction effects on Openness ($\beta < 0$; $p = 0.007$). Finally, *OPRD1*^{SNP27} had effects on Agreeableness, modified by sex ($\beta < 0$ in females, $\beta > 0$ in males).

Genotypewise Roy-Bargmann Stepdown analyses showed that the genotype T/T of *OPRD1*^{SNP28} decreased Extraversion scores in females ($\beta < 0$; $p = 0.028$). Similarly, the genotype A/A of *OPRD1*^{SNP28} decreased Neuroticism scores in females ($\beta < 0$; $p = 0.005$). Finally, the genotype A/T of *OPRK1*^{SNP30} increased Neuroticism scores in cases ($\beta > 0$; $p = 0.00039$).

If corrected for multiple testing, setting α at 0.01 for the above stepwise Roy-Bargmann ANCOVAs, only the diplotype TTAGGA/TTCAGA at *OPRM1* had main effects on Extraversion ($\beta = -3.559$, $p = 0.008$), and the diplotypes *OPRM1*^{TTCAGA/TTCAGA} and *OPRD1*^{CAC/TAC} had interaction effects on Openness ($\beta = 5.360$, $p = 0.010$) (details not shown).

4. Fisher Exact tests for case-control comparisons showed that there were nominally significant differences ($p < 0.05$) in genotype and/or allele frequencies between cases and healthy subjects for many markers, including SNPs 9–14, 18–19, 21, 23–25, 27, and 34 in EAs, and SNPs 13 and 19 in AAs, which is consistent with the results in previous studies in the parent samples (introduced above; Luo et al., 2003; Zhang et al., 2006; 2008). Allelewise backward stepwise logistic regression analysis (using cases vs. healthy subjects as the dependent variable) showed that SNPs 27, 28, and 30–32 remained in the final equation. Genotypewise backward stepwise logistic regression analysis showed that SNPs 10, 27–28, 30 and 32 remained in the final equation. Among all of the markers, *OPRD1*^{SNP28} was most strongly associated with trait. Haplotypewise and diplotypewise analyses showed that some specific haplotypes and diplotypes were positively or inversely associated with risk for SD [details not shown; partially reported by Luo et al., 2003; Zhang et al., 2006; 2008]. No markers were in significant HWD in controls, but many markers were in significant HWD in cases (which could indicate valid marker-phenotype associations), including SNPs 17, 20–21, 23–24, 26, 28–29, 31, and 33–34 in EAs, and SNPs 14, 26, 28–29, 31 and 34–35 in AAs ($p < 0.05$) (Table 2).

Discussion

The findings in the present study suggest that *OPRM1*, *OPRD1* and *OPRK1* may play important roles in the development of personality traits, with *OPRM1* making the greatest contribution. In addition to having main effects, the three *OPR* genes appear to have interaction effects on personality traits.

MANCOVA is a powerful and robust method of analysis. MANCOVA models account for the correlations between different personality factors; reduce the familywise error rate; control for confounding effects of such variables as affection status, age, ethnicity, and sex; avoid multiple tests; take into account gene-gene interactions; allow diplotype phase to be uncertain (in the present study, the proportion of individuals with unambiguous diplotypes (i.e., probability=1) were 16.2%, 38.7% and 66.0% in *OPRM1*, *OPRD1* and *OPRK1*,

respectively); control for population stratification and admixture effects on association analysis; and allow analysis even in the presence of deviation from HWE. These advantages have been detailed by us previously [Luo et al., 2007a]. We thus draw conclusions mainly from MANCOVAs.

Haplotype-wise and diplotype-wise MANCOVAs (at the “whole gene” level) showed that: (i) Some specific haplotypes and diplotypes, most of which contain the haplotypes *OPRMI*^{TTCAGA}, *OPRDI*^{TAC}, and *OPRK1*^{ACA}, have main or interaction effects on personality (see Table 4). In contrast, the contributory haplotype *OPRMI*^{TTCAAA} does not appear in any of the significantly associated diplotypes (probably because the diplotypes including this haplotype were too rare). (ii) Diplotype-wise analysis provided much more significant results than haplotype-wise analysis, probably because more diplotypes were involved in the analysis, leading to greater sensitivity for detection. This also supports our previous work showing that diplotype-wise analysis is more powerful than haplotype-wise analysis in the absence of HWE for markers [Luo et al., 2005a;b]. (iii) The inter-haplotype and inter-diplotype interaction effects and the allele-wise and genotype-wise inter-marker interactions were much stronger than their main effects, most of which were not significant. The strongest interaction effect was between two inter-gene diplotypes (i.e., *OPRMI*^{TTCAG/TTCAGA} and *OPRDI*^{CAC/TAC}; $p=0.004$). Genetically complex traits like personality are multigenic – effects of a single gene might be minor ($0.01 < p < 0.05$) (particularly given the comparatively small sample size), as observed in the present study (see Table 4). The interaction effect of two genes (or markers) could be greater, since they may exert multiplicative effects that are stronger than two doses of either of them, as was observed in the present study (see Table 4). These findings suggest that all of the three *OPR* genes might contribute to the development of personality traits via the interactions between them or otherwise.

Analyses aimed at fine-mapping the contributory loci within the three *OPR* genes showed that the responsible loci are contained within two *OPRMI* haplotype blocks (one of which contains SNP9 to SNP12 and the other of which contains SNP17 to SNP18), one *OPRDI* haplotype block (which contains SNP27 to SNP28), and one *OPRK1* block (which contains SNP30). As greater than 2-way interactions among markers were not considered in these analyses (because they were not computationally feasible), these “marker” level findings are not as reliable as those from the “whole gene” level. These contributory loci might fluctuate with the candidate marker numbers and the analytic approaches; i.e. it would be not surprising if other “non-risk” loci (per this study) in LD with the contributory loci detected in the present study were to be identified in the future as true contributory loci for personality traits.

To decompose the composite personality trait set into separate personality factors, MANCOVA was followed by Roy-Bargmann Stepdown ANCOVA. This analysis has most of the advantages of MANCOVA, so that the findings from Roy-Bargmann Stepdown ANCOVA are also considered to be reliable. Additionally, this analysis provides us with the signs of regression coefficients to judge whether the gene variants had positive or negative associations with the personality factors. One of the disadvantages of this analysis is that the five personality factors are each tested separately, which inflates the familywise error rate and thus decreases the power. We therefore set $\alpha=0.05$, despite the multiple test issue, to avoid an excess of false negative findings.

In summary, the Roy-Bargmann Stepdown ANCOVA demonstrated that *OPRMI*, *OPRDI*, and *OPRK1* haplotypes, diplotypes, alleles and/or genotypes (including their interactions) increase ($\beta > 0$) or decrease ($\beta < 0$) the likelihood of different personality factors (as detailed in the Results section). Among the three *OPR* genes, we obtained the evidence that *OPRMI*

contributes most to personality – this may be because more *OPRM1* markers were included in this study, resulting in greater power or more comprehensive coverage of the gene, or perhaps because the role played by genetic variation at *OPRM1 per se* is greater than that played by other *OPR* genes. Among the five personality factors, Extraversion and Openness are most significantly associated with *OPR* genes (effects that survive after conservative correction for multiple testing). Two particular notes here include: (i) The allele SNP27^T at *OPRD1* decreases Agreeableness scores in females but increases them in males; and the genotype SNP28^{T/T} at *OPRD1* decreases Neuroticism scores in females but increases them in males – these are not necessarily conflicting findings, because females have higher Agreeableness and Neuroticism scores than males, and lower scores in females are close to higher scores in males, both of which are associated with SNP27^T or SNP28^{T/T} (i.e., these variants have “moderating” effects on these traits in both sexes). (ii) The contributory factors (shown in the “variables” column in Table 5) that contain the *OPRK1* gene (including haplotypes, diplotypes, and genotypes) have effects on personality factors opposite to those containing only the *OPRM1* and/or *OPRD1* genes. This is probably because the “minor” haplotypes, diplotypes, and genotypes have effects opposite to the “major” ones, or otherwise, because *OPRK1 per se* has effects opposite to *OPRM1* and *OPRD1*. We note that among those contributory variables, *OPRM1*^{TTCAGA/TTCAGA} interacting with *OPRD1*^{CAC/TAC} ($f=0.106$) increases Neuroticism scores ($\beta>0$), but interacting with *OPRK1*^{ACA/ACG} ($f=0.101$) decreases Neuroticism scores ($\beta<0$). The difference between these two interactions is attributable to *OPRK1*^{CAC/TAC} and *OPRK1*^{ACA/ACG}, which have similar frequencies. This evidence supports the preceding presumption that *OPRK1 per se* has opposite effects to *OPRM1* and *OPRD1*, consistent with the effects of their agonists introduced above. This interpretation is consistent with neurobiological findings on a number of effects of these genes, i.e., agonists at mu- and delta- receptors have effects opposite to agonists at kappa-receptor.

In the present study, there was no significant difference in gene effects between different population and age subgroups (i.e., the gene-personality associations were not modified by ancestry proportion or age; see Tables 4 and 5), suggesting that the positive associations in EAs could be replicated in AAs (which are mostly genetically distinct from EAs), and the positive associations in older adults could be replicated in younger adults. There was no significant difference in the gene effects in many (although not all) specific personality factors between different sex and phenotype subgroups either, suggesting that generally many positive associations in males could be replicated in females, and many positive associations in substance dependent subjects could be replicated in healthy subjects (see Table 5). Validation of these findings is required in subsequent studies, preferably of large study samples that permit separate examination of these findings in groups differentiated by age, sex, population, and affection status. Additionally, replication in an independent sample is obviously required to validate our findings.

From the genotype-phenotype association analyses (conventional case-control comparison, regression analysis, and HWE test), all of the contributory markers for personality traits are at least nominally associated with SD as well. We therefore conclude that personality traits and SD have a partially shared genetic basis, consistent with the extant literature. This could be because personality traits have some underlying opioidergic mechanisms that are intrinsically related to those influencing risk for SD.

Some *OPR* gene haplotypes, diplotypes (data not shown; partially reported in Luo et al., 2003; Zhang et al., 2006; 2008), alleles, or genotypes increase risk for SD, which suggests that these genetic factors might enhance opioidergic activity. Increased opioidergic effects can facilitate dopaminergic, serotonergic, noradrenergic, and other neurotransmitter system activity [Kenna et al., 2004], which can partially account for the increased euphoric and

rewarding effects of psychoactive substances in some individuals. Conversely, some *OPR* gene haplotypes, diplotypes [data not shown; partially reported by Luo et al., 2003; Zhang et al., 2006; 2008], alleles or genotypes decrease risk for SD, which suggests that these genetic factors might inhibit opioidergic activity, leading to the suppression of the neurotransmission systems and inhibiting euphoric effects and reducing risk for SD.

Enhanced dopaminergic, serotonergic, or noradrenergic activity, or low levels of the enzyme monoamine oxidase (associated with high levels of those neurotransmitters) are hypothesized to be related to positive emotion, social engagement, and life satisfaction, which are features of extraversion [e.g., Stein et al., 2004; 2005]. Individuals with high extraversion scores are more sensitive to reward cues, are motivated to obtain future rewards [Gray, 1970; Depue and Collins, 1999], and tend to seek stimulating environments, which could lead them to use alcohol or drugs. Enhanced dopaminergic activity may be related to novelty seeking [Cloninger, 1987], which is a characteristic of openness. Individuals who are high in novelty seeking may be more likely to seek and use alcohol or drugs, and thus be at increased risk for SD. Enhanced serotonergic activity is hypothesized to relate to compulsive behaviors, obsessive tidiness, and inhibition of impulse/affect, which are related to conscientiousness. Enhanced noradrenergic activity may also be related to dependency (i.e., high agreeableness scores), anxiety-proneness, and emotional reactivity (i.e., high neuroticism scores). Many of these personality factors are considered “negative emotionality” (anxious, inhibited, moody, and unhappy) and often accompany SD. This is a mechanism by which both high scores on these personality factors and risk for SD may be associated with similar *OPR* gene variation, as observed to some degree in the present study.

Some *OPR* gene variation might inhibit opioidergic activities, leading (speaking very schematically) to the relative suppression of opioidergic neurotransmission. However, individuals with these gene variants might still use alcohol or drugs because of their specific personality features, which could represent compensatory behavior, to stimulate the hypo-potential of the neurotransmission systems. Hypo-potential of the serotonergic or noradrenergic systems can result in disinhibition (i.e., low conscientiousness scores), aggressive behaviors (i.e., low agreeableness scores), chronic pessimism, depression, impulsivity, obsessive worry, irrational anger, and hypersensitivity to rejection (i.e., high neuroticism scores) – some of these personality characters have been labeled as “behavioral undercontrol” (impulsivity, thrill seeking, rebelliousness, irresponsibility, nonconformity, and aggressiveness); all of these personality characters may make some individuals more likely to use alcohol or drugs. This is a mechanism by which both low scores on these personality factors and risk for SD may be associated with similar *OPR* gene variation, also as observed in the present study.

In conclusion, the present study demonstrated that *OPRM1*, *OPRD1*, and *OPRK1* may contribute to the genetic component of variation in personality traits. These three genes have significant interaction effects on personality traits. Since personality traits and SD have a partially overlapping genetic basis, common neurobiological mechanisms may account for the contributions of *OPR* gene variation to both phenotypes.

Acknowledgments

Funding. This work was supported in part NIH grants R01-DA12849, R01-AA016015, R01-DA12690, K24-DA15105, R01-AA11330, P50-AA12870, K08-AA13732, K24-AA13736, K02-MH01387, and M01-RR06192 (University of Connecticut General Clinical Research Center), by funds from the U.S. Department of Veterans Affairs (the VA Medical Research Program, and the VA Connecticut–Massachusetts Mental Illness Research, Education and Clinical Center [MIRECC], and the VA Research Enhancement Award Program [REAP] research center), and Alcoholic Beverage Medical Research Foundation (ABMRF) grant award R06932 (X Luo).

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Figure 1.

The analysis procedure from comprehensive analysis to decomposing analysis (which corresponds to Data analysis section).

Figure 2. Pairwise LD analysis for *OPR* markers in EAs and AAs. [Numbers in the squares are $D' \times 100$. Empty red squares mean $D' = 1.0$ (i.e. complete LD between two SNPs). Blue squares represent low r^2 values]

Table 1

Marker information

Marker	rs#	Alias	Substitution	Chrom. pos.	Contig pos.	Distance(bp)	Location
<i>OPRM1</i> *SNP1	rs17180961	-2044C/A	C/A	Chr06:154400331	58463067	0	5'
<i>OPRM1</i> *SNP2	rs17180968	-1793T/A	T/A	Chr06:154400581	58463317	250	5'
<i>OPRM1</i> *SNP3	rs17180982	-1699insT	T/-	Chr06:154400675	58463411	344	5'
<i>OPRM1</i> *SNP4		-1469T/C	T/C	Chr06:154400905	58463641	574	5'
<i>OPRM1</i> *SNP5	rs17174629	-1320A/G	A/G	Chr06:154401054	58463790	723	5'
<i>OPRM1</i> *SNP6	rs17174638	-111C/T	C/T	Chr06:154402262	58464998	1930	promoter
<i>OPRM1</i> *SNP7	rs1799972	+17C/T	C/T	Chr06:154402389	58465125	2058	exon1
<i>OPRM1</i> *SNP8	rs1799971	+118A/G	A/G	Chr06:154402490	58465226	2159	exon1
<i>OPRM1</i> *SNP9	rs511435		C/T	Chr06:154410240	58472976	9909	intron1
<i>OPRM1</i> *SNP10	rs524731		A/C	Chr06:154416785	58479521	16454	intron1
<i>OPRM1</i> *SNP11	rs3823010		A/G	Chr06:154420845	58483581	20514	intron1
<i>OPRM1</i> *SNP12	rs495491		A/G	Chr06:154424235	58486971	23904	intron1
<i>OPRM1</i> *SNP13	rs1381376		C/T	Chr06:154434951	58497687	34620	intron1
<i>OPRM1</i> *SNP14	rs3778156		A/G	Chr06:154446006	58508742	45675	intron1
<i>OPRM1</i> *SNP15	rs2075572		C/G	Chr06:154453697	58516433	53366	intron2
<i>OPRM1</i> *SNP16	rs548646		C/T	Chr06:154459840	58522576	59509	intron3
<i>OPRM1</i> *SNP17	rs9322447		A/G	Chr06:154466013	58528749	65682	intron3
<i>OPRM1</i> *SNP18	rs609148		A/G	Chr06:154472707	58535443	72376	intron3
<i>OPRM1</i> *SNP19	rs648893		A/G	Chr06:154480321	58543057	79990	intron3
<i>OPRM1</i> *SNP20	rs671531		A/G	Chr06:154482434	58545170	82103	3'
<i>OPRD1</i> *SNP21	rs1042114	+81T/G	T/G	Chr01:29011562	11963317	0	exon 1
<i>OPRD1</i> *SNP22	rs678849		C/T	Chr01:29017775	11969530	6213	Intron1
<i>OPRD1</i> *SNP23	rs2236857		C/T	Chr01:29034196	11985951	22634	intron1
<i>OPRD1</i> *SNP24	rs2298896		G/T	Chr01:29038725	11990480	27163	intron1
<i>OPRD1</i> *SNP25	rs421300		G/A	Chr01:29042180	11993935	30618	intron1
<i>OPRD1</i> *SNP26	rs12749204		A/G	Chr01:29048800	12000555	37238	intron1
<i>OPRD1</i> *SNP27	rs2234918	+921T/C	C/T	Chr01:29062184	12013939	50622	Exon 3

Marker	rs#	Alias	Substitution	Chrom. pos.	Config pos.	Distance(bp)	Location
<i>OPRD1</i> /SNP28	rs204076		A/T	Chr01:29062977	12014732	51415	3'
<i>OPRK1</i> /SNP29	rs7820807		C/T	Chr08:54301414	5992213	0	3'
<i>OPRK1</i> /SNP30	rs963549		C/T	Chr08:54304377	5995176	2963	exon3
<i>OPRK1</i> /SNP31	rs702764		C/T	Chr08:54304710	5995509	3296	exon3
<i>OPRK1</i> /SNP32	rs997917		C/T	Chr08:54314931	6005730	13517	intron1
<i>OPRK1</i> /SNP33	rs6985606		C/T	Chr08:54323669	6014468	22255	intron1
<i>OPRK1</i> /SNP34	rs1051660	+36A/C	A/C	Chr08:54326115	6016914	24701	exon1
<i>OPRK1</i> /SNP35	rs12675595		A/G	Chr08:54330478	6021277	29064	5'

Chrom., chromosome; Pos., position; Distance, map distance from the first marker within the same gene.

Table 2

a. (supplementary) Genotype and allele frequencies of *OPRM1* markers

Marker	Genotype or Allele	European-Americans				African-Americans			
		Case		Control		Case		Control	
		N	f	N	f	N	f	N	f
SNP9	TT	17	0.100	14	0.067	5	0.081	0	0.000
	TC	64	0.3768	57	0.273	16	0.258	10	0.250
	CC	89	0.524	138	0.660	41	0.661	30	0.750
	T	98	0.288 ^a	85	0.203	26	0.210	10	0.125
	C	242	0.712	333	0.797	98	0.790	70	0.875
SNP10	AA	17	0.096	12	0.057	2	0.032	0	0.000
	AC	66	0.3738	59	0.281	9	0.143	8	0.200
	CC	94	0.531	139	0.662	52	0.825	32	0.800
	A	100	0.282 ^a	83	0.198	13	0.103	8	0.100
	C	254	0.718	337	0.802	113	0.897	72	0.900
SNP11	AA	11	0.065	9	0.043	2	0.033	0	0.000
	AG	51	0.302	47	0.226	8	0.133	7	0.175
	GG	107	0.633	152	0.731	50	0.833	33	0.825
	A	73	0.216 ^a	65	0.156	12	0.100	7	0.088
	G	265	0.784	351	0.844	108	0.900	73	0.913
SNP12	AA	84	0.477	118	0.581	19	0.288	11	0.282
	AG	68	0.386	70	0.345	30	0.455	22	0.564
	GG	24	0.136	15	0.074	17	0.258	6	0.154
	A	236	0.670	306	0.754	68	0.515	44	0.564
	G	116	0.330 ^a	100	0.246	64	0.485	34	0.436
SNP13	TT	11	0.063	8	0.037	5	0.078	0	0.000
	TC	51	0.290	47	0.217	21	0.328	9	0.225
	CC	114	0.648	162	0.747	38	0.594	31	0.775
	T	73	0.207 ^a	63	0.145	31	0.242 ^a	9	0.113
	C	279	0.793	371	0.855	97	0.758	71	0.888

a. (supplementary) Genotype and allele frequencies of *OPRM1* markers

Marker	Genotype or Allele	European-Americans			African-Americans		
		Case		N	Control		N
		N	f		N	f	
SNP14	AA	109	0.619	155	0.735	47	0.723
	AG	57	0.324 ^g	49	0.232	14	0.215 ^h
	GG	10	0.057	7	0.033	4	0.062
A	A	275	0.781	359	0.851	108	0.831
	G	77	0.219 ^a	63	0.149	22	0.169
SNP17	AA	44	0.250	49	0.234	22	0.355
	AG	70	0.398 ^h	102	0.488	30	0.484
	GG	62	0.352	58	0.278	10	0.161
A	A	158	0.449	200	0.478	74	0.597
	G	194	0.551	218	0.522	50	0.403
SNP18	AA	7	0.041	9	0.044	0	0.000
	AG	42	0.244	81	0.393	13	0.203
	GG	123	0.715 ^g	116	0.563	51	0.797
A	A	56	0.163	99	0.240	13	0.102
	G	288	0.837 ^a	313	0.760	115	0.898
SNP19	AA	122	0.705 ^g	115	0.561	61	0.953 ^g
	AG	42	0.243	80	0.390	3	0.047
	GG	9	0.052	10	0.049	0	0.000
A	A	286	0.827 ^a	310	0.756	125	0.977 ^a
	G	60	0.173	100	0.244	3	0.023

b. (supplementary) Genotype and allele frequencies of *OPRD1* and *OPRK1* markers

Marker	Genotype or Allele	European-Americans			African-Americans		
		Case		N	Control		N
		N	f		N	f	
SNP21	GG	8	0.048 ^g	1	0.005	0	0.000
	GT	31	0.188 ^h	54	0.258	4	0.062

b. (supplementary) Genotype and allele frequencies of *OPRD1* and *OPRK1* markers

Marker	Genotype or Allele	European-Americans				African-Americans			
		Case		Control		Case		Control	
		N	f	N	f	N	f	N	f
SNP23	TT	126	0.764	154	0.737	61	0.938	28	0.966
	G	47	0.142	56	0.134	4	0.031	1	0.017
	T	283	0.858	362	0.866	126	0.969	57	0.983
	TT	100	0.592 ^g	104	0.479	31	0.500	14	0.359
	TC	52	0.308 ^h	100	0.461	26	0.419	19	0.487
SNP24	CC	17	0.101	13	0.060	5	0.081	6	0.154
	T	252	0.746	308	0.710	88	0.710	47	0.603
	C	86	0.254	126	0.290	36	0.290	31	0.397
	TT	94	0.550 ^g	85	0.392	20	0.317	13	0.325
	TG	55	0.322 ^h	105	0.484	34	0.540	19	0.475
SNP26	GG	22	0.129	27	0.124	9	0.143	8	0.200
	T	243	0.711 ^a	275	0.634	74	0.587	45	0.563
	G	99	0.289	159	0.366	52	0.413	35	0.438
	AA	119	0.704	136	0.627	53	0.855	33	0.846
	AG	41	0.243 ^h	73	0.336	6	0.097 ^h	6	0.154
SNP27	GG	9	0.053	8	0.037	3	0.048	0	0.000
	A	279	0.825	345	0.795	112	0.903	72	0.923
	G	59	0.175	89	0.205	12	0.097	6	0.077
	TT	59	0.378	63	0.280	5	0.079	2	0.051
	TC	66	0.423	106	0.471	30	0.476	13	0.333
SNP28	CC	31	0.199	56	0.249	28	0.444	24	0.615
	T	184	0.590 ^a	232	0.516	40	0.317	17	0.218
	C	128	0.410	218	0.484	86	0.683	61	0.782
	AA	69	0.421	82	0.394	43	0.717	25	0.714
	AT	64	0.390 ^h	94	0.452	14	0.233	9	0.257
SNP29	TT	31	0.189	32	0.154	3	0.050	1	0.029
	A	202	0.616	258	0.620	100	0.833	59	0.843
	T	126	0.384	158	0.380	20	0.167	11	0.157

b. (supplementary) Genotype and allele frequencies of *OPRD1* and *OPRK1* markers

Marker	Genotype or Allele	European-Americans				African-Americans			
		Case		Control		Case		Control	
		N	f	N	f	N	f	N	f
SNP30	AA	129	0.759	170	0.749	22	0.386	7	0.179
	AT	37	0.218	52	0.229	25	0.439	25	0.641
	TT	4	0.024	5	0.022	10	0.175	7	0.179
	A	295	0.868	392	0.863	69	0.605	39	0.500
	T	45	0.132	62	0.137	45	0.395	39	0.500
SNP31	TT	148	0.841	155	0.760	36	0.563	14	0.359
	TC	25	0.142 ^h	41	0.201	16	0.250 ^h	17	0.436
	CC	3	0.017	8	0.039	12	0.188	8	0.205
	T	321	0.912 ^a	351	0.860	88	0.688	45	0.577
	C	31	0.088	57	0.140	40	0.313	33	0.423
SNP34	AA	5	0.030 ^g	0	0.000	2	0.033	1	0.026
	AC	23	0.138 ^h	32	0.162	7	0.117 ^h	7	0.179
	CC	139	0.832	165	0.838	51	0.850	31	0.795
	A	33	0.099	32	0.081	11	0.092	9	0.115
	C	301	0.901	362	0.919	109	0.908	69	0.885

N, number of individuals (for genotypes) or chromosomes (for alleles); f, frequency.

^g Genotypewise and/or

^a allelewise case-control comparison: p<0.05;

^h Deviation from HWE in cases; p<0.05. Only the markers with Pa, Pg, or Ph<0.05 are listed in Table 2.

N, f, a, g, h: same as table 2a.

Table 3

Frequencies of common haplotypes and diplotypes

	Haplotype	<i>f</i>	Diplotype	<i>f</i>
<i>OPRM1</i>	TTCAGA	0.412	TTCAGA/TTCAGA	0.202
	TTCAAG	0.170	TTCAAG/TTCAGA	0.143
	TTAGAA	0.123	TTAGAA/TTCAGA	0.088
	TTCGAA	0.077	TTCAAA/TTCAGA	0.055
	TTCAAA	0.073	TTAGAA/TTCAAG	0.049
	TTAGGA	0.068	TTCAGA/TTCGAA	0.046
	TACGAA	0.028	TTAGGA/TTCAGA	0.045
	TTCGGA	0.010	TTCAAG/TTCAAG	0.035
			TTAGGA/TTCAAG	0.025
			TTAGAA/TTAGGA	0.023
			TACGAA/TTCAGA	0.022
			TTCAAG/TTCGAA	0.021
			TTAGAA/TTAGAA	0.019
			TTCAAA/TTCAAG	0.017
			TTAGAA/TTCGAA	0.016
			TTAGAA/TTCAAA	0.015
<i>OPRD1</i>	CAC	0.254	CAC/TAC	0.106
	TAT	0.243	CAC/TAT	0.092
	TAC	0.196	TAT/TAC	0.085
	CAT	0.138	CAC/CAC	0.084
	CGT	0.099	CAT/CAC	0.077
	CGC	0.042	CAT/TAT	0.076
	TGC	0.015	TAT/TAT	0.075
	TGT	0.012	CAT/TAC	0.056
			CGT/TAT	0.052
			CGT/TAC	0.045
			TAC/TAC	0.036
			CAC/CGT	0.035
<i>OPRK1</i>	ACG	0.690	ACG/ACG	0.492
	TCG	0.151	ACG/TCG	0.176
	ACA	0.062	ACA/ACG	0.101
	AAG	0.054	AAG/ACG	0.066
	TAG	0.033	ACG/TAG	0.041
			TCG/TCG	0.038
			AAG/TCG	0.019

Haplotypes are constructed only by "tagSNPs": SNPs 1, 7, 10, 12, 17, and 19 at *OPRM1*, SNPs 22, 26, and 27 at *OPRD1*, and SNPs 30, 34, and 35 at *OPRK1*. Only those haplotypes and diplotypes with $f > 0.01$ are listed.

Table 4MANCOVAs on the relationships between personality traits and *OPR* genes

Variables	Haplotypewise	Diploypewise	Allelewise	Genotypewise
Age	9.3E-10	2.2E-09	1.2E-13	3.8E-06
Diagnosis (Case vs. Control)	6.3E-37	9.9E-37	4.9E-50	2.3E-16
European ancestry	3.8E-06	2.5E-06	8.0E-12	1.8E-04
Sex (Male=1, Female=0)	9.8E-06	1.0E-05	8.4E-06	0.012
<i>OPRM1</i> [^] TTCAGA × TTCAAA	0.045			
<i>OPRD1</i> [^] TAC × <i>OPRK1</i> [^] ACA	0.020			
<i>OPRM1</i> [^] TTAGAA/TTAGGA		0.027		
<i>OPRM1</i> [^] TTAGGA/TTCAGA		0.015		
<i>OPRM1</i> [^] TTCAAG/TTCAGA × <i>OPRD1</i> [^] CAC/TAC		0.004		
<i>OPRM1</i> [^] TTCAGA/TTCAGA × <i>OPRD1</i> [^] CAC/TAC		0.040		
<i>OPRM1</i> [^] TTCAGA/TTCAGA × <i>OPRK1</i> [^] ACA/ACG		0.031		
Sex × SNP27			0.007	
SNP9 × SNP10			0.011	
SNP11 × SNP12			0.021	
SNP17 × SNP18			0.002	
Sex × SNP28				0.046
Diagnosis × SNP30				0.043

“E”, scientific format of 10^E; “×”, interaction between.

Table 5
Roy-Bargmann Stepdown ANCOVAs on the relationships between every personality factor and predictor variables

Variables	F1		F2		F3		F4		F5	
	β	p	β	p	β	p	β	p	β	p
Haplotypewise										
Diagnosis(case=1,control=0)			-2.110	0.001	-1.520	0.018	11.045	5.6E-37	-1.665	0.005
Sex(male=1,female=0)			-1.564	0.002			-3.119	1.0E-05		
Age	-0.148	5.2E-09	0.080	0.003			-0.086	0.026	-0.076	0.008
European ancestry	2.323	0.001	1.777	0.007			2.344	0.011	2.008	0.003
Agreeableness					0.167	0.001				
Conscientiousness	0.102	0.007								
Extraversion									0.195	9.8E-07
Neuroticism	-0.250	1.3E-14	-0.307	8.6E-23	-0.342	2.5E-19				
<i>OPRMI</i> ⁺ <i>TTCAGA</i> × <i>TTCAAA</i>			9.061	0.048					-10.477	0.028
<i>OPRDI</i> ⁺ <i>TAC</i> × <i>OPRKI</i> ⁺ <i>ACA</i>			-12.161	0.043					14.364	0.022
Diploypewise										
Diagnosis(case=1,control=0)			-2.100	0.001	-1.555	0.016	11.163	7.8E-38	-1.705	0.004
Sex(male=1,female=0)			-1.533	0.002			-3.134	8.1E-06		
Age	-0.149	4.1E-09	0.079	0.003			-0.089	0.020	-0.079	0.006
European ancestry	2.448	2.6E-04	1.790	0.006			2.180	0.017	2.169	0.001
Agreeableness					0.172	3.8E-04				
Conscientiousness	0.097	0.011								
Extraversion									0.197	7.2E-07
Neuroticism	-0.246	2.9E-14	-0.293	6.7E-21	-0.346	7.8E-20				
<i>OPRMI</i> ⁺ <i>TTAGAA</i> / <i>TTAGGA</i>			-3.724	0.027			4.926	0.039		
<i>OPRMI</i> ⁺ <i>TTAGGA</i> / <i>TTACGA</i>	-3.354	0.012	-2.796	0.028						
<i>OPRMI</i> ⁺ <i>TTCAGG</i> / <i>TTTCAGA</i> × <i>OPRDI</i> ⁺ <i>CAC</i> / <i>TAC</i>			-5.869	0.011			6.737	0.040		
<i>OPRMI</i> ⁺ <i>TTCAGA</i> / <i>TTTCAGA</i> × <i>OPRDI</i> ⁺ <i>CAC</i> / <i>TAC</i>							5.547	0.049	5.360	0.010
<i>OPRMI</i> ⁺ <i>TTTCAGA</i> / <i>TTTCAGA</i> × <i>OPRKI</i> ⁺ <i>ACA</i> / <i>ACG</i>					-4.629	0.020	-4.939	0.042		
Allelewise										
Diagnosis(case=1,control=0)										
Case										
Sex(male=1,female=0)			-2.881	6.5E-08					-2.639	9.7E-09
Male			-0.140	0.042						

Variables	F1		F2		F3		F4		F5	
	β	p	β	p	β	p	β	p	β	p
Age			0.078	3.3E-04					-0.060	0.006
European ancestry			1.403	0.007					2.513	1.2E-06
Extraversion									0.188	2.4E-10
Neuroticism			-0.258	1.4E-24						
<i>OPRM1</i> ' <i>SNP9</i> × <i>SNP10</i>				0.008						
<i>SNP9</i> ' <i>T</i> × <i>SNP10</i> ' <i>A</i>			-2.935	0.001						
<i>OPRM1</i> ' <i>SNP17</i> × <i>SNP18</i>										
<i>SNP17</i> ' <i>G</i> × <i>SNP18</i> ' <i>A</i>										
Sex × <i>OPRD1</i> ' <i>SNP27</i>										
Female × <i>OPRD1</i> ' <i>SNP27</i> ' <i>T</i>				0.046						0.048
Male × <i>OPRD1</i> ' <i>SNP27</i> ' <i>T</i>			-0.969	0.081					-7.081	0.007
Genotypewise			0.989	0.097						
Diagnosis(case=1,control=0)										
Case										
Sex(male=1,female=0)										
Male										
Age	-0.137	9.2E-07								
European ancestry	2.647	4.5E-04					9.539	6.3E-15		
Conscientiousness	0.096	0.020					-4.922	3.8E-16		
Neuroticism	-0.278	1.5E-14					-0.096	0.013		
Sex × <i>OPRD1</i> ' <i>SNP28</i>		0.041								
Female × <i>OPRD1</i> ' <i>SNP28</i> ' <i>T/T</i>	-2.645	0.028								
Female × <i>OPRD1</i> ' <i>SNP28</i> ' <i>A/A</i>							-3.489	0.030		
Diagnosis × <i>OPRK1</i> ' <i>SNP30</i>							-3.243	0.005		
Case × <i>OPRK1</i> ' <i>SNP30</i> ' <i>A/T</i>							4.611	0.011		
								3.9E-04		

F1 to F5; personality factors, i.e., F1=Extraversion, F2=Agreeableness, F3=Conscientiousness, F4=Neuroticism, and F5=Openness. "E", scientific format of 10ⁿ. β , regression coefficient; p, p-value; the indented lines in "Variables" column correspond to *post-hoc* decomposition analysis. The bold corresponds to the gene effects.