HHS Public Access

Author manuscript

J Opioid Manag. Author manuscript; available in PMC 2020 March 01.

Published in final edited form as:

J Opioid Manag. 2019 ; 15(2): 103–109. doi:10.5055/jom.2019.0491.

Four single nucleotide polymorphisms in genes involved in neuronal signaling are associated with Opioid Use Disorder in West Virginia

Shane W. Kaski, BS [MD/PhD Dual Degree Scholar],
Department of Physiology & Pharmacology, West Virginia University School of Medicine, Morgantown, WV

Stephan Brooks, MPH [Coordinator],
Clarion University of Pennsylvania, Clarion, PA

Sijin Wen, PhD [Associate Professor],
Department of Biostatistics, West Virginia University School of Public Health, Morgantown, WV

Marc W. Haut, PhD [Professor and Chair],
Department of Behavioral Medicine & Psychiatry, West Virginia University School of Medicine, Morgantown, WV

David P. Siderovski, PhD [Professor and Chair],
Department of Physiology & Pharmacology, West Virginia University School of Medicine, Morgantown, WV

James H. Berry, DO [Associate Professor/Medical Director],
Chestnut Ridge Center and Inpatient Acute Dual Diagnosis Program, West Virginia University School of Medicine, Morgantown, WV

Laura R. Lander, MSW[#] [Associate Professor], and
West Virginia University School of Medicine, Morgantown, WV

Vincent Setola, PhD[#] [Assistant Professor]
Departments of Behavioral Medicine & Psychiatry, Neuroscience, and Physiology & Pharmacology, West Virginia University School of Medicine, Morgantown, WV

[#] These authors contributed equally to this work.

Abstract

Objective: Pilot study to assess utility in opioid use disorder (OUD) of a panel of single nucleotide polymorphisms in genes previously related to substance use disorder (SUD) and/or phenotypes that predispose individuals to OUD/SUD.

Design: Genetic association study.

To whom correspondence should be addressed: **Dr. Vincent Setola**, Robert C. Byrd Health Sciences Center, 64 Medical Center Dr., Room 3054A HSN 3rd Floor, Morgantown, WV 26506-9229 USA; Tel: (304) 293-1512; Fax: (304) 293-3850; vssetola@hsc.wvu.edu.

Setting: West Virginia University's Chestnut Ridge Center Comprehensive Opioid Abuse Treatment (COAT) clinic for individuals diagnosed with OUD.

Patients: Sixty patients 18 years of age or older with OUD undergoing medication (buprenorphine/naloxone)-assisted treatment (MAT); all sixty patients recruited contributed samples for genetic analysis.

Outcome Measure(s): Minor allele frequencies for single nucleotide polymorphisms.

Results: Four of the fourteen single nucleotide polymorphisms examined were present at frequencies that are statistically significantly different than in a demographically-matched general population.

Conclusions: For the purposes of testing WV individuals via genetic means for predisposition to OUD, at least four single nucleotide polymorphisms in three genes are likely to have utility in predicting susceptibility. Additional studies with larger populations will need to be conducted to confirm these results before use in a clinical setting.

Keywords

Opioid use disorder (OUD); substance use disorder (SUD); single nucleotide polymorphisms (SNPs); genetic testing

INTRODUCTION

Opioid use disorder (OUD) has reached epidemic proportions in America.[1, 2] Non-medical use of opioid analgesics and heroin is on the rise.[3–5] Opioid overdose is now the leading cause of death of people under 50 years old.[6] Mindful of these developments, many addiction researchers are focusing combatting the epidemic with prevention and treatment.[7–10] A shared aspiration is to obtain an individual's genetic "fingerprint" and identify whether they are at increased risk for developing OUD (*e.g.*, ref. [11]). Such testing is likely to interrogate several genes, as a multitude of factors[12–23] contribute to OUD predisposition. Several gene variations are associated with predisposition to OUD and/or OUD treatment response[12–16]. Environmental factors also contribute to OUD[19–22], and some environmental factors can affect the influence of genetic factors on predisposition to developing addiction[23]. The ability to identify *a priori* individuals at increased OUD risk would help greatly in prevention. For instance, using opioid analgesics with these individuals could be avoided or strictly monitored.

West Virginia is the epicenter of this opioid crisis, leading in opioid-related overdose deaths[26]. The state is also demographically homogenous[27]*, being predominantly whites (94%) of European Caucasian ancestry (2010 US Census Data; http://censusviewer.com/state/WV). These factors make West Virginia ideal for genetic studies aimed at curbing the opioid epidemic. West Virginia University's Chestnut Ridge Center is home to a successful

*Demographic homogeneity in West Virginia in no way reflects any heightened degree of consanguinity. Tincher [82] examined 140 years' worth of marriage records in a remote four-county Appalachian region. While cousin marriage was found to occur in Appalachia, it was not conspicuously more prevalent than in other geographical locales: by 1970, the cousin marriage rate was no higher than in the general U.S. population.

medication-assisted treatment program, known as the Comprehensive Opioid Addiction Treatment (COAT) clinic[7, 28–30]. Given access to this WV patient population, we sought to address whether there are single nucleotide polymorphisms (SNPs) more or less prevalent in WV persons with OUD than in the general population.

METHODS

Recruitment

60 volunteers, diagnosed with OUD using DSM-V criteria[31], were recruited from WVU's COAT clinic under IRB 1506733605. All participants were patients of the COAT clinic in Morgantown, West Virginia. The COAT clinic's service area (and hence our research subject area) is geographically diverse, as it draws from the entire state.

Tissue Collection

Consenting volunteers provided buccal swabs, which were placed in 15-ml conical centrifuge tubes (Corning) and stored at −80ºC.

Genomic DNA Extraction

DNA was extracted using QIAamp DNA Mini Kits according to manufacturer's protocol. Yield was assessed by measuring 260-nm absorbance of DNA extracts on a QIAxpert microfluidic reader. Purity was assessed by measuring 260-nm and 280-nm absorbance; all samples had a 260-nm/280-nm quotient greater than 1.8 units.

Genotyping

Genotyping was performed using TaqMan (FisherSci #4351379) primer-probe sets for each SNP (Table 1) and Type-it Fast SNP Probe PCR Kits (Qiagen) according to manufacturer's protocol. Samples were run on a Rotor-Gene Q (Qiagen) in duplicate, and no-DNA negative controls were also performed for each TaqMan primer-probe set.

Statistical Analysis

Allele frequencies were compared to those of 1006 individuals of European descent (1000 Genomes Project[32]); if no information was available from the 1000 Genomes Project, allele frequencies were compared to ExAc database[33] of ~60,000 genomes. Statistical analyses for differences of SNP variant frequency between groups were performed using Pearson's chi-square statistical test. The null hypothesis was that the allele frequencies from WV COAT clinic participants were the same as those from public databases. Therefore, p-value < 0.05 implies that the allele frequencies from WV COAT clinic participants are significantly different from the general public.

RESULTS

A literature search for SNPs associated with OUD/SUD and/or related traits (*e.g.*, anxiety) identified fourteen of known or suspected relevance to the biological actions of opioids (*i.e.*, variations within genes encoding G protein-coupled receptors [the GPCRs dopamine D_2 receptor[34, 35], serotonin 5-HT_{2B} receptor[36–38], and μ-opioid receptor[39–62]]; Regulators of G

protein Signaling [RGS2, RGS17][63–70]; a nerve growth factor [brain-derived neurotrophic factor][71, 72]; a voltage-gated potassium channel [KCNC1][73]; and, a second messenger-regulated transcription factor [cyclic-AMP responsive element binding protein-1][74]; Table 1). We isolated buccal swabs from 60 WV COAT clinic participants, prepared genomic DNA, and performed genotyping of the following SNPs.

OPRM1 rs9479757 (mu opioid receptor gene variant[39–41])

Xu *et al.*[40] identified rs9479757 as linked to heroin addiction severity among Han Chinese males; the G-to-A transition facilitates exon 2 skipping, leading to altered *OPRM1* splice-variant mRNAs and encoded mu opioid receptor (MOR) isoforms. All sixty participants were heterozygous for *OPRM1* rs9479757; in contrast, the minor allele frequency in the general European population is only 0.097 (Table 1).

OPRM1 rs1799971 (mu opioid receptor gene variant[41–62])

Woodcock *et al.*[46] published that Caucasian male carriers of the non-synonymous *OPRM1* rs1799971 minor allele (G) reported significantly more heroin use-related consequences and quit attempts, and were more likely to seek OUD treatment, than individuals homozygous for the ancestral A allele. Schwantes-An *et al.*[48], in a meta-analysis of ~28,000 Europeans, investigated non-specific risk for SUD and compared cases dependent on any substance to controls not dependent on all assessed substances. The rs1799971 G allele showed a modest protective effect on general substance dependence[48]. The rs1799971 G allele was significantly less frequent in WV COAT clinic participants than in the population at large, by a factor of ~2 (Table 1; 0.083 *vs* 0.162, $p = 0.018$).

RGS2 rs4606 (Regulator of G protein Signaling-2 gene variant[63–69])

One of us[75] previously reported that loss of RGS2 in mice engenders heightened anxiety and diminished aggression. Subsequently, Smoller *et al.*[63] found that *RGS2* variations, including rs4606, were associated with introversion (a core personality trait in social anxiety disorder)[63]. Associations between the *RGS2* SNP rs4606 and panic disorder[68], suicidal ideation[64], post-traumatic stress disorder[69], and generalized anxiety disorder[65] have also been reported. We found that rs4606 was more frequent in COAT clinic volunteers than in the general population by a factor of ~2 (Table 1; 0.533 *vs* 0.276, $p < 0.001$).

HTR2B rs6736017 (serotonin [5-HT] receptor-2B gene[36–38] variant)

There are presently no published details on the *HTR2B* SNP rs6736017. However, *HTR2B* variations likely play a role in drug abuse. Doly *et al.*[36, 37] showed that the serotonin 5-HT_{2B} receptor, encoded by the *HTR2B* gene, is required for 3,4-methylene-dioxymethamphetamine (MDMA)-induced hyperlocomotion, serotonin and dopamine release, and conditioned place preference, the latter a rodent-based metric of reinforcing properties. Furthermore, Lin *et al.*[38] conducted a genome-scan identifying the human *HTR2B* gene as a candidate for drug abuse liability. The *HTR2B* SNP rs6736017 was markedly more frequent in COAT clinic participants than in the population at large, when comparing to the 60,000 genomes of the ExAc database (Table 1; 0.500 *vs* 0.0078, $p < 0.01$).

None of the other 10 SNPs investigated exhibited a significantly different frequency in the COAT clinic volunteers *versus* the population at large (Table 1).

DISCUSSION

We identified four SNPs present at different frequencies in persons with OUD compared with a demographically-matched general population. It is currently difficult to quantify the degree of significance of each of these altered frequencies on OUD in the COAT population. One reason for this difficulty is that many gene variants are likely to play an integrated role in predisposition to OUD, and these variants may have additive or synergistic effects; here, we have only interrogated 14 SNPs in 8 candidate genes. Also, possible gene x environment effects were not assessed in the present study.

Two SNPs were in the *OPRM1* gene encoding MOR -- the main molecular target for the euphoric effects of opioids, as well as for the therapeutic effects of buprenorphine[7, 30] and methadone in OUD treatment. Therefore, it is not surprising that *OPRM1* variations would be associated with OUD.

We also identified an *RGS2* variant associated with OUD. This finding is novel, but it is also not surprising. Opioids like oxycodone and buprenorphine generate neuronal signals by activating opioid-binding GPCRs, altering receptor conformation to cause nucleotide exchange[76] by the associated G protein heterotrimer (Galpha·GDP/Gbeta/Ggamma). GTP-loaded G-alpha then dissociates from the Gbeta/gamma heterodimer; both subunits become free to modulate enzymes that generate intracellular second messengers (*e.g.*, cyclic AMP, calcium) or stimulate potassium channels, leading to neuronal membrane hyperpolarization and thus action potential inhibition. G-alpha proteins have intrinsic GTPase activity, leading to self-inactivation and reassociation with Gbeta/gamma subunits[76]. RGS proteins like RGS2 greatly enhance the GTP hydrolysis rate of G-alpha subunits[76–78], thereby inhibiting GPCR signaling. Thus, one might indeed expect that a polymorphism in an *RGS* gene – encoding a negative regulator of GPCR signaling -- would be implicated in OUD. Moreover, *RGS2* function is particularly germane to multiple anxiety disorders[63–69] that could predispose an individual toward OUD.

HTR2B encodes the serotonin 5-HT_{2B} receptor, implicated in the rewarding actions of MDMA[36, 37] and cocaine[79], and thought to be involved in impulsivity[80] and resistance to antidepressants[81], conditions that could predispose to OUD. Other polymorphisms in *HTR2B* have also been associated with vulnerability to illegal drug use[38]. Therefore, our identification of a *HTR2B* polymorphism significantly more frequent in persons with OUD compared with a demographically-matched general population is not surprising.

There are several goals for identifying SNPs associated with OUD. One of these goals is to identify markers that, in genetic screens, could identify people at risk for developing OUD (*e.g.*, before prescribing opioid analgesics). Another goal is to identify potential novel "druggable" targets for preventing and/or treating OUD. For instance, the published role of the 5-HT_{2B} receptor in the actions of illicit drugs, coupled with our finding that rs6736017 is so frequent in OUD individuals, suggests that 5-HT_{2B} receptor-active pharmaceuticals (of

which many are already approved for human use) might have efficacy in preventing/treating OUD. Future studies with larger cohorts are required to validate our findings, both in West Virginia and in other, more demographically heterogeneous regions. Such studies should identify genes to interrogate in order to predict individuals with susceptibility to OUD/SUD and in doing so provide an opportunity for the prevention of the disease.

Acknowledgments:

Research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number 2U54GM104942-02 to the West Virginia Clinical & Translational Science Institute. Funding support was also provided by the E.J. Van Liere Endowed Medicine Professorship (to D.P.S.) and by NIDA F30 fellowship DA044711 (to S.W.K.).

REFERENCES

1. Rudd RA, Seth P, David F, et al. Increases in Drug and Opioid-Involved Overdose Deaths - United States, 2010–2015. MMWR Morb Mortal Wkly Rep. 2016; 65(5051): 1445–52. [PubMed: 28033313]
2. McBain R, Rose AJ, LaRochelle MR. The U.S. opioid epidemic: One disease, diverging tales. Prev Med. 2018; 112: 176–8. [PubMed: 29684417]
3. Fischer B, Keates A, Buhringer G, et al. Non-medical use of prescription opioids and prescription opioid-related harms: why so markedly higher in North America compared to the rest of the world? Addiction. 2014; 109(2): 177–81. [PubMed: 23692335]
4. Jordan AE, Blackburn NA, Des Jarlais DC, et al. Past-year prevalence of prescription opioid misuse among those 11 to 30years of age in the United States: A systematic review and meta-analysis. J Subst Abuse Treat. 2017; 77: 31–7. [PubMed: 28476268]
5. Pergolizzi JV Jr., LeQuang JA, Taylor R Jr., et al. Going beyond prescription pain relievers to understand the opioid epidemic: the role of illicit fentanyl, new psychoactive substances, and street heroin. Postgrad Med. 2018; 130(1): 1–8. [PubMed: 29190175]
6. Dowell D, Arias E, Kochanek K, et al. Contribution of Opioid-Involved Poisoning to the Change in Life Expectancy in the United States, 2000–2015. JAMA. 2017; 318(11): 1065–7. [PubMed: 28975295]
7. Marshalek PJ, Sullivan CR. Buprenorphine clinics: an integrated and multidisciplinary approach to treating opioid dependence. W V Med J. 2010; 106(4 Spec No): 60–3. [PubMed: 21932755]
8. Schmid CL, Kennedy NM, Ross NC, et al. Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics. Cell. 2017; 171(5): 1165–75 e13. [PubMed: 29149605]
9. Vashishtha D, Mittal ML, Werb D. The North American opioid epidemic: current challenges and a call for treatment as prevention. Harm Reduct J. 2017; 14(1): 7. [PubMed: 28494762]
10. Ratycz MC, Papadimos TJ, Vanderbilt AA. Addressing the growing opioid and heroin abuse epidemic: a call for medical school curricula. Med Educ Online. 2018; 23(1): 1466574. [PubMed: 29708863]
11. Blum K, Chen ALC, Thanos PK, et al. Genetic addiction risk score (GARS) , a predictor of vulnerability to opioid dependence. Front Biosci (Elite Ed). 2018; 10: 175–96. [PubMed: 28930612]
12. Kreek MJ, Levran O, Reed B, et al. Opiate addiction and cocaine addiction: underlying molecular neurobiology and genetics. J Clin Invest. 2012; 122(10): 3387–93. [PubMed: 23023708]
13. Bawor M, Dennis BB, Tan C, et al. Contribution of BDNF and DRD2 genetic polymorphisms to continued opioid use in patients receiving methadone treatment for opioid use disorder: an observational study. Addict Sci Clin Pract. 2015; 10: 19. [PubMed: 26437921]
14. Curtis K, Viswanath H, Velasquez KM, et al. Increased habenular connectivity in opioid users is associated with an alpha5 subunit nicotinic receptor genetic variant. Am J Addict. 2017; 26(7): 751–9. [PubMed: 28857330]

15. Crist RC, Clarke TK, Berrettini WH. Pharmacogenetics of Opioid Use Disorder Treatment. CNS Drugs. 2018; 32(4): 305–20. [PubMed: 29623639]
16. Fonseca F, Torrens M. Pharmacogenetics of Methadone Response. Mol Diagn Ther. 2018; 22(1): 57–78. [PubMed: 29168075]
17. Mistry CJ, Bawor M, Desai D, et al. Genetics of Opioid Dependence: A Review of the Genetic Contribution to Opioid Dependence. Curr Psychiatry Rev. 2014; 10(2): 156–67. [PubMed: 25242908]
18. Nielsen DA, Kreek MJ. Common and specific liability to addiction: approaches to association studies of opioid addiction. Drug Alcohol Depend. 2012; 123 Suppl 1: S33–41. [PubMed: 22542464]
19. Lu L, Shepard JD, Hall FS, et al. Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neurosci Biobehav Rev. 2003; 27(5): 457–91. [PubMed: 14505687]
20. Badiani A, Robinson TE. Drug-induced neurobehavioral plasticity: the role of environmental context. Behav Pharmacol. 2004; 15(5–6): 327–39. [PubMed: 15343056]
21. Scherbaum N, Specka M. Factors influencing the course of opiate addiction. Int J Methods Psychiatr Res. 2008; 17 Suppl 1: S39–44. [PubMed: 18543361]
22. Valentine K, Fraser S. Trauma, damage and pleasure: rethinking problematic drug use. Int J Drug Policy. 2008; 19(5): 410–6. [PubMed: 17875389]
23. Ducci F, Goldman D. The genetic basis of addictive disorders. Psychiatr Clin North Am. 2012; 35(2): 495–519. [PubMed: 22640768]
24. McCarberg B Tramadol extended-release in the management of chronic pain. Ther Clin Risk Manag. 2007; 3(3): 401–10. [PubMed: 18488071]
25. Smith HS, Raffa RB, Pergolizzi JV, et al. Combining opioid and adrenergic mechanisms for chronic pain. Postgrad Med. 2014; 126(4): 98–114.
26. Seth P, Scholl L, Rudd RA, et al. Overdose Deaths Involving Opioids, Cocaine, and Psychostimulants - United States, 2015–2016. MMWR Morb Mortal Wkly Rep. 2018; 67(12): 349–58. [PubMed: 29596405]
27. Gall BJ, Wilson A, Schroer AB, et al. Genetic variations in GPSM3 associated with protection from rheumatoid arthritis affect its transcript abundance. Genes Immun. 2016; 17(2): 139–47. [PubMed: 26821282]
28. Lander LR, Marshalek P, Yitayew M, et al. Rural healthcare disparities: challenges and solutions for the pregnant opioid-dependent population. W V Med J. 2013; 109(4): 22–7.
29. Zheng WH, Wakim RJ, Geary RC, et al. Self-reported Sleep Improvement in Buprenorphine MAT (Medication Assisted Treatment) Population. Austin J Drug Abuse Addict. 2016; 3(1).
30. Zheng W, Nickasch M, Lander L, et al. Treatment Outcome Comparison Between Telepsychiatry and Face-to-face Buprenorphine Medication-assisted Treatment for Opioid Use Disorder: A 2-Year Retrospective Data Analysis. J Addict Med. 2017; 11(2): 138–44. [PubMed: 28107210]
31. Association AP. Diagnostic and Statistical Manual of Mental Disorders. 5th ed: American Psychiatric Association Publishing; 2013.
32. Genomes Project C, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature. 2015; 526(7571): 68–74. [PubMed: 26432245]
33. Song W, Gardner SA, Hovhannisyan H, et al. Exploring the landscape of pathogenic genetic variation in the ExAC population database: insights of relevance to variant classification. Genet Med. 2016; 18(8): 850–4. [PubMed: 26681313]
34. Blomqvist O, Gelernter J, Kranzler HR. Family-based study of DRD2 alleles in alcohol and drug dependence. Am J Med Genet. 2000; 96(5): 659–64. [PubMed: 11054774]
35. Ikeda M, Yamanouchi Y, Kinoshita Y, et al. Variants of dopamine and serotonin candidate genes as predictors of response to risperidone treatment in first-episode schizophrenia. Pharmacogenomics. 2008; 9(10): 1437–43. [PubMed: 18855532]
36. Doly S, Valjent E, Setola V, et al. Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. J Neurosci. 2008; 28(11): 2933–40. [PubMed: 18337424]

37. Doly S, Bertran-Gonzalez J, Callebert J, et al. Role of serotonin via 5-HT2B receptors in the reinforcing effects of MDMA in mice. PLoS One. 2009; 4(11): e7952. [PubMed: 19956756]
38. Lin Z, Walther D, Yu XY, et al. The human serotonin receptor 2B: coding region polymorphisms and association with vulnerability to illegal drug abuse. Pharmacogenetics. 2004; 14(12): 805–11. [PubMed: 15608559]
39. Beer B, Erb R, Pavlic M, et al. Association of polymorphisms in pharmacogenetic candidate genes (OPRD1, GAL, ABCB1, OPRM1) with opioid dependence in European population: a case-control study. PLoS One. 2013; 8(9): e75359. [PubMed: 24086514]
40. Xu J, Lu Z, Xu M, et al. A heroin addiction severity-associated intronic single nucleotide polymorphism modulates alternative pre-mRNA splicing of the mu opioid receptor gene OPRM1 via hnRNPH interactions. J Neurosci. 2014; 34(33): 11048–66. [PubMed: 25122903]
41. Olesen AE, Sato H, Nielsen LM, et al. The genetic influences on oxycodone response characteristics in human experimental pain. Fundam Clin Pharmacol. 2015; 29(4): 417–25. [PubMed: 26042474]
42. Adrian M, Kiff C, Glazner C, et al. Examining gene-environment interactions in comorbid depressive and disruptive behavior disorders using a Bayesian approach. J Psychiatr Res. 2015; 68: 125–33. [PubMed: 26228411]
43. Bartosova O, Polanecky O, Perlik F, et al. OPRM1 and ABCB1 polymorphisms and their effect on postoperative pain relief with piritramide. Physiol Res. 2015; 64 Suppl 4: S521–7. [PubMed: 26681082]
44. Laugsand EA, Skorpen F, Kaasa S, et al. Genetic and Non-genetic Factors Associated With Constipation in Cancer Patients Receiving Opioids. Clin Transl Gastroenterol. 2015; 6: e90. [PubMed: 26087058]
45. Wachman EM, Hayes MJ, Sherva R, et al. Variations in opioid receptor genes in neonatal abstinence syndrome. Drug Alcohol Depend. 2015; 155: 253–9. [PubMed: 26233486]
46. Woodcock EA, Lundahl LH, Burmeister M, et al. Functional mu opioid receptor polymorphism (OPRM1 A(118) G) associated with heroin use outcomes in Caucasian males: A pilot study. Am J Addict. 2015; 24(4): 329–35. [PubMed: 25911999]
47. Matic M, van den Bosch GE, de Wildt SN, et al. Genetic variants associated with thermal pain sensitivity in a paediatric population. Pain. 2016; 157(11): 2476–82. [PubMed: 27541715]
48. Schwantes-An TH, Zhang J, Chen LS, et al. Association of the OPRM1 Variant rs1799971 (A118G) with Non-Specific Liability to Substance Dependence in a Collaborative de novo Meta-Analysis of European-Ancestry Cohorts. Behav Genet. 2016; 46(2): 151–69. [PubMed: 26392368]
49. Choi SW, Lam DMH, Wong SSC, et al. Effects of Single Nucleotide Polymorphisms on Surgical and Postsurgical Opioid Requirements: A Systematic Review and Meta-Analysis. Clin J Pain. 2017; 33(12): 1117–30. [PubMed: 28379874]
50. Gao X, Wang Y, Lang M, et al. Contribution of Genetic Polymorphisms and Haplotypes in DRD2, BDNF, and Opioid Receptors to Heroin Dependence and Endophenotypes Among the Han Chinese. OMICS. 2017; 21(7): 404–12. [PubMed: 28692418]
51. Levran O, Peles E, Randesi M, et al. The mu-opioid receptor nonsynonymous variant 118A>G is associated with prolonged abstinence from heroin without agonist treatment. Pharmacogenomics. 2017; 18(15): 1387–91. [PubMed: 28976288]
52. Gong XD, Wang JY, Liu F, et al. Gene polymorphisms of OPRM1 A118G and ABCB1 C3435T may influence opioid requirements in Chinese patients with cancer pain. Asian Pac J Cancer Prev. 2013; 14(5): 2937–43. [PubMed: 23803057]
53. Haerian BS, Haerian MS. OPRM1 rs1799971 polymorphism and opioid dependence: evidence from a meta-analysis. Pharmacogenomics. 2013; 14(7): 813–24. [PubMed: 23651028]
54. Kolesnikov Y, Gabovits B, Levin A, et al. Chronic pain after lower abdominal surgery: do catechol-O-methyl transferase/opioid receptor mu-1 polymorphisms contribute? Mol Pain. 2013; 9: 19. [PubMed: 23566343]
55. Manini AF, Jacobs MM, Vlahov D, et al. Opioid receptor polymorphism A118G associated with clinical severity in a drug overdose population. J Med Toxicol. 2013; 9(2): 148–54. [PubMed: 23318993]

56. Song Z, Du B, Wang K, et al. Effects of OPRM1 A118G polymorphism on epidural analgesia with fentanyl during labor: a meta-analysis. Genet Test Mol Biomarkers. 2013; 17(10): 743–9. [PubMed: 23909491]
57. Cajanus K, Kaunisto MA, Tallgren M, et al. How much oxycodone is needed for adequate analgesia after breast cancer surgery: effect of the OPRM1 118A>G polymorphism. J Pain. 2014; 15(12): 1248–56. [PubMed: 25239082]
58. Solak O, Erdogan MO, Yildiz H, et al. Assessment of opioid receptor mu1 gene A118G polymorphism and its association with pain intensity in patients with fibromyalgia. Rheumatol Int. 2014; 34(9): 1257–61. [PubMed: 24671502]
59. Hancock DB, Levy JL, Gaddis NC, et al. Cis-Expression Quantitative Trait Loci Mapping Reveals Replicable Associations with Heroin Addiction in OPRM1. Biol Psychiatry. 2015; 78(7): 474–84. [PubMed: 25744370]
60. Pfeifer P, Sariyar M, Eggermann T, et al. Alcohol Consumption in Healthy OPRM1 G Allele Carriers and Its Association with Impulsive Behavior. Alcohol Alcohol. 2015; 50(4): 379–84. [PubMed: 25836994]
61. Oertel BG, Doehring A, Roskam B, et al. Genetic-epigenetic interaction modulates mu-opioid receptor regulation. Hum Mol Genet. 2012; 21(21): 4751–60. [PubMed: 22875838]
62. Carpentier PJ, Arias Vasquez A, Hoogman M, et al. Shared and unique genetic contributions to attention deficit/hyperactivity disorder and substance use disorders: a pilot study of six candidate genes. Eur Neuropsychopharmacol. 2013; 23(6): 448–57. [PubMed: 22841130]
63. Smoller JW, Paulus MP, Fagerness JA, et al. Influence of RGS2 on anxiety-related temperament, personality, and brain function. Arch Gen Psychiatry. 2008; 65(3): 298–308. [PubMed: 18316676]
64. Amstadter AB, Koenen KC, Ruggiero KJ, et al. Variation in RGS2 is associated with suicidal ideation in an epidemiological study of adults exposed to the 2004 Florida hurricanes. Arch Suicide Res. 2009; 13(4): 349–57. [PubMed: 19813112]
65. Koenen KC, Amstadter AB, Ruggiero KJ, et al. RGS2 and generalized anxiety disorder in an epidemiologic sample of hurricane-exposed adults. Depress Anxiety. 2009; 26(4): 309–15. [PubMed: 18833580]
66. Dunn EC, Solovieff N, Lowe SR, et al. Interaction between genetic variants and exposure to Hurricane Katrina on post-traumatic stress and post-traumatic growth: a prospective analysis of low income adults. J Affect Disord. 2014; 152–154: 243–9.
67. Stein MB, Keshaviah A, Haddad SA, et al. Influence of RGS2 on sertraline treatment for social anxiety disorder. Neuropsychopharmacology. 2014; 39(6): 1340–6. [PubMed: 24154666]
68. Hohoff C, Weber H, Richter J, et al. RGS2 ggenetic variation: association analysis with panic disorder and dimensional as well as intermediate phenotypes of anxiety. Am J Med Genet B Neuropsychiatr Genet. 2015; 168B(3): 211–22. [PubMed: 25740197]
69. Amstadter AB, Koenen KC, Ruggiero KJ, et al. Variant in RGS2 moderates posttraumatic stress symptoms following potentially traumatic event exposure. J Anxiety Disord. 2009; 23(3): 369–73. [PubMed: 19162436]
70. Zhang H, Wang F, Kranzler HR, et al. Variation in regulator of G-protein signaling 17 gene (RGS17) is associated with multiple substance dependence diagnoses. Behav Brain Funct. 2012; 8: 23. [PubMed: 22591552]
71. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003; 112(2): 257–69. [PubMed: 12553913]
72. de Cid R, Fonseca F, Gratacos M, et al. BDNF variability in opioid addicts and response to methadone treatment: preliminary findings. Genes Brain Behav. 2008; 7(5): 515–22. [PubMed: 18182069]
73. Nascimento FA, Andrade DM. Myoclonus epilepsy and ataxia due to potassium channel mutation (MEAK) is caused by heterozygous KCNC1 mutations. Epileptic Disord. 2016; 18(S2): 135–8. [PubMed: 27629860]
74. Pal A, Chakraborty J, Das S. Association of CREB1 gene polymorphism with drug seeking behaviour in eastern Indian addicts. Neurosci Lett. 2014; 570: 53–7. [PubMed: 24704376]

75. Oliveira-Dos-Santos AJ, Matsumoto G, Snow BE, et al. Regulation of T cell activation, anxiety, and male aggression by RGS2. Proc Natl Acad Sci U S A. 2000; 97(22): 12272–7. [PubMed: 11027316]

76. Siderovski DP, Willard FS. The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int J Biol Sci. 2005; 1(2): 51–66. [PubMed: 15951850]

77. Ingi T, Krumins AM, Chidiac P, et al. Dynamic regulation of RGS2 suggests a novel mechanism in G-protein signaling and neuronal plasticity. J Neurosci. 1998; 18(18): 7178–88. [PubMed: 9736641]

78. Kimple AJ, Soundararajan M, Hutsell SQ, et al. Structural determinants of G-protein alpha subunit selectivity by regulator of G-protein signaling 2 (RGS2). J Biol Chem. 2009; 284(29): 19402–11. [PubMed: 19478087]

79. Doly S, Quentin E, Eddine R, et al. Serotonin 2B Receptors in Mesoaccumbens Dopamine Pathway Regulate Cocaine Responses. J Neurosci. 2017; 37(43): 10372–88. [PubMed: 28935766]

80. Bevilacqua L, Doly S, Kaprio J, et al. A population-specific HTR2B stop codon predisposes to severe impulsivity. Nature. 2010; 468(7327): 1061–6. [PubMed: 21179162]

81. Diaz SL, Narboux-Neme N, Boutourlinsky K, et al. Mice lacking the serotonin 5-HT2B receptor as an animal model of resistance to selective serotonin reuptake inhibitors antidepressants. Eur Neuropsychopharmacol. 2016; 26(2): 265–79. [PubMed: 26727039]

82. Tincher RB. Night Comes to the Chromosomes: Inbreeding and Population Genetics in Southern Appalachia. Central Issues in Anthropology. 1980; 2(1): 27–49.

Table 1.

Genetic markers and minor allele frequency (MAF) data obtained from public databases (1000 Genomes Project and/or ExAc) and WV COAT clinic patients (N = 60).

Gene	dbSNP ID	Minor Allele	TaqMan Probe	MAF via 1000Genomes*	MAF via ExAc†	MAF from COAT clinic‡
OPRM1	rs9479757	A	C_25472011_10	0.097	0.082	**0.500 p < 0.001**
OPRM1	rs1799971	G	C_8950074_1	0.162	0.185	**0.083 p = 0.018**
RGS2	rs4606	G	C_2498717_10	0.276	n.a.	**0.533 p < 0.001**
HTR2B	rs6736017	C	C_25614588_20	n.a.	0.0078	**0.500 p < 0.001**
OPRM1	rs2075572	G	C_1691815_1	0.413	n.a.	0.483 p = 0.12
RGS17	rs596359	T	C_7830523_10	0.517	n.a.	0.467 p = 0.27
DRD2 / ANKK1	rs1800497	A	C_7486676_10	0.188	0.256	0.217 p = 0.41
DRD2	rs1799978	C	C_7486599_20	0.060	n.a.	0.075 p = 0.44
BDNF	rs6265	T	C_11592758_10	0.197	0.194	0.167 p = 0.49
KCNC1	rs60349741	C	C_89088414_10	0.001	n.a.	0.000
HTR2B	rs77982984	A	C_99996051_10	0.003	0.0012	0.000
HTR2B	rs78484969	C	C_99996081_10	0.001 (N = 1030 Finns)	n.a.	0.000
HTR2B	rs77570025	G	C_99996068_10	0.006	0.002	0.000
CREB1	rs35349697	A	C_25636228_10	n.a.	0.000008	0.000

*Minor allele frequency reported on N = 1006 Europeans via 1000 Genomes (except as otherwise noted); n.a. = not available.

†Minor allele frequence reported on N ~ 60,000 people via ExAc database of the Broad Institute; n.a. = not available.

‡p-value, denoting significance of difference between MAFs, is derived by comparison between WV COAT clinic and European (if not, then ExAc) MAF using binomial test (null hypothesis is "*no difference between WV COAT patients and Europeans*"; alternative is "*there is difference between WV COAT patients and Europeans*")