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Review Article

A brief review on genetics of opioid receptors in opioid addiction

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Abstract

Opioid addiction is a chronic mental illness that manifests itself through several relapses and remissions throughout the course of an addict's life. Over the last few decades, opioid addiction has developed into a significant public health epidemic. Classical molecular genetics research has limited the opioid receptor family down to three major subtypes: mu (μ), kappa (κ), and delta (δ) opioid receptors, which are encoded by the *OPRM1, OPRD1,* and *OPRK1* genes. Individuals' opioid addiction is regulated by this *OPRM1, OPRD1,* and *OPRK1* genes via a reward system route, including the dopaminergic system. Furthermore, when opioid ligands attach to these receptors, it produces euphoric and rewarding effects. Numerous research has been conducted on the single nucleotide variations (SNVs) of these genes in several ethnic groups.

The majority of the studies had focused on the *OPRM1* gene and its variants. Therefore, this article will summarize recent research on opioid receptor genetic variants associated with opioid addiction and emphasize the relevance and importance of investigating gene variants to ascertain genetic predisposition.

Keywords: *OPRM1, OPRD1, OPRK1,* opioid addiction, rs1799971

Introduction

Addiction to opioids is a significant public health problem that has reached epidemic proportions in many parts of the world. It is a chronic and multifaceted neurobiological disorder characterized by frequent relapses to the use of drugs (Kakko et al., 2019). In 2019, approximately 275 million (5.5%) of the world's population aged 15–64 years reported using drugs at least once during the year, where around 62 million of them have used opioids. In 2019, approximately 36.3 million people suffered from substance use disorders (WHO, 2021).

Addiction to opioids develops due to the use of illegal opioids such as heroin, morphine, or illicit prescription opioids as the treatment of chronic or acute pain. Following self-exposure, two major factors contribute to an individual's susceptibility to developing an opiate addiction. These are genetic factors, which account for 40%–60% of the variability in risk of developing an opioid addiction, the highest of any substance of abuse (Mura et al., 2013; Orna Levran et al., 2012; Kendler, Jacobson, et al., 2003) and the environment's influence on the individual (Prom-Wormley et al., 2017; Kendler et al., 2008). Opioids continue to bear the burden of the majority of drug-related diseases. Opioids are defined by their ability to bind to G protein-coupled opioid receptors mu (μ), kappa (κ), and delta (δ), which are encoded by distinct genes (*OPRM1, OPRK1,* and *OPRD1*) and are stimulated by endogenous opioid peptides: β-endorphin, prodynorphin, enkephalin, and orphanin/nociceptin encoded by *POMC, PDYN* genes (Kopinsky & Hyman, 2002).

Exogenous opioids (such as morphine and heroin) and endogenous opioid peptides (such as β-endorphin, enkephalin, and dynorphin) are thought to exert their pharmacological and physiological effects via binding to μ-, δ-, and κ-opioid receptors. Both central and peripheral nervous systems contain opioid receptors (Zhang et al., 2008). They are found in varying concentrations throughout the brain depending on their classification, but all receptors are highly abundant in the amygdala, nucleus accumbens (NAc), and caudate putamen (CP) (Zhang et al., 2008). These areas, along with the ventral tegmental area (VTA), are densely packed with gamma-aminobutyric acid (GABA)-ergic interneurons that form the intricate neural circuitry underlying opioid dependence (Kalivas, 2009; Kalivas & Volkow, 2005). The opioid system mediated by μ-, δ-, and κ-opioid receptors is implicated in the reward circuitry's dopaminergic activity. Numerous substances, including opioids, nicotine, alcohol, and stimulants, alter the opioid system significantly. In general, activation of the brain opioid system contributes to the reward effect and modulates the neurochemical and behavioral effects of various addictive substances (Lopez-Leon et al., 2021).
Numerous studies have established that these three opioid receptors are involved in the analgesic and addictive properties of opioid drugs. Among these three receptors, it has been suggested that the μ-receptor is the primary target for opioid addiction (Matthes et al., 1996). μ-receptor is the most receptive to morphine, and its stimulation results in pain relief and euphoria (Chiara & Alan North, 1992). It has also been found that the δ-receptor mediates antinociception at both the spinal and supraspinal levels (Heyman et al., 1988).

Although opiate addiction has reached epidemic proportions in recent years, opiates have remained relatively unstudied. Investigating the role of genetic variants in the etiology of addiction may improve treatment response and disease prevention. Identifying genes involved in neuroadaptation is being used in conjunction with genome-wide and candidate gene association studies to elucidate the genetic factor's underlying role in drug addiction. This further paves the way for a better understanding of what drives opioid addiction and specifically addresses it. This brief review summarizes recent research evidence on the genetic variants of opioid receptors associated with opioid addiction.

Genetic susceptibility to opioid addiction

Genome-wide association studies in opioid addiction

Genome-wide association studies (GWAS) examine genetic variants across multiple individuals' genomes to identify genotype-phenotype associations. GWAS have revolutionized the field of complex disease genetics over the last decade, revealing numerous compelling associations for human complex traits and diseases (Tam et al., 2019). GWAS has taken the lead surpassing the previous candidate gene-driven approaches. GWAS has paved the way for the identification of common genetic variants such as single nucleotide variants (SNVs), rare variants, and structural variants [e.g., copy number variants (CNVs)] (Gaddis et al., 2021).

Numerous large-scale GWAS of opioid addiction have been published in previous years (Berrettini, 2017). Opioids act primarily by activating μ-opioid receptors on GABAergic interneurons, inhibiting GABA release and thus disinhibiting mesolimbic dopamine neurons. Thereby, opioids increase dopamine levels in the nucleus accumbens (Johnson & North, 1992). In addition to previously identified genes involved in dopaminergic signaling (e.g., *ANKK1/DDRD2, DRD1,* and *DBH*) (Clarke et al., 2013; Garrido et al., 2011; Hoenicka et al., 2010; Perez de los Cobos et al., 2007), the specific variant encoding the μ-opioid receptor (*OPRM1*, rs1799971, A118G) has been extensively studied (Prom-Wormley et al., 2017).

Twin and family studies have estimated that additive genetic factors account for 50% of the risk of opioid dependence among closely related family members (Berrettini, 2017; Kendler, Prescott, et al., 2003; Tsuang, 2001).

Hancock et al., (2015) has discovered a common missense functional SNV (rs1799971) in the exon 1 of *OPRM1* gene (A encodes the wild-type asparagine allele, whereas G encodes the aspartate allele). Through a GWAS, an opioid addiction haplotype comprising of the C allele of rs3778150 and the A allele of rs1799971 described by Hancock et al., (2015) was associated with opioid addiction. This finding may support the hypothesis of the association of rs1799971 with opioid addictions (Schwantes-An et al., 2016; Haerian & Haerian, 2013). The most recent meta-analysis included three samples (8529 affected European American individuals and 71 200 opioid-exposed European American controls and 4032 affected African American individuals and 26029 opioid-exposed African American controls) totaling 82,707 Europeans confirmed that SNV rs1799971 was associated with opioid use disorder while there was no association among African Americans (Zhou et al., 2020). A significant association between variant rs1799971 and opioid/cocaine/heroin dependence was found in Asian populations, where no association was observed in African American or Caucasian people. Thus, the *OPRM1* rs1799971 variant may be a risk factor for Asians being vulnerable to an addiction to opioids or heroin (Haerian & Haerian, 2013).

In contrast to the above-described studies, Glatt et al., (2007) reported no significant role of rs1799971 in opioid addiction.

In addition to *OPRM1* gene SNVs, other genes related to the dopaminergic system have also been investigated. However, analyses of prodynorphin *(PDYN),* proenkephalin *(PENK),* and the κ *(OPRK1)* and δ-opioid receptors *(OPRD1)* have not consistently yielded reliable results with respect to opioid addiction (Zhang et al., 2008; Gerra et al., 2007; Franke et al., 1999; Mayer et al., 1997).

Previous research revealed that heterodimerization of the *OPRM1* and *OPRD1* genes can alter the opioid signaling pathway more than individual activation of the OPRM1 and OPRD1 receptors. More research revealed that heterodimerization of *OPRD1–OPRM1* resulted in physiological repercussions, necessitating further studies to determine how the coexistence of both receptors contributes to an increased risk of opioid addiction (Wu et al., 2021).

There are promising GWAS reports in opioid addiction that identify genome-wide significant risk alleles, but inconsistent results cast doubt on the association of these genes across ethnic groups. Larger samples with opioid addiction must be evaluated in different ethnic groups through candidate gene studies of opioid addiction.

Candidate gene association studies of opioid receptors

Numerous studies have established a link between opioid system gene variants and drug addiction-related phenotypes, but the findings are inconsistent. The inconsistency of genetic studies may be explained by multiple factors such as: inconsistency in phenotyping the variations, severity stage of diagnosis and diagnosis criteria of addiction, more studies with smaller sample size, insufficient statistics, ethnic heterogeneity, stratification strategy of population and wide phenotype range. The majority of studies used single nucleotide variations (SNVs) analysis, and several studies used hypothesis-based multi-SNP arrays that detect a significant proportion of common genetic variations (Orna Levran et al., 2012; Maher et al., 2011; Hodgkinson et al., 2008; Levran et al., 2008).

The μ, κ, and δ opioid receptors are all G protein-coupled receptors that work in conjunction with inhibitory G proteins and dopaminergic neurons to generate the physiological effects of opioids, according to one of the candidate gene studies (Chiara & Alan North, 1992).

***OPRM1* gene**

The opioid receptor gene 1 *(OPRM1)* is located on chromosome 6q25.2 (NCBI database, 2021c). Four exons comprise the primary subtype. The *OPRM1* gene, which encodes the opioid receptor, has been implicated in respiration, gastrointestinal motility, physical dependence, euphoria, and analgesia (Mistry et al., 2014). The μ- opioid receptor, *OPRM1* (G protein-coupled) is the primary site of action for endogenous opioids, opiate and opioid analgesics, and exogenous opioids such as methadone, heroin, and morphine (Orna Levran et al., 2012). β-endorphin binding to the μ- opioid receptor results in the disinhibition of dopaminergic neurons, which has been linked to reward and reinforcement and is thought to contribute to the development of drug dependence (Johnson & North, 1992). Numerous SNVs in this gene have been associated with opioid dependence and ethnic variation in opioid dependence (Baldacchino et al., 2019).

Various studies in diverse populations have demonstrated an association between the rs1799971 variant and opioid dependence and other substance dependencies. According to Bond et al., (1998) the most frequently occurring SNPs in the *OPRM1* gene are rs1799971 (A118G) and rs1799972 (C17T). The most studied *OPRM1* variant rs1799971 was shown to eliminate a potential N-glycosylation site in the extracellular domain, increase beta-endorphin binding affinity, and decrease receptor signaling efficacy (Bond et al., 1998). The rs1799971 variant of the *OPRM1* gene is prevalent among Europeans (15-30%) and Asians (40-60%). However, the variant is less prevalent among African Americans and Hispanics (1-3%) (Tan et al., 2003; Gelernter et al., 1999; Bergen et al., 1997). The variant rs1799971 was less prevalent among Africans (Kreek et al., 2005).

The rs1799971 minor allele variant was associated with opioid dependence in an Indian population (Kapur et al., 2007) and with heroin dependence among Sri Lankans (Dissabandara et al., 2021). The rs1799971 was associated with opioid addiction in Swedish (Bart et al., 2004), Chinese (Szeto et al., 2001), European Americans (Drakenberg et al., 2006) and Indian patients (Deb et al., 2010; Kapur et al., 2007; Tan et al., 2003) where some of the studies have contradicted this finding (Orna Levran et al., 2012; Glatt et al., 2007; Kapur et al., 2007; Tan et al., 2003; Shi et al., 2002).

The *OPRM1* gene variant, rs1799971, was not

associated with opiate addiction in a recent study in a Bulgarian population, which included 1842 opiate-addicted subjects (Bulgarians (18% allelic frequency of 118G) and Romas (Romani population) (20.2 percent allelic frequency of 118G)) and 1451 healthy volunteers (Nikolov et al., 2011). The study conducted by Kreek et al., (2005) showed a significant association between the rs1799971 variants, but in contrast, no association was seen between the rs1799972 variant and opioid dependence.

Due to the contrasting findings regarding the association of the rs1799971 SNV with opioid dependence across different ethnic groups, it is critical to further investigate this variant in multiethnic cohort genetic association studies.

OPRD1 gene

The opioid receptor delta 1 *(OPRD1)* gene encodes for the δ-opioid receptor is located on chromosome 1p35.3 (NCBI database, 2021a). Numerous studies have established a link between variations in the allelic frequencies of *OPRD1* SNVs and opioid addiction. There are hundreds of variants of the *OPRD1* gene that have been studied with association to opioid addiction, but only a few variants have been studied with respect to different ethnic groups (Baldacchino et al., 2019).

Hunag et al. investigated the rs2234918 SNV among the Han Chinese and discovered that the minor C allele of rs2234918 in *OPRD1* is considered a risk allele for heroin dependence (Huang et al., 2019). While Mayer et al. also reported that rs2234918 SNV of the *OPRD1* gene is associated with opioid addiction among the German Caucasian heroin addicts (Mayer et al., 1997). In contrast to the above studies, Zhang et al., (2008) (1063 European Americans: 620 cases of alcohol, cocaine, and opioid dependence and 443 control subjects) reported that there were no associations between the silent mutation rs2234918 with opioid addiction among the European Americans in a candidate gene association study.

In addition to the SNV mentioned above, Zhang et al., (2008) also studied several SNVs among the European Americans, including eleven *OPRD1* variants and seven *OPRK1* variants. Among the studied variants, rs1042114 was the only variant detected in exon 1 of the *OPRD1* gene, demonstrating a significantly increased frequency of the minor G allele in opioid-dependent subjects compared to the controls. Similarly, Crist et al., (2013) also found that rs1042114 was significantly associated with opioid addiction among European Americans and African Americans. Based on the results, both the rs1042114 (G80T) and rs2234918 (T921C) in the *OPRD1* gene are risk factors for opioid drug addiction among mixed ethnic groups (Crist et al., 2013; Zhang et al., 2008).

Levran et al., (2008) conducted a study among the Americans and Israel people and found that rs2236861, rs2236857, and rs3766951 of the *OPRD1* gene were suggestively associated with heroin addiction. Similarly, Nelson et al., (2014) also concluded that rs2236861 and rs3766951 were considered risk factors for opioid addiction but did not observe any significant association for the common rs2236857 SNV for opioid addiction. A few *OPRK1* gene variants have been associated with SUDs; however, most are silent and do not affect gene expression (Mayer & Höllt, 2006).

OPRK1 gene

The opioid receptor kappa 1 *(OPRK1)* gene is located on chromosome 8q11.23 (NCBI database, 2021b). Earlier, the studies reported that the *OPRK1* gene has been playing a role as an anti-addictive effect and produces dysphoria, but recent evidence suggests that prolonged exposure to drugs activate the *OPRK1* gene, which may play a key role in motivational aspects of dependence through modulation of basal and drug-induced dopaminergic tone (Wee & Koob, 2010; Kreek et al., 2002).

Despite numerous studies examining the role of the κ opioid receptor, the evidence for a link between *OPRK1* SNPs and opioid dependency is inconsistent, with results considerably varying

between ethnic groups (Mistry et al., 2014).

Gerra et al., (2007) genotyped 106 heroin - dependent subjects and 70 healthy controls for rs1051660 variant in the *OPRK1* gene among Western Europeans. The study concluded that the rs1051660 variant is more prevalent among heroin addicts. Meanwhile, Yuferov et al., (2004) also concluded that the rs1051660 variant is a risk factor for opioid use disorders. Mistry et al., (2014) genotyped 202 healthy individuals and 202 opium addicts with rs997917, rs6985606, and rs6473797 variants of the *OPRK1* gene and found these variants reported significant association with susceptibility to opioid dependence among Iranians. In contrast to these studies, Zhang et al., (2008) reported that rs997917 SNV was not associated with opioid addiction.

Table 1: Summary of selected opioid receptor genes

Gene	Receptor	Reward pathway effect	Variants	Location	Findings	References
OPRM1	μ opioid receptor	euphoria and respiratory depression	rs1799971	Exon 1	rs1799971 variant has been reported as a risk factor for opioid addiction among Europeans, Indian, Caucasians, Malaysian, Chinese.	(Schwantes-An et al., 2016)
			rs1799972	Exon 1	rs1799972 (T) allele is less consistent across ethnic groups and is not widely linked to opioid dependence despite being widely studied.	(Brattwall et al., 2010)
OPRD1	δ opioid receptor	anxiolysis	rs2234918	Exon 3	The *OPRD1* exon III rs2234918 is associated with opioid addiction among Han Chinese, Caucasians.	(Huang et al., 2019; Compton & Volkow, 2006; Mayer et al., 1997)
			rs1042114	Exon 1		
			rs2236861	Intron 1	*ORPD1* SNP, rs2236861, was associated with non-dependent opioid use	(Nelson et al., 2014; Levran et al., 2008)
			rs2236857	Intron 1	Associated with OUD in individuals of European descent	
			rs3766951	Intron 1	Associated with opioid addiction risk among Caucasian ancestry	
OPRK1	κ opioid receptor	dysphoria	rs1051660	Exon 2	Associated with opioid addiction as a risk factor among African American, Caucasian, Hispanic, Asian-American, and mixed ethnic groups.	(Gerra et al., 2007; Yuferov et al., 2004)
			rs997917	Intron 2	Associated among Iranian but not associated among Americans and Europeans.	(Mistry et al., 2014; Zhang et al., 2008)

Summary

The review aimed to summarize the evidence for opioid receptor genes associated with opioid addiction. μ, κ, and δ opioid receptor subunits are encoded by the *OPRM1, OPRD1,* and *OPRK1* genes, respectively. The majority of the studies included in this review were retrospective genome-wide association studies in which opioid addiction-related genotypes and SNVs were investigated.

Previous studies included African Americans, Han Chinese, Hispanics, Europeans, and Caucasians as common ethnic groups with significant ethnic variations in the association between genetic variants and opioid dependence (Baldacchino et al., 2019). There were several larger studies which had been studied among varying ethnic groups, including the Zhang et al., (2008) study, which included 1063 European Americans, Nagoya et al., (2018) included 1002 Malay males, and Nelson et al., (2014) included 2954 Australians. Although compelling results have been obtained from the genetic studies of opioid addiction, it is necessary to investigate the association of opioid receptor gene variants among varying ethnic groups, both with small scale and large-scale studies, to find the genetic susceptibility. A detailed analysis of the effects of these genes on the pathophysiology and metabolism of opioids will provide additional insight into the aetiology of such disorders.

The *OPRM1, OPRD1,* and *OPRM1* genes were all implicated in the development of opioid addiction. These genes encode receptors and signaling molecules involved in the pathophysiology of substance use disorders (Mistry et al., 2014). Despite a high genetic predisposition to opioid dependence, environmental factors play a significant role in this opioid use disorder, as they do in many other multifactorial diseases. Hence, studies should focus on interactions between genes and the environment, or epigenetics, to understand more about opioid and other substance use disorders.

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References

Baldacchino, A. M., George, O., Ciccocioppo, R., Belcher, A., Martinez, D., Wang, G.J., Burns, J. A., Kroll, D. S., Feldman, D. E., Liu, C. K., Manza, P., Wiers, C. E., & Volkow, N. D. (2019). Molecular Imaging of Opioid and Dopamine Systems: Insights Into the Pharmacogenetics of Opioid Use Disorders. *Psychiatry,* 10: 626. https://doi.org/10.3389/fpsyt.2019.00626

Bart, G., Heilig, M., LaForge, K., Pollak, L., S. L.-M., & 2004, U. (2004). Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. *Molecular Psychiatry*, 9(6), 547–549. doi: 10.1038/sj.mp.4001504

Bergen, A. W., Kokoszka, J., Peterson, R., Long, J. C., Virkkunen, M., Linnoila, M., & Goldman, D. (1997). Mu opioid receptor gene variants: lack of association with alcohol dependence. *Molecular Psychiatry,* 2(6), 490–494. https://doi.org ≈/10.1038/SJ.MP.4000331

Berrettini, W. (2017). A brief review of the genetics and pharmacogenetics of opioid use disorders. *Dialogues in Clinical Neuroscience,* 19(3), 229. https://doi.org/10.31887/DCNS.2017.19.3/WBERRETTINI

Bond, C., Laforge, K. S., Tian, M., Melia, D., Zhang, S., Borg, L., Gong, J., Schluger, J., Strong, J. A., Leal, S. M., Tischfield, J. A., Kreek, M. J., & Yu, L. (1998). Single - nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proceedings of the National Academy of Sciences of the United States of America,* 95(16), 9608–9613. https://doi.org/10.1073/PNAS.95.16.9608

Brattwall, M., Turan, I., & Jakobsson, J. (2010). Musculoskeletal pain: prescription of NSAID and weak opioid by primary health care physicians in Sweden 2004; 2008 a retrospective patient record review. *Journal of Pain Research,* 3, 131–135. https://doi.org/10.2147/JPR.S12052

Chiara, G. Di, & Alan North, R. (1992). Neurobiology of opiate abuse. *Trends in Pharmacological Sciences,* 13(5), 185–193. https://doi.org/10.1016/0165-6147(92)90062-B

Clarke, T. K., Crist, R. C., Kampman, K. M., Dackis, C. A., Pettinati, H. M., O'Brien, C. P., Oslin, D. W., Ferraro, T. N., Lohoff, F. W., & Berrettini, W. H. (2013). Low frequency genetic variants in the μ-opioid receptor (OPRM1) affect risk for addiction to heroin and cocaine. *Neuroscience Letters,* 542, 71–75. https://doi.org/10.1016/J.NEULET.2013.02.018

Compton, W. M., & Volkow, N. D. (2006). Major increases in opioid analgesic abuse in the United States: concerns and strategies. *Drug and Alcohol Dependence,* 81(2), 103–107. https://doi.org/10.1016/J.DRUGALCDEP.2005.05.009

Crist, R. C., Ambrose-Lanci, L. M., Vaswani, M., Clarke, T. K., Zeng, A., Yuan, C., Ferraro, T. N., Hakonarson, H., Kampman, K. M., Dackis, C. A., Pettinati, H. M., O'Brien, C. P., Oslin, D. W., Doyle, G. A., Lohoff, F. W., & Berrettini, W. H. (2013). Case-control association analysis

of polymorphisms in the delta-opioid receptor, OPRD1, with cocaine and opioid addicted populations. *Drug and Alcohol Dependence,* 127(1–3), 122–128. https://doi.org/10.1016/J.DRUGALCDEP.2012.06.023

Deb, I., Chakraborty, J., P. G. J. of, & 2010, undefined. (2010). Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the. *Wiley Online Library,* 112(2), 486–496. https://doi.org/10.1111/j.1471-4159.2009.06472.x

Dissabandara, L. O., Loxton, N. J., Ho, A. M., Wu, H. M., Dodd, P. R., Daglish, M., & Stadlin, A. (2021). Direct, Indirect and Epistatic Associations of Reward System Genes with Heroin Dependence. *Ashdin Publishing Journal of Drug and Alcohol Research,* 10(2021). https://doi.org/10.4303/jdar/236116

Drakenberg, K., Nikoshkov, A., Cs, M., Th, H., Fagergren, P., Gharibyan, A., Saarelainen, K., Rahman, S., Nylander, I., Bakalkin, G., Rajs, J., Keller, E., & Hurd, Y. L. (2006). μ Opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. *Proc Natl Acad Sci,* 103(20), 7883–7888.

Franke, P., Nöthen, M. M., Wang, T., Neidt, H., Knapp, M., Lichtermann, D., Weiffenbach, O., Mayer, P., Höllt, V., Propping, P., & Maier, W. (1999). Human δ-opioid receptor gene and susceptibility to heroin and alcohol dependence. *American Journal of Medical Genetics,* 88(462–464). https://doi.org/10.1002/(SICI)1096-8628(19991015)88:5%3C462::AID-AJMG4%3E3.0.CO;2-S

Gaddis, N., Mathur, R., Marks, J., Zhou, L., Quach, B., Waldrop, A., Levran, O., Agrawal, A., Randesi, M., Adelson, M., Jeffries, P. W., Johnson, E. C., Martin, N. G., Degenhardt, L., Montgomery, G. W., Wetherill, L., Lai, D., Bucholz, K., Foroud, T., Johnson, E. O. (2021). Multi-trait genome-wide association study of opioid addiction: OPRM1 and Beyond. *MedRxiv,* https://doi.org/10.1101/2021.09.13.21263503

Garrido, E., Palomo, T., Ponce, G., García-Consuegra, I., Jiménez-Arriero, M. A., & Hoenicka, J. (2011). The ANKK1 protein associated with addictions has nuclear and cytoplasmic localization and shows a differential response of Ala239Thr to apomorphine. *Neurotoxicity Research,* 20(1), 32–39. https://doi.org/10.1007/S12640-010-9219-6

Gelernter, J., Kranzler, H., & Cubells, J. (1999). Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Molecular Psychiatry,* 4(5), 476–483. https://doi.org/10.1038/SJ.MP.4000556

Gerra, G., Leonardi, C., Cortese, E., D'Amore, A., Lucchini, A., Strepparola, G., Serio, G., Farina, G., Magnelli, F., Zaimovic, A., Mancini, A., Turci, M., Manfredini, M., & Donnini, C. (2007). Human Kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics,* 144B(6), 771–775. https://doi.org/10.1002/AJMG.B.30510

Glatt, S. J., Bousman, C., Wang, R. S., Murthy, K. K., Rana, B. K., Lasky-Su, J. A., Zhu, S. C., Zhang, R., Li, J., Zhang, B., Li, J., Lyons, M. J., Faraone, S. V., & Tsuang, M. T. (2007). Evaluation of OPRM1 variants in heroin dependence by family - based association testing and meta-analysis. *Drug and Alcohol Dependence,* 90(2–3), 159–165. https://doi.org/10.1016/J.DRUGALCDEP.2007.02.022

Haerian, B. S., & Haerian, M. S. (2013). OPRM1 rs1799971 polymorphism and opioid dependence: evidence from a meta-analysis. *Pharmacogenomics,* 14(7), 813–824. https://doi.org/10.2217/PGS.13.57

Hancock, D. B., Levy, J. L., Gaddis, N. C., Glasheen, C., Saccone, N. L., Page, G. P., Hulse, G. K., Wildenauer, D., Kelty, E. A., Schwab, S. G., Degenhardt, L., Martin, N. G., Montgomery, G. W., Attia, J., Holliday, E. G., McEvoy, M., Scott, R. J., Bierut, L. J., Nelson, E. C., Johnson, E. O. (2015). Cis-Expression Quantitative Trait Loci Mapping Reveals Replicable Associations with Heroin Addiction in OPRM1. *Biological Psychiatry,* 78(7), 474. https://doi.org/10.1016/J.BIOPSYCH.2015.01.003

Heyman, J. S., Vaught, J. L., Raffa, R. B., & Porreca, F. (1988). Can supraspinal delta-opioid receptors mediate antinociception? *Trends in Pharmacological Sciences,* 9(4), 134–138. https://doi.org/10.1016/0165-6147(88)90195-2

Hodgkinson, C. A., Yuan, Q., Xu, K., Shen, P. H., Heinz, E., Lobos, E. A., Binder, E. B., Cubells, J., Ehlers, C. L., Gelernter, J., Mann, J., Riley, B., Roy, A., Tabakoff, B., Todd, R. D., Zhou, Z., & Goldman, D. (2008). Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol and Alcoholism,* 43(5), 505–515. https://doi.org/10.1093/ALCALC/AGN032

Hoenicka, J., Quiñones-Lombraña, A., España-Serrano, L., Alvira-Botero, X., Kremer, L., Pérez-González, R., Rodríguez-Jiménez, R., Jiménez-Arriero, M. Á., Ponce, G., & Palomo, T. (2010). The ANKK1 gene associated with addictions is expressed in astroglial cells and upregulated by apomorphine. *Biological Psychiatry,* 67(1), 3–11. https://doi.org/10.1016/J.BIOPSYCH.2009.08.012

Huang, C. C., Kuo, S. C., Yeh, T. C., Yeh, Y. W., Chen, C. Y., Liang, C. S., Tsou, C. C., Lin, C. L., Ho, P. S., & Huang, S. Y. (2019). OPRD1 gene affects disease vulnerability and environmental stress in patients with heroin dependence in Han Chinese. *Progress in Neuro-Psychopharmacology and Biological Psychiatry,* 89, 109–116. https://doi.org/10.1016/J.PNPBP.2018.08.028

Johnson, S. W., & North, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *The Journal of Neuroscience,* 12(2), 483. https://doi.org/10.1523/JNEUROSCI.12-02-00483.1992

Kakko, J., Alho, H., Baldacchino, A., Molina, R., Nava, F. A., & Shaya, G. (2019). Craving in opioid use disorder: From neurobiology to clinical practice. *Frontiers in Psychiatry,* 10(AUG), 592. https://doi.org/10.3389/FPSYT.2019.00592/BIBTEX

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nature Reviews Neuroscience* 10(8), 561–572. https://doi.org/10.1038/nrn2515

Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: A pathology of motivation and choice. *American Journal of Psychiatry,* 162(8), 1403–1413. https://doi.org/10.1176/APPI.AJP.162.8.1403/ASSET/IMAGES/LARGE/P42F5.JPEG

Kapur, S., Sharad, S., Singh, R. A., & Gupta, A. K. (2007). A118G polymorphism in mu opioid receptor gene (OPRM1): Association with opiate addiction in subjects of Indian origin. *Journal of Integrative Neuroscience,* 6(4), 511–522. https://doi.org/10.1142/S0219635207001635

Kendler, K. S., Jacobson, K. C., Prescott, C. A., & Neale, M. C. (2003). Specificity of Genetic and Environmental Risk Factors for Use and Abuse/Dependence of Cannabis, Cocaine, Hallucinogens, Sedatives, Stimulants, and Opiates in Male Twins. *Am J Psychiatry,* 160(4), 687–695. http://ajp.psychiatryonline.org

Kendler, K. S., Prescott, C. A., Myers, J., & Neale, M. C. (2003). The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Archives of General Psychiatry,* 60(9), 929–937. https://doi.org/10.1001/ARCHPSYC.60.9.929

Kendler, K. S., Schmitt, E., Aggen, S. H., & Prescott, C. A. (2008). Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. *Archives of General Psychiatry,* 65(6), 674–682. https://doi.org/10.1001/ARCHPSYC.65.6.674

Kopinsky, K. L., & Hyman, S. E. (2002). Molecular and cellular biology of addiction. Neuropsychopharmacology: *The Fifth Generation Of Progress,* 8(6), 194–199. https://doi.org/10.1002/bies.950080605

Kreek, M. J., Bart, G., Lilly, C., Laforge, K. S., & Nielsen, D. A. (2005). Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. *Pharmacological Reviews,* 57(1), 1–26. https://doi.org/10.1124/PR.57.1.1

Kreek, M. J., LaForge, K. S., & Butelman, E. (2002). Pharmacotherapy of addictions. Nature Reviews. *Drug Discovery,* 1(9), 710–726. https://doi.org/10.1038/NRD897

Levran, O., Londono, D., O'Hara, K., Nielsen, D. A., Peles, E., Rotrosen, J., Casadonte, P., Linzy, S., Randesi, M., Ott, J., Adelson, M., & Kreek, M. J. (2008). Genetic susceptibility to heroin addiction: a candidate gene association study. Genes, *Brain, and Behavior,* 7(7), 720–729. https://doi.org/10.1111/J.1601-183X.2008.00410.X

Levran, Orna, Yuferov, V., & Kreek, M. J. (2012). The genetics of the opioid system and specific drug addictions. *Human Genetics,* 131(6), 823. https://doi.org/10.1007/S00439-012-1172-4

Lopez-Leon, S., González-Giraldo, Y., Wegman-Ostrosky, T., & Forero, D. A. (2021). Molecular genetics of substance use disorders: An umbrella review. *Neuroscience & Biobehavioral Reviews,* 124, 358–369. https://doi.org/10.1016/J.NEUBIOREV.2021.01.019

Maher, B. S., Vladimirov, V. I., Latendresse, S. J., Thiselton, D. L., McNamee, R., Kang, M., Bigdeli, T. B., Chen, X., Riley, B. P., Hettema, J. M., Chilcoat, H., Heidbreder, C., Muglia, P., Murrelle, E. L., Dick, D. M., Aliev, F., Agrawal, A., Edenberg, H. J., Kramer, J., Vanyukov, M. M. (2011). The AVPR1A gene and substance use disorders: association, replication, and functional evidence. *Biological Psychiatry,* 70(6), 519–527. https://doi.org/10.1016/J.BIOPSYCH.2011.02.023

Matthes, H. W. D., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., Befort, K., Dierich, A., Le Meur, M., Dolie, P., Tzavara, E., Hanoune, J., Roques, B. P., & Kieffer, B. L. (1996). Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature,* 383(6603), 822–823. https://doi.org/10.1038/383819A0

Mayer, P., Rochlitz, H., Rauch, E., Rommelspacher, H., Hasse, H. E., Schmidt, S., & Höllt, V. (1997). Association between a delta opioid receptor gene polymorphism and heroin dependence in man. *Neuroreport,* 8(11), 2547–2550. https://doi.org/10.1097/00001756-199707280-00025

Mayer, Peter, & Höllt, V. (2006). Pharmacogenetics of opioid receptors and addiction. *Pharmacogenetics and Genomics,* 16(1), 1–7. https://doi.org/10.1097/01.FPC.0000182781.87932.0D

Mistry, C. J., Bawor, M., Desai, D., Marsh, D. C., & Samaan, Z. (2014). Genetics of Opioid Dependence: A Review of the Genetic Contribution to Opioid Dependence. *Current Psychiatry Reviews,* 10(2), 156. https://doi.org/10.2174/1573400510666140320000928

Mura, E., Govoni, S., Racchi, M., Carossa, V., Nadia Ranzani, G., Allegri, M., & van Schaik, R. H. N. (2013). Consequences of the 118A>G polymorphism in the OPRMI gene: Translation from bench to bedside? *Journal of Pain*

Research, 3(6), 331–353. https://doi.org /10.2147/JPR.S42040

Nagaya, D., Zahari, Z., Saleem, M., Yahaya, B. H., Tan, S. C., & Yusoff, N. M. (2018). An analysis of genetic association in opioid dependence susceptibility. *Journal of Clinical Pharmacy and Therapeutics,* 43(1), 80–86. https://doi.org /10.1111/JCPT.12585

NCBI database. (2021a). OPRD1 opioid receptor delta 1 [Homo sapiens (human)]. https://www. ncbi.nlm.nih.gov/gene/4985

NCBI database. (2021b). OPRK1 opioid receptor kappa 1 [Homo sapiens (human)]. https://www .ncbi.nlm.nih.gov/gene/4986

NCBI database. (2021c). OPRM1 opioid receptor mu 1 [Homo sapiens (human)]. https:// www.ncbi.nlm.nih.gov/gene/4988

Nelson, E. C., Lynskey, M. T., Heath, A. C., Wray, N., Agrawal, A., Shand, F. L., Henders, A. K., Wallace, L., Todorov, A. A., Schrage, A. J., Madden, P. A. F., Degenhardt, L., Martin, N. G., & Montgomery, G. W. (2014). Association of OPRD1 polymorphisms with heroin dependence in a large case-control series. *Addiction Biology,* 19(1), 111–121. https: //doi.org/10.1111/J.1369-1600.2012.00445.X

Nikolov, M. A., Beltcheva, O., Galabova, A., Ljubenova, A., Jankova, E., Gergov, G., Russev, A. A., Lynskey, M. T., Nelson, E. C., Nesheva, E., Krasteva, D., Lazarov, P., Mitev, V. I., Kremensky, I. M., Kaneva, R. P., & Todorov, A. A. (2011). No evidence of association between 118A>G OPRM1 polymorphism and heroin dependence in a large Bulgarian case-control sample. *Drug and Alcohol Dependence,* 117(1), 62. https: //doi.org/10.1016/J.DRUGALCDEP.2010.12.0 26

Perez de los Cobos, J., Baiget, M., Trujols, J., Sinol, N., Volpini, V., Banuls, E., Calafell, F., Luquero, E., del Rio, E., & Alvarez, E. (2007). Allelic and genotypic associations of DRD2 TaqI A polymorphism with heroin dependence in Spanish subjects: a case control study. *Behavioral and Brain Functions :* BBF, 3(25). https://doi.org/10.1186/1744-9081-3-25

Prom-Wormley, E. C., Ebejer, J., Dick, D. M., & Bowers, M. S. (2017). The genetic epidemiology of substance use disorder: A review. *Drug and Alcohol Dependence*, 180, 241–259. https://doi.org/10.1016/J.DRUGA LCDEP.2017.06.040

Schwantes-An, T. H., Zhang, J., Chen, L. S., Hartz, S. M., Culverhouse, R. C., Chen, X., Coon, H., Frank, J., Kamens, H. M., Konte, B., Kovanen, L., Latvala, A., Legrand, L. N., Maher, B. S., Melroy, W. E., Nelson, E. C., Reid, M. W., Robinson, J. D., Shen, P. H., Saccone, N. L. (2016). 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. *Behavior Genetics,* 46(2), 151–169. https://doi.org/ 10.1007/S10519-015-9737-3

Shi, J., Hui, L., Xu, Y., Wang, F., Huang, W., mutation, G. H.-H., & 2002, undefined. (2002). Sequence variations in the mu-opioid receptor gene (OPRM1) associated with human addiction to heroin. *Wiley Online Library,* 497(4), 459–460. https://doi.org/10.1002 /humu.9026

Szeto, C., Tang, N., Lee, D., Neuroreport, A. S.-, & 2001, U. (2001). Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. *Neuroreport,* 12(6), 1103–1106. https://doi.org/doi:10.1097/000 01756-200105080-00011.

Tam, V., Patel, N., Turcotte, M., Bossé, Y., Paré, G., & Meyre, D. (2019). Benefits and limitations of genome-wide association studies. *Nature Reviews Genetics* 20(8), 467–484. https://doi.org/10.1038/s41576-019 -0127-1

Tan, E. C., Tan, C. H., Karupathivan, U., & Yap, E. P. H. (2003). Mu opioid receptor gene polymorphisms and heroin dependence in Asian populations. *Neuroreport,* 14(4), 569–572. https://doi.org/10.1097/00001756-200303240-00008

Tsuang, M. T. (2001). The Harvard Twin Study of Substance Abuse: What We Have Learned. *Harvard Review of Psychiatry,* 9(6), 267–279. https://doi.org/10.1093/HRP/9.6.267

Wee, S., & Koob, G. F. (2010). The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse. *Psychopharmacology,* 210(2), 121–135. https://doi.org/10.1007/S00213-010-1825-8

WHO. (2021). Opioid overdose. WHO. https://www.who.int/news-room/fact-sheets/detail/opioid-overdose

Wu, B., Hand, W., & Alexov, E. (2021). Opioid addiction and opioid receptor dimerization: Structural modeling of the oprd1 and oprm1 heterodimer and its signaling pathways. *International Journal of Molecular Sciences,* 22(19), 22. https://doi.org/10.3390/IJMS221910290/S1

Yuferov, V., Fussell, D., LaForge, K. S., Nielsen, D. A., Gordon, D., Ho, A., Leal, S. M., Ott, J., & Kreek, M. J. (2004). Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. *Pharmacogenetics,* 14(12), 793. https://doi.org/10.1097/00008571-200412000-00002

Zhang, H., Kranzler, H. R., Yang, B. Z., Luo, X., & Gelernter, J. (2008). The OPRD1 and OPRK1 loci in alcohol or drug dependence: OPRD1 variation modulates substance dependence risk. *Molecular Psychiatry,* 13(5), 531. https://doi.org/10.1038/SJ.MP.4002035

Zhou, H., Rentsch, C. T., Cheng, Z., Kember, R. L., Nunez, Y. Z., Sherva, R. M., Tate, J. P., Dao, C., Xu, K., Polimanti, R., Farrer, L. A., Justice, A. C., Kranzler, H. R., & Gelernter, J. (2020). Association of OPRM1 Functional Coding Variant With Opioid Use Disorder: A Genome-Wide Association Study. *JAMA Psychiatry,* 77(10), 1072–1080. https://doi.org/10.1001/JAMAPSYCHIATRY.2020.1206