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PERSPECTIVE

# The dopamine 3 receptor as a candidate biomarker and therapeutic for opioid use disorder

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## Abstract

Here, we present recent studies suggesting that specific *DRD3* single nucleotide polymorphisms (SNPs, e.g. rs324029 and rs2654754) might serve as prognostic biomarkers for opioid use disorder (OUD). Additionally, preclinical studies with novel dopamine 3 receptor (D3R) partial agonists and antagonists have been evaluated as candidate OUD therapeutics and have shown a reduced risk of cardiovascular toxicity compared with the original D3R antagonist. From these findings, we argue that *DRD3* SNPs could serve as a diagnostic tool for assessing OUD risk and that more research is warranted examining the D3R as a safe and effective therapeutic target for treating OUD.

**KEYWORDS**
genetic risk, opioid, opioid use disorder, pharmacogenetics

## 1 | INTRODUCTION

In the United States, between 6.7 and 7.6 million adolescents and adults are estimated to have opioid use disorder (OUD).[1] Consequently, there has been a resurgence in research efforts to both identify novel methods for predicting the risk of developing OUD and develop novel OUD treatment targets outside opioid receptors. Although the three current medications for OUD (MOUDs) therapeutics approved by the U.S. Food and Drug Administration are effective: naltrexone, buprenorphine and methadone also directly target opioid receptors and have undesirable effects that limit their overall clinical utility. Here, we propose that the dopamine 3 receptor (D3R) and the *DRD3* gene which regulate D3R expression levels have now emerged as candidate targets for OUD treatment and potential biomarker, respectively.

Opioid agonists such as fentanyl or oxycodone are hypothesized to produce their addictive effects through the direct activation of medium-sized spiny neurons of the nucleus accumbens (NAc) core and shell[2] and through the indirect activation of the mesolimbic dopamine reward pathway by binding to μ-opioid receptors within the ventral tegmental area (VTA) and the closely associated brain region, the rostromedial tegmental nucleus (RMTg).[3] Mu-opioid receptors are predominately expressed on GABAergic interneurons, not on dopaminergic cell bodies within the VTA and RMTg. Because μ-opioid receptors belong to a family of G-protein-coupled receptors (GPCRs) that inhibit adenylyl cyclase, activation of μ-opioid receptors on these GABAergic neurons by an opioid receptor agonist such as fentanyl inhibits the release of the inhibitory neurotransmitter GABA acting on dopaminergic neurons. The net effect is a disinhibition of the inhibitory GABAergic tone resulting in an increase in dopaminergic neurotransmission of the mesolimbic dopamine reward pathway.[4] The neurochemistry and neurobiology associated with OUD is complex and the role of dopamine in opioid reward/reinforcement remains a highly debated scientific area.[5] Nonetheless, the increased neurotransmission within the mesolimbic dopamine reward pathway by opioids is theorized to be a crucial pathway involved in the addictive effects of opioids and thus a candidate target for both biomarker and candidate medication development research.

In humans and rodents, there are five known dopamine receptors (D1–D5). Dopamine receptors are also GPCR and are classified as D1-class receptors (D1 and D5) coupled to $G\alpha_s$ or D2-class receptors (D2, D3 and D4), which are coupled to $G\alpha_{i/o}$.[3] Of particular interest, D3R mRNA is expressed at high levels within the ventral striatum/NAc, dentate gyrus and striate cortex.[3,6] *DRD3* regulates the expression level of D3R, which are predominately pre-synaptic autoreceptors that are preferentially expressed in brain regions associated with drug reward and addictive processes.[7] Two recent clinical studies of candidate genes have identified two single nucleotide polymorphisms (SNPs) of *DRD3* (rs324029 and rs2654754) that were associated with an increased risk of developing OUD.[8,9] In addition, two other *DRD3* (rs6280 and rs9825563) variants have been found to be associated with the early development of heroin dependence.[7,10] *DRD3* SNPs associated with OUD and population cohorts are summarized in Table 1. Consistent with these clinical studies, animal studies where the D3R has been genetically deleted report enhanced sensitivity to opioid reward and reinforcement indicative of a vulnerable phenotype analogous to the clinical studies.[11] Taken together, these clinical and preclinical studies suggest that *DRD3* SNPs might serve as prognostic biomarkers for OUD risk.

Recent development of more selective D3R compounds has facilitated preclinical research efforts in the evaluation of D3R partial agonists and antagonists as candidate OUD therapeutics. For example, acute pretreatment with the D3R partial agonist/antagonist (±) VK4-40 decreased oxycodone self-administration and oxycodone-induced reinstatement in both rats and monkeys.[12,13] However, chronic pharmacological treatment to model current clinical OUD medication dosing regimens with the D3R partial agonist cariprazine failed to decrease remifentanil self-administration in monkeys.[14] Potential reasons for the differential results between cariprazine and the VK4-40 D3R partial agonist studies could be related to either experimental design considerations such as acute vs. repeated dosing of the D3R partial agonist or differences in test compound pharmacological selectivity for the D3R versus D2R. Additionally, the R-enantiomer of VK4-40 has been determined to be an antagonist and the S-enantiomer a partial agonist of the D3R.[15] Cariprazine is currently being evaluated in a phase IIa, randomized, placebo-controlled clinical study as a candidate medication for comorbid cocaine and OUD.[16] Although human laboratory drug self-administration studies and/or clinical trials such as the one ongoing will ultimately be needed to confirm or refute these preclinical results with D3R compounds, these later monkey results using a translationally relevant opioid-vs-food choice procedure and repeated cariprazine dosing experimental design do not provide compelling evidence for the D3R partial agonist cariprazine as an effective OUD therapeutic.

The development and evaluation of D3R partial agonists and antagonists as candidate medications for various substance use disorders has been an active area of research for over two decades.[3] The translation of D3R partial agonists and antagonists from the bench to the bedside as substance use disorder therapeutics has further been hindered because of concerns over cardiovascular toxicity interactions between these D3R ligands and addictive drugs such as cocaine or methamphetamine that have sympathomimetic effects. Recently, acute *R*-VK4-40 and *R*-VK4-116 administration were shown to have a positive effect on oxycodone or cocaine-induced increases in heart rate and blood pressure in rats.[17] Further research on these promising results with repeated D3R ligand administration and opioid or cocaine interactions on cardiovascular safety endpoints will be critical towards their development as candidate substance use disorder medications.

Our working hypothesis on the mechanism by which SNPs within the *DRD3* might be implicated in an increased OUD risk would be if these presynaptic D3R were either downregulated (i.e. less receptor expression) or were not signalling correctly (through SNP altered function) when bound by an endogenous agonist such as dopamine. Dopamine binding to D3R autoreceptors under non-diseased conditions should result in attenuation of further synaptic dopamine release via a negative feedback loop (Figure 1A). Dopamine binding to a reduced function D3R (as a result of an SNP) or a D3R antagonist would be predicted to result in an increase in dopamine within the synaptic cleft due to an impaired negative feedback loop (Figure 1B). Whereas a partial agonist under non-diseased conditions would lead to decreased dopamine in the synaptic cleft through activation of the negative feedback loop, under conditions

**TABLE 1** DRD3 single nucleotide polymorphisms associated with OUD.

| Source | Sample size | Study population | rs number |
|---|---|---|---|
| Levran et al.[10] | 828 former heroin addicts and 232 healthy controls | Predominately European ancestry | rs2654754<br>rs9288993<br>rs1486009 |
| Kuo et al.[7] | 566 heroin dependent and 501 controls | Han Chinese | rs324029<br>rs6280<br>rs9825563 |
| Bright et al.[8] | 200 OUD patients and 194 controls | Predominately European ancestry | rs324029<br>rs9288993<br>rs6280 |
| Freiermuth et al.[9] | 250 OUD patients and 1051 controls | European and African American/Afro-Caribbean | rs324029<br>rs2654754 |

Abbreviation: OUD, opioid use disorder.

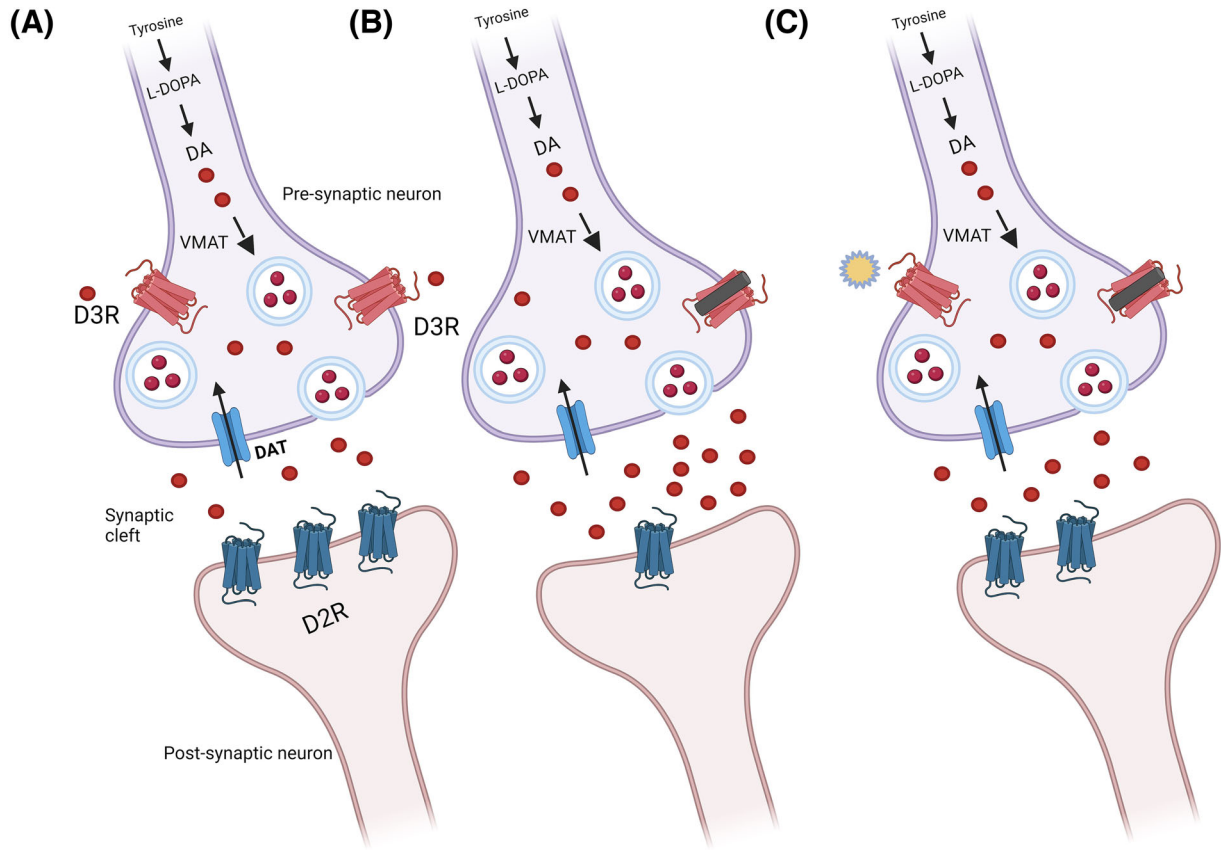


**FIGURE 1** Hypothesized models of dopamine levels in the synaptic cleft under various healthy or genetic mutation conditions. (A) The dopamine 3 receptor (D3R) agonist, dopamine (red circle), activates the pre-synaptic D3R to reduce dopamine release in the synaptic cleft through a negative feedback loop. (B) A reduced function single nucleotide polymorphism (SNP) within the D3R or a D3R antagonist (grey cylinder) would increase dopamine within the synaptic cleft due to impaired negative feedback loop function that may confer a vulnerability to addictive drugs such as opioids. (C) A partial agonist (yellow circle) under healthy conditions would decrease dopamine in the synaptic cleft similar to Panel (A). However, if there was reduced D3R expression or reduced function because of a SNP, dopamine levels following D3R partial agonist administration alone would be predicted to remain the same or may slightly increase. Furthermore, D3R partial agonist occupancy of the D3R may also function as a competitive antagonist for DA at the D3R and thus preventing D3R agonist-induced activation of this negative feedback loop resulting in increased or sustained dopamine in the synaptic cleft that may blunt subsequent opioid agonist-induced dopamine effects. Created with BioRender.com.

of reduced D3R expression or reduced function, dopamine levels would be predicted to remain the same or may slightly increase following partial agonist binding to the D3R (Figure 1C). These hypothesized effects of D3R partial agonists acting on D3R that have reduced function or expression are based on receptor theory, which supports the fact that partial agonists have reduced receptor activation following changes in either signal transduction function or the total number of receptors. As one hypothetical example, an OUD patient with one of the potential SNPs impacting D3R discussed above would be maintained on a D3R partial agonist or antagonist for OUD treatment. Maintenance on a D3R partial agonist or antagonist in this context may increase basal dopaminergic tone in the synaptic cleft, blunt the effects of opioid-induced dopamine increases, resulting in a reduced opioid reinforcing effect and subsequent extinction of opioid-taking behaviours and reallocation of behaviour towards more adaptive, nondrug reinforcers (e.g. social interaction or employment).

In conclusion, the current preclinical and clinical data available suggest an association between *DRD3* and OUD that warrants further research into *DRD3* as a potential biomarker for OUD risk. Genetic association studies with the identified candidate SNPs presented here are necessary for further validation. Furthermore, D3R expression could be measured in humans and nonhumans with a D3R PET ligand. However, the expression does not equate to function, which is proposed to be impaired in the *DRD3* SNPs identified to date. Moreover, the D3R function would be harder to measure in humans and would require post-mortem tissue. Therefore, the candidate gene SNPs (e.g. rs324029 and rs2654754) in *DRD3* identified to date would be logical biomarkers for OUD risk assessment.[9] Alternatively, D3R function changes could be determined using pharmacological probes such as D3R agonist-induced yawning.[18,19] Although partial agonist/antagonist studies targeting the D3R have shown promising but equivocal results as novel candidate therapeutics for treating OUD, further preclinical research utilizing a proposed preclinical screening

algorithm[20] and mainly clinical research is warranted in this area. Moreover, combining D3R partial agonists/antagonists with approved MOUDs that have demonstrated protection against opioid overdose in the event of a relapse of opioid use,[21] could further provide a therapeutic benefit to OUD patients.

## AUTHOR CONTRIBUTIONS

MLB and JES contributed equally to the writing of this manuscript.

## CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests in this work.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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