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Validating Zhx2 in oxycodone metabolite (oxymorphone) brain concentration and behavior via reciprocal gene editing and viral manipulation of gene expression in BALB/c substrains

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Opioid Use Disorder **(OUD)** maintains epidemic proportions in the U.S., with current pharmacological treatments limited to opioid substitution therapy. Sensitivity to the subjective and physiological responses to opioids has a genetic component that could influence addiction liability. We identified *Zhx2* as a candidate gene underlying increased oxycodone **(OXY)** metabolite brain concentration in BALB/cJ (**J**) vs. BALB/cByJ (**By**) females. The metabolite, oxymorphone (**OMOR**), is more potent and efficacious and could enhance state-dependent learning and recall of OXY-induced conditioned place preference (CPP) in J vs. By females. A structural intronic variant causes a significant reduction in Zhx2 expression in J vs. By mice.

Thus, here, we tested the role of this variant in OMOR levels and OXY behaviors through gene editing of the variant, through modeling Zhx2 loss-of-function via exon 3 deletion, and through virally manipulating *Zhx2.* We are still validating the Zhx2 variant on OMOR and behavior.

Following AAV-mediated liver overexpression of Zhx2, J females showed an increase in state-dependent OXY reward learning and a decrease in OXY-induced locomotor sensitivity.

We also observed an increase in Cyp2d22 RNA, thus providing a potential intermediary mechanism linking Zhx2 with differential brain OMOR concentration. Complementary to these results, there was an increase in OXY-induced locomotor sensitivity when *Zhx2* was knocked out and an increase in state-dependent reward learning. Our work supports validation of *Zhx2* as a quantitative trait gene underlying brain OMOR concentration and behavior, which could increase our understanding of OXY addiction liability in humans.