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Validating Zhx2 as a candidate gene underlying Oxycodone Metabolite (Oxymorphone) Brain Concentration and Behavior via Gene Editing and -Omics analyses in BALB/cByJ mice

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Opioid Use Disorder **(OUD)** maintains epidemic proportions in the U.S. with limited pharmacological treatments. Sensitivity to the opioids' rewarding properties has a genetic component and can predict 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 than OXY and could explain the enhancement of state-dependent learning of OXY conditioned place preference (**CPP**) in J vs. By females. A structural intronic variant significantly reduces in Zhx2 expression in J vs. By mice, which we hypothesize could enhance OMOR levels and OXY addiction-model behaviors. We are currently testing this hypothesis in Zhx2 knockout mice and measuring OXY metabolite levels and addiction model behaviors. Consistent with our hypothesis, Zhx2 KO females showed an increase in brain OMOR levels compared to WT females with no genotype effect observed in males. However, in contrast to our hypothesis, we found that state-dependent expression of OXY-CPP was decreased in KO females and increased in males. Brain proteomic analysis of Zhx2 KO mice identified multiple proteins implicated in small-molecule metabolism and inflammatory processes that could contribute to behavioral differences. We are currently conducting brain CUT&RUN-seq and bulk RNA-seq to complement the proteomic analyses and identify functional DNA-binding targets of Zhx2. Our work supports validation of *Zhx2* as a quantitative trait gene underlying brain OMOR concentration and behavior and candidate quantitative trait mechanisms, which could increase our understanding of *Zhx2* brain function and OXY addiction liability in humans.