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Using Large-Scale Genetics to Decipher Signaling Networks that Regulate Opioid Behavioral Responses

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Abstract

Large-scale, unbiased genetics with engineered *C. elegans* behavioral models is substantially expanding our understanding of how μ-opioid receptor (MOR) signaling and opioid drug responses are regulated. We previously performed large-scale forward genetics with an engineered *C. elegans* chemo-genetic model of μ-opioid receptor (MOR) signaling. This led to the discovery of multiple receptor systems that regulate opioid sensitivity and tolerance from *C. elegans* through rodents[1,2]. We have now developed a next generation, humanized tgMOR *C. elegans* model (HtgMOR). Here, we combined our HtgMOR *C. elegans* model with CRISPR engineering to facilitate a large-scale reverse genetic screen targeting ubiquitin ligases for effects on opioid-induced behavior.

Previous cell-based studies have shown the ubiquitin-proteome system (UPS) can affect MOR signaling, and pharmacologically impairing the UPS alters opioid tolerance in rodents. At present, it is unclear how ubiquitin ligases (key components of the UPS) affect opioid responses. Moreover, genetic evidence that ubiquitin ligases and the UPS affect opioid behavioral responses in organismal models remains absent. Using the *C. elegans* single-cell neural transcriptome, we identified 49 ubiquitin ligases that were evolutionarily conserved and expressed in the nervous system. We then used CRISPR engineering to generate molecular nulls for 36 ubiquitin ligases in the HtgMOR model. Automated, unbiased behavioral assays were then used to evaluate HtgMOR ubiquitin ligase mutants for changes in opioid sensitivity. We identified three HtgMOR ligase mutants that were hypersensitive to fentanyl, and two further mutants with hyposensitive responses. We are presently evaluating potential downstream ubiquitination substrates of these ligases for effects on opioid sensitivity. Thus, large-scale reverse genetics has revealed a genetic mini-network composed of 5 ubiquitin ligases that negatively and positively tune MOR signaling. Importantly, this mini-network contains a cadre of potential new molecular targets for managing opioid use disorder.

1) Wang…Grill[#], Martemyanov[#]. Genetic behavioral screen identifies an orphan anti-opioid system. *Science*, 2019 [#] co-corresponding authors

2) Maza N*, Wang D*, Kowalski C*… Grill B[#], Martemyanov KA[#]. Ptchd1 mediates opioid tolerance via cholesterol-dependent effects on μ-opioid receptor trafficking. *Nat Neurosci*. 2022. [#] co-corresponding authors * co-first authors