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Exploring molecular mechanisms underlying fentanyl overdose-induced toxicities via *Oprm1* exon 1-associated variants using tandem mass tag-based phosphoproteomics/proteomics

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Fentanyl is a potent synthetic mu analgesic. Fentanyl and its analogs have been associated with two thirds of the United States opioid overdose deaths in recent years. The single-copy MOR gene, *OPRM1,* undergoes extensive alternative mRNA splicing, generating an array of splice variants conserved from rodents to humans. One set of the *OPRM1* variants contains exon 1-associated 7TM carboxyl terminal variants (E1 variants), including the original MOR-1. Recently, we generated a rat *Oprm1* E1 conditional KO models ($rE1^{f/f}$), in which the E1 coding and adjacent intron were floxed with loxPs. Like WT rats, fentanyl overdose induced severe respiratory depression (FOISRD), persistent apnea (FOIPA), muscle rigidity (FOIMR) and lethality in $rE1^{f/f}$ rats. However, these fentanyl toxicities were not seen in the global rE1-KO ($rE1^{d/d}$) rats generated by breeding $rE1^{f/f}$ with a CAG-Cre rat, suggesting that E1 variants primarily mediate these toxicities. Using both $rE1^{f/f}$ and $rE1^{d/d}$ models, we have explored the molecular mechanisms underlying fentanyl overdose-induced toxicities via E1 variants in the preBötzinger complex (preBöC) and the parabrachial nucleus (PBN) at protein phosphorylation level by using tandem mass tag-based phosphoproteomics/proteomics (TMT PP/P-omics). Our results revealed differential expression of many phosphoproteins the PBC and PBN that are associated with many important signaling pathways between acute fentanyl overdose and chronic fentanyl/acute fentanyl overdose, between male and female rats, between the PBC and PBN, and between $rE1^{f/f}$ and $rE1^{d/d}$ models. We also identified several upstream kinase regulators, which may lead to identifying potential targets for developing novel therapeutic strategies to combat fentanyl overdose death.