# Synaptic mechanisms of OPRM1 A118G (MOR N40D) gene variants in human neurons

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Mu opioid receptor (MOR) signaling modulates synaptic transmission and thus plays a pivotal role in regulating reward behaviors relevant to addiction. However, the molecular and synaptic mechanisms of opioid signaling in the context of drug addiction are poorly understood. Interestingly, the single nucleotide polymorphism (SNP) rs1799971 (OPRM1 A118G) in the human MOR has been linked to impaired MOR trafficking and signal transduction and has been implicated in increased predisposition to drug abuse disorders and alcohol use disorders. The A118G SNP produces a non-synonymous amino acid substitution in the human MOR, replacing an asparagine with an aspartate at position 40 (MOR N40D). To investigate the molecular and cellular mechanisms of this SNP, we derived human neurons, using the induced neuronal (iN) cell technology, from induced pluripotent stem (iPS) cell lines generated from multiple human subjects carrying either homozygous N40 or D40 alleles.

Our compelling preliminary data reveal that D40 MOR mediates stronger suppression of synaptic releases compared to N40 MOR in inhibitory human neurons. Interestingly, pre-exposure of these human neurons to DAMGO ([D-Ala$^2$, NMe-Phe$^4$, Gly-ol$^5$]-enkephalin) for 24 hours diminishes their sensitivity to DAMGO, possibly owing to the desensitization or internalization of membrane MORs following prolonged activation of the receptors. Moreover, N40 MOR carrying human neurons regained partial sensitivity to DAMGO during a 7-day pre-exposure paradigm, whereas D40 failed to re-sensitize. We thus hypothesize that human N40 and D40 MORs have differential membrane recycling dynamics. To control for the inherent line-to-line variability and background genetic variation which potentially occludes detection of a clear phenotype in human stem cell systems, using CRISPR/Cas-9 system, we have genetically engineered the G118 SNP in NIH registered human H1 embryonic stem cells (AA118

carrier) and converted a GG118 iPS cell line into the AA118 genotype. The two pairs of human pluripotent stem cells differ at A118G locus but have isogenic background with the parental cell line. We hypothesize that the iNs from these isogenic cell lines will isolate the specific role of the A118G SNP in altered synaptic transmission and MOR trafficking. Our system using iNs carrying human MOR gene variants provides important insights into the neurophysiology of MOR regulation on synaptic transmission in a human system and will likely provide novel information about the neurocircuitry of reward behavior.