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Differential expression and transcription factor binding associated with genotype at a pharmacogenetic variant in OPRD1

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There is a growing list of genetic variants associated with substance use disorder phenotypes, but information on the underlying functional mechanisms is lacking for most of these associations. Rs678849, an intronic variant in the delta-opioid receptor gene (*OPRD1*) has been found to predict regional brain volume, dependence risk, and the efficacy of buprenorphine/naloxone in treating opioid use disorder. The variant has also been implicated as an expression quantitative trait locus (eQTL) for several genes. The objective of this study was to identify functional differences between the two alleles of rs678849 in vitro. 15bp regions containing the C or T alleles of rs678849 were cloned into luciferase constructs and transfected into BE(2)C neuroblastoma cells. At 24 hours post-transfection, the C allele construct had significantly lower luciferase expression than the T allele construct and empty vector control (ANOVA $p < 0.001$). Electrophoretic mobility shift assays (EMSA) using BE(2)C nuclear lysate indicated that the two alleles of rs678849 differentially bound distinct protein complexes. Proteomic analysis and supershift assays identified XRCC6 as a transcription factor specifically binding the C allele, whereas hnRNP D0 was found to specifically bind the T allele. XRCC6 and hnRNP D0 were also found to bind the C and T probes, respectively, when EMSA and supershift assays were performed with nuclear lysate from human postmortem medial prefrontal cortex. These functional differences between the C and T alleles may help explain the psychiatric and neurological phenotype differences predicted by rs678849 genotype and the potential role of the variant as an eQTL.