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TSS-MPRA to decode regulatory functions of genetic variants associated with substance use disorders.

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Substance use disorders (SUD) are highly heritable, and numerous genetic variants associated with SUD have been identified through genome-wide association studies (GWAS). Many of these non-coding variants likely affect transcriptional regulation through enhancers and promoters containing key transcription factor (TF) motifs. However, predicting the effects of these variants on gene regulation is challenging due to the complexity of the cis-regulatory code.

To address this, we leveraged a newly developed method, TSS-MPRA, to profile transcription initiation sites with single-nucleotide precision from a massively parallel reporter assay (MPRA). Unlike traditional MPRA methods, the TSS-MPRA method leverages an innovative design that captures transcription start sites (TSS), allowing the identification of specific TSSs affected by sequence variants. This information facilitates interpreting and validating the biological role of non-coding variants.

We tested TSS-MPRA in primary neuronal cultures using a lentiviral MPRA library of ~18,000 inserts. This library comprised several designs: 1) We assayed neuronal regulatory elements enriched for neuronal activity-dependent transcription factor (TF) motifs. 2) We included elements in which TF motifs were either inserted at different positions relative to the TSS or mutated. 3) We performed saturation mutagenesis for key activity-dependent regulatory elements. 4) We screened GWAS variants associated with neuropsychiatric disorders.

Our findings highlight the importance of TF motif positioning relative to transcription start sites and the effect of genetic variants on the activity of regulatory elements. Overall, TSS-MPRA identifies active regulatory sequences and the functional impacts of genetic variants on gene regulation.