A Genome-Wide Approach to Efficiently Identify Replicable DNA Methylation Regions Associated with Alcohol Use

Rita Cervera-Juanes, Kathleen A. Grant, Betsy Ferguson

Division of Neuroscience, Oregon National Primate Research Center, Oregon Health & Sciences University, Beaverton, OR 97006

One of the challenges of genome-wide differential DNA methylation (DM) analysis, is identifying valid, functional effects from among the hundreds of thousands or millions of CpGs analyzed. We report on a method we used to identify DM associated with chronic alcohol use in the nonhuman primate (macaque) model of alcohol self-administration. This well-established model provides macaques open access to 4% unflavored alcohol for 22 hours/day for 12 months. The daily, automated data collection enables the precise, real-time measurement of all alcohol consumed, and it has revealed a broad range of macaque alcohol consumption levels similar to those reported among humans with alcohol use disorders (AUDs). Moreover, modeling analysis of the macaque drinking patterns revealed four stable, and distinguishable categories (low, binge, heavy and very heavy drinkers). We used pair-wise DM analysis among 24 subject that were either alcohol naïve (AN), low/binge (L/B) or heavy/very heavy (H/VH) drinkers. We analyzed nucleus accumbens core (NAcc) tissue, because of the recognized role of the mesocorticolimbic system in modulating motivated behaviors, and its association with the development of drug and alcohol dependence. Using the Agilent SureSelect MethylSeq enrichment system, combined with bisulfite deep sequencing, we measured an average of 2.6 million CpG methylation levels per individual. The significantly differentially methylated CpGs (DMCs) were then analyzed using CombP software to identify differentially methylated regions (DMRs). We identified 50 DMRs (spanning 4 to 19 contiguous CpGs) that distinguished AN, L/B and H/VH drinkers. The vast majority of the DMRs identified were located within gene bodies, and mapped to genes encoding synaptic proteins, ion channels, transcriptional regulators, ncRNAs or genes of unknown function. The DMR-linked genes included those with known relevance to AUDs (e.g., *PDE10A*), and genes not previously associated with alcohol use, but that encode functions of direct relevance to alcohol-associated neuroadaptation (e.g., *ARHGEF7, RIMS1*). Twenty of the DMRs were selected for validation analysis using an expanded set of subjects and bisulfite amplicon sequencing (BSAS). In every case, the alcohol-associated DMR was supported by the validation study. Moreover, transcriptional analysis of the DMR linked genes identified a correlation between DMR methylation level and transcript expression, supporting a functional role of DMRs in regulating gene and alternative transcript expression. In addition, alcohol dose-correlation analysis suggested that the DMRs were associated with differing roles relative to AUD risk. First, a small set of the DMRs were uniquely detected among stable, low-binge drinkers, raising the possibility that they identify protective mechanisms, preventing the escalation of alcohol use. Second, another set of DMRs had methylation levels that were significantly associated with the average daily amount of alcohol consumed, suggesting that the CpG methylation are modified in an alcohol dose-dependent manor. Third, a set of DMRs were uniquely detected among heavy drinkers, potentially identifying mechanisms associated with alcohol tolerance or dependence. A fourth set of DMRs were equally modified in L/B and H/VH drinkers, but differed from alcohol naïve subjects, implicating alcohol dose-independent effects. Taken together, our study demonstrates the application of a genome-wide DM analysis approach that efficiently identifies reproducible, functional DNA methylation signals of high relevance to alcohol use.