High-Throughput Transcriptome Sequencing to Identify Genetic Modifiers of Sensitivity to Alcohol

G.R. Flentke, M.E. Berres, A. Garic, S.M. Smith
Nutrition Research Institute, University of North Carolina at Chapel Hill, 500 Laureate Way, Kannapolis NC 28081

Alcohol exposure to the developing brain causes transcriptional changes that affect neural function and differentiation and increase the risk for addictive behaviors in later life. We have characterized the transcriptomes of a unique genetic resource: two closely related avian strains (*Gallus gallus* W98S and W98D) whose neural progenitor cells differ dramatically in their alcohol sensitivity and outcomes. W98S neural progenitors exhibit strong calcium transients and substantial apoptosis following exposure to 52 mM ethanol, whereas these responses are significantly blunted in W98D neural progenitors. High-throughput sequencing of these neural progenitors' transcriptomes identified the KEGG clusters of Ribosome Biogenesis (*p*=1.3E-17, N=36 genes), Oxidative Phosphorylation (*p*=6.5E-7, N=31), and spliceosome (*p*=1.0E-3, N=20) as having the greatest differential expression between the alcohol-vulnerable and alcohol-resistant cell lineage. To identify pathways within this gene set with a mechanistic contribution, we then analyzed the transcriptomes of alcohol-vulnerable neural progenitors 6hr post-alcohol challenge (52mM). Again, the greatest differentially expressed gene clusters involved Ribosome Biogenesis (*p*=1.2xE-17, N=67), Oxidative Phosphorylation (*p*=4.8E-12, N=60), and spliceosome (*p*=2.6E-2, N=39). Nearly all the transcripts represented in these clusters were repressed by alcohol exposure. Comparison of the alcohol-vulnerable (1201 genes) and alcohol-treated gene sets (3422 genes) identified 525 overlapping genes of which 257 had the same directional change. These included 36 ribosomal (p=6.8E-30), 25 oxidative phosphorylation (p-1.8E-10) and 7 spliceosome genes (8.6E-1). We tested the mechanistic contribution of three of these ribosomal proteins (RP; *zrpl11, zrpl5a, zrps3a*) using a morpholino approach in zebrafish. While a moderate alcohol dose or RP haploinsufficiency separately did not increase neural progenitor apoptosis or disrupt craniofacial development, their combination caused high apoptosis and ablated cranial development, suggesting genetic RP insufficiency sensitizes neural progenitors to alcohol. In humans, genetic syndromes of RP haploinsufficiency impair cranial development through aberrant activation of MDM2/p53 dependent signaling, suggesting a potential mechanism for alcohol's action. To understand the basis underlying alcohol's repression of RP expression, *in silico* analysis revealed abundant CpG islands within many RP promoter regions. Exon evaluation within the alcohol-treated transcriptome showed that alcohol uniformly repressed exons encoding RPs, suggesting transcriptional silencing. At the conference, we will report the methylation status of RP genes in response to alcohol, for which sequencing is underway. In summary, the chick model and unbiased approach of whole transcriptome and genome analysis is a powerful tool in which to discover and identify novel genes and pathways that modify alcohol vulnerability. [Supported by R37 AA11085 to SMS]