Cortisol-related Genes and Substance Use Severity at age 16 years

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Introduction: Substance use (SU) disorders are among the most familial of mental health disorders, showing substantial genetic influence. The majority of work on genetic influences on SU have used twin and family studies to infer genetic influences, or directly assessed the role of specific genes on SU outcomes. A key gap in the literature is in understanding *how* genes influence SU. It is critical to include biological mechanisms by which genes may affect behavior in models of the development of SU. Here we consider cortisol responsivity, a primary steroid hormone of the hypothalamic-pituitary-adrenal axis linked to stress response. Genes influence cortisol production, and cortisol can bind within cell nuclei and change transcription and expression of genes to regulate further hormone production, brain function and behavior. Biosocial theories of externalizing problems posit that youth who were exposed to prolonged stressors may experience a "down-regulation" of cortisol at the level of the adrenal and in the brain, evidenced as low basal levels of cortisol and blunted reactivity in response to challenge or stress. It has been posited that children and adolescents who engage in externalizing behaviors may do so, in part, in an attempt to increase or return arousal to more typical levels. We build on this theory to hypothesize that the combined, polygenic, influence of genes that have been related to cortisol function in the literature and biosystems databases are associated with reduced cortisol responsivity to an event related potential task protocol (ERP), and that this polygenic risk facilitates the transition from externalizing problems to SU severity in adolescence differentially in a sample of youth at-risk for SU disorder and controls.

Sample: The sample was drawn from the Center for Education and Drug Abuse Research (CEDAR), a northern US-based study following 775 families longitudinally (at ages 10-12 [W1], 12-14 [W2], 16 [W3]). Biological children of men with a SU disorder (n = 344), or no disorder (n = 350) were included (71% male). Prior work shows that study attrition is unrelated to externalizing problems, ethnicity, SES, or education.

Measures: Relevant to the current study, cortisol responsivity to ERP was collected at W1 (cortisol generally showing declines in response to the protocols; 26% showed an increase). A composite score reflecting externalizing problems on multiple measures (http://www.pitt.edu/~cedar/TLIdocument.html) was assessed at W1, and a multi-method, multi-rater composite indicator of SU severity was assessed at W3. Genetic data was available for a subset of 237 individuals (n = 106 in the SUrisk and n = 131 in Control group).

Analysis: We randomly split the sample into discovery (50%, n=119) and test sets (50% n=118). In the discovery set we correlated each SNP with cortisol responsivity independently, after standard quality control and pruning for LD > .70. Generated beta-weights for each SNP were used for creating the polygenic score (PGS) in the test sets by multiplying the matrix of SNPs (coded for number of minor alleles) by the vector of beta-weights from the discovery sample and summing across all of the SNPs for each person. The PGS is then modeled to test its association with cortisol responsivity in the test set using a K-fold (K=2) cross-validation approach, which avoids non-independence of testing the effect of the polygenic score in the same sample from which the score was derived, over-fitting, and inflated effects. Limitations of this approach include that only one test sample (50% of the subset with genetic data, n = 118) can be used for hypothesis testing. Regressions wherein PGS, externalizing problems, and cortisol responsivity, predicted SU were tested within the K-fold framework – there were two sets of results, the first replicate as described, and the second replicate, where the discovery and test sets were swapped. Finally, structural equation models were fit to the full data (n = 693,) to test the direct effects of PGS and externalizing problems on SU as well as the indirect effects of PGS and externalizing through cortisol responsivity, first in the entire sample, and then separately by risk group. Again there were two replicates, where the non-missing PGS data were drawn from the test set from the first and then the second replicates.

Results: Results from the K-fold cross-validation approach found no direct effect of genes theoretically linked to cortisol function on cortisol responsivity to the ERP with either fold as test sample. Results within the K-fold framework yielded a significant effect of the PGS on SU such that a negative PGS (indicating individuals with more minor alleles contributing to lower cortisol responsivity) predicted higher SU severity in one replicate. This effect was not replicated when the discovery and test sets were swapped. Results from the structural equation models confirmed the K-fold regression results, with no indirect effects. The main effect of PGS on SU in the initial replicate were driven by the SUrisk group and not found in the control group (and not replicated).