**EXPRESSION OF SLC6A3 IN PERSISTENT ADHD**

**ABSTRACT**

Attention Deficit Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder that begins in early childhood and is associated with impulsive behavior and inability to concentrate. Currently, ADHD diagnosis is based solely on survey responses provided by the patient. Without the definitive information provided by a physiological biomarker, ADHD is often misdiagnosed, leading to unwarranted over-prescriptions of associated medications. This study aims to address this issue by identifying a genomic biomarker for ADHD, which would allow the physician to make a more definitive diagnosis. Mutations of the SLC6A3 gene have been suspected to have an association with persistent ADHD in adults. The SLC6A3 gene, also known as the dopamine transporter gene, codes for the DAT1 protein. The DAT1 protein is a neuronal transmembrane protein that aids in the transmission of dopamine and prevents excessive dopamine re-uptake. Genetic analyses were performed to evaluate the presence of a mutation in SLC6A3, specifically in sequences found in the 3’ UTR and Intron 8 locus of the SLC6A3 gene. Volunteers were provided with the ASRS-V1.1 survey and two vials of whole blood. Volunteers’ survey responses were compared with the resulting gene product yielded by PCR and gel electrophoresis. Cochran-Armitage and Odds Ratio analyses yielded a significant association with ADHD and the 3’ UTR polymorphism.

**INTRODUCTION**

Attention Deficit Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder that begins in early childhood. ADHD is characterized by hyperactivity, inability to concentrate, and abnormally impulsive behavior. These symptoms often persist into adulthood (Garner-Dyksra, Pinchesvsky, Caldeira, Vincent, & Amia; 2010, October). Currently, ADHD diagnosis is performed by survey-based criteria, which has been argued to be inconclusive. Many have argued that the ambiguity of ADHD diagnostic criteria is leading to overdiagnosis and over-prescription of its associated medications. For this study, evaluation of two potential genomic biomarkers for ADHD will be carried out on the dopamine transporter gene (DAT1). It was surmised that the combined expression of the 10-repeat allele of the 3’UTR (untranslated region) VNTR (variable number tandem repeat) and the six-repeat allele of the intron 8 VNTR on the DAT1 gene would prove to be connected with adults diagnosed with ADHD. The DAT1 gene codes for a protein that aids the reuptake of dopamine at the synaptic cleft of the neuron. Various alleles on the DAT1 gene have been evaluated in previous ADHD studies, including the 10-repeat allele of the 3’UTR (10) and the six-repeat allele of the intron 8 VNTR (6). The 10-, relative to 9- allele, is associated with the amount of dopamine transporter gene expressed and smaller brain volume in areas of the brain connected with ADHD. The 6- allele, relative to the 5- allele, is associated with an altered gene expression that links to symptoms commonly observed in ADHD (Spencer, Biederman, Faraone, Madras, Bonab, Dougherty, Batchelder, Clarke, Fischerman, 27 Dec. 2012). Genotyping adults with ADHD in Northern Utah will allow identification of these alleles to evaluate whether a difference in allelic frequency exists when compared with individuals without symptoms associated with ADHD.

**MATERIALS AND METHODS**

Testing was performed to verify the probability of each participant having ADHD given their genetic biomarkers found in gel electrophoresis. The following results were obtained as shown in Figure 1 and Table 1.

**RESULTS**

It was surmised that patients with the 10 base pair repeat allele would have a correlation with ADHD positive participants. As seen in Figure 1, a p-value of 0.8282 was calculated using the Cochran-Armitage Trend Test. Using the odds ratio, a p-value of 0.8249 was calculated. This data suggests no significant association between the 10 base pair repeat allele of the 3’UTR and ADHD positive participants. However, there were a few limitations to this study that may have affected the results. One issue was the inability to test for the 6 repeat allele of intron 8. PCR conditions that were used in previous studies associated with the repeat in intron 8 were attempted in this study, but were unsuccessful. Annealing temperatures were altered and the concentrations in multiple different assays, neither which yielded the needed banding patterns for gel electrophoresis. An additional limitation to the study was the administration of the ASRS-V1.1. The study did not control for gender or pre-existing conditions. The scoring of the survey only allowed participants to be placed into two qualitative groups. Further investigation should be performed to establish whether or not a quantitative number can be derived from the survey. In the future, studies will be performed in an attempt to evaluate whether participants have a 6 repeat in tandem with the 10 repeat. The tandem repeat would provide further validity to the current findings. It would also be beneficial to evaluate the mature-mRNA products of the 10/6 haplotype to evaluate the possibility of an error in transcription or translation that may be causing the symptoms shown in ADHD.

**DISCUSSION**

Statistical analysis was performed using a Cochran-Armitage Trend test and an odd’s ratio. The Cochran Armitage Trend test was used and the aim is to assess for the presence of an association between a variable with two categories and a variable with K categories. This proved beneficial for the study because results were being compared between ADHD positive and ADHD negative participants when looking at homozygous dominant, recessive and heterozygous participants. An odd’s ratio was performed to verify the probability of each participant having ADHD given their genetic biomarkers found in gel electrophoresis.