EXPRESSION OF SLC6A3 IN PERSISTENT ADHD

Ryan D. Anderson (MLT) ASCP[CM], Lucas J. Waddoups (MLT) ASCP[CM],
David W. Merkley (MLT) ASCP[CM]
Mentor: Matthew Nicholau, DrPH (MT) ASCP[CM]

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ABSTRACT

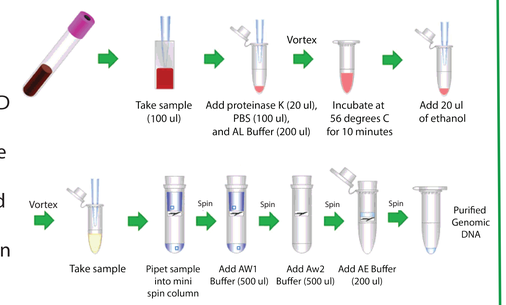
Attention Deficit Hyperactivity Disorder (ADHD) is a common neuropsychiatric disorder that begins in early childhood and is associated with impulsive behavior and inability to concentrate. Currently, ADHD diagnosis is based solely on survey responses provided by the patient. Without the definitive information 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 gene mutation in SLC6A3, specifically in sequences found in the 3' UTR and Intron 8 loci 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 no 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 (Garnier-Dykstra, Pinchevsky, Caldeira, Vincent, & Arria; 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.

METHODS

This study had a total of 84 participants. Forty-two participants met ADHD criteria and 42 were used for control. A consent form and an ADHD self-report scale was provided prior to obtaining their samples. Categorization of the samples into case or control groups was based on their responses to the Adult ADHD Self-Report Scale (ASRS-V1.1; see below for scoring criteria). Participants with four or more responses in the 'A' section of the survey were categorized as ADHD positive. Two samples of whole blood were collected from each participant. DNA was extracted from white blood cells using the Qiagen DNA extraction kit. The samples were put into microfuge tubes given by the kit. After which, AL buffer and Proteinase K were added to lyse the cells. Once the cells had lysed, ethanol was added to precipitate out the DNA. Samples were then transferred into a mini spin column where they underwent wash cycles with AW1 and AW2 buffer. Following the wash cycles, AE buffer was added to extract the now purified DNA. Analysis of the DAT1 intron 8 VNTR and 3' UTR VNTR was performed using the TaqmanTM Genotyping Master Mix. Taqman uses Polymerase Chain Reaction (PCR) to amplify the target region of the DNA. Gel electrophoresis was performed to verify which allele each participant had. A solution of two-percent agarose gel was prepared for electrophoretic migration. The samples were mixed with Coomassie blue to stain the DNA and make it visible on the gel. Following straining, samples were pipetted onto the gel and allowed to migrate in a 1X TEA buffer solution. A molecular weight standard, applied to the gel, was used to verify the size of the DNA. A blank sample with no DNA was also added to ensure purity of the samples added.



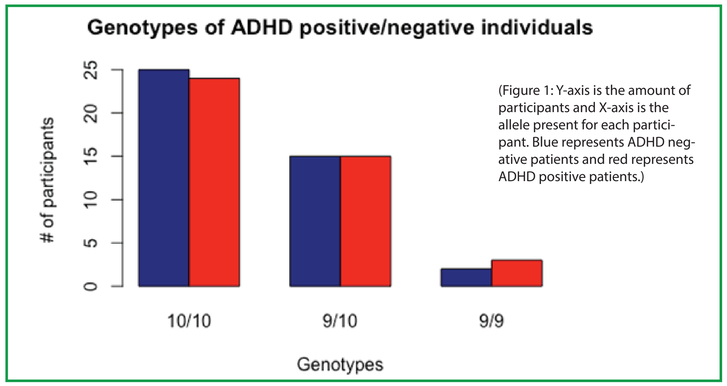
RESULTS

Results were found using R studio. For the 3'UTR participants were tested to see if they had the 9 or 10 allele present in their gene. The following results were obtained as shown in Figure 1 and Table 1.

	10/10	9/10	9/9	Sum
ADHD neg	25	15	2	42
ADHD pos	24	15	3	42
Sum	49	30	5	84

Odds ratio	0.9067
95 % CI:	0.3807 to 2.1594
z statistic	0.221
Significance level	P = 0.8249

(Table 1 results used to calculate p-value)



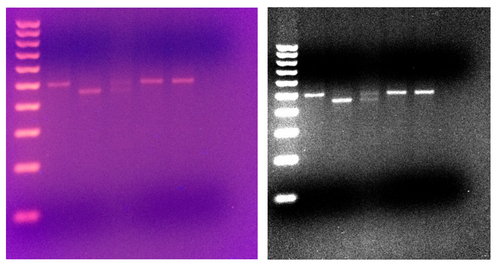
(Figure 1: Y-axis is the amount of participants and X-axis is the allele present for each participant. Blue represents ADHD negative patients and red represents ADHD positive patients.)

STATISTICAL ANALYSIS

Statistical analysis was performed using a Cochran Armitage Trend test and an odd's ratio. The Cochran Armitage Trend test is used when 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.

DISCUSSION

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 yielding the needed banding pattern 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 summary, 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.



3'UTR Gel Electrophoresis

Using a 2X3 contigency table a CATT score of 0.4615 was found (CATT=Cochran Armitage trend test) and a P-value of 0.8282. Results for intron 8 are not included due to problems with PCR and gel electrophoresis with the intron 8 sequence.

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Dumke College of Health Professions
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