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THE INFLUENCE OF GENETIC VARIANCE ON ATTENTION DEFICIT HYPER ACTIVITY DISORDER

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ABSTRACT

Genetics databases were reviewed to determine if there is a correlation between specific genes and the severity of attention deficit hyperactivity disorder (ADHD) because the condition affects the emotional, social, and academic performance of 2 to 18 % of children worldwide. Datasets from Gene Expression Omnibus Database were analyzed using Gene Spring software to compare gene variations and expressions between ADHD and non-affected individuals. Results showed an increase in the expression of CXCL8 and DDX6, while there was a decreased expression of RHOT1, RPS24, and TMEM59 in the ADHD patients. The differences in gene expressions of the genes suggests that the disorder may have genetic disorders.

Keywords: ADHD, CNVS, hyperactivity, disorder, hyperactive, attention deficit

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) manifests in childhood with symptoms of hyperactivity, impulsivity, and/or inattention. The symptoms affect cognitive, academic, behavioral, emotional, and social functioning (American Psychiatric Association, 2013). While more common in boys than girls, the reported prevalence in children varies from 2 to 18% depending on diagnostic criteria and the population studied (Boyle, 2011).

The pathogenesis of ADHD is not definitively known. A genetic imbalance of catecholamine metabolism in the cerebral cortex appears to play a primary role, as illustrated by functional brain imaging, animal studies, and the response to drugs with noradrenergic activity (Pliszka, 2007; Millichap, 2008; National Institute for Health and Clinical Excellence, 2008).

Several genes have been identified that appear to play a role in the development of ADHD including, dopamine D2, D4, and D5 receptor genes (DRD2, DRD4, and DRD5), serotonin transporter genes (SLC6A3 and SLC6A4), serotonin 1B receptor gene (HTR1B), dopamine beta-hydroxylase gene (DBH), synaptic associated protein 25 kDa (SNAP25), and glutamate receptors, metabotropic (GRM1, GRM5, GRM7, GRM8).

Copy Numbers Variations (CNVs) are prevalent structural genetic variations that involve DNA segments, spanning thousands to millions of bases, whose copy number varies between different individuals. These submicroscopic genomic differences in the number of copies of one or more sections of DNA result in DNA expression changes. CNVs may be associated with phenotypic effects, but some cases are clinically silent (Redon, 2006; Tuzun, 2005). In numerous studies, ADHD individuals have been found to have higher rates of CNVs than control subjects (Williams, 2010).

Neuroimaging has revealed smaller prefrontal cortical volume, reduced thickness of the anterior cingulated cortex, and cortical thinning in superior frontal brain regions of ADHD patients. In addition, ADHD patients have differences in the caudate nucleus, smaller cerebral and cerebellar volume, and smaller posterior corpus callosum regions when compared to non-ADHD patients. Functional brain imaging has also revealed reduced global activations in the basal ganglia and anterior frontal lobe. (Bush, 1999; Rubia, 1999; Rubia, 2005; Zang, 2005; Hart, 2013; McCarthy, 2014; Spencer, 2013).

Dietary factors generally do not impact behavior except in a small subset of children. However, prenatal exposure to tobacco is associated with development of ADHD, while association is inconsistent for prematurity, low birth weight, prenatal exposure to alcohol, and head trauma (Millichap, 2008; Barrett, 2007).

The genetic variants that are associated with ADHD have yet to be fully identified. Therefore, to predict the disorder, genetic databases were reviewed to determine if there is a correlation between specific genes and ADHD.

METHODS

Sample Acquisition

Subjects were obtained from an autism spectrum disorder mapping experiment during which the blood samples of individuals with different parts of the autism spectrum were collected and analyzed to see if there was a correlation between individuals on similar parts of the spectrum. Samples, selected based on ADHD status and other disease status, were marked as control or ADHD subjects. To ensure diversity of the samples, chosen individuals ranged in ages from 10 to 15 years old, were both male and female, and represented a multitude of races.

Input and organization of samples

Genespring 14.5, by Agilent of California, was used to organize and visualize the expression of the genes between the ADHD samples and the control samples. Once gene spring was opened, the tab to create a new experiment was clicked and the experiment type "Association by expression" was selected.

To organize the samples and the expressions of the genes, the Quick start guide under the Experiment setup was selected. The experimental group was called the "ADHD group" and the control group was the "Non-ADHD group." To compare the genes, the two groupings were organized to calculate the fold change values relative to the average expression in the Non-ADHD group.

Significance Analysis

The intensity values of the ADHD group were electronically compared with the expression of the Non-ADHD group and were organized in a volcano chart. To determine biological significance a fold change in the expression of the data was set to 2.0 making two green lines which signified the cutoff of the 2-fold change. The Interpretations section under the Experiment setup tab was then selected. A p value cutoff of .05 was selected, such that a 95% chance that the differences in the genetic makeup were associated with ADHD prevalence was considered statistically significant.

Fold Change Analysis

To determine significance, the Fold change was set to 2.0, meaning the average intensity of the genes in the ADHD group was found to be either twice as highly expressed or two times lower than the average expression of the gene in the Non-ADHD group. A fold change of 2.0 indicates a significant difference of expression between the subjects.

RESULTS

DDX6

Dead box helicase six (DDX6) is responsible for regulating the number of the P-bodies (Ayache, Bénard, Ernoult-Lange, 2015) which regulate excess mRNA. As expression of DDX6 increases, the number of p bodies decrease, allowing the excess mRNA to be produced and to disrupt normal neurological function (Ayache, Bénard, Ernoult-Lange, 2015). As displayed in Figure 1 the large change in expression between the two groups shows that DDX6 is heavily expressed

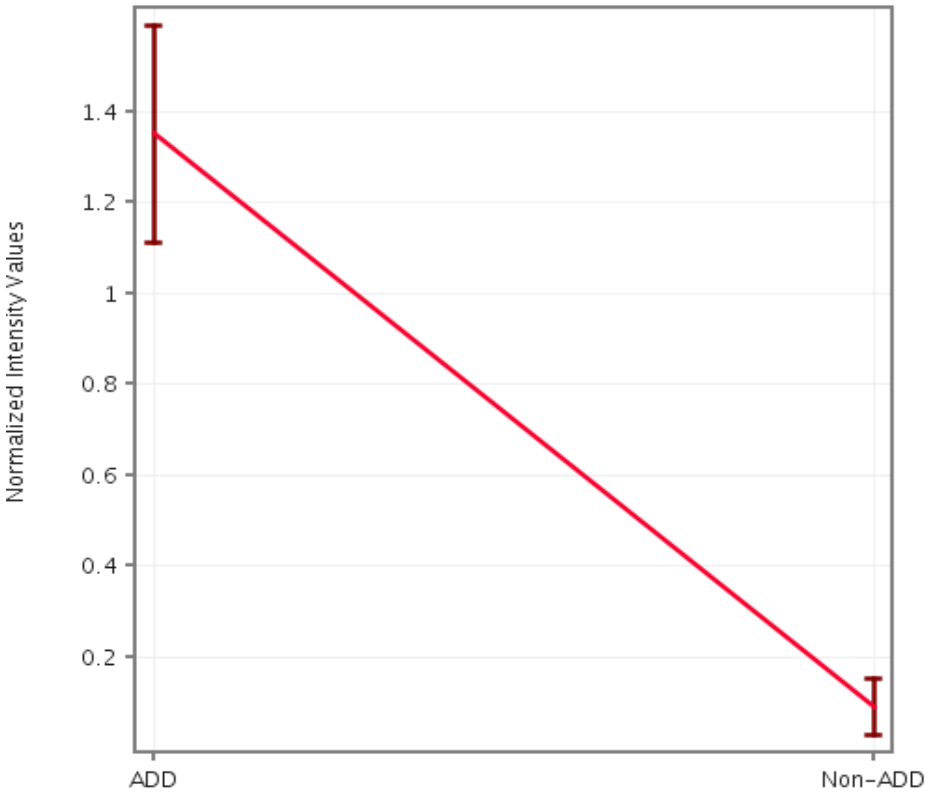

Figure 1 Intensity values of the DDX6 gene in the ADHD group was over a 2.0-fold change in expression compared to the non-ADHD group

CXCL8

CXC motif chemo ligand 8, also known as IL-8, is a major mediator of inflammatory response. As the expression of the gene increases, the activity of the microglial cells also increases. Action from IL-8 and hyperactivity of the microglial cells is apparent in diseases such as Alzheimer's (Birsa, N., Norkett, R., Wauer, T., Mevissen, 2014). As seen in Figure 2, the increased expression of CXCL8 is present in the ADHD group when compared to the non-ADHD group.

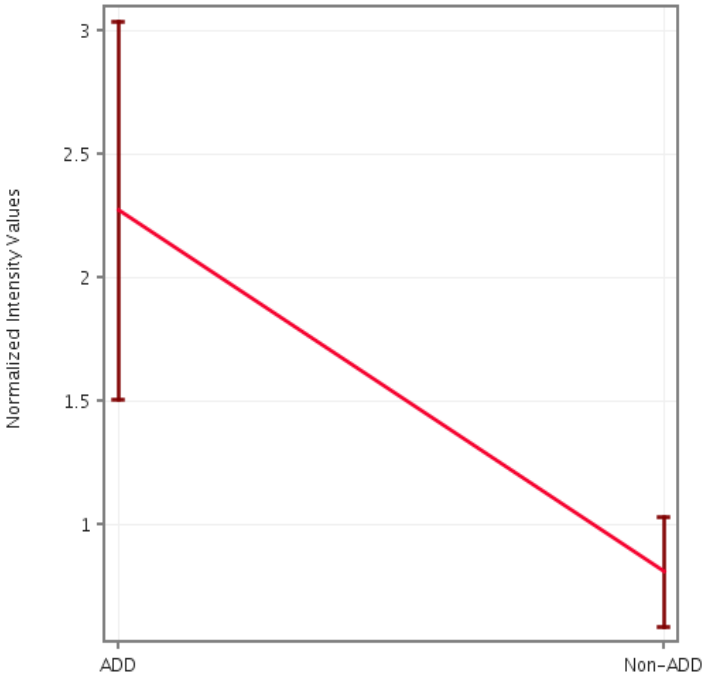

Figure 2 The expression value of CXCL8 has a 2.0-fold change in expression in the ADHD group compared to the non-ADHD group

RHOT1

Ras Homolog family member T1 (RHOT1), is a gene responsible for the removal of damaged mitochondria. When the gene is downregulated, the ability to remove damaged mitochondria is weakened (Schwarz, 2013). As displayed in figure 3, the expression of RHOT1 is twofold lower in the ADHD group than in the non-ADHD group.

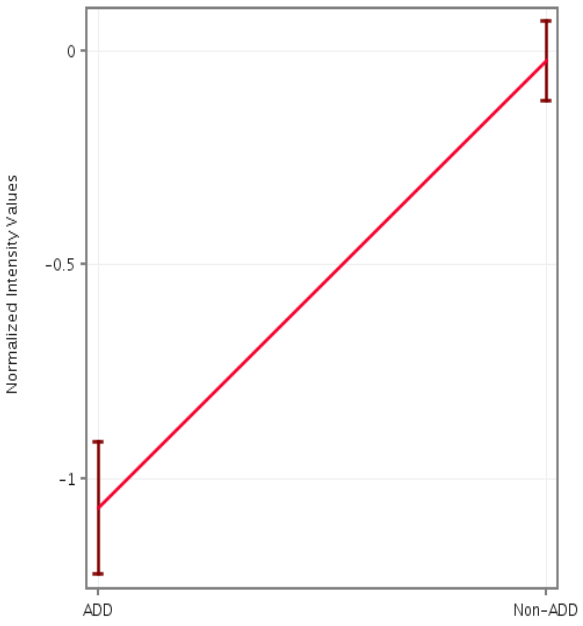

Figure 3 The intensity values for RHOT1 in the ADHD group are over 2.0-fold lower than in the Non-ADHD group

TMEM59

Transmembrane protein 59 is responsible for the regulation of amyloid precursor protein. As the expression of TMEM59 decreases, the chance of amyloid plaque formation increases (Ullrich, Münch, Neumann, Kremmer, 2010). In other neurological diseases, such as Alzheimer's, plaque formation has been linked to disruption of normal brain function. As shown in Figure 4, there was a threefold decrease in expression of TMEM59 in the ADHD samples compared to the non-ADHD samples.

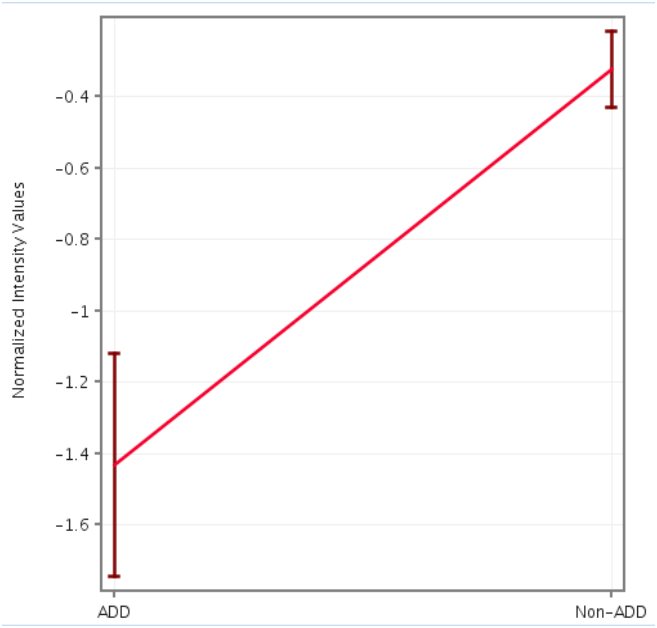

Figure 4 The intensity value of TMEM59 is 3.0-fold lower in the ADHD group than the Non-ADHD group

RPS24

Ribosomal Protein S24 (RPS24) has been linked to autism spectrum disorder. In cases where gene mutations result in complete non-function of the gene, the individual has a high chance of developing autism (Inoue, Watanabe, Egawa 2015). As shown in Figure 5, the gene has more than a twofold lower expression in the ADHD group than in the non-ADHD group.

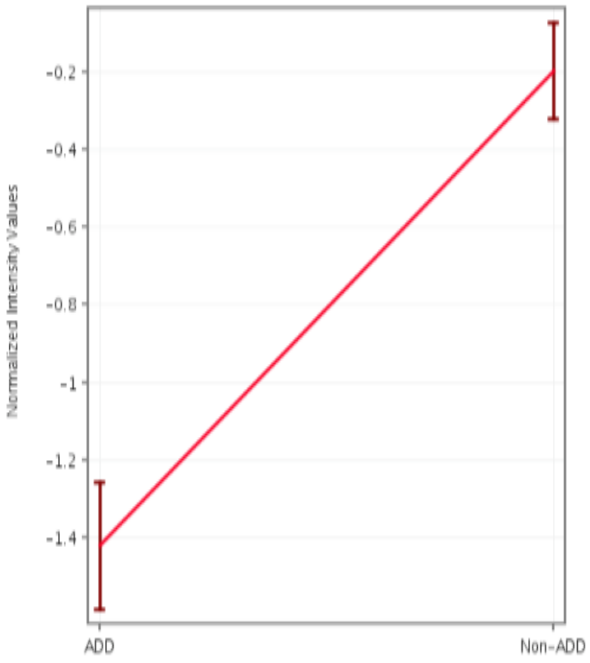

Figure 5 The intensity value of RPS24 is over 2.0-fold lower in the ADHD group when compared to the Non-ADHD group

CONCLUSION

DDX6

*T*he increased expression of DDX6 results in a decreased amount of P-bodies in the brain. These P bodies are needed to regulate the production of the excess mRNA bodies (Ayache, Bénard, Ernoult-Lange, 2015). However, as illustrated in Figure 1, due to the increased expression of DDX6, the P-body level is lower and may disrupt normal neurological function in the ADHD group.

CXCL8

As shown, CXCL8 is responsible for the activity of the microglial cells. As seen in Figure 2, there is an increased expression of the gene leading to a possible hyperactivity of the microglial cells in the brain (Birsa, N., Norkett, R., Wauer, T., Mevissen, 2014). Thus, the hyperactivity in the cells is possible due to the increased expression of IL-8 in the ADHD group. There was a large standard deviation of the data for expression of CXCL8, suggesting that although the general trend is for there to be an increased expression in ADHD patients, there is a large variation from patient to patient suggesting that ADHD may have different effects on different people.

RHOT1

RHOT1 is a gene responsible for the removal of damaged mitochondria. If the gene has decreased expression as displayed in the ADHD group from Figure 3, there can be difficulties with removing damaged mitochondria (Schwarz, 2013). The expression of the gene may result in the damaged mitochondria disrupting neurological function of the ADHD group, suggesting the gene could be used to identify ADHD

TMEM59

TMEM59 is responsible for the regulation of the formation of amyloid plaque. The decreased expression, as shown by the ADHD sample of Figure 4, it is possible for enough of the plaque to accumulate and disrupt neurological function, as seen in Alzheimer's patients (Ullrich, Münch, Neumann, Kremmer, 2010). The large standard deviation for the expression of the gene in the ADHD group suggests that the expression would vary from person to person. However, on average the expression was lower for individuals with ADHD than without ADHD.

RPS24

RPS24 is a gene that is commonly linked to Autism Spectrum Disorder. The level of effect the disorder has on an individual depends on the state of the gene expression. If there is a complete malfunction of the gene, then there is a high chance of the individual developing autism (Inoue, Watanabe, Egawa 2015). However as displayed in Figure 5, there is a decreased expression of RPS24 result in a disorder lower on the Autism spectrum, such as ADHD, to occur instead of autism as the gene still retains some function.

Future Research

There may be genes that can be used to determine an individual's ADHD status. However, more tests with a larger sample size should be conducted to confirm the relationships. Perhaps some information could be used to develop a diagnostic technique. The expression of some

genes had large standard deviations suggesting that varying levels of expression can result in different types of ADHD, such as hyperactivity and inattentiveness. Meanwhile, those genes with lower standard deviations could be used to identify if a patient has ADHD. Finally, ADHD could be linked to other disorders genetically by finding common changes in gene expression between disorders.

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