



Published in final edited form as:

Mol Psychiatry. 2018 August ; 23(8): 1787–1793. doi:10.1038/mp.2017.242.

Why is there Selective Subcortical Vulnerability in ADHD? Clues from Postmortem Brain Gene Expression Data

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Abstract

Sub-cortical volumetric differences were associated with attention-deficit/hyperactivity disorder (ADHD) in a recent multi-site, *mega*-analysis of 1713 ADHD persons and 1529 controls. Because there was a wide range of effect sizes among the sub-cortical volumes, it is possible that selective neuronal vulnerability plays a role in these volumetric losses. To address this possibility, we used data from Allen Brain Atlas to investigate variability in gene expression profiles between subcortical regions of typically developing brains. We tested the hypothesis that the expression of genes in a set of curated ADHD candidate genes and five *a priori* selected, biological pathways would be associated with the ENIGMA findings. Across the subcortical regions studied by ENIGMA, gene expression profiles for three pathways were significantly correlated with ADHD-associated volumetric reductions: apoptosis, oxidative stress, and autophagy. These correlations were strong and significant for children with ADHD, but not for adults. Although preliminary, these data suggest that variability of structural brain anomalies in ADHD can be explained, in part, by the differential vulnerability of these regions to mechanisms mediating apoptosis, oxidative stress, and autophagy.

Keywords

ADHD; autophagy; apoptosis; oxidative stress; neurodevelopment; brain volumes; transcript expression; imaging genomics; microarray

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Supplementary Information

Supplementary information is available at *Molecular Psychiatry*'s website.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder characterized by impairing symptoms of hyperactivity, impulsivity, and inattention. The prevalence is estimated at 5–7% of school-age children and 2.5–5% of adults.¹ Decades of research have documented environmental² and genetic¹ risk factors along with both structural and functional brain abnormalities.³ These data suggest that ADHD is a multifactorial condition caused by the confluence of many environmental and genetic risks that affect neurodevelopment and, ultimately, lead to symptoms of the disorder.¹ It is unknown, however, why some brain regions are impacted by environmental and/or genetic risks and others are not.

Structural magnetic resonance imaging (MRI) studies revealed small but consistent volumetric reductions in ADHD brains compared to those of typically developing subjects. Meta-analyses of structural MRI studies have reported area and volume reductions of cerebellar regions, the splenium of the corpus callosum, total and right cerebral volume, and the right caudate in ADHD children e.g.,⁴. Whole-brain voxel-based morphometry meta-analyses have consistently demonstrated gray matter reductions of the caudate, globus pallidus, and putamen in both children and adults with ADHD. There is also evidence for effects of pharmacological treatment and age: volumetric changes are more pronounced in people with ADHD who have not been treated with medications and these changes tend to diminish with increasing age.⁵

The ADHD subgroup of the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium investigated subcortical brain volumes in data from 23 cohorts with a total sample size of over 3,200 subjects, including children and adults (age range: 4 – 63 years, median = 14, 66% males).⁶ ENIGMA ADHD used both meta-analyses (relying on summary statistics) and mega-analyses (relying on raw data) to test for group differences in subcortical volumes. Mega-analysis demonstrated that ADHD probands had significantly smaller volumes for accumbens (Cohen's $D = 0.15$), amygdala ($D = 0.19$), caudate ($D = 0.11$), hippocampus ($D = 0.11$), and putamen ($D = 0.14$) when compared to typically developing individuals. Meta-analysis yielded similar results and same ordering of effect sizes, however there was no significant difference detected for the volumes of the hippocampus or accumbens between ADHD cases and unaffected comparison subjects in the meta-analysis. In both the mega- and meta-analyses, no significant differences were observed for volumes of the pallidum or thalamus. Subcortical volume differences were strongest for children, with no significant findings for adults. The subcortical volumes were not influenced by lifetime stimulant medication, presence of comorbid psychiatric disorders, or ADHD symptom severity.⁶

The ENIGMA ADHD results raise intriguing questions: Why are the different subcortical regions affected to different degrees? Is this evidence of selective neuronal vulnerability (SNV), i.e., the selective sensitivity of particular neuronal populations to stressors?⁷ Neurons present a wide variety of distinct morphological and functional characteristics reflecting the unique gene expression profile of each neuronal population. As a consequence, different neurons respond differently to damaging stimuli regardless of whether these stimuli derive

from genetic variants or environmental insults. There is evidence of SNV for neurodegenerative disorders. For example, neurons from the entorhinal cortex, hippocampus CA1 region, frontal cortex, and amygdala are the most sensitive to neurodegeneration in Alzheimer's disease. These neurons are selectively vulnerable to conditions such as energy deprivation and oxidative stress. Activating cell stress pathways accumulates beta-amyloid protein and tau-based tangles in these vulnerable neurons. In Parkinson's disease, dopaminergic neurons from the substantia nigra pars compacta are particularly vulnerable to mitochondrial dysfunction and elevated levels of reactive oxygen species. In Huntington's, striatal medium spiny GABAergic neurons (MSNs) are the main neuronal population affected by the mutant *Huntingtin*, possibly leading to mitochondrial dysfunction. The selective loss of the neuronal populations cited above are responsible in large part for the clinical manifestations observed in these neurodegenerative disorders.^{8,9}

SNV could explain why the ENIGMA study found some subcortical structures to show greater ADHD-associated volumetric loss than others. If so, understanding SNV in ADHD could point to biological pathways involved in the disorder. To assess this possibility, we tested hypotheses about the expression profiles of candidate genes and pathways in the subcortical structures evaluated by the ENIGMA ADHD Working Group. We, *a priori*, selected five pathways previously implicated in ADHD, which we hypothesized would be associated with SNV: neurotransmission regulation, oxidative stress, neurodevelopment, apoptosis, and autophagy.^{10–13} We also selected a set of ADHD candidate genes that had been proposed by a panel of ADHD experts and further implicated via GWAS.¹⁴

Materials and Methods

Identification of ADHD Candidate Genes and Pathways

For each pathway, we identified relevant genes by surveying gene sets made available through data sets curated by Ingenuity Pathway Analysis (IPA) (version 26127183, November-30–2015, <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/>) and the Molecular Signature Database (MSigDB) (version 5, August-15–2015, <http://software.broadinstitute.org/gsea/msigdb>). We downloaded the following biological pathways from IPA: (1) axon guidance signaling, (2) dopamine degradation, (3) dopamine receptor signaling, (4) GABA receptor signaling, (5) glutamate receptor signaling, (6) glutamate degradation, (7) noradrenergic/adrenaline degradation, (8) neuron signaling, (9) NRF2-guided oxidative stress response, (10) production of reactive oxygen species, (11) serotonin degradation, and (12) serotonin receptor signaling. In addition, we extracted similar gene sets and pathways available through MSigDB.

We combined gene sets from IPA and MSigDB and eliminated duplicate gene identifiers after merging discrete annotations into five biological pathways: (1) autophagy, (2) apoptosis, (3) neurodevelopment (*e.g.*, axon guidance, axonogenesis), (4) neurotransmitter regulation (*e.g.*, neurotransmitter release, receptor activity, and metabolism), and (5) oxidative stress (*e.g.*, production of reactive oxygen species, oxidative stress responses). The following 43 ADHD candidate genes comprised a gene set for a separate analysis: *ADRA1A*, *ADRA1B*, *ADRA2A*, *ADRA2C*, *ADRB2*, *ADRBK2*, *ARRB1*, *BDNF*, *CDH13*, *CHRNA4*, *COMT*, *CSNK1E*, *DBH*, *DDC*, *DRD1*, *DRD2*, *DRD3*, *DRD4*, *FADS1*, *FADS2*,

HES1, HTR1B, HTR1E, HTR2A, HTR2C, HTR3B, *MAOA*, *MAOB*, *NFIL3*, *NR4A2*, *PER1*, *PER2*, *SLC18A2*, *SLC6A1*, *SLC6A2*, *SLC6A3*, *SLC6A4*, *SLC9A9*, *SNAP25*, *STX1A*, *SYT1*, *TPH1*, and *TPH2*. These genes had been previously nominated as ADHD risk genes by the International Multisite ADHD Genetics (IMAGE) group.¹⁵ As a group, these genes were associated with ADHD in a meta-analysis of genome-wide association studies.¹⁴ These 43 ADHD candidate genes overlap with pathways for neurodevelopment, neurotransmitter regulation, and apoptosis, and provide additional genes that are not implicated by the remaining pathways.

Microarray Data Import and Management

We downloaded publicly available phenotypic and transcriptome-wide molecular data for typically developing human subjects released through the Allen Brain Atlas website (<http://www.brain-map.org>).¹⁶ We targeted microarray data of *postmortem* brain samples from six adult donors (24 years to 55 years) whose brain tissues were eligible for inclusion in the Allen Brain Atlas microarray survey as these subjects had no known psychiatric or neurological disorder, brain injury or disease, history of epilepsy, drug or alcohol dependence, prion disease, infectious disease, cancer deaths, chronic renal failure, on a ventilator for > 1 hour, or had a time of death > 30 hours. Microarray data were acquired with existing normalization and adjustment for batch effects included. Expression data conformed to the log₂-scale. We applied median summarization to collapse many-to-one mappings of probe sets into single estimates of gene expression within samples.

High resolution sub-cortical gene expression data were targeted for the following brain regions: (1) nucleus accumbens, (2) amygdalohippocampal transition zone, (3) body, head, and tail of caudate nucleus, (4) internal and external segments of globus pallidus, (5) bank and lateral bank of parahippocampal gyrus, (6) putamen, and (7) reticular nucleus of thalamus. Each of the sub-cortical regions that were dissected from adult samples included left and right hemispheres. The median expression of genes was derived across hemispheres and within micro-dissected sub-cortical structures to obtain an average spatial measure of gene expression across 7 regions (Supplementary Figure 1). Sample sizes for each brain region and the total number of genes detected in the Allen Brain cohort are provided in Supplementary Table 1. Note that we omitted samples from a separate Allen Brain Atlas cohort used for RNA-sequencing experiments with coverage of pre- and postnatal (perinatal to adulthood) ages, because those data had only a limited number of brain regions examined by ENIGMA. The number of genes overlapping between pathways is shown in Supplementary Table 2. We found that the normalized pathway expression levels within brain regions were highly correlated between the Allen Brain Atlas and two additional cohorts (Genotype Tissue Expression Project and Human Brain Transcriptome, Supplementary Figure 2).

Statistical Analyses

We compared the average effect sizes between the eight brain regions included in ENIGMA's meta-analysis⁶ using two-tailed *T*-tests. Results from our analysis are provided in Supplementary Table 3. The largest difference between regions was amygdala and pallidum ($P = 1.72e-04$). Despite there being small effect sizes overall, statistically

significant regional differences observed in the average ADHD brain suggests that this variability is meaningful and warrants further investigation.

Our primary analyses tested for an association between the average gene expression levels in each pathway and the magnitude of sub-cortical volumetric changes in ADHD participants as measured by the standardized mean difference (Cohen's D) as computed by the ENIGMA Consortium, correcting for age, sex, and total intracranial volume.⁶ Using microarray data from the Allen Brain Atlas and pathway-based annotations acquired from IPA and MSigD, we arrived at a single estimate of pathway expression per targeted brain region by calculating the mean expression of genes within pathways (\pm 95% confidence intervals). Pearson's correlation coefficients tested the association of pathway expression levels with volumetric changes in ADHD across sub-cortical regions. We adjusted *p*-values using the Bonferroni procedure to control for testing multiple pathways.

Gene set analysis with GWAS data for ADHD

We supplied summary statistics from the largest available GWAS of ADHD¹⁷ to the software MAGMA (v1.06)¹⁸ to perform a gene set enrichment analysis focusing on the six pathways evaluated in our study. The NCBI hg19 reference genome was used to map SNPs to genes with a \pm 20 kilobase window added to the start and end position of every gene. Gene significance scores were calculated by MAGMA based on the mean SNP significance. A linear regression model was then used to test for a difference in mean gene significance scores between genes inside and outside of a defined pathway. The regression models included covariates for minor allele frequency, SNP density, and linkage disequilibrium between genes.

Code availability

The code (*R* script) used to run the analysis is available from the authors upon request.

Results

Pathways Associated with Sub-Cortical Volume Changes in ADHD

For each pathway, Figure 1 plots the mean and 95% confidence intervals of the mean expression level against Cohen's D for each pathway along with the best fitting linear regression. We found large and significant correlations between Cohen's D (children and adults combined) and expression of three pathways: apoptosis, autophagy, and oxidative stress (Table 1A). The significance was strongest for the apoptosis pathway ($r = -0.953$, uncorrected *p*-value = $9.0E-04$, Bonferroni *p*-value = $5.4e-03$). These findings were the same when analyses were limited to the child subset (Table 1B). In contrast, we found no significant correlations between pathway expression levels and Cohen's D effect sizes for adults with ADHD (Table 1C). We performed a *post hoc* analysis by combining expression values for genes in apoptosis and autophagy pathways, in addition to apoptosis, autophagy, and oxidative stress pathways. The combined pathways showed significant correlations with Cohen's D effect sizes for adults or children with ADHD (apoptosis + autophagy: $r = -0.942$, *p*-value = $1.5e-03$; apoptosis + autophagy + oxidative stress: $r = -0.942$, *p*-value = $1.5e-03$).

Given these findings, we then performed a *post hoc* test for each of 704 genes constituting the statistically significant pathways for the combined Cohen's D effect sizes for subcortical volume differences in children and adults with ADHD. We identified two genes that remained statistically significant after correcting for testing 704 genes (Figure 2): *TNFRSF1A* (Pearson's $r = -0.986$, p -value = $3.84e-05$, Bonferroni p -value = 0.027) and *MGMT* (Pearson's $r = -0.983$, p -value = $6.67e-05$, Bonferroni p -value = 0.046). None of the 704 genes examined were significantly correlated with subcortical volume changes in children with ADHD after multiple testing correction. The correlation of *TNFRSF1A* with Cohen's D estimates for children with ADHD was consistent in direction and nominally significant before correction ($r = -0.945$, uncorrected p -value = 0.0014, Bonferroni p -value = 0.95). However, the top ranking gene by correlation with subcortical volume differences in children with ADHD was *HMOX1* ($r = -0.975$, uncorrected p -value = $1.9e-04$, Bonferroni p -value = 0.13).

Pathways Not Enriched with GWAS Signals for ADHD

We observed no significant enrichment of GWAS associations among genes contained in the apoptosis gene set from our analysis with MAGMA (Beta = 0.198, $SE = 0.164$, uncorrected p -value = 0.56). In total, none of the pathways we examined showed a significant enrichment of GWAS signals for ADHD (Supplementary Table 4).

Discussion

Our analyses support our hypothesis that pathways mediating apoptosis, autophagy, and oxidative stress might play a role in the variability of volumetric differences between people with and without ADHD. These findings, albeit preliminary, suggest that normal variability in these pathways may regulate sensitivity to the genetic and environmental events that lead to ADHD. Because we lack gene expression and brain volumetric data from the same samples, we cannot infer whether pathways are up- or down-regulated between people with and without ADHD. Because the significant correlations we reported cannot be interpreted as causal relationships, experimental work in animal models investigating the functional role of genetic variants linked with brain volume regulation is needed. Ideally, studies of ADHD brain tissue would be used to confirm these results but such data are not possible to obtain because tissue resource centers have not made ADHD a focus of collection efforts. Therefore, we caution against extrapolating our findings from "normal" brain tissue to the ADHD brain.

Pathways mediating oxidative stress, autophagy, and apoptosis showed the strongest correlations with the magnitude of volumetric anomalies associated with ADHD. We had selected these pathways *a priori* based on prior studies of ADHD (see Introduction). These findings suggest that the expression of genes and environmental factors regulating programmed cell death may mediate risk for ADHD and that their differential effects on subcortical regions are due to regional differences in susceptibility to these effects.

The gene expression results cannot parse genetic and environmental sources of SNV. To get some leverage on this issue, we tested for significant enrichment of common variant GWAS signals for ADHD among the gene sets examined in this study. These analyses were not

significant, which suggests that genetic sources of SNV may be limited or lie in rare variants. Consistent with this, according to the LD Hub database, the genetic correlations between ADHD the subcortical volumes for brain regions examined in this study are low and not significant.¹⁹ Estimates of genetic correlations produced by LD Hub are based on effect sizes of common variation from GWAS. Taken together these finding suggest that future work should investigate environmental risk factors as a source of SNV in subcortical structures. Consistent with this, a study of monozygotic twins discordant for ADHD found exhibit epigenetic differences among genes highly expressed in the developing cerebellum, striatum, and thalamus, in addition to genes functionally related to neurodevelopment and neurotransmitter regulation.²⁰

We found lower expression of oxidative stress pathway genes in regions with greater ADHD-associated volumetric loss. This may indicate that regions which are *a priori* less able to process reactive oxygen species (ROS) are more susceptible to their effects. ROS cause cellular damage through oxidation of critical cellular components, such as enzymes, membrane lipids, and DNA.^{21, 22} To avoid such damage, the enzymatic antioxidant system within cells maintains a low concentration of ROS by generating reducing agents and ROS-scavengers, such as glutathione and ascorbic acid.^{22, 23} Because the brain metabolizes over 20% of the body's oxygen and does not contain a robust antioxidant system, it is disproportionately susceptible to ROS damage.^{21, 24}

Our hypothesis about oxidative stress had been motivated by a meta-analysis that found a significant association between ADHD and peripheral measures of oxidative stress that could not be accounted for by publication biases.¹⁰ Because the association between ADHD and peripheral antioxidant levels was not significant, the authors concluded that patients with ADHD have normal levels of anti-oxidant production but that their response to oxidative stress is insufficient, leading to oxidative damage. Indirect evidence for oxidative stress in ADHD also comes from studies showing treatment efficacy for anti-oxidant compounds e.g.,^{25, 26, 27}. Copy number variants associated with ADHD are also significantly enriched for pathways relevant for ROS regulation.¹² Increased oxidative stress has been found in the cortex, striatum, and hippocampus of established animal models of ADHD (spontaneously hyperactive rats).²⁸

Cellular stressors, such as ischemia, excitotoxicity, ROS, reduced cell nutrient availability and decreased growth factor signaling, cause macromolecular and organelle damage and increase the production and aggregation of misfolded proteins. These stressors initiate autophagy to remove damaging molecules and provide cellular nutrients.²⁹ Autophagy ("self-eating") is a bulk degradative, largely pro-survival pathway³⁰. It degrades cellular components, such as proteins and mitochondria, through the endo-lysosomal system. Through this mechanism it protects cells from nutrient and growth factor deprivation, protein aggregation, apoptotic signaling and damaged cellular organelles.²⁹ Its proper function is critical and considered a necessary homeostatic mechanism for neuronal development and function.^{31, 32}

The rate of autophagy during development modulates the proliferation and differentiation of neural progenitor cells³³ and is instrumental in regulating neuronal size.⁷ Thus, neurons with

decreased autophagic function are more susceptible to cellular stressors^{34, 35} and are likely to have perturbations in size and morphology.³³ Consistent with this, our finding suggest that low expression of autophagic genes is associated with greater ADHD-associated loss in regional sub-cortical brain volumes.

Apoptosis is a programmed cellular death pathway that indiscriminately degrades cellular proteins and causes cell shrinkage and pyknosis.³⁶ This orderly removal of cells does not cause inflammation to nearby tissue³⁷ and is critical during development.³⁶ In neurons, apoptotic molecules play an important role in synaptic scaling and pruning.^{38–41} Apoptotic dysregulation has been associated with attention disorders^{41, 42} and is regulated by methylphenidate.¹¹ Lo et al.⁴¹ hypothesized that lower apoptotic signals in attention disorders cause a failure in synaptic scaling and thus information processing. They further suggested that decreased apoptotic pathway expression leads to decreased neuronal downscaling and thus overstimulation of neuronal networks when processing novel stimuli. Consistent with this, we found gene expression in apoptotic pathways to correlate with ADHD-associated decreases in regional sub-cortical brain volumes.

Autophagy and apoptosis act together to determine the fate of cells.^{43–46} However, the biological context is important, as they can activate, antagonize or assist one another in their activities.^{30, 45, 46} Thus, it is not surprising that these pathways are co-regulated. Since autophagy acts to regulate apoptotic signals,⁴⁷ decreased expression of critical autophagic proteins, such as Beclin-1 and other autophagy genes, would make cells more sensitive to apoptosis.^{30, 46} Moreover, autophagy is upregulated by ROS and protects against its cytotoxic effects.^{24, 48–51}

Lower autophagic gene expression could predispose neurons to apoptotic cell death in critical brain regions and lead to the region-specific volumetric reductions we found in the ADHD-ENIGMA study. Lower autophagic expression would make neurons more susceptible to ROS, due to the lack of appropriate defective protein degradation and mitochondrial turnover.^{21, 24} In addition, decreased autophagy would disinhibit apoptotic signaling, further adding to selective neuronal vulnerability.⁴⁶ Because ROS, autophagy and apoptosis are co-regulated in a compensatory fashion, it is especially notable that all were implicated in our study. In addition, significant overlaps between apoptosis and autophagy, and apoptosis and ROS gene sets were found, which might explain why all three pathways were significant in our analysis.

Additional analysis of individual genes in the apoptosis, autophagy, and ROS pathways identified the *TNFRSF1A* gene as significant after correcting for multiple comparisons. *TNFRSF1A* encodes the tumor necrosis factor receptor 1 (TNFR1), one of the major receptors for the pro-inflammatory cytokine TNF. The activation of the TNFR1 receptor results in the formation of two opposing signaling complexes depending on the circumstances upon which it occurs. While Complex I regulates the expression of anti-apoptotic proteins, Complex II triggers cell death processes.⁵² As a result of complex downstream signaling processes, TNF presents both neuroprotective and neurodegenerative effects that varies according to brain regions.⁵³ Consistent with our findings, a neuroimaging study on typically developing adults reported significant associations between *TNFRSF1A*

polymorphisms and volume of the caudate and putamen. However, there was no evidence of association with hippocampal volume. *TNFRSF1A* is a genome-wide significant locus for multiple sclerosis⁵⁴, mothers with multiple sclerosis are at risk for having ADHD children⁵⁵ and diffusion tensor imaging suggests that aberrant myelination is associated with ADHD.⁵⁶ Thus, *TNFRSF1A* should be considered for further study in ADHD.

The *MGMT* gene, which encodes O-6-Methylguanine-DNA methyltransferase, was the only other gene that showed a strong correlation with ADHD brain volumetric differences after multiple testing correction. The enzyme encoded by *MGMT* protects cells from DNA damage caused by alkylation, which is significant for maintaining cell integrity.⁵⁷ No literature on *MGMT* relationship to ADHD has been found, however, it would be intriguing to explore the relationship in the context of DNA-damage repair and ADHD susceptibility.

The correlations supporting our conclusions were strong and significant for children with ADHD, but not for adults. This pattern is consistent with the original ENIGMA report which found significant case-control volumetric differences for children but not adults.⁶ One explanation for these findings is that environmental risk factors early in development (e.g., anoxia, maternal smoking) lead to increased oxidative stress, autophagy and apoptosis which, in turn, lead to reduced brain volumes. If the molecular defenses against these risk factors are upregulated and persist through development, it is possible that they slowly lead to a reduction in oxidative stress and, in some patients, a normalization of brain volumes. Consistent with this hypothesis, a meta-analysis found that the association between ADHD and peripheral measures of oxidative stress was significant for children but not adults.¹⁰

Our results, albeit intriguing, are limited in several ways. We cannot make causal statements about the relationship of expression levels and volumetric changes as the two sources of data were from different samples. In addition, gene expression studies do not provide evidence about protein functioning. For example, a gene with low transcript number does not necessarily have decreased function, as post-translational modification or other pathway modifiers can increase activity. The Allen Brain data are based on a very small sample of typically developing adults that will not generalize to all populations of interest. Also, expression data for the pallidum was not available in the Allen Brain Atlas. Instead, we used data for the globus pallidus which, with the ventral pallidum, forms the larger pallidum examined in the ENIGMA ADHD study.

Despite these limitations, our work is the first to propose a mechanism to explain the differential vulnerability of brain regions to the as yet unknown mechanisms that cause ADHD. This leads us to further hypothesize that future etiologic studies of ADHD will implicate genetic and environmental risk factors implicating pathways regulating oxidative stress, autophagy and apoptosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Dr. Faraone is supported by the K.G. Jebsen Centre for Research on Neuropsychiatric Disorders, University of Bergen, Bergen, Norway, the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 602805, the European Union's Horizon 2020 research and innovation programme under grant agreement No 667302 and NIMH grants 5R01MH101519 and U01 MH109536-01. Dr. Akutagava-Martins' contribution was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil.

Disclosures

In the past year, Dr. Faraone received income, potential income, travel expenses and/or research support from Lundbeck, Rhodes, Arbor, Pfizer, Ironshore, Shire, Akili Interactive Labs, CogCubed, Alcobra, VAYA Pharma and NeuroLifeSciences. With his institution, he has US patent US20130217707 A1 for the use of sodium-hydrogen exchange inhibitors in the treatment of ADHD.

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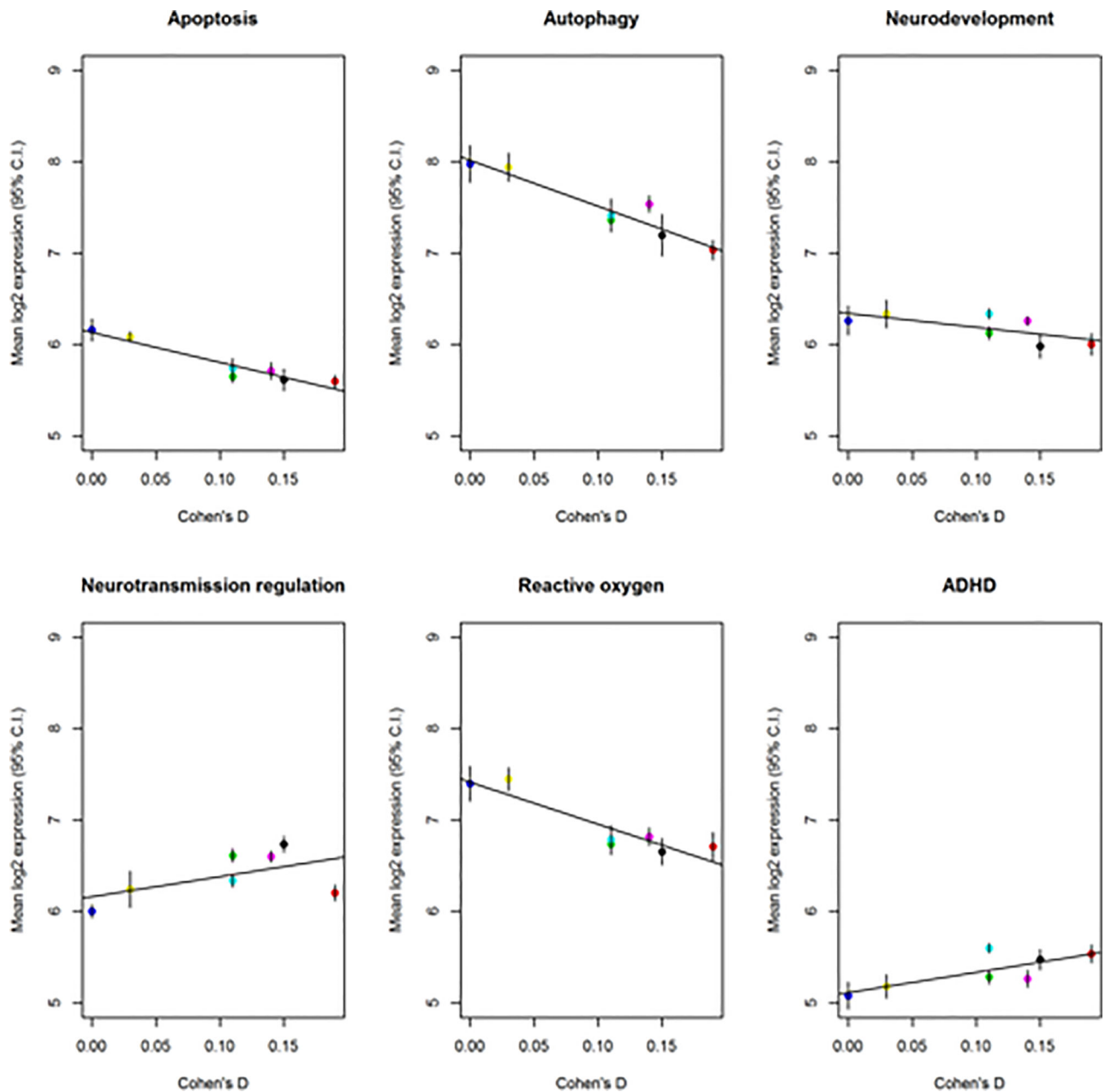


Figure 1: Comparison of pathway expression levels and the Cohen's d effect sizes from ENIGMA's ADHD vs. control comparisons of subcortical brain regions. Points correspond to mean expression of pathway with 95% confidence intervals indicated by vertical bars. The best fitting regression is denoted by a red line. The Cohen's d for each brain region are as follows: (1) pallidum = 0.00, (2) thalamus = 0.03, (3) hippocampal formation = 0.11, (4) caudate = 0.11, (5) accumbens = 0.15, (6) putamen = 0.14, and (7) amygdala = 0.19.

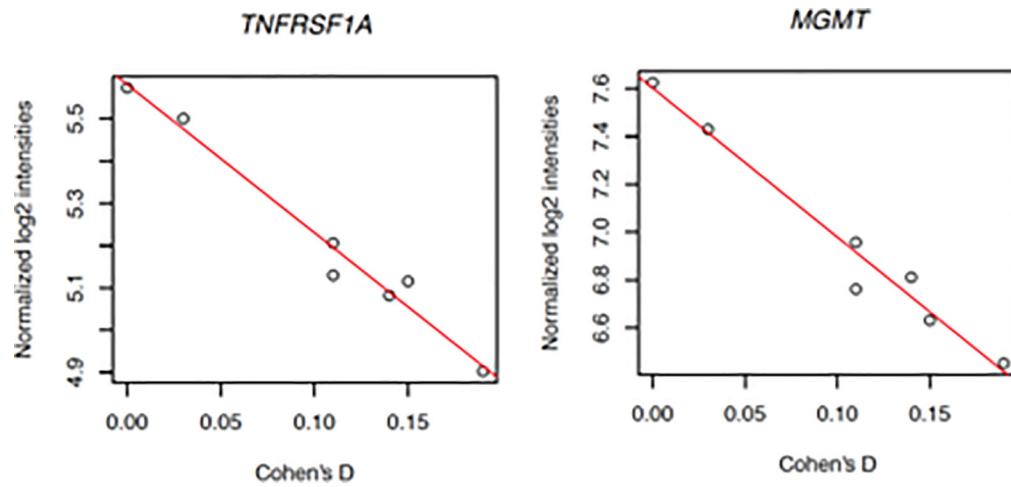


Figure 2:

A strongly negative correlation of *TNFRSF1A* expression and Cohen's d was identified in adult brain samples (Pearson's $r = -0.986$, p -value $< 3.84e-05$, Bonferroni p -value < 0.027). A similar correlation was found for *MGMT* with the same samples (Pearson's $r = -0.983$, p -value $= 6.67e-05$, Bonferroni p -value $= 0.046$). A total of 704 genes were examined across pathways for apoptosis, autophagy, and oxidative stress.

Table 1A.

Correlation of Sub-cortical Volumetric Changes in ADHD with Pathway Expression Levels across Seven Brain Regions in Adult Microarray Samples.

Pathway label	Pearson's <i>r</i>	p-value	Bonferroni p-value
ADHD candidate genes	0.771	4.22E-02	0.253
Apoptosis	-0.953	9.00E-04	0.0054
Autophagy	-0.948	1.20E-03	0.007
Neurodevelopment	-0.676	9.52E-02	0.57
Neurotransmitter regulation	0.557	1.94E-01	1.0
Oxidative Stress	-0.915	3.90E-03	0.0243

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Table 1B.

Correlation of Sub-cortical Volumetric Changes in Children with ADHD with Pathway Expression Levels across Seven Brain Regions in Adult Microarray Samples.

Pathway label	Pearson's <i>r</i>	p-value	Bonferroni p-value
ADHD candidate genes	0.688	0.87	0.52
Apoptosis	-0.953	0.00086	0.0052
Autophagy	-0.899	0.0059	0.036
Neurodevelopment	-0.662	0.11	0.63
Neurotransmitter regulation	0.699	0.08	0.48
Oxidative stress	-0.949	0.0011	0.0066

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Table 1C.

Correlation of Sub-cortical Volumetric Changes in Adults with ADHD with Pathway Expression Levels across Seven Brain Regions in Adult Microarray Samples.

Pathway label	Pearson's <i>r</i>	p-value	Bonferroni p-value
ADHD candidate genes	0.584	0.17	1.00
Apoptosis	-0.676	0.095	0.54
Autophagy	-0.792	0.033	0.20
Neurodevelopment	-0.761	0.047	0.28
Neurotransmitter regulation	0.226	0.626	1.00
Oxidative stress	-0.579	0.173	1.00

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