The relationship between type 1 hereditary hemochromatosis and ADHD symptoms within adolescents using mental health assessments and neuroimaging

Author: Cherisse Tompkins**,** University of California San Diego, Cognitive Science Department, Honors Program 2020-2021

Defend date: June 11, 2021

Committee: Dr. Mary ET Boyle, Dr. Lisa Delano-Wood, Robert Loughnan

Abstract

Hereditary hemochromatosis (HH) is an autosomal recessive genetic disease that leads to excess iron absorption from the diet causing inflammation. HH was used as a model disease in this study to look at inflammation within the body caused by iron. Previous studies have found associations between attention-deficit-hyperactive-disorder (ADHD) and inflammation, but it is unclear as to what the source of inflammation is. HH symptoms typically begin displaying in middle-age since iron accumulates with age, making the disease difficult to diagnose early. In this study, we investigated whether inflammation from excess iron in adolescents with HH could manifest as increased ADHD symptoms compared to healthy controls (adolescents with no genetic markers for HH) or similarities in T2 weighted intensities for specific brain regions compared to adolescents with ADHD. Adolescent participant data was accessed from the Adolescent Brain Cognitive Development (ABCD) database and individuals were parsed into separate groups if they were homozygous recessive (+/+), heterozygous (+/-), or a healthy control (-/-) for a specific HH gene (HFE). Our data illustrated that there is no significant effect of inflammation caused by iron on ADHD symptoms or brain regions implicated in ADHD. Although no associations were discovered between ADHD symptoms or ADHD brain abnormalities for individuals with type 1 HH, the inflammation from iron can affect participants' brains at this early stage as there was a significantly lower T2 intensity in the putamen for individuals with HH compared to controls. We discovered that early manifestation is possible in a disease typically diagnosed from the ages of 40-60 years. Comparative neuroimaging studies can give us a greater insight into how inflammation can affect the brain and body from adolescence to old age.

Introduction

Hereditary hemochromatosis (HH) is the most common autosomal recessive disorder in Caucasians, but not very well known to society (Porter, 2021). There are different types of HH which develop as a result of different mutated genes: HFE/C282Y, HAMP, TRF2, HJV, H63D, SLC40A1, HFE2, BMP2, BMP6 (Pantopoulos, 2018 & Brissot, 2018). Any type of HH leads to an increased absorption of iron from the diet into the body. The excess iron that is absorbed is stored in the body's vital organs like the liver, pancreas, and heart (Porter, 2021). Too much iron storage in these organs causes inflammation (Wessling-Resnick, 2010). This increased inflammation can lead to the development of metabolic diseases such as diabetes mellitus,

cardiovascular disease, liver disease, and even neurodegenerative diseases later in life (Basuli, 2014 & Gerlach, 1994). Classic hemochromatosis (type 1) results due to a homozygous recessive mutation of the HFE gene (+/+) which affects hepcidin levels (Pantopoulos, 2018). The mutation on the HFE gene is commonly noted as C282Y because at position 282 a cysteine (C) becomes a tyrosine (Y) which leads to a disruption in the formation of a disuflide bond in the HFE protein which is a major histocompatibility (MHC) protein on the cell surface (Hollerer, 2017). This disruption hinders the protein from binding to B2-microglobulin, and the HFE protein cannot reach the cell surface causing impaired signaling which leads to a reduced amount of mRNA expression of hepcidin (Hollerer, 2017). The decreased expression of hepcidin leads to increased systemic iron accumulation because hepcidin is an iron regulatory hormone (Rossi, 2005).

Previous research has illustrated that type 1 HH largely affects individuals of European ancestry with a prevalence ranging from 1 in 300-500 individuals (Porter, 2021). There are additional versions of HH including type 2 which is also known as juvenile hemochromatosis because symptoms typically show up before the age of 30, however the mutated gene(s) (HJV, HAMP) are extremely rare with an allele frequency of 0.000316-0.00074 (Piperno, 2020). We were unable to find any participants with this rare genotype in the population of adolescents we had access to, and therefore could not further investigate type 2 HH.

Type 1 HH is often not diagnosed until middle age (40-60 years) as the typical symptoms do not begin to present until later in life, which is a result of the accumulation of iron as we age. Symptoms include diabetes mellitus, liver cirrhosis, fatigue, hyperpigmentation of the skin, arthropathy (joint pain without joint destruction), hypogonadism (impotence), and heart failure (Porter, 2021).

ADHD is a very common hereditary neurodevelopmental disorder that affects attention, motivation, and hyperactivity, but also has very high comorbidity rates with depression, anxiety and mood disorders, and substance use disorders (Wilens & Spencer, 2010). It is largely diagnosed in children, as symptoms are distinguishable at a relatively young age, but diagnoses can continue into adolescence and young adulthood as ADHD is a chronic disorder with impairment spanning into adulthood (Wilens & Spencer, 2010). Symptoms include restlessness, occasional aggression, temper tantrums, inability to pay attention or focus on a single task for very long, overly chatty, hyperactivity, and rebelliousness in multiple different settings (American Psychiatric Association, 2013).

Associations between inflammation and ADHD have been discovered through observational studies showing strong comorbidity of ADHD with inflammatory or autoimmune disorders as well as evidence of increased inflammation during early development (Leffa, 2018). It is still unclear where the inflammation within ADHD individuals stems from. In this study, we planned to investigate the effect of being

homozygous recessive (+/+) for the HFE gene on the outcome of several mental health assessments highly correlated with ADHD diagnoses and symptoms (Palmer, 2020).

In addition, we also looked at the effect of being homozygous recessive (+/+) for the HFE gene on other mental health assessments for depression, anxiety, PTSD, psychosis, bipolar disorder, as well as intelligence measures.

ADHD research has also shown some brain abnormalities in certain regions of the brain including the caudate, hypothalamus, putamen, pallidum, cerebellum, and hippocampus (Hoogman, 2017 & Stoodley, 2016). We planned to analyze T2 weighted brain images of adolescents with HH to investigate whether they had any similar brain abnormalities. T2 weighted imaging was chosen as it is one of the most effective ways to visualize iron in the brain: lower T2 intensity, higher iron levels (Drayer, 1986). Additionally, recent research discovered that in older individuals (40-70 years old), T2 intensity abnormalities can be seen in the putamen and thalamus (Atkins, 2021).

There has been some research on type 1 HH and its association with metabolic diseases and neurodegenerative diseases later in life, but not a lot of research looking at the disease in adolescence (9-18 years). This could be because the disease is difficult to diagnose at this age without specific genetic information, and that the iron may not have accumulated enough to disrupt the body and produce symptoms. However, we wanted to see if the iron absorbed as a result of type 1 HH could disrupt the body and brain at this early age. Studies have looked at markers for inflammation and ADHD, but not specifically iron as a marker for inflammation. And while studies have been conducted that discovered brain abnormalities in patients with type 1 HH mutations, it is typically done on patients aged 40-70 years (Atkins, 2021). Through this study we expect to see that having type 1 HH is positively correlated with ADHD symptoms, and that the brain regions implicated in ADHD are similarly implicated in adolescents with type 1 HH.

Methods

Subjects: Adolescent participant data was collected from the Adolescent Brain Cognitive Development (ABCD) database. The ABCD study is a longitudinal study following adolescents aged 9-10 years old for ten years. The ABCD database holds genetic information, neuroimaging data, and mental health assessment data for 11,878 adolescents across 21 different sites in the United States. Within this research paper, only baseline data was analyzed.

Because this disease is better characterized in a European ancestry pool, we controlled for this in our analysis by retaining only those with European genetic makeup (European Genetic Ancestry Factor (EUR-GAF) > 0.8) (N= 5,327). This is an important step as allele frequency can differ across different ancestral groups and could lead to false results. Family relatedness was not taken into consideration for this reduction in sample size, and therefore singletons were not selected.

From there it was identified that 4,542 individuals were healthy controls (-/-), 663 were heterozygous (+/-), and 16 were homozygous (+/+) for the HFE gene (Table 1). Heterozygous individuals were identified so as not to include them in the healthy control sample when comparing between controls and homozygous individuals, but tests comparing heterozygous and homozygous or heterozygous with controls were not conducted as the penetrance of HH in heterozygous individuals is only 3% (Hollerer, 2017), and past studies haven't seen much of an effect on iron levels from having only one mutated HFE allele (+/-).

HFE Genotype	**Percentage**	**Total Number**
Control (-/-)	87%	4542
Heterozygous (+/-)	12.7%	663
Homozygous (+/+)	0.3%	16

Table 1: Breakdown of the genotype groups analyzed in this study. Shows the total number of adolescent participants for each genotypic category once ancestry, age, sex, top ten principal components, household income, and higher education have been controlled for.

ABCD Mental Health Battery: Within ABCD, there is a large battery of questionnaires and somewhat structured interviews to assess diagnostic and dimensional measures of substance use, impulsivity, psychosis, activation and prosociality, measures of mania, psychopathology, and behavioral inhibition. Responses are taken from both the adolescent participants and their parent/guardian or caregiver at baseline. Within this paper, only mental health assessments highly correlated with ADHD diagnoses or symptoms were utilized: lack of planning, fluid intelligence, crystalized intelligence, rule breaking, total problems, thoughts, somatic issues, social skills, externalizing problems, inattention, aggression, reward, fun-seeking, drive, a diagnostic oppositional conduct disorder questionnaire , and a diagnostic ADHD questionnaire (Palmer, 2020).

Analysis: Generalized Linear Models (GLMs) were fit in order to predict behavioral phenotypes based on homozygosity through the HFE SNP (rs1800562). All models controlled for fixed covariates such as sex, age, top 10 principal components of genetic analysis, and ABCD study site. Models run for the mental health assessments correlated with ADHD additionally controlled for household income and higher education. Models run on the T2 weighted brain images controlled for an MRI scanner ID to ensure that differences between MRI scanners did not sully the results.

Once participant genotype information was collected, univariate models, which include one independent variable (IV) of interest (HFE Genotype or ADHD) were fit for each behavioral phenotype or T2 weighted brain scan (dependent variable, DV). For the T2 weighted brain regions, the left and right hemisphere were averaged.

Each dependent variable was z-scored so coefficients could be interpreted in units of standard variation. The distribution of every DV fell into three possible categories: normal, right skewed or zero inflated, or binary. Each distribution was modelled appropriately. Normal distributions were

further normalized through rank normalized, and GLMs were fit using the default gaussian family. Right skewed, zero inflated distributions were fit using a gamma distribution with a log link function to make certain that each distribution was non-negative therefore ensuring the correct bounds for the link function. Binary variables were fit using a binomial log link function. Some of the mental health assessments: Kiddie Schedule for Affective Disorders and Schizophrenia for DSM-5 (KSADS-5) symptom scores were not stable as a continuous measure (models did not converge), so they were treated as binary.

Results

Generalized linear models (GLMs) were used to predict each behavioral phenotype from participant HFE Genotype and covariates of no interest. The univariate models contained a single genetic predictor (HFE Genotype). This approach permitted the identification of behavioral variance associated with this particular genetic predictor, when controlling for all other measures of genetic risk. A forest plot showing the effect of the homozygous HFE genotype on behavioral phenotypes are shown in Figure 1.

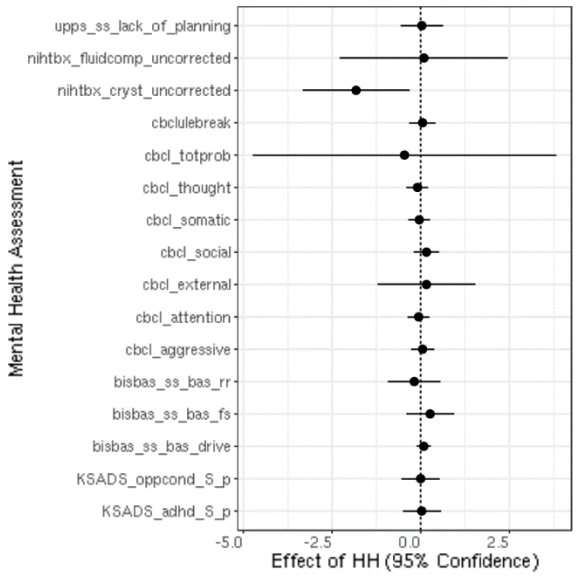

Figure 1: Forest plot showing the effect of HFE (+/+) genotype on behavioral phenotypes correlated with ADHD. The effect shown in this plot for crystalized intelligence (nihtbx_cryst_uncorrected) looks nominally significant but does not pass the multiple comparison correction.

The low beta (□) values and large regions of error indicate that there is no effect of being homozygous (+/+) for the HFE gene on the outcome for these mental health assessments.

For the comparison of the T2 weighted brain images of cerebellum white matter, cerebellum cortex, caudate, thalamus, pallidum, and putamen, GLMs were used to predict T2 intensity from one of the mental health assessments: KSADS_adhd_S_p that utilizes the DSM-V to diagnose ADHD. This enabled us to identify the variance in T2 intensity for each brain region associated with this ADHD predictor. A forest plot indicating the effect of ADHD on the T2 intensities of these brain regions is shown in Figure 2. ADHD showed a significant effect on the T2 intensities of the cerebellum cortex (□=-0.0024, p=0.0008, $p_{FDR}<0.01$), pallidum (□=0.0024, p=0.014, $p_{FDR}<0.05$),

and thalamus (□=0.0019, p= 0.032, $p_{FDR} > 0.05$).

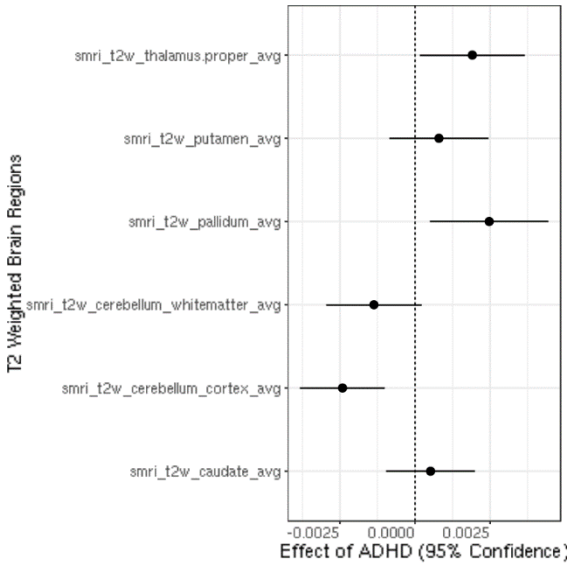

Figure 2: Forest plot showing the effect of ADHD on the T2 intensities of the thalamus (p=0.032), putamen, pallidum (p=0.014), cerebellum white matter, cerebellum cortex (p=0.0008), and the caudate.

P-values were calculated using the Wald test statistic for fixed effects and significant associations were determined using a false discovery rate (FDR).

With this information on the effect of ADHD on these regions implicated in both type 1 HH (later in life), and ADHD, a univariate GLM was fit to predict the T2 intensities of these brain regions from participant HFE genotype and covariates of no interest. This allowed us to identify the T2 intensity of each brain region associated with the genetic predictor for type 1 HH (HFE). A forest plot illustrating the effect of the HFE homozygous genotype on the T2 intensities of the thalamus, putamen, pallidum, cerebellum white matter, cerebellum cortex, and caudate is shown in Figure 3. A homozygous genotype for HFE showed a significant negative effect on the T2 intensities in the putamen (□=-0.37, p=0.018).

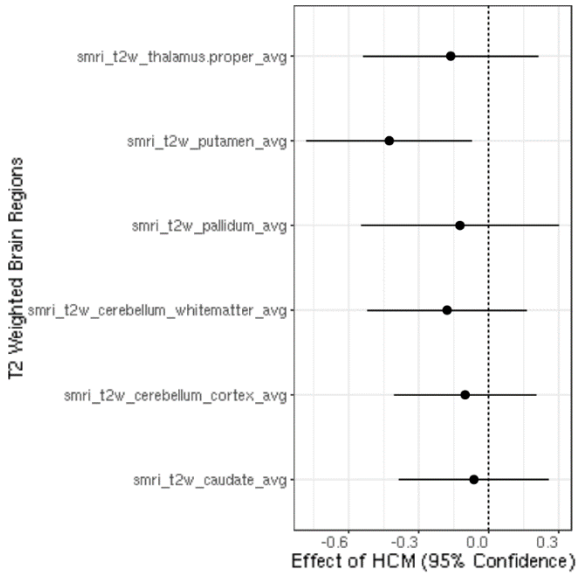

Figure 3: A forest plot demonstrating the effect of a (+/+) HFE Genotype on the T2 intensities of the thalamus, pallidum, putamen (p=0.018), cerebellum white matter, cerebellum cortex, and caudate.

Discussion

This study hypothesized that iron inflammation caused by a genetic mutation in the HFE gene leading to the probable development of type 1 HH by the age of 40 years may be associated with ADHD in adolescents. By running univariate GLMs, we were unable to uncover any association between the type 1 HH mutation and ADHD symptoms or ADHD brain abnormalities in the cerebellum, thalamus, putamen, pallidum, or caudate.

Previous research looked at the relationship between inflammation and ADHD (Leffa, 2018), but did not include iron overload as a possible source of inflammation in their study. Additionally, there has been research indicating that type 1 HH mutations can lead to low T2 intensity brain abnormalities in regions like the putamen and thalamus (Atkins, 2021). However, these studies have

been conducted on older individuals after symptoms of HH have already presented.

While no association was discovered linking ADHD to type 1 HH, this study did discover that the mutation for type 1 HH can disrupt the brain as early as 9-10 years old manifesting in abnormally low T2 intensities/high iron deposits in the putamen (*p=0.018)* (Figure 3). For a disease that goes widely undiagnosed until the age of 40-60 years old, this is a significant finding as it demonstrates that HH can have an effect on the brain at such a young age. While iron accumulation over the years is necessary for the traditional HH symptoms to manifest, it is clear that iron build up can manifest in other ways in adolescence.

More studies need to be conducted on larger samples of adolescents and young adults across a greater range of ages to look at the gradual effect of HH on the brain. HH may not be manifesting as ADHD, but it is manifesting in adolescents as young as 9 years old. I expect that in future studies we will see an increased amount of iron deposits as adolescents grow older, and once females begin menstruation, there will be a larger effect in men compared to women. Longitudinal comparative neuroimaging studies can greatly impact our understanding of how type 1 HH can progress and affect the body from adolescence to old age. This would allow for earlier diagnoses and treatment that could potentially decrease the probability of developing the typical HH symptoms or developing the metabolic, movement disorders (Parkinson's), or neurodegenerative diseases later in life.

Acknowledgements

I am incredibly appreciative of my primary advisor, Dr. Mary ET Boyle, for mentoring, supporting, guiding me, and pushing me to do my best throughout my honors thesis project. I would also like to thank my secondary advisor Dr. Lisa Delano-Wood for her support as well as my graduate student advisor Robert Loughnan for his patience, kindness, and teaching abilities. Additionally, thank you to Dr. Marta Kutas for preparing me for the end presentation, and thank you to the following people who assisted in a variety of different ways: Jonathan Ahern, Devansh Agarwal, Katherine Coiner, Marana Kuhn, Mariela Lopez-Valencia, Patrick Liu, and Katie Morton.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Atkins, J. L., Pilling, L. C., Heales, C. J., Savage, S., Kuo, C.-L., Kuchel, G. A., Steffens, D. C., & Melzer, D. (2021). Hemochromatosis Mutations, Brain Iron Imaging, and Dementia in the UK Biobank Cohort. *Journal of Alzheimer's Disease: JAD*, *79*(3), 1203–1211. https://doi.org/10.3233/JAD-201080

Basuli, D., Stevens, R. G., Torti, F. M., & Torti, S. V. (2014). Epidemiological associations between iron and cardiovascular disease and diabetes. *Frontiers in Pharmacology*, *5*. https://doi.org/10.3389/fphar.2014.00117

Brissot, Pierre & Pietrangelo, Antonello & Adams, Paul & de Graaff, Barbara & Mclaren, Christine & Loreal, Olivier. (2018).

Haemochromatosis. Nature Reviews Disease Primers. 4. 18016. 10.1038/nrdp.2018.16.

Drayer, B., Burger, P., Darwin', R., Riederer', S., Herfkens', R., & Johnson', G. A. (1986). *Magnetic Resonance Imaging of Brain Iron*. 8.

Gerlach, M., Ben-Shachar, D., Riederer, P., & Youdim, M. B. (1994). Altered brain metabolism of iron as a cause of neurodegenerative diseases? *Journal of Neurochemistry*, *63*(3), 793–807. https://doi.org/10.1046/j.1471-4159.1994.63030793.x

Hollerer, I., Bachmann, A., & Muckenthaler, M. U. (2017). Pathophysiological consequences and benefits of HFE mutations: 20 years of research. *Haematologica*, *102*(5), 809–817. https://doi.org/10.3324/haematol.2016.160432

Hoogman, M., Bralten, J., Hibar, D. P., Mennes, M., Zwiers, M. P., Schweren, L. S. J., Hulzen, K. J. E. van, Medland, S. E., Shumskaya, E., Jahanshad, N., Zeeuw, P. de, Szekely, E., Sudre, G., Wolfers, T., Onnink, A. M. H., Dammers, J. T., Mostert, J. C., Vives-Gilabert, Y., Kohls, G., … Franke, B. (2017). Subcortical brain volume differences in participants with attention deficit hyperactivity disorder in children and adults: A cross-sectional mega-analysis. *The Lancet Psychiatry*, *4*(4), 310–319. https://doi.org/10.1016/S2215-0366(17)30049-4

Information (US), N. C. for B. (1998). Hereditary hemochromatosis. In *Genes and Disease [Internet]*. National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK22223/

Leffa, D. T., Torres, I. L. S., & Rohde, L. A. (2018). A Review on the Role of Inflammation in Attention-Deficit/Hyperactivity Disorder. *Neuroimmunomodulation*, *25*(5–6), 328–333. https://doi.org/10.1159/000489635

National Center for Biotechnology Information (US). Genes and Disease [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 1998-. Hereditary hemochromatosis. Available from: https://www.ncbi.nlm.nih.gov/books/NBK22223/

Palmer, C. E., Loughnan, R., Makowski, C., Thompson, W. K., Barch, D., Jernigan, T., Dale, A., & Fan, C. C. (2020). Delineating genetic and familial risk for psychopathology in the ABCD study. *MedRxiv*, 2020.09.08.20186908. https://doi.org/10.1101/2020.09.08.20186908

Pantopoulos, K. (2018). Inherited Disorders of Iron Overload. *Frontiers in Nutrition*, *5*, 103. https://doi.org/10.3389/fnut.2018.00103

Petitti DP. Hereditary hemochromatosis: population screening for gene mutations. In: Khoury MJ, Bedrosian S, Gwinn M, editors. Human Genome Epidemiology. Oxford University Press; New York: 2009. pp. 639–676.

Piperno, A., Bertola, F., & Bentivegna, A. (1993). Juvenile Hemochromatosis. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. Bean, G. Mirzaa, & A. Amemiya (Eds.), *GeneReviews®*. University of Washington, Seattle. http://www.ncbi.nlm.nih.gov/books/NBK1170/

Porter, J. L., & Rawla, P. (2021). Hemochromatosis. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK430862/

Rossi, E. (2005). Hepcidin—The Iron Regulatory Hormone. *Clinical Biochemist Reviews*, *26*(3), 47–49.

Schipper, H. M. (2012). Neurodegeneration with brain iron accumulation—Clinical syndromes and neuroimaging. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1822*(3), 350–360. https://doi.org/10.1016/j.bbadis.2011.06.016

Stoodley, C. J. (2016). The cerebellum and neurodevelopmental disorders. *Cerebellum (London, England)*, *15*(1), 34–37. https://doi.org/10.1007/s12311-015-0715-3

Wessling-Resnick, M. (2010). Iron Homeostasis and the Inflammatory Response. *Annual Review of Nutrition*, *30*, 105–122. https://doi.org/10.1146/annurev.nutr.012809.104804

Wilens, T. E., & Spencer, T. J. (2010). Understanding Attention-Deficit/Hyperactivity Disorder From Childhood to Adulthood. *Postgraduate Medicine*, *122*(5), 97–109. https://doi.org/10.3810/pgm.2010.09.2206