

**The Combined Effects of Mercury Exposure and DNA Methylation on the Developing
Human Brain**

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Toxicology)
in the University of Michigan
2024

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Dedication

This dissertation is dedicated to my grandparents Eunice and Roger Verstegen. Your undying belief in me and continued support are what kept me going, especially when times were tough. At every milestone and every setback, you were the first call I would make. No one was more invested in my success and general wellbeing than the two of you. Although I lost you both before I could quite finish this race, it is your love and support that will carry me across this finish line. Thank you for everything you have done for me over the years, for the lessons you've taught me, and for the good genes you passed down to me. Words cannot express how much I love and miss you. I will carry you with me in my heart now and always.

Acknowledgements

In recognition of their contributions to this dissertation, I would like to thank my primary advisor Dr. Jackie Goodrich, who was one of my biggest advocates throughout these past five years. Her time, guidance, and support were instrumental in the successful completion of this work. I would also like to thank Dr. Dana Dolinoy, who co-advised me and provided me with essential training, resources, and funding throughout this process. I would also like to officially recognize the remaining members of my dissertation committee, Drs. Karen Peterson, Laurie Svoboda, and Deb Watkins who consistently devoted their time, energy, and expertise to the development of this dissertation. In utilizing such different research designs, your diverse experiences and perspectives were invaluable to the success of this work. Another important contributor to this research deserving of a special thank you was Dr. Justin Colacino. He allowed me access to much of his laboratory equipment and provided me with valuable advice that informed how I conducted my *in vitro* experiments. I would also like to thank any Colacino lab members who helped me throughout this process, all of whom were exceptionally supportive. Dr. Matthew Rand and members of his lab at the University of Rochester also deserve my sincerest gratitude, as they provided me with the methylmercury I needed to complete my final experiments. Further recognition should be attributed to two specific post-doctoral fellows, Drs. Mathia Colwell and Rachel Morgan, who provided foundational work in establishing new procedures in our lab from which I benefited. They provided me with additional training and guidance while developing my dissertation that helped me optimize my *in vitro* protocols. I

would also like to acknowledge some dear friends who I met during my doctoral program who helped keep me sane, supported me through even the most stressful times, and helped me have some fun along the way. My former roommate Sasha, my officemates Tomoko and Katelyn, and my peer mentee Shannon are only some of the amazing friends that have been there for me throughout these past five years.

This research would not have been possible without the support of the ELEMENT research staff, study participants, Dolinoy/Goodrich lab members, and funding from the U.S. Environmental Protection Agency (grant: RD83543601), and National Institute for Environmental Health Sciences (NIEHS) (grants: P01 ES02284401, R01 ES007821, R01 ES014930, R01 ES013744, P30 ES017885, U2 CES026553, and R35 ES031686). These studies were also supported and partially funded by the National Institute of Public Health/Ministry of Health of Mexico. We also acknowledge the American British Cowdray Hospital in Mexico City for providing research facilities for part of this work. This work is also in part supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under the Environmental Toxicology and Epidemiology training grant (T32ES007062).

Lastly, I would like to thank my family. My parents and grandparents, for example, were my first teachers and have supported me from the very beginning. I am also grateful for all the amazing dogs in my life, whose energy and positive natures are something to aspire to. Most importantly I would like to acknowledge my sisters Autumn, Andrea, and Jenna, who are always there for me. You are my best friends, and I am so thankful that are in my life. Finally, I'd like to officially recognize the friends and family members scattered across multiple time zones who have devoted time and cellular data to persistently calling me when I have important deadlines to ensure I don't accidentally oversleep. You are the unsung heroes of my life.

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Abstract

The diagnosis of Attention-deficit/hyperactivity disorder (ADHD) is continuously increasing in child and adult populations worldwide. Despite its marked prevalence, the complex etiology of ADHD remains poorly understood. Previous research suggests aberrant DNA methylation (DNAm) and mercury (Hg) exposure are potential ADHD risk factors. Still, combined assessments of these two proposed ADHD predictors are rare. The first factor, DNAm, is a mitotically heritable epigenetic mechanism characterized by the addition of methyl groups to cytosine bases of DNA without altering the genetic sequence. This stable modification is dynamic and potentially reversible, making it an excellent target for potential interventions. The second main risk factor assessed in this study is exposure to Hg, a potent neurotoxicant that people are commonly exposed to through consumption of contaminated seafood. Past research predominantly focuses on the effects of prenatal methyl-Hg exposures and DNAm on subsequent neurodevelopmental outcomes, leaving other relevant windows of susceptibility under-studied. Childhood through adolescence, for example, is a critical period in the development of executive functioning skills, when individuals may be particularly vulnerable to epigenetic and toxic influences. In a two-tiered approach, leveraging both *in vitro* (SH-SY5Y cell line) and epidemiological methodologies (Early Life Exposures in Mexico to ENvironmental Toxicants (ELEMENT) cohort), this dissertation addressed gaps in the literature, by assessing the effects of both post-natal DNAm and Hg exposures on the developing human brain.

We conjectured that epigenetic susceptibility to toxicant-related health effects may help explain ADHD etiology by interacting with childhood Hg exposure to impact ADHD-like behaviors. This dissertation's over-arching hypothesis states that childhood Hg exposure and DNAm marks are both important predictors of ADHD symptoms. This hypothesis was tested in three aims, designed to (1) examine associations between adolescent DNAm and performance on the Conners Continuous Performance Test, 3rd edition (CPT3), (2) analyze the role of DNAm in adolescence in the relationship between childhood Hg exposure and CPT3 performance, and (3) identify mercury-induced gene expression and DNAm changes in cultured human neurons. Using data generated from ELEMENT children, we identified significant associations between both predictors and CPT3 scores individually, as well as significant interactions between the two terms when modeled together. In Aim 1, we discovered *ZNF814*, *ELF4*, and *OR6K6* DNAm levels were significantly associated with vigilance and sustained attention CPT3 scores. Functional enrichment for ferroptosis, inflammation, immune response, and neurotransmission pathways were also significantly related to multiple CPT3 outcomes. In Aim 2, we first identified significant positive and negative associations between hair and urine Hg and CPT3 performance, which depended upon the CPT3 and Hg variable used. When the effects of the two main predictors were modeled together, significant interactions were identified for inattention and vigilance. Associations described in Aims 1 and 2 were often modified by gender. Notably, in interaction models from Aim 2, hair Hg significantly interacted with DNAm at 157 CpG sites in females for the inattentiveness models; three significant interactions were identified for the perseveration models in males. In Aim 3, significant differential expression and methylation in dopaminergic-like neurons were observed for several genes, including those encoding two novel proteins differentially responding to low/biologically relevant methyl-Hg exposures. This aim

provided mechanistic insight suggesting epigenetics, immune response, and cell motility are all related to Hg-induced neurotoxicity. Inflammation and epigenetic mechanisms were related to neurobehavior and/or function across findings from all three aims of this dissertation.

Chapter 1

Introduction

General Overview

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, affecting roughly 5.3% of children globally. Two thirds of ADHD patients diagnosed in childhood experience symptoms into adulthood [1]. The disorder can impact multiple parts of the brain and is associated with increased risk of cardiovascular diseases, accidental self-injury, substance abuse, depression, anxiety, eating disorders, suicides, incarcerations, and obesity. ADHD is characterized by symptoms of impulsivity, hyperactivity, and inattentiveness. It is not solely a mood disorder, but rather a grave public health concern. Because its effects on executive functioning often result in poor self-regulation and decision making, the affected individual often engages in risky behavior and poor life habits that predispose them to a number of adverse health effects. In fact, recent studies have found that people with childhood ADHD are twice as likely to die prematurely than those without ADHD, and that if it persists into adulthood, they have a reduced estimated life expectancy (ELE) of about 13 years with a 9.5-year reduction in healthy ELE [2, 3]. Despite these findings, ADHD remains underacknowledged as a public health concern, and continues to increase in prevalence [4]. To confront the concerns surrounding ADHD in our society, care must be taken to not only conceptualize this disorder as a risk factor for accidental injuries, seemingly unrelated diseases, and/or mental health conditions, but also to more fully comprehend the complex set of risk factors for ADHD itself.

This present study focuses on the assessment of two potential players in the etiology of this disorder: exposure to mercury during child development and epigenetics. Mercury is a known environmental neurotoxicant that is considered by the World Health Organization to be among the top ten chemicals/ groups of chemicals of major public health concern due to its

environmental prevalence and potency [5]. Notably, associations between exposure to this heavy metal and DNA methylation, one of the best characterized epigenetic mechanisms, have been documented [6-8]. For these modifications, methyl groups are added to cytosines in CpG sites (adjacent cytosine and guanine bases within the genome) and regulate the expression of genes without revising the genetic code itself, as is characteristic of epigenetic mechanisms. Both DNA methylation and mercury toxicity are implicated in the dysregulation of normal brain development and the etiology of ADHD [8].

The ADHD Brain

ADHD is a neurocognitive disorder that affects neurodevelopment in multiple areas of the brain and is associated with impairments in executive functioning and motivation. Symptoms, etiology, and affected brain regions may vary from person to person, but generally there are three main subtypes for ADHD: predominantly inattentive (ADHD-PI), predominantly hyperactive-impulsive (ADHD-HI), and combined (ADHD-C) presentations [9]. Developmental timing for different regions of the brain is often abnormal in ADHD patients, experiencing delayed development in some regions and rapid development in others, which is why it is not uncommon for symptom profiles to change throughout an individual's development. For instance, individuals with ADHD-HI during childhood frequently exhibit ADHD-PI as adults. Another important factor driving common differences in ADHD is gender, with males more frequently exhibiting hyperactivity than females [10]. ADHD is complex, not only in the manifestation of its symptoms, but also in its pathology. While the anatomical changes in ADHD patients compared to neurotypical controls is highly variable between individuals, the most consistent findings state that ADHD is generally associated with reduced dimensions of prefrontal cortex white matter, caudate nucleus, corpus callosum and the cerebellar vermis, as well as an overall reduction in brain size into adolescence [9, 11]. Although structural differences in the hippocampus are inconsistent across studies, this section of the brain is suspected to be very important in ADHD etiology [12]. This is particularly relevant to this dissertation, because data suggests the hippocampus is exceptionally vulnerable to methylmercury (meHg) toxicity in childhood extending into prepubescence [13]. If meHg targets the hippocampus in children at this selected timepoint, it may result in associations with inattention. Dopamine signaling in this hippocampal region is critical in regulating spatial learning, memory, attention, and synaptic

plasticity. In fact, methylphenidate, one of the most commonly prescribed ADHD medications, works by increasing synaptic dopamine availability in the hippocampus and prefrontal cortex [12]. Notably, the cell line used in this dissertation has the unique ability to be differentiated to represent dopaminergic-like neurons, which we leveraged to gain a better understanding of how dopamine may be dysregulated in the brain.

Because ADHD is such a broad term, constraining it to a fixed set of symptoms and treating it as one single condition can be limiting, which is why Conners Continuous Performance Test (CPT) III, as was used in this study, is such a helpful tool. While there are other objective, lab-based tests that can complement clinical diagnoses in ADHD assessments, CPTs are among the most commonly cited and reliable in the literature [14]. For this computerized test, children are presented with a series of stimuli to which they are supposed to respond by either hitting a button or not. From the response data, variables are generated to reflect four different aspects of attention: inattentiveness, impulsivity/hyperactivity, sustained attention, and vigilance. These variables are described in Table 1-1 which notes what aspect(s) of attention each is likely to reflect, with higher scores for each indicating poorer performance. In several cohorts, published data suggests meHg exposure is associated with certain CPT test variables. In example, for children from the Faroe Islands aged 7 and 14 years, CPT variables omissions errors and mean reaction times were significantly increased with greater prenatal meHg exposure [15, 16]. It is important to note not all CPT variables should be taken at face value, as some may be more meaningful when analyzed in concert with another (see Table 1-1 for notes on interpretation). Research also cautions against using mean reaction time as an overall average, but rather as blocks of time relative to test initiation. The different blocks likely reflect different things, for instance the first few minutes are primarily influenced by factors related to orientation, learning and habituation. The subsequent stage is more reflective of speed processing and selective focused attention, as their performance of the task may not yet be affected by mental fatigue. In the third stage, on the other hand, latencies in hit reaction times (HRT) are likely to reflect one's sustained attention functioning. By assessing these blocks separately, Julvez *et al* were able to identify associations that imply meHg is particularly deleterious to sustained attention and probable dysfunction in the frontal lobes [17]. To follow this recommendation, whenever available this study utilized block change variables, which are reported as slope across block changes (Table 1-1). Of the variables generated from this test, the four variables omissions

errors, perseverations, hit reaction time (HRT) interstimulus interval change, and HRT block change were selected for use in this dissertation to respectively reflect inattentiveness, impulsivity, vigilance, and sustained attention. These variables are described below in Table 1-1, along with HRT, the variable from which many other CPT3 variables are derived. Despite the fact this variable is commonly cited in the literature, it was not included in the primary analysis for this dissertation, because it can either be associated with impulsivity or inattention, dependent upon a secondary variable. For ease of interpretation, it was excluded. Its use in the literature may contribute to the lack of consensus in the scientific community regarding the exact effects of mercury on neurodevelopment and executive functioning.

Environmental Mercury Exposure

Throughout history, the neurotoxicant mercury has been used for a diverse set of purposes, including tooth amalgams, early treatments of syphilis, gold mining, photography, and electronic devices. The heavy metal exists environmentally in three main forms— elemental, inorganic, and organic—all of which can exert a toxic effect on humans [21-23]. Elemental mercury is the least toxic form of the metal. Humans are predominantly exposed to elemental mercury via inhalation of mercury vapors that can be produced through natural or anthropogenic sources [1, 6]. Once inhaled, the toxicant is rapidly absorbed and distributed throughout the body, with the kidneys and brain as the primary target organs [24]. Because of its liposolubility, elemental mercury can cross biological membranes like the blood-brain barrier and can be oxidized to inorganic mercury. This oxidation process occurs a few minutes after inhalation of the vapor. Inorganic mercury salts are more toxic than elemental mercury. They are corrosive and water soluble in nature, making them highly permeable to the gastrointestinal tract. Because they are not lipid soluble, they are unlikely to cross the blood brain barrier or placenta to exert adverse neurological or developmental effects [22, 25]. The arguably most toxic species of this non-essential metal is its organic form, namely meHg. It is the most prevalent exposure, predominantly consumed through eating contaminated seafood. The metal in any form can collect in water sources following emissions (e.g. from coal-fired power plants), where it is metabolized by micro-organisms to its more toxic meHg form and bioaccumulates in the tissues of shellfish, fish, and other important types of seafood. Even with recent efforts to reduce total Hg emissions, unfortunately for vulnerable populations that depend on fish for their livelihood,

this reduction in emissions has not occurred yet in all parts of the world and thus is not readily reflected in the biota [23].

In recent years, much attention has been placed on studying meHg due to known associations between human exposure and adverse neurological health effects. The Minamata Bay disaster, occurring in the 1950s in Japan, demonstrated the neurological effects of high-dose, acute exposure to meHg. This event was caused by the Chisso Minamata Factory's improper disposal of wastewater that contained high levels of meHg which they dumped in the bay. As a result, numerous humans and animals started to exhibit adverse neurological effects which could include ataxia, slurred speech, constricted field of vision, paresthesia, impaired hearing, disequilibrium, tremors, and erratic eye movement. Most severe cases experienced madness, coma, or even death. The famous "Dancing Cats of Minamata", local felines whose impaired motor control gave them their name, helped scientists to identify meHg as the causal agent and to begin exploring the pathology of this strange illness, which was later referred to as Minamata disease [23, 24]. In later years, the disease was reported again in Niigata and regions of Ontario, also due to environmental meHg exposure, and congenitally in individuals whose mothers were acutely exposed during gestation [23, 26, 27]. These cases tended to be quite severe, a testament to the vulnerability of this developmental period.

While the effects of acute, high-dose meHg exposures are well-understood, the impacts of chronic low-dose exposures are not as straight-forward. Over the years, a number of epidemiological studies have explored relationships between meHg exposure and neurological outcomes. Two examples of such studies include the Inuit cohort in arctic Canada and a Faroe Islands birth cohort located in the North Atlantic Ocean between Norway and Iceland. In both cases, researchers reported associations between prenatal meHg exposures and symptoms of ADHD in childhood and adolescence [16, 28]. Pregnancy is not the only window vulnerable to the effects of meHg. The brain is still developing during childhood and adolescence and is susceptible to meHg toxicity [29-31]. For the INMA cohort in Valencia, Spain, researchers reported that childhood Hg exposure is associated with poorer neurobehavioral development at ages 9 and 11 years. Interestingly, these associations were modified by sex and the presence of distinct genetic polymorphisms [32].

Not all studies in the literature were able to identify adverse associations between exposure to this heavy metal and neurodevelopment, thus creating some controversy in the field [33, 34]. In

fact, some studies even suggest a beneficial relationship between chronic low dose mercury exposure, resulting in improved attention, however, most scientists disagree with this theory [34]. This beneficial relationship is more likely the result of researchers not considering in their models important protective exposures like PUFA (polyunsaturated fatty acids) and selenium levels, which can play protective roles in neurodevelopment and the detoxification of Hg. Studies have shown that the inclusion of these variables in statistical models can improve the ability to detect associations between meHg exposure and neurological effects [28, 35]. Selenium and PUFA nutrients are especially important in populations with high seafood consumption, because like mercury they are highly abundant in fish [36, 37]. Notably, within ELEMENT (Early Life Exposures in Mexico to ENvironmental Toxicants), the cohort used in this dissertation, Food Frequency Questionnaires (FFQ) were completed by participants from which dietary data was used to estimate childhood PUFA and selenium intake at the same timepoints that mercury was measured [36]. Based on existing data, we hypothesized that the consideration of these variables would provide a clearer, more accurate estimate of the association between Hg exposure and neuro-development & -behavior.

Other factors that may help explain discrepancies in literature findings include lack of appropriate consideration of differences in cohort characteristics and the effects of sex, race, age, concomitant exposures to other toxicants, and genetic/epigenetic variations. Further complicating the interpretation of results in the literature is the developmental timing of the exposure and the outcome. The effects of a prenatal chemical exposure may be entirely different than those of a postnatal exposure, and the association with the outcome may differ depending on the timepoint at which it is measured. For instance, in one study in the USA, prenatal mercury was not associated with adverse neurobehavioral outcomes in early infancy and even suggested Hg was able to improve attention [34]. This timepoint, however, may be too early to discern anything meaningful from the results, because development of executive functioning skills (closely related to frontal lobe maturation in the brain) do not generally begin to emerge until about six years of age and continue to develop through adolescence [38]. It is largely for this reason that we assessed performance on the Conners Continuous Performance Test (CPT) at childhood and adolescence in this dissertation. The timepoints of exposures and outcomes were carefully selected to yield the most meaningful results based on availability of data, biological vulnerability, developmental relevance, and temporal relationship between the variables. They

also address major gaps in the literature, which tends to emphasize the effects of prenatal exposures over those in childhood and adolescence, which are also very important, sensitive stages in neurodevelopment.

Epigenetic Involvement

The epigenetic mechanism of DNA methylation, comprising both 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) marks, can often help researchers gain mechanistic understanding of bodily response to a chemical exposure and may also serve as a biomarker of a past toxicant exposure and/or current or future disease state. DNA methylation at specific loci (i.e. CpG site) is predominantly associated with the silencing of that gene, especially in promoter regions of genes. It is a modification that alters gene expression but does not change the genetic code and is heritable, at least across cell divisions (mitosis). DNA methylation is an important consideration in this dissertation, because it is known to help regulate brain development and is hypothesized to play a role in ADHD [8, 39]. Peripheral blood DNAm (DNA methylation) at *LIME1* and *SPTBN2* genes, for example, were found to be associated with ADHD in children [39]. Furthermore, in human studies, site specific differential DNAm was documented in response to both inorganic and organic mercury exposures [6, 40]. Two of such examples include hypomethylation at *SEPP1* (Selenoprotein P) measured in saliva DNA and hypermethylation at *GSTM1* (Glutathione S-Transferase Mu 1) measured in blood DNA following exposure in adulthood [40, 41]. Changes in DNA methylation in response to mercury exposure were also reported at genes encoding brain-derived neurotrophic factor (*BDNF*) in rat hippocampus and Rho Family GTPase 2 (*Rnd2*) in mouse embryonic stem cells [7, 42]. One cohort of striking interest is the Seychelles Child Development study (SCDS). It is considered the largest prospective cohort studying the effects of prenatal mercury exposure on human development. A recent study within SCDS conducted by Ulloa *et al*, however, documented clear associations between prenatal meHg exposure and childhood DNA methylation measured in saliva at *NR3C1*, *GRIN2B*, and *BDNF* CpG sites. All three of these genes are crucial for normal neuro-development & -function, and contained CpG sites for which higher mercury levels were significantly associated with hypermethylation [43]. One limitation is that this study did not also relate these changes to a behavioral outcome, such as performance on a CPT test. Additionally,

Ulloa *et al* conducted their analysis in a site-specific manner, selecting genes based on their functional relevance and focused on prenatal exposure. Within the scope of this dissertation, we aim to identify genes whose methylation status is associated with adolescent performance on the CPT3 test. We conjectured that the true nature of the controversial relationship between mercury exposure and neuro-behavioral outcomes would be elucidated by the consideration of the potential interaction between DNA methylation and childhood exposure to the toxicant.

In recent years, researchers have started to explore DNA methylation as a probable mechanism underlying environmental toxicant-associated neurodevelopmental disorders [8, 44]. In fact, a recent study by Maccani *et al* found hypomethylation in human infants at the *EMID2* gene to be associated with placental mercury levels and adverse neurobehavioral outcomes measured 24 to 96 hours after birth [45]. This may reflect a novel mechanism facilitating mercury induced neurotoxicity. Similar to this aforementioned study, Cardenas *et al* used an epigenome-wide screen to identify genes, namely *PON1*, *WBP11P1*, and *TOR4A*, differentially methylated in cord blood DNA based on prenatal mercury exposure. They then assessed the relationship between methylation at these genes with cognitive performance in early childhood (using PPVT and WRAVMA tests). Importantly, many of these associations were modified by gender. Unlike the Maccani *et al* study, these researchers also evaluated the persistence of this DNA methylation into early- and mid-childhood [46].

Fetal developmental stages are characterized by extensive epigenetic reprogramming events and heightened vulnerability to toxic insult, which is why the bulk of the literature assesses epigenetic profiles at birth and later characterizes the persistence of those findings into childhood and adolescence. Because DNA methylation continues to change throughout one's life course and is important in regulating brain development, learning, and memory, associations seen at birth may differ from those seen in adolescence and may signify different things [47]. It is for this reason it is important to assess not only the persistence of DNA methylation marks present at birth, but also those that are distinctive to childhood and/or adolescence. In this dissertation, an epigenome-wide analysis (EWAS) was conducted to identify regions where DNA methylation predicts the CPT3 outcome in late childhood/early adolescence.

Strengths and Weaknesses of Different Study Designs

Most of the studies referenced in this dissertation thus far are epidemiological in nature, but the present research consists of both human and cell culture data in a two-tiered approach. In the first tier of this dissertation, comprising aims one and two, we leverage the benefits of epidemiological study designs. In tier two, or aim three of this dissertation, we address the weaknesses of the first tier by employing an entirely different *in vitro* research design. There are pros and cons to both approaches. Epidemiology studies are beneficial in assessing the effect of a chemical exposure on human health in the presence of other co-existing risk factors and present a more realistic view of how the chemical behaves in a larger system. However, due to the innate complexity of such systems (both on the organismal and ecosystem levels), it can be difficult to understand all the variables influencing one's findings and isolate the effects of the chemical itself from the multifaceted web of risk factors. For *in vitro* studies, researchers have greater control over the system and can more readily discern causal relationships between the toxicant and outcome. Whether or not these conclusions hold true in a whole organism system or are environmentally relevant is not so easy to answer. Regardless, most epidemiological studies are limited to biologically available tissues, such as blood or saliva, but epigenetic profiles can vary by cell type, making cell culture a very helpful tool. While DNAm status may be similar in human blood and brain tissues, there is no way to confirm this in an ethical, non-invasive manner. It is for this reason the present dissertation considers mercury's effect on cultured human neurons.

Additionally, *in vitro* data is an excellent way to glean insight about mechanisms of toxicity. For instance, while there is still much to learn regarding meHg- induced neurotoxicity in humans, by using cell models we now know that meHg induces oxidative stress and excitotoxicity in neurons. Dysregulation of intracellular calcium and glutamate homeostasis have also been documented in cultured neurons exposed to meHg. The toxicant can oxidize and bind with high affinity to proteins and non-protein molecules via their thiol and selenol groups to exert a toxic effect [48, 49]. Interestingly, glutathione (GSH), an important antioxidant rich in thiol groups, is often thought of as the body's first defense against heavy metal-induced cytotoxicity. MeHg specifically, however, has been known to target the GSH-GSSG redox cycling system and can either aid in processes of detoxification or toxicity by shifting the ratio of oxidized (GSSG) and active (GSH) glutathione in the cell to toxic levels [50, 51]. Glutathione

peroxidase (GPx) and glutaredoxins (Grx) are two families of enzymes involved in this system and can also play a role in disruption of cell redox state. MeHg can interact both directly with these proteins' isoforms, and indirectly, by targeting post-transcriptional and pre-translational phenomena, resulting in decreased GPx and Grx activity [51-53].

More research is needed to better understand the dynamic interactions within the GSH redox cycling process and the potential roles of metallothionines and selenium in detoxification of meHg in human brain tissues [50, 54]. Importantly, understanding the interactions between these related meHg-induced phenomena and their downstream effects can not only elucidate molecular mechanisms, but also how they translate to neuro-developmental & -behavioral outcomes in humans. To conceptualize this complete picture, information from both *in vitro* and epidemiological studies is essential.

Cohort

From 1994 to 2003 1,643 mother–child pairs were recruited into the ELEMENT (Early Life Exposures in Mexico to ENvironmental Toxicants) cohort either during pregnancy or at birth from maternity hospitals in Mexico City, Mexico [21]. The purpose of this prospective cohort was to assess the consequences of early life exposures on the recruited children later in life. It has been followed for over 20 years, measuring important exposures and outcomes at different timepoints during development, including childhood and adolescence. Because of the large sample size, availability of data, and high cohort retention rate through time-points relevant to our research question, ELEMENT was an ideal cohort for use in this dissertation. Furthermore, it allows us to explore our central hypotheses in an underrepresented population of Hispanic children. The major timepoints and variables of interest to this study are outlined in Figure 1-1.

Study Design and Objective

The overall objective of this two-tiered dissertation is to examine the combined effects of DNAm and Hg exposure on the developing human brain during the under-explored developmental period of childhood through adolescence. Similarly, the over-arching hypothesis states that both the effects of childhood mercury exposure and DNAm are important influences

in the etiology of ADHD and ADHD-like symptoms in childhood/adolescence. We examined these influences in three specific aims, two of which are based on epidemiological analysis using the ELEMENT birth cohort (tier 1) and the third is based in experiments in cultured human neurons (tier 2). Additionally, we employed the use of big data methodologies through both the cohort study, which examined epigenome wide associations between DNA methylation and cognitive performance in children, and the *in vitro* data, which assessed differential expression in mercury-exposed neurons using RNA sequencing technologies. Then, using candidate genes identified via these full-genome methods, we also considered the effects of a third variable—mercury exposure in the ELEMENT tier and DNA methylation in the *in vitro* tier. While mercury and DNA methylation cannot directly relate to a behavioral outcome *in vitro*, to a degree one could conceptualize the expression data generated through RNAseq as a proxy for a functional outcome. In this way, both tiers of the project incorporate mercury exposure, DNA methylation, and draw inferences regarding functional relevance. A brief overview of each specific aim is outlined below.

AIM 1: To examine the associations between adolescent DNA methylation and performance on the CPT 3 test.

Within this aim, we hypothesized that site-specific blood DNA methylation marks present in adolescence would be associated with attention-related CPT3 test variables, as identified through an epigenome-wide screen. In this cross-sectional analysis using data generated from the ELEMENT cohort, we explored the individual effects of DNAm on the CPT3 performance at a neurodevelopmental timepoint where epigenetics is said to be highly relevant. This timepoint is particularly important, because executive functioning skills are honed during adolescence in tight correlation with maturation of the frontal lobe. Past research predominantly focuses on the associations between DNAm measured at birth and future ADHD-like behaviors, but some associations have been identified with adolescent DNAm, as well. Notably, these studies tend to fixate on DNAm of candidate genes identified at birth to discern if the methylation state was maintained through adolescence. Because the present study utilizes an epigenome-wide assessment, it has the capacity to detect differential methylation marks that are specific to the underexplored neurodevelopmental timepoint of adolescence.

AIM 2: To analyze the role of DNA methylation in adolescence in the relationship between childhood Hg exposure and performance on the CPT3 test.

This aim expands upon the findings of Aim 1, using a subset of the same ELEMENT participants that also provided hair and urine samples for quantification of Hg levels. The central hypothesis explored by this aim is that the relationship between childhood Hg and adolescent CPT3 performance will be modified by blood DNAm measured during adolescence. This hypothesis was assessed in an innovative approach that modeled interaction between hair or urine Hg levels and DNAm at the 1000 CpG sites with the lowest raw p-values for each CPT3 outcome identified in Aim 1. These sites were selected because they are believed to be functionally relevant to neurodevelopment based on the results of functional pathways enrichment assessment in Aim 1. In past research DNAm and Hg have both been implicated in the development of ADHD, though combined assessments of these factors are uncommon and usually explore the potential mediating role of epigenetic factors in the relationship between Hg exposure and adverse neurodevelopment. This aim takes a novel approach to explore the combined effects in an assessment of epigenetic x environment (E x E) interaction. While past gene x environment interaction studies have identified genetic variants that may predispose an individual to the neurotoxic effects of Hg, E x E studies may be more relevant in a clinical setting, because epigenetics, unlike genetics, is modifiable. This presents a novel concept which we refer to as “epigenetic susceptibility.” Studying how DNAm at specific sites and Hg exposure interact to affect CPT3 performance may help us gain a better understanding of ADHD etiology, serve as a model for future E x E studies, and inform future research and risk assessment.

AIM 3: To identify mercury-induced gene expression and DNA methylation changes in cultured human dopaminergic-like neurons.

This *in vitro* exploration is examined through two sub aims, which tested the central hypotheses that differential (Aim 3A) gene expression AND (Aim 3B) DNAm at specific genes believed to be involved in neurodevelopment or toxicity will be present in differentiated SH-SY5Y cells treated with varying levels of meHg. The six candidate genes used in Aim 3B were selected by combining findings from the transcriptomic analysis of Aim 3A, the results from the previous tier of this dissertation, and functional relevance described in previous publications.

This is a particularly impactful aim, because unlike Aims 1 and 2, it utilizes a model of human brain cells rather than blood. It also aims to assess the effects of low biologically relevant and cytotoxic doses of meHg in dopaminergic-like neurons, rather than introducing the exposure during the differentiation process of the SH-SY5Y cells, which is a common model of *in utero* exposures. In this experiment, cells are treated with meHg after the differentiation protocol is completed in efforts to model the toxic effect in mature neurons meant to represent the human brain during childhood through adolescence. Lastly, this aim distinguishes between 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). Traditional methodologies of DNAm tend to quantify both marks jointly in assessments of total methylation. These marks, especially in brain tissue, have been shown to have distinct functions and should be modeled separately.

Overall, this dissertation presents innovative and impactful research that can inform future studies and interventions. The two-tier approach synthesizes concepts from epidemiological and *in vitro* methodologies that can be used to fortify our understanding of the combined effects of DNAm and Hg exposure on the developing human brain. The targeted methylation analysis of Aim 3A uniquely complements analyses of both tiers outlined in this dissertation. It allows us to explore the idea that DNAm may be driving some of these alterations in gene expression identified in aim 3A. Additionally, it brings together concepts from the previous aims by addressing weaknesses in their experimental design and examining mechanistic implications in greater depth. One weakness from these aims is that the DNA methylation analysis cannot distinguish between 5mC and 5hmC, which are distinct marks that may mask true associations if analyzed together. Analyzing these marks separately, as is done in this final targeted methylation analysis, may help elucidate some findings from tier one. Lastly, by selecting genes where DNAm and Hg interacted in the tier one analyses, we can help confirm the involvement of both of the key variables on actual brain cells rather than drawing inferences based on data generated in blood leukocyte DNA measured in a cohort of Mexican children.

Figures and Tables

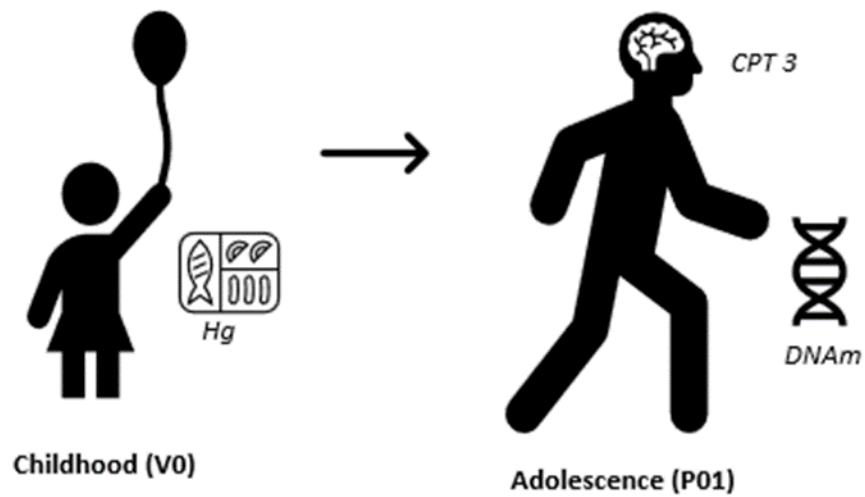


Figure 1-1: Schematic of the timing of key predictors and outcomes in ELEMENT that are the focus of this dissertation.

Table 1-1: Conners Continuous Performance Test (CPT 3) Variables.

Variable	Definition/Explanation	Aspects of Attention	Interpretations
Omissions	Percent of missed targets	Inattentiveness	Higher T scores reflect greater rate of omission errors (more inattentive)
Perseverations	Percent of random or anticipatory responses (HRT<100ms, the minimum processing time for informed response)	Impulsivity	Higher T scores reflect greater rate of perseveration errors (heightened impulsivity).
HRT Block Change	Change in response speed across the 6 blocks	Sustained Attention*	Higher T scores reflect slower response speeds in the later blocks, which reflects a reduction in information processing efficiency over time (loss of sustained attention)
HRT ISI Change (Inter-Stimulus Interval)	Change in response speeds at various Inter-Stimulus Intervals (ISI: 1sec, 2sec, and 4sec)	Vigilance*	Higher T score signify greater slowing at longer ISIs, which reflects a reduction in information processing efficiency (loss of vigilance)
Response Style (C)	Signal detection variable assessing an individual's natural tendencies to respond to stimuli based on a speed versus accuracy tradeoff		Higher T scores indicate conservative response styles (emphasis on accuracy over speed) and lower scores indicate liberal response styles (emphasis on speed over accuracy)
Hit Reaction Time (HRT)	Mean response speed (in ms)	Inattentiveness; Impulsivity	Higher T scores reflect slower HRT (lower scores = faster response speeds)

* **Note:** Some scientists consider vigilance and sustained attention to be synonymous, but CPT 3 recently acknowledged them as distinct items with vigilance describing one's ability to remain alert even in situations with less stimulation (ie. with a longer ISI) [18-20].

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Chapter 2

Aim 1: Associations between Blood Leukocyte DNA Methylation and Sustained Attention in Mid-to-Late Childhood

Abstract

Aims: To identify associations between DNA methylation (DNAm) across the epigenome and symptoms related to Attention Deficit Hyperactivity Disorder (ADHD) in a population of Hispanic children. **Materials & Methods:** Among 517 participants in the Early Life Exposures in Mexico to ENvironmental Toxicants (ELEMENT) study aged 9-18 years, we conducted an EWAS examining associations between blood leukocyte DNAm and performance on the Conners Continuous Performance Test (CPT3). **Results:** DNAm at loci in or near *ZNF814*, *ELF4*, and *OR6K6* and functional enrichment for gene pathways pertaining to ferroptosis, inflammation, immune response, and neurotransmission were significantly related to CPT3 scores. **Conclusion:** DNAm was associated with CPT3 performance. Further analysis is warranted to understand how these genes and enriched pathways contribute to ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, affecting roughly 5.3% of children globally. Two thirds of people diagnosed with ADHD in childhood experience symptoms into adulthood [1]. The disorder can impact multiple parts of the brain and is associated with increased risk of cardiovascular diseases, accidental self-injury, substance abuse, depression, anxiety, eating disorders, suicides, incarcerations, and obesity. ADHD is characterized by symptoms of impulsivity, hyperactivity, and inattentiveness. Because its effects on executive functioning often result in poor self-regulation and decision making, the affected individual may engage in risky behavior that predisposes them to adverse health effects. People with childhood ADHD are twice as likely to die prematurely than those without ADHD, and if ADHD persists into adulthood, they have a

reduced estimated life expectancy of about 13 years with a 9.5-year reduction in healthy estimated life expectancy [1, 2]. Despite these findings, ADHD remains underacknowledged as a public health concern, and continues to increase in prevalence [3]. To address this issue, we need to not only conceptualize ADHD as a risk factor for other health conditions, but also understand the risk factors for the disorder itself.

Epigenetic mechanisms, including DNA methylation (DNAm), are potential contributors to ADHD susceptibility and etiology. Epigenetic modifications may alter gene expression but do not change the underlying genetic code. They are fairly stable and heritable, at least across cell divisions (mitosis), but are still modifiable, making them an excellent target for potential intervention. In the field of epigenetics, DNAm is the most commonly studied process in humans. It occurs when a methyl group is added to a cytosine base located 5 prime of a guanine base referred to as a CpG site. A body of literature exists relating DNAm to neuro-developmental and -behavioral outcomes [4-8]. DNAm at CpG sites, especially in promoter regions of genes, is predominantly associated with gene silencing. DNA methylation is known to help regulate brain development and is hypothesized to play a role in ADHD [4, 5]. In a recent study conducted by Li *et al*, researchers used CPT (continuous performance test), a commonly used method to assess ADHD-like behaviors in children, to explore the relationship between DNAm and attention-related symptoms in a sample of Taiwanese children. They found significant associations between higher DNAm levels (hypermethylation) at the *LIME1* (Lck Interacting Transmembrane Adaptor 1) gene and poorer CPT test performance. They also documented a significant relationship between hypomethylation at the *SPTBN2* (Spectrin Beta, Non-Erythrocytic 2) gene and worse CPT scores [5]. Both *LIME1* and *SPTBN2* are implicated in neurological disorders. *LIME1* plays a major role in inflammatory pathways like MAPK signaling and has been linked to cerebral palsy pathophysiology [9]. *SPTBN2*, which helps regulate the glutamate signaling pathway through stabilization of the glutamate transporter EAAT4 (excitatory amino acid transporter 4), is associated with spinocerebellar type 5 (SCA5) in humans [10, 11]. The Li *et al* study was conducted using candidate genes whose DNAm levels were identified as ADHD relevant genes through a pilot EWAS (epigenome-wide association study; data from 12 ADHD cases and 9 controls). Selected candidate sites that were associated with ADHD in the EWAS and related to any neurological process were subjected to further analysis via pyrosequencing using a sample of 198 children (126 ADHD patients and 72 controls)[5]. In a larger EWAS by

Mooney *et al* (391 cases and 213 neurotypical controls) DNAm was found to be associated with ADHD diagnosis and polygenic risk score. This study was conducted in a US population of children aged 7-12 years [6]. Other EWAS studies have been conducted, exploring the relationship between DNAm and ADHD-like symptoms in children [7, 8]. Collectively these studies suggest that DNAm may be part of the etiology of ADHD. However, past studies are limited to primarily Asian and White populations. Larger studies in more ethnically diverse populations are needed to validate past findings and identify differences across cohorts of varying demographics. One of such populations was used in the present study employing data from similarly aged participants of the Mexican ELEMENT (Early Life Exposures in Mexico to ENvironmental Toxicants) cohort.

Childhood and adolescence are important neurodevelopmental periods. Development of executive functioning skills, which is closely related to maturation of the frontal lobe in the brain, does not generally begin to emerge until about six years of age and continues to develop through adolescence [12]. Not only do epigenetic mechanisms help regulate brain development in childhood and adolescence, but they are hypothesized to play a role in ADHD [4, 5]. Only a handful of EWAS studies currently explore the relationship between DNAm and executive function at these highly relevant developmental periods [5-8]. Identifying genes that are differentially methylated in relation to ADHD may help us identify potential biomarkers and enable us to gain insight about mechanistic pathways. To address these gaps, we assessed the associations between blood leukocyte DNAm using an epigenome-wide screen and CPT3 performance in children and adolescents of Latino origin from the Mexico City-based ELEMENT cohorts.

Methods

Cohort

1012 pregnant mothers in Mexico City were recruited (1994-2004) from prenatal clinics from the Mexican Social Security Institute, serving low- to middle-income populations. Mother/child dyads were followed through present day. Data was collected at various timepoints, including in 2015, when a subset of 550 children (between 9 and 18 years of age) participated in a follow up visit [13]. This study focusses on 526 (51% female and 49% male) participants at

this timepoint, for whom epigenome-wide DNAm was quantified in blood leukocytes. At this timepoint, most participants also completed the Conners Continuous Performance Test, 3rd Ed. (CPT3). Approximately 517 participants had both CPT3 performance data and DNAm data collected and are included in this analysis. Research protocols were approved by the institutional review boards of the Mexico National Institute of Public Health and the University of Michigan. Parental informed consent and child assent was obtained for all participants in this study.

Epigenetic Analysis

All participants attending the adolescent study visit and providing a blood sample were selected for epigenome-wide DNA methylation analysis as previously described [14]. To generate methylation data, we extracted DNA from blood leukocytes via Qiagen Flexigene kits, performed bisulfite conversion on the samples, and subsequently analyzed them on an Illumina Infinium Methylation EPIC BeadChip (referred to as the EPIC array). This probe-based assay quantifies methylation at more than 850,000 CpG sites across the human genome [15]. While a fraction of those comprising the entire genome (~28 million CpG sites), it is representative of a vast distribution of regions within and outside of genes, including promoters, enhancers, and intergenic regions, providing coverage at loci within every known gene [16].

Bisulfite converted DNA samples from the ELEMENT participants were randomized across chips and positions and hybridized to BeadChips. Signals were read, processed, and subjected to rigorous quality controls at the UM Advanced Genomics Core. To analyze this data, we followed a standardized pipeline for this technology, previously described [14]. In brief, we read raw image data files into R using the *minfi* package and corrected for background noise and dye bias using RELIC [17, 18]. To account for unwanted technical variability, estimated from control probes on each chip, quantile normalization was performed [18, 19]. We excluded probes with poor detection above background (in less than 5% of samples), probable cross-reactivity, and/or polymorphisms in the CpG or single base extension sites [20]. Additional quality control measures included comparison of documented gender to that estimated from X and Y chromosomal DNA methylation profiles, low intensity across all probes, and/or >5% probes failing. An initial quality control check showed that all 526 adolescent samples undergoing this analysis passed quality control. As heterogeneous populations of cells comprise whole blood (the sources from which the DNA was extracted), cell type proportions were estimated based on data

from tissue-specific differentially methylated regions included on the chips [21]. Surrogate variable analysis was performed using the intensity values from the non-negative control probes to create variables representing technical variation influencing the DNA methylation data [22]. The final data used in downstream statistical analysis reflects average beta values for the proportion of total methylated cytosine at each of the 762,657 CpG sites included in this analysis.

Cognitive Performance Test

At the adolescent study visit, Conners CPT, 3rd edition (CPT3) was conducted by a trained research staff. For this computerized test, children are presented with a series of stimuli to which they respond by either hitting a button or not. From the response data scores, variables are generated to reflect four different aspects of attention: inattentiveness, impulsivity/hyperactivity, sustained attention, and vigilance [23, 24], as shown in Chapter 1-Table 1-1. Four representative variables for each aspect of attention were selected as outcomes based on ease of interpretation and prevalence of use in the literature. Response style, generated by the CPT3 test to account for individualistic style of responding, was considered a covariate in this study [23-25].

Covariates

Additional variables, including child gender, age, CPT3 response style, and socioeconomic status (SES) were considered as potential confounders in the relationship between DNA methylation and CPT3 outcomes on the basis of their biological relevance and/or correlation (Spearman) with the outcomes (Appendix: Table 2-6 appx). SES was quantified using a scaling system specifically designed for Mexican populations (AMAI) as a metric [26, 27]. In subsequent analyses, principal component 4 (PC4), representing batch effects from the EPIC data, and cell type proportions in whole blood (CD8+ T cells, B cells, and granulocytes) were added to the models. These variables were selected as described below. Child Tanner staging, a measure of pubertal status determined by a trained physician at the study visit, was also considered but not included in the models, as this variable was not associated with the outcome variables and its inclusion in the model did not improve fit.

Statistical Analysis

All statistical analyses were performed in R version 4.0.3. Linear regression models were employed to identify CpG sites with methylation associated with Conners CPT3 performance (using outcome variables omission errors, perseverations, hit reaction time (HRT) interstimulus interval change, and HRT block change in independent analyses). Main Models adjusted for age, gender, SES (AMAI), and response style. For sensitivity analyses, an additional model was run for each of the 4 outcomes, controlling for age, gender, response style, principal component 4 (PC4) representing batch effects from the EPIC data, and cell type proportions (CD8+ T cells, B cells, and granulocytes). These variables were selected from a singular value decomposition analysis, which assessed the correlation between each potential covariate and PCs representing DNAm across all probes (Appendix: Figure 2-1 appx). These additional variables explain a large portion of the DNAm data variance but are not expected to have a substantial influence on the outcome, which is why precedence was placed on biological relevance and correlation with CPT3 outcomes in selection of covariates for the main model analyses. Gender-stratified models were also conducted, accounting for age, SES and CPT3 response style. CpG sites were considered statistically significantly associated with outcomes using Benjamini-Hochberg false discovery rate (q-values less than 0.2) [28]. This significance threshold is only moderately stringent, so results that are statistically significant at the more conventional $q < 0.05$ are also highlighted. The 0.2 cutoff was selected because it has been used in other epigenome-wide studies of neurologically-related outcomes [29-33]. The *missMethyl* R package, accounting for unequal representation of CpG sites within genes on the EPIC array, was used to identify functional pathways for differentially methylated genes enriched among the loci with the smallest raw p-values (top 1000) for each outcome using both KEGG and GO pathways.

Gene Expression Data

To quantify the relative expression of genes across the entire genome, RNA sequencing (RNA-seq) was conducted for a subset of participants with RNA isolated from blood ($n = 72$). Expression data was obtained using paired-end 50 cycle sequencing on an Illumina HiSeq 4000 at the University of Michigan Advanced Genomics Core, and was processed and normalized as previously described [14]. For the 69 participants for whom both gene expression and DNAm

data exists, correlations between DNAm at each of these probes and expression of the gene annotated to these loci were calculated. In addition, correlations between DNAm at significant differentially methylated sites (associated with any of the 4 outcomes) and genome-wide expression was identified via Spearman correlation using a Bonferroni corrected critical value of 3.138×10^{-6} ($0.05/15932$).

Blood-Brain DNA Methylation Comparison

Employing a publicly available tool, generated by Hannon *et al*, comparisons were made between DNAm in blood and DNAm in various brain regions for each of the statistically significant probes identified to be differentially methylated by CPT3 performance in the present study. This Blood Brain DNA Methylation Comparison Tool was created using DNAm data generated via the Illumina 450K array (previous version of the EPIC array) from matched blood and brain samples collected postmortem from adults [34]. Out of the 5 significant loci identified in our study, 3 were available in the database and evaluated.

Results

Of the 517 adolescent participants for whom epigenetic data and CPT3 results were available, 51% were female and 49% were male. CPT3 scores exhibited a broad range of values and were comparable to cohorts of similarly aged children across the globe (Table 2-1). After correcting for multiple comparisons via the Benjamini-Hochberg method using a significance threshold of 0.2, four significant probes were identified that were associated with interstimulus interval change—cg02280912, cg12603272, cg22510337, and cg22601108 (Table 2-2 and Appendix: Table 2-7 appx). One of these probes (cg22510337 in gene *ZNF814*) was also significantly associated with block change in both the primary model and sensitivity analysis. DNAm at this probe, along with cg23825347, was significantly associated with perseverations, but only in the sensitivity model (Table 2-2, Appendix: Tables 2-7 and 2-8 appx). P-values from the models for the outcome quantifying inattention (omissions error), were likely inflated ($\lambda=1.99$). For all outcomes other than omissions error, a general overview of the number of statistically significant findings for Main Models and Sensitivity Models is outlined in Appendix Table 2-7 appx. All statistically significant results from the Main Models and Sensitivity models

are presented in Table 2-2 and Appendix Table 2-8 appx, respectively. When models were stratified by gender, we identified only 2 probes (cg13097337 and cg23024411) whose methylation status was significantly associated with CPT3 score, specifically the block change outcome. Both were significant in females (cg13097337: $\beta=-3.014$ (SE=0.582) $q=0.17$ & cg23024411: 1.380 (0.266) 0.17), but not in males (cg13097337: -0.070 (0.24) 1 & cg23024411: -0.209 (0.432) 1) (Appendix: Table 2-9 appx).

For full participant models, we tested for enrichment in functional gene pathways from the KEGG and GO databases. For the ISI outcome, the top KEGG pathways and GO terms (up to top 25 lowest p-values where $p<0.05$) are presented in tables 2-3 and 2-4, respectively. The results for the remaining outcomes are included in the appendix (Appendix: Tables 2-10 appx – 2-15 appx). Across all outcomes, enrichment for pathways related to neurotransmission, immune response, and inflammation was observed. Specific KEGG pathways were enriched for multiple outcomes. For instance, the glutamatergic synapse term was enriched in the block change and ISI change EWASs (Table 2-3, Appendix: Table 2-10 appx), ferroptosis was enriched for perseveration and ISI change EWASs (Table 2-3, Appendix: Table 2-12 appx), and MAPK signaling pathway was enriched for block change and perseveration EWASs (Appendix: Tables 2-10 and 2-12 appx). Notably, after employing a q-value cutoff of $q<0.2$, few pathways were significantly enriched for any outcome. The KEGG pathway ferroptosis was enriched among results from the ISI change analysis ($q\text{-value}=0.048$).

For a subset of ELEMENT participants, transcriptomics gene expression data was also obtained from whole blood [35]. Correlations between DNAm at loci significantly associated with outcomes and gene expression of their annotated genes were generally not statistically significant (Table 2-5). When examining Spearman correlations between DNAm at these significant probes and expression across all genes, significant correlations (Bonferroni corrected $p<3.138\cdot10^{-6}$) were only identified between DNAm at one probe (cg12603272) and gene expression of 19 genes (Appendix: Table 2-16 appx). Because this probe was located on the X chromosome, the correlation analysis was repeated in males and females separately. When stratified by gender, no correlations were statistically significant.

Using the Blood Brain DNA Methylation Comparison Tool, we identified significant correlations between DNAm from matched blood and brain samples that are part of the database

at some of the loci associated with CPT3 outcomes in our study. DNAm in blood at both of the CpG sites for *ZNF814* that we found to be significantly associated with aspects of attention were correlated with DNAm from different brain regions: whole blood DNAm at cg02280912 and cg22510337 were correlated with DNAm in the superior temporal gyrus ($r=0.355$, $p=0.002$) and the entorhinal cortex ($r=0.348$, $p=0.003$), respectively. Lastly, for probe cg22601108, located in the *OR6K6* gene, DNAm in whole blood and the prefrontal cortex were correlated ($r=0.209$, $p=0.073$) [34].

Discussion

The etiology of ADHD continues to be poorly understood, but DNAm in peripheral tissues of children has been associated with this disorder. In a cohort of Mexican children aged 9 to 18 years, we identified relationships between peripheral blood DNAm at specific loci and performance on the CPT3 test, which quantifies measures of inattention, impulsivity, sustained attention, and vigilance. At a significance threshold of $q=0.2$, we identified 5 significant probes, including loci annotated to the genes *ZNF814*, *ELF4*, and *OR6K6*. The direction and magnitude of effect sizes for these loci were comparable in the primary model and in a sensitivity analysis adjusting for batch and cell type proportions, providing further confidence in our results.

One gene of particular interest is *ZNF814*, as two loci annotated to this gene were significantly associated with outcomes (probe IDs cg22510337 and cg02280912, both loci are located near each other in the first intron of the gene in a known CpG island and active regulatory region; Appendix: Figure 2-2 appx) [36]. One of these, cg22510337, was associated with 3 out of 4 CPT3 outcomes—block change, ISI change, and perseverations. *ZNF814* is a protein-coding gene encoding Zinc Finger Protein 814, which is believed to help facilitate DNA-binding transcription activator activity, as well as RNA polymerase II-specific and RNA polymerase II cis-regulatory region sequence-specific DNA binding activity. It is also suspected to be involved in regulating transcription via RNA polymerase II. Genetic variations in *ZNF814* have been documented in patients with familial Hemangioblastomas—rare, slow-growing tumors of the central nervous system [37]. Polymorphisms in several other zinc finger proteins in this family (C_2H_2 -type zinc finger proteins— C_2H_2 -ZNFs) have been associated with

neurodevelopmental disorders, including bipolar disorder, schizophrenia, and autism spectrum disorders [38, 39]. To our knowledge, zinc finger proteins have not been shown to be associated with ADHD specifically, but C₂H₂-ZNFs are known to be highly expressed in developing human brain tissue and help regulate early CNS gene expression patterning [40, 41]. In one study, a single nucleotide polymorphism in the *ZNF804A* gene was found to be associated with executive control of attention in healthy volunteers, but in subsequent studies within ADHD participants researchers were unable to identify significant associations between this zinc finger protein variant and ADHD [38, 42, 43]. This discrepancy could be due to the fact that one study broke down attention into symptom profiles while the other utilized a broader clinical diagnosis of ADHD. Differences in demographic characteristics between the study populations, e.g. age, race/ethnicity, SES, and disease state, may also have played a role. Regardless, evidence from this and other published literature supports the idea that C₂H₂-ZNFs, which are the largest family of transcription factors in humans, may be important targets for the pathologic processes associated with mood and neurodevelopment disorders, including ADHD.

Whole blood is often used as a proxy for the target tissue of interest in epigenetic studies. However, DNAm can vary by tissue and even cell type, so assessing whole blood DNAm may not reflect the same findings we would see in brain regions and cells. We accessed publicly available resources comparing DNAm from matched blood and brain samples to infer whether associated genes have correlated or divergent DNAm between the surrogate and target tissue of interest. This Blood Brain DNA Methylation Comparison Tool generated by Hannon *et al* is a unique resource; however, it must be noted that brain tissue was collected post-mortem and may not be representative of DNAm profiles in healthy brain tissue. Regardless, utilization of this tool for probes we found to be significantly associated with aspects of attention generated some interesting findings. At the *ZNF814* probe cg02280912, for example, blood DNAm was correlated with DNAm in the superior temporal gyrus [34]. The superior temporal gyrus is a section of the brain that is involved in auditory processing, language, and social cognition [44]. Abnormalities in the superior temporal gyrus (i.e., increased radial diffusivity) have been documented in children with ADHD [45, 46]. In another example, whole blood DNAm at cg22510337, a probe that is also annotated to *ZNF814*, and DNAm at the same probe in the entorhinal cortex were associated with each other [34]. The entorhinal cortex plays a major role in regulation of the neuro circuitry of the hippocampus, the part of the brain responsible for

learning and memory; both structures are implicated in the etiology of neuropsychiatric disorders such as ADHD [47, 48]. Another important structure implicated in the etiology of ADHD is the prefrontal cortex [48-50]. We found that blood and prefrontal cortex DNAm were correlated at cg22601108 (loci in the *OR6K6* gene) ($r=0.209$, $p=0.073$) [34]. While blood leukocytes and brain cells are innately designed to perform different functions and are known to have different epigenetic regulation at many genes, the correlations identified between blood and brain tissues may represent a common set of genes with similar epigenetic patterns in both tissues. These genes may serve as better biomarkers of ADHD-like symptoms than others in blood. Another potential explanation of the association between blood DNAm and ADHD symptoms comes from emerging evidence suggesting that various immune cells in blood may help regulate neurophysiology and behavior[51]. While these correlations between blood and brain DNAm are interesting to think about, the results should not be overstated. However, if paired with *in vitro* or *in vivo* analyses where brain tissue can be studied directly, we may be able to gain stronger mechanistic insight probing questions identified in peripheral blood analyses.

A number of interesting findings appeared in both the GO and KEGG pathways analyses. These were exploratory analyses since we used the top loci by raw p-value, and not all results would withstand correction for multiple hypothesis testing ($q<0.2$). Even so, pathways related to neurotransmission, immune response, and inflammation were common observations across analyses for all outcomes. The KEGG term for glutamatergic synapses was enriched for both the block change and ISI change analyses (Tables 2-3 and 2-4). The dysregulation of glutamatergic neuronal functioning is implicated in the etiology of ADHD [52, 53]. Its enrichment in our study for block change and ISI change outcomes suggests glutamatergic neurons may be more relevant to sustained attention and vigilance, respectively.

Among top loci associated with preservation, pathways analysis using the KEGG database revealed enrichment for cGMP-PKG signaling as well as a number of calcium-related signaling pathways. It has been shown that cGMP-PKG signaling acts as an intrinsic modulatory system by regulating neuronal calcium levels [54]. This can be accomplished by activating cyclic nucleotide-gated channels or releasing calcium stores via ryanodine receptors [55, 56]. Calcium pathways were also enriched among preservation results when using the GO database. These findings suggest that calcium dysregulation in the brain may influence impulsivity. This claim is

further supported by Varmis *et al* who found that increased calcium levels were associated with ADHD, as were reduced parathyroid hormone levels, which helps regulate calcium levels [57]. In a childhood ADHD EWAS conducted by Mooney *et al*, the top-ranked differentially methylated position, showing increased DNA methylation in ADHD cases compared with non-ADHD controls, was located in the promotor region of *SLC7A8* [6]. This gene is involved in several functions, including response to elevated platelet cytosolic calcium levels and thyroid hormone transport.

Another KEGG pathway enriched among loci genes associated with perseveration, as well as with ISI change, was ferroptosis. This was the only pathway that was significantly enriched at the FDR adjusted significance value. Ferroptosis is a highly regulated, complex, iron-dependent form of cell death that is characterized by the oxidative destruction of the cell's lipid bilayer. Recent studies suggest that dysregulation of ferroptosis may be closely related to the pathogenesis of neurological disorders including neurodegenerative diseases, strokes, and glioma [58-60]. Interestingly, iron deficiency in various brain regions, including the globus pallidus, caudate nucleus, and hippocampus, was characteristic of children with ADHD according to a quantitative susceptibility mapping study conducted by Tang *et al* [61]. This, partnered with our study's findings, suggests that iron deficiency in the brain and subsequent dysregulation of the ferroptosis pathway may be associated with ADHD in children. Growing recognition by the scientific community that iron deficiency may be associated with ADHD and the findings of a recent randomized clinical controlled trial suggest that iron supplementation may reduce ADHD symptom severity in children, at least when partnered with methylphenidate treatment [62, 63]. Additional research is needed to discern the true effects of iron supplementation both in isolation and in concert with methylphenidate treatment or other pharmacological interventions. Furthermore, the role of ferroptosis in this process until this point has only been implied; mechanistic studies of this process in relation to ADHD may be warranted.

Abnormal inflammatory response and subsequent immune dysregulation are implicated in the etiology of ADHD [64]. Numerous studies have linked other psychiatric illnesses to aberrant inflammatory pathways [65, 66]. Additionally, anxiety and stress are important risk factors and comorbidities of ADHD and are also associated with inflammation and immune dysregulation [67-69]. Results from the present study support the idea that inflammation and

immune dysregulation may play a role in the development of ADHD. The CpG site cg12603272 corresponds the 5'UTR (untranslated region) of *ELF4*, an X-linked ETS transcription factor gene that plays an important role in regulating inflammation and immune response, was statistically significantly associated with the ISI change outcome. Generally, *ELF4* suppresses macrophage response and regulates anti- and pro-inflammatory genes in macrophages. Deficiency in *ELF4* increases inflammatory T_H17 cell responses [70]. In the context of our study, increased DNAm at this CpG site was associated with higher CPT3 scores, or a decrease in vigilance. DNAm at a given gene is often associated with transcriptional suppression of that gene [71]. When we used a subset of ELEMENT participants (N=69) who also had transcriptomic data available to quantify the associations between DNA methylation and expression at the genes of interest; though not statistically significant, the expected inverse relationship between DNAm and expression was observed for *ZNF814* and *OR6K6* genes (Table 2-5). For the *ELF4* gene, no relationship was observed ($r=0.08$, $p=0.52$) so we cannot draw any inferences regarding how expression levels of this gene may be related to CPT3 performance or the etiology of ADHD. Notably, this transcriptomic data was quantified in blood and not in brain, which is the biologically relevant tissue to the outcome. Additionally, we were not able to distinguish between transcriptional variants through this analysis. The gene counts data we generated from the RNA sequencing results represent expression of any transcriptional variant of a given gene. It is entirely possible that methylation at a specific probe can affect not only gross transcriptional levels, but also the ratio of variants transcribed. This is biologically relevant, because distinct variants may be associated with different levels of transcript stability, translational efficiency, or altered protein structure that could affect the gene product's activity without significantly impacting gross expression levels themselves. At least for the example of *ZNF814*, according to both Gencode and Refseq, transcriptional variants are similar in the region where the differentially methylated loci are (Appendix: Figure 2-2 appx) [72, 73].

Inflammatory and immune pathways were enriched for all 4 CPT3 outcomes. Biological process and molecular function terms related to various types of interleukins or leukocytes were present among the top GO pathways for all outcomes. Interleukins are a class of cytokines comprising glycoproteins produced by white blood cells that aid in immune response regulation. Because our EWAS used whole blood to quantify DNAm, pathways analyses were also conducted using the sensitivity models for all 4 outcomes, as they controlled for cell type. As

noted in the final columns of tables 2-3, 2-4, and Appendix Tables 2-10 – 2-15 appx, several of these immune-related pathways remained enriched in the sensitivity analyses, especially for the ISI outcome. Examples include negative regulation of leukocyte degranulation, negative regulation of myeloid leukocyte mediated immunity, and CD8-positive, alpha-beta T cell lineage commitment. The MAPK signaling pathway, playing a role in inflammation and immune cell activation in the brain, is another important example of a pathway that was enriched for KEGG analyses for both block change and perseveration outcomes. One study suggests that disruption of MAPK signaling may result in autism spectrum disorder ASD [74]. Preliminary evidence also exists supporting ADHD as a neuroinflammatory disease characterized by activation of immune cells in the brain, perturbation of the blood brain barrier, and activation of MAPK and NF- κ B signaling [75]. One EWAS assessing relationships with externalizing behavior (characterized by aggression, disobedience, impulsivity, and poor emotional regulation) in children and adolescents noted significant differential methylation at ADHD-related genes and saw enrichment for pathways related to neuroinflammation. The results of their study suggest that interactions between neuroinflammation and dopaminergic pathways may underlie externalizing behaviors, such as those exhibited by children with ADHD [7]. As several of the immune/inflammation pathways identified by our study were enriched in both main and sensitivity models, we believe the results of our present research are consistent with the fount of literature supporting ADHD as a neuroinflammatory disease.

Previous research has explored the relationship between DNAm across the epigenome and ADHD (or ADHD-like symptoms). The significant loci identified in our study did not overlap with those previously reported, but these studies vary in the age of study participants, tissue used for DNAm analysis (e.g., blood, saliva), method of assessment for attention (e.g., clinical diagnosis of ADHD, neuropsychological test, self/parent-reported documentation of symptoms, etc.), and more. Studies focusing on the relationship between DNAm and neurodevelopmental outcomes, particularly ADHD symptoms and externalizing behaviors, include (1) Mooney *et al.* 2020, (2) Li *et al.* 2021, (3) Sun *et al.* 2023, (4) Neumann *et al.* 2020, and (5) Meijer *et al.* 2023[5-8, 76]. Mooney *et al.* 2020 conducted an EWAS of childhood ADHD using peripheral saliva DNA from 603 children (391 with ADHD and 213 without ADHD). The study found epigenome-wide significant associations between ADHD polygenic risk and DNAm at sites annotated to the promoter of genes *GART* and *SON*. The study also

identified DNAm markers that were associated with clinical diagnosis of ADHD, though not via Bonferroni-corrected p -value threshold of $8.8\text{e-}8$. Hypermethylation in ADHD cases relative to controls was observed at the two top-ranked dinucleotide CpG sites, cg17478313 ($\Delta\beta = 0.93\%$, $p = 1.54\text{e-}6$) and cg21609804 ($\Delta\beta = 1.38\%$, $p = 2.82\text{e-}6$). These sites are respectively located in the promotor region of *SLC7A8* and the 3'-UTR of *MARK2*[6].

One of the major sources of variability across ADHD EWAS studies is the method used for phenotyping the outcome and for quantifying methylation. The parent-, teacher-, or self-rated Strengths and Difficulties Questionnaire (SDQ), for example, is a widely used screening tool for behavioral and emotional problems in children and more recently adolescents but is innately subject to bias and human error. For this reason, it is preferable to cross-validate self-report data with parent- or teacher-reports, but this is often not feasible[77, 78]. This was the case for the longitudinal Sun *et al.* 2023 study and some of the studies considered in a combined analysis conducted by Meijer *et al.* that used SDQ for their assessments[7, 8]. Lacking in clinical ADHD diagnosis data for a sufficient sample-size for use in their models, many researchers must use the hyperactivity-inattention domain of the SDQ or similar assessments to quantify ADHD and ADHD-like symptoms, and only confirm ADHD status in participants with clinical diagnosis when data is available[8, 76]. While the present study in ELEMENT participants is also lacking in clinical ADHD diagnosis data, it used data from the computerized CPT3 test for its models. Using continuous outcomes such as this may provide more power to detect small changes in symptoms but cannot technically be related to ADHD without a clinical diagnosis. Li *et al.* 2021 included both CPT outcomes and diagnostic data to complement one another in studying the relationships with DNAm. While this study is an epigenome-wide screen (followed by site-specific pyrosequencing analysis on selected candidate genes) like the other studies discussed in this section, it is distinct in that instead of using a more traditional regression model for its statistical method it employs ANOVA and MANCOVA models, which is likely to underestimate standard error and makes it more difficult to compare results across studies. Furthermore, its sample size is notably small (12 cases and 9 controls), thus reducing its power [5]. To its credit, this study, along with Mooney *et al.* 2020, is among the first to use the EPIC array to study potential biomarkers of ADHD. This provides a greater coverage of the epigenome, as it contains >850,000 probes, whereas the more commonly cited assay for prior EWAS studies, Illumina Human Methylation 450K array, only contains ~450,000 probes[5, 6].

Interestingly, Sun *et al.* 2023 used both the EPIC and the 450K arrays, pulling data from the IMAGEN project from 8 different sites across Europe[7]. The researchers used the SDQ to investigate the relationship between DNAm and internalizing behavior (IB) or externalizing behavior (EB). In the EB-EWAS, only 1 probe (cg01460382) attained epigenome-wide significance ($r = 0.18$, $t_{1009} = 5.74$, $p_{\text{uncorrected}} = 1.26 \times 10^{-8}$, $p_{\text{Bonferroni}} = 4.7 \times 10^{-3}$). No significant relationships between DNAm and IB were identified. They also related DNAm to gray matter volumes, discovered differentially methylated regions (DMRs) associated with behavioral problems and suggested these DMRs may play a role in facilitating brain development [7]. Neumann *et al.* 2020 also employed data from both Illumina EPIC and 450K arrays. They conducted a prospective meta-analysis of research assessing the association between DNAm and ADHD symptoms from birth to school age (12 years old) as determined by parent-reported questionnaires. DNAm in school age children was not associated with ADHD symptoms[76]. One potential factor that could be contributing to the lack of significant findings in these multi-array analyses is the fact that even though there is an overlap in probes across these assays, DNAm from the EPIC and 450k array are not readily comparable to one another and do not reliably replicate results on the individual probe level even within the same samples[79]. This poses a challenge to replication studies and meta-analyses such as these, but fortunately computational methods have been designed to address this issue and harmonize probes across arrays[80, 81]. The research outlined by Neuman *et al.* included cohorts based in Europe, including Denmark, England, Finland, the Netherlands, and Spain, but did not include any from Latin America, as is seen in this current analysis[76]. This also sets the present research aside from the previously discussed studies such as, Mooney *et al.*, Meijer *et al.*, and Sun *et al.*, whose cohort participants are predominantly of North American and European descent[6-8, 13]. It is important to note that relationships between specific DNAm markers and ADHD symptoms observed in the Neumann *et al* meta-analysis varied across different cohorts and time points, suggesting the role of DNAm in ADHD etiology may differ based on developmental timepoint, demographics, and other risk factors of the underlying population[76].

Meijer *et al.* 2023 conducted a combined analysis and review of epigenome-wide DNAm and externalizing behaviors in children, adolescents and adults, with ten studies meeting their inclusion criteria. Across multiple studies, this review identified several DMRs that were consistently associated with externalizing behaviors in children and adolescents and proposed ten

candidate genes for ADHD: *GNG7*, *ADAMTS2*, *TNXB*, *ATP11A*, *BRD2*, *CACNA1H*, *SDK2*, *ACOXL*, *FLJ30306*, and *ARID4A*. Meijer *et al.* also highlighted the importance of considering the age range of participants, tissue used for DNAm quantification, and methods employed to assess their externalizing behavior outcomes for appropriate interpretation of results. The studies included in their analysis measured externalizing behaviors in different ways, including the SDQ, behavioral observations, and diagnostic interviews [8]. While this combined analysis does not solely relate DNAm to ADHD diagnosis or ADHD-specific symptoms and instead uses the broader term of externalizing behavior, ADHD is one of the most commonly occurring comorbidities of externalizing behavior disorders and the findings from this study are comparable to more ADHD-specific studies[8, 82]. One study in a Swedish birth cohort suggested externalizing behaviors in middle childhood were predictive of ADHD-like symptoms in early adolescence, and saw associations between ADHD-like traits and externalizing behaviors increased over time [83]. This underscores the interrelationship between these commonly concurring symptoms and disorders.

Overall, previous studies suggest that DNAm may be a promising biomarker for predicting and understanding neurodevelopmental outcomes such as ADHD symptoms and externalizing behaviors. Several proposed gene biomarkers of ADHD, as identified by Meijer *et al.* 2023, contain significant findings across different studies. The relevant probes annotated to these genes were extracted from the results files of this current investigation. Only probes that were identified as associated with ADHD in child or adolescent populations were extracted for comparison and compiled into document Appendix Table 2-17 appx. While none of these results were significant on the epigenome-wide level, some raw p-values were significant ($p < 0.05$) and many of the probes had similar direction of effect as observed in prior studies. The probe cg15829622, for instance, was significant for both ISI change ($\beta = -0.582$, $SE = 0.20$, $p = 0.004$) and block change ($\beta = -0.429$, $SE = 0.19$, $p = 0.024$) EWASs. Not only was the direction of effect similar for both analyses, with lower DNAm associating with worse CPT3 performance (higher scores), but it was also similar to what we would expect from Goodman *et al.* 2020, which document an association between lower levels of DNAm at this probe and ADHD ($\beta = -0.96$, $p = 4.57E-4$)[8, 84].

Epigenomic research focused on ADHD to date is met with many challenges. For one, the lack of consistency in assay type utilized makes comparisons across studies difficult. As previously mentioned, the Illumina 450k and EPIC arrays, despite covering many of the same CpG sites and quantifying DNAm through the same principles, fail to produce consistent findings even when identical DNA sources are used[79-81]. This could be attributed to un-equal representation of type I (containing 2 sequences per probe: one for methylated and the other for unmethylated versions) and type II probes (containing only one sequence, occupying half the physical space as type I probes). For this reason, one should take caution in drawing conclusions and interpreting results when making cross-study comparisons. In addition to variability between the different iterations of the Illumina Infinium platforms, there can also be batch effects across experiments of the same type and even between probes on an individual chip[16, 79, 81, 85]. Another challenge to the field is the lack of a standard threshold for significance. This study uses a Bonferroni-Hochberg method to correct for multiple comparisons with a q-value cutoff of 0.2. This is moderately stringent, with prior studies documenting significant findings based on far more lenient cutoffs and others far stricter[8, 33, 86]. This could be considered a weakness of our study, which is why results with a more conventional q-value cutoff of 0.05 are emphasized in our discussion.

Prior research also emphasizes the relevance of considering participant age and tissue source when interpreting results and provide insight into potential mechanisms underlying the development of these outcomes [76]. While these studies offer a starting point to uncover the associations of DNAm with ADHD etiology, more research is warranted in diverse populations with more ADHD-specific outcomes. Unlike previous studies, this research is conducted in a Hispanic population. This investigation also builds upon past research by using an ADHD-specific test completed by the participant which employs computerized quantifications of varying aspects of attention, rather than survey responses from external parties like parents or teachers. Future research may continue to explore these associations and their potential implications for diagnosis and treatment in diverse cohorts.

As with any study, ours is met with both strengths and weaknesses. Notably, this study is among the first to employ an epigenome-wide screen to identify associations between DNAm and ADHD-like symptoms in adolescence, a relevant neurodevelopmental timepoint [5-8] . To

our knowledge it is also the first study to do so in a Hispanic population. Another strength is the large sample size of 517 participants. While this provides power to detect small changes in DNAm in the full sample, it is limited in its power to detect gender-specific differences. Notably, past studies have documented gender-specific associations between DNAm and ADHD-related phenotypes, but only 2 significant associations were identified in this study with one outcome[6, 8]. The present study also utilizes the results from the CPT3 test, which quantifies symptoms related to ADHD as continuous variables but does not equate them to a diagnosis [23-25]. While using this test in our analyses impedes our ability to draw a relationship between DNAm and a clinical diagnosis for ADHD, the continuous nature of these CPT3 variables strengthens our study's power to identify associations with small differences in test performance reflecting behavioral changes that might not signify an official diagnosis. One limitation is the use of blood leukocyte DNA for EWAS analyses instead of brain DNA, the ideal tissue for a neurological outcome. While methylation profiles in blood correlate with those of the brain at some genes, they differ at many others [34]. Notably, all CPT3 outcomes met the assumptions of linearity and were modelled using linear regression. It is possible, however, that the relationship between DNAm at some loci and outcomes is not linear and was not identified here. Non-linear model types should be explored in future analyses to entertain the possibility of different types of relationships between DNAm at certain genes and CPT3 performance. As previously mentioned, the EPIC array has a number of technical limitations, such as the unequal representation of type I and type II probes. It also has an over-representation of cancer and other disease related sites. It only covers a portion of the CpG sites in the entire epigenome. Computational techniques have been developed to address some of these concerns. Regardless of its challenges, the EPIC array has a larger coverage than past versions of Illumina Infinium arrays, spans the entire genome, and remains to be a useful tool for epigenome-wide analyses[16, 80, 81, 85, 87]. To that extent, DNAm in any tissue type is not the only epigenetic mechanism modulating gene expression. Other factors, such as chromatin architecture, histone modification, and non-coding RNA such as piRNA, also play a role and may be impacting the development of this highly complex neuropsychological disorder [4]. These mechanisms should be explored in relation to ADHD in children in future research. Regardless, the present EWAS provides a foundation for future explorations of the relationship between epigenetic mechanisms and the etiology of ADHD.

Conclusion

We conducted an EWAS of ADHD-like symptoms using blood leukocyte DNA from Mexican adolescents. We identified associations between DNAm at specific CpG sites and three out of four CPT3 variables selected to reflect ADHD symptom profiles: sustained attention (block change), vigilance (ISI change), and impulsivity (perseverations). Differential DNAm at sites located in the gene body of *ZNF814* (Zinc Finger Protein 814) were associated with these three CPT3 outcomes. Additionally, hypermethylation at *ELF4* and *OR6K6* were individually associated with an increase in ISI change score (i.e., a decrease in vigilance). Across all CPT3 outcomes, we observed enrichment for pathways related to neurotransmission, immune response, and inflammation among differentially methylated sites.

Epigenetics, including DNAm, has great potential to serve as biomarkers of ADHD and help unravel its underlying etiology. ADHD is a complex disorder that may be influenced by many different factors, including the social environment, diet, and exposure to toxic chemicals. Future research would benefit from analysis of the joint effect of DNAm and environmental exposures on the etiology of ADHD in children. Prior to that, analyses like this current study, which analyze the effects of each predictor on ADHD etiology independent of the other, are essential. While the ELEMENT cohort was not designed to include clinical ADHD diagnostic data, analyses in similarly aged populations may consider a case-control design to best identify DNAm biomarkers of ADHD. Regardless, this research laid the groundwork for future biomarker and mechanistic studies. For example, the potential role of Zinc Finger Protein 814, as well as specific pathways, such as those related to inflammation, immune response, and neurotransmission, could be considered in *in vitro* or *in vivo* experiments focused on ADHD pathogenesis.

Summary Points

- ADHD is a highly prevalent neurodevelopmental disorder in children and a risk factor for numerous future health outcomes.
- The complex etiology of ADHD is not well understood, but DNAm has been suggested to play a role.

- In an EWAS of 517 ELEMENT participants, DNAm was related to childhood performance on the CPT3 test.
- DNA methylation at *ZNF814* was associated with vigilance, sustained attention, and impulsivity.
- *ELF4* and *OR6K6* DNA methylation was individually associated with an increase in ISI change score, or a decrease in vigilance.
- Inflammation, immune response, and neurotransmission pathways were enriched among differentially methylated genes across all CPT3 outcomes.
- Future *in vitro* and *in vivo* studies regarding potential ADHD etiology are needed to validate any mechanistic implications discussed in this study.
- DNAm may potentially serve as a biomarker for predicting and understanding neurodevelopmental outcomes such as externalizing behaviors and ADHD symptoms, but currently more research is warranted.

Figures and Tables

Table 2-1: Descriptive statistics for adolescent ELEMENT participants included in this analysis.

	N	Median	IQR	Mean	Standard Deviation	Range
Age (years)	517	14.4	12.8 – 16.7	14.59	2.12	9.8 - 18.1
Response Style	517	52	47 – 58	52.45	9.27	27 - 90
Omissions Error	517	47	44 – 53	50.57	10.48	41 - 90
Perseveration	517	46	45 – 52	51.17	11.35	44 - 90
Block Change	516	51	45.75 – 57	51.5	9.84	13 - 90
ISI Change	517	50	44 – 56	51.03	10.23	27 - 90
Gender		N (%)				
Male		243 (47%)				
Female		274 (53%)				
AMAI						
A/B		3 (0.58%)				
C+		35 (6.77%)				
C		94 (18.18%)				
C-		126 (24.37%)				
D+		125 (24.18%)				
D		132 (25.53%)				
E		2 (0.39%)				

Note: For the AMAI variable, categories are listed sequentially with A/B representing the highest SES level (A/B=7) and category E reflecting the lowest SES level (E=1)

Table 2-2: Loci with DNAm statistically significantly associated with CPT outcomes.

Probe ID	Outcome	Estimate	SE	P value	Q value	Gene Name	Chr.	Position	Relation to Island	USCS RefGene Annotation
cg02280912	ISI Change	0.719	0.142	5.49E-07	0.122	<i>ZNF814</i>	chr19	58400095	Island	Body
cg12603272	ISI Change	0.873	0.173	6.38E-07	0.122	<i>ELF4</i>	chrX	129221241	Open Sea	5'UTR
cg22510337	ISI Change	0.909	0.18	5.80E-07	0.122	<i>ZNF814</i>	chr19	58399967	Island	Body
cg22601108	ISI Change	1.815	0.358	5.54E-07	0.122	<i>OR6K6</i>	chr1	158723939	Open Sea	TSS1500
cg22510337	Block Change	0.971	0.169	1.55E-08	0.012	<i>ZNF814</i>	chr19	58399967	Island	Body

Table 2-3: Top KEGG terms enriched among results from the ISI change model.

KEGG term	Description	N	DE	P-value	FDR q-value	Significant in sensitivity model, too?
path:hsa04216	Ferroptosis	41	8	1.00E-04	0.048 *	Y
path:hsa00061	Fatty acid biosynthesis	17	5	0.001	0.193	N
path:hsa03040	Spliceosome	130	11	0.002	0.193	Y
path:hsa04658	Th1 and Th2 cell differentiation	90	10	0.005	0.467	N
path:hsa04723	Retrograde endocannabinoid signaling	141	13	0.009	0.554	N
path:hsa04114	Oocyte meiosis	121	11	0.01	0.554	N
path:hsa00071	Fatty acid degradation	43	5	0.011	0.554	N
path:hsa04914	Progesterone-mediated oocyte maturation	92	9	0.02	0.799	N
path:hsa05142	Chagas disease	102	9	0.021	0.799	N
path:hsa04936	Alcoholic liver disease	139	10	0.03	0.802	N
path:hsa01212	Fatty acid metabolism	56	6	0.03	0.802	N
path:hsa04912	GnRH signaling pathway	93	9	0.035	0.802	N
path:hsa04933	AGE-RAGE signaling pathway in diabetic complications	100	9	0.038	0.802	Y
path:hsa04218	Cellular senescence	155	12	0.041	0.802	N
path:hsa05150	Staphylococcus aureus infection	86	5	0.042	0.802	N
path:hsa04520	Adherens junction	71	8	0.044	0.802	N
path:hsa04724	Glutamatergic synapse	114	11	0.049	0.802	N

Note: In table headings N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest. Up to the 25 terms with the lowest p-values (must be $p < 0.05$) were included in this table.

Table 2-4: Top GO terms enriched among results from the ISI change model.

Description	Term Identifier	ONTOLOGY	N	DE	P-value	FDR q-value	Significant in Sensitivity model?
Negative regulation of leukocyte degranulation	GO:0043301	BP	12	4	4.32E-04	1	Y
Cellular response to histamine	GO:0071420	BP	8	4	5.45E-04	1	N
Recycling endosome	GO:0055037	CC	187	18	8.18E-04	1	N
Negative regulation of exocytosis	GO:0045920	BP	32	6	1.08E-03	1	Y
Ethanol catabolic process	GO:0006068	BP	11	3	1.34E-03	1	N
Response to histamine	GO:0034776	BP	11	4	1.53E-03	1	N
Primary alcohol catabolic process	GO:0034310	BP	14	3	2.61E-03	1	N
CD4-positive, CD25-positive, alpha-beta regulatory T cell lineage commitment	GO:0002362	BP	2	2	3.07E-03	1	Y
Negative regulation of myeloid leukocyte mediated immunity	GO:0002887	BP	10	3	3.48E-03	1	Y
Nuclear speck	GO:0016607	CC	393	27	3.78E-03	1	N
Regulation of atrial cardiac muscle cell membrane repolarization	GO:0060372	BP	8	3	4.12E-03	1	N
Synapse pruning	GO:0098883	BP	11	3	4.72E-03	1	N
Negative regulation of leukocyte mediated immunity	GO:0002704	BP	52	6	5.16E-03	1	N
Cell junction disassembly	GO:0150146	BP	19	4	5.26E-03	1	N
Positive regulation of protein lipidation	GO:1903061	BP	4	2	5.40E-03	1	N
Regulation of vascular permeability	GO:0043114	BP	45	7	5.58E-03	1	N
SH3 domain binding	GO:0017124	MF	130	14	5.75E-03	1	N
Double-strand break repair involved in meiotic recombination	GO:1990918	BP	5	2	5.99E-03	1	N
Positive regulation of reactive oxygen species metabolic process	GO:2000379	BP	104	10	6.46E-03	1	N

Negative regulation of cellular extravasation	GO:0002692	BP	9	3	6.47E-03	1	N
Negative regulation of neutrophil degranulation	GO:0043314	BP	2	2	6.87E-03	1	Y
CD8-positive, alpha-beta T cell lineage commitment	GO:0043375	BP	2	2	6.88E-03	1	Y
Regulation of reactive oxygen species metabolic process	GO:2000377	BP	197	15	7.25E-03	1	Y
Positive regulation of mitochondrial ATP synthesis coupled electron transport	GO:1905448	BP	5	2	7.30E-03	1	N
Negative regulation of vascular permeability	GO:0043116	BP	16	4	7.39E-03	1	N

Note: In table headings N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest and the 25 terms with the lowest p-values that also had $p < 0.05$ were included in this table.

Table 2-5: Spearman Correlation Between DNAm at a given probe and expression of its nearest gene.

Gene\ProbeID	cg22510337	cg02280912	cg12603272	cg22601108
ZNF814	-0.21 (0.08) ▪	-0.13 (0.3)	NA	NA
ELF4	NA	NA	0.08 (0.52)	NA
OR6K6	NA	NA	NA	-0.15 (0.21)

Note: $r(p)$; ▪ signifies $p < 0.10$; NA did not test

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Appendix

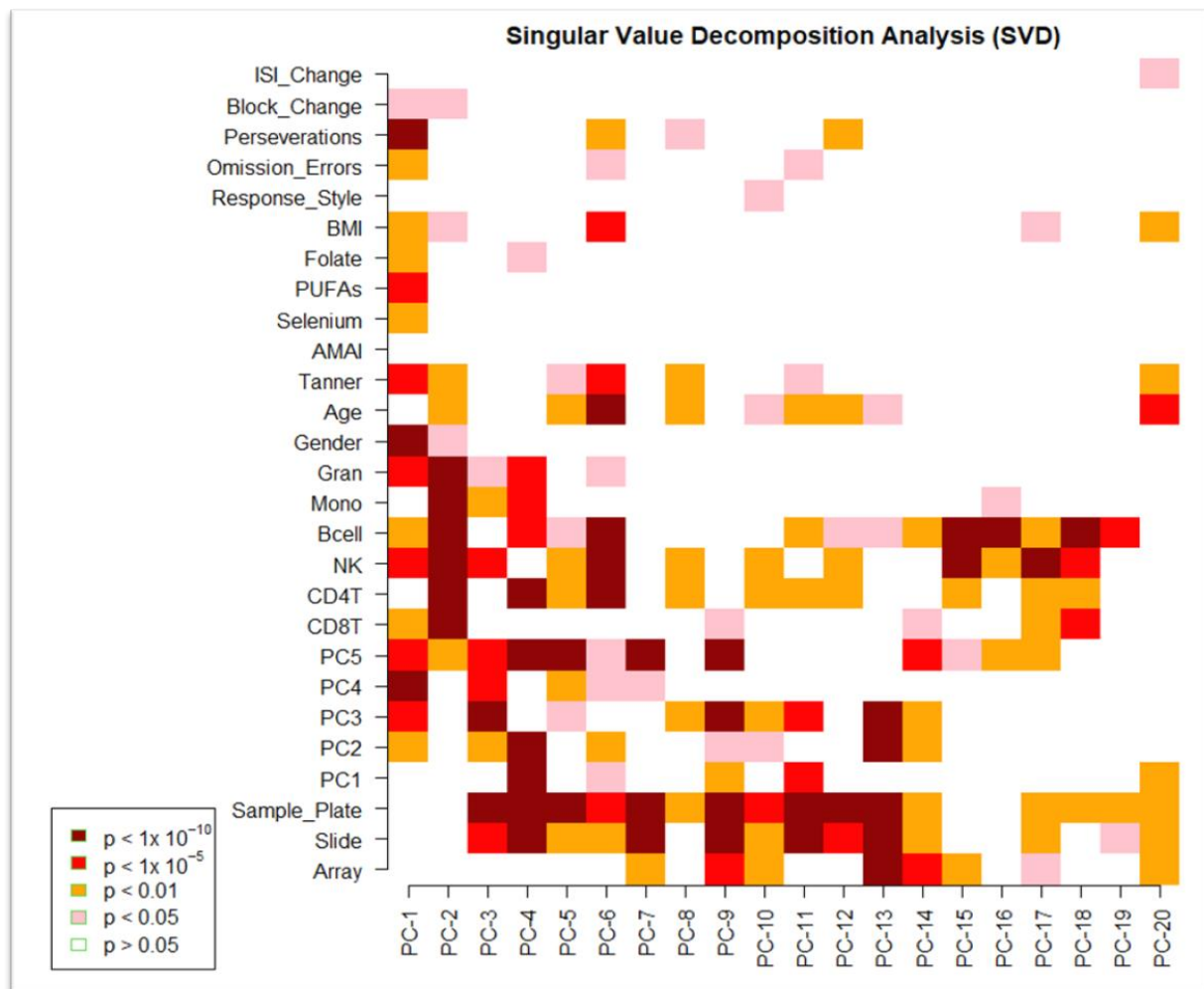


Figure 2-1 appx: Singular value decomposition analysis outlining correlations between relevant variables and DNAm profiles of the ELEMENT participants considered in this EWAS.

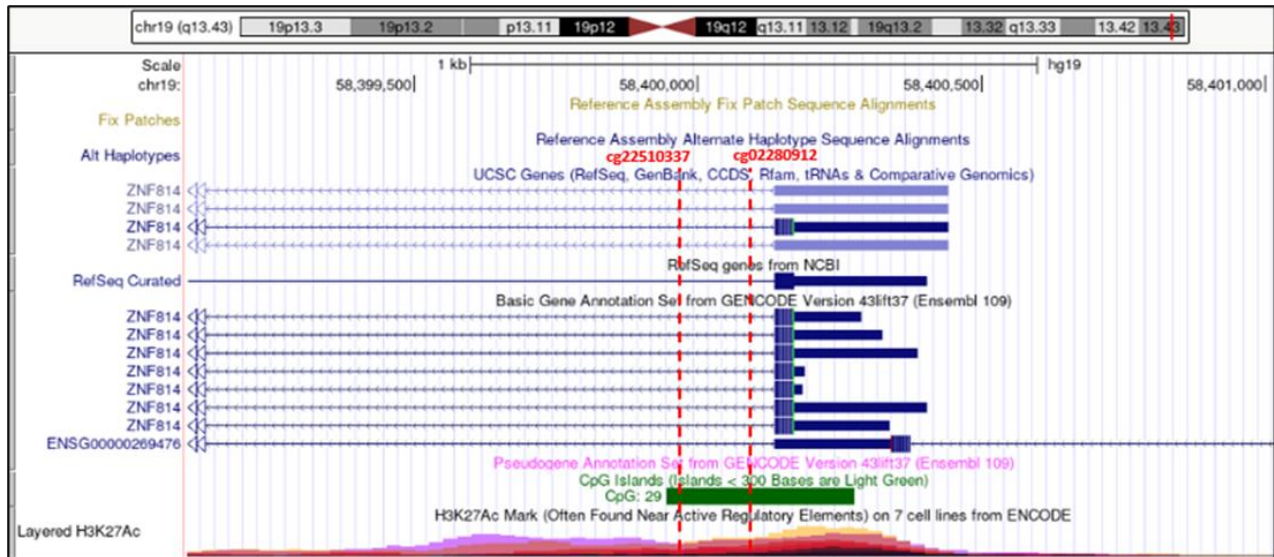


Figure 2-2 appx: Location of significant probes within the ZNF814 gene.

Red dashed line indicates the location of each probe along the gene within intron 1 and arrows denote the direction of transcription. Image modified from UCSC Genome Browser track https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr19%3A58399095%2D58401095&hgid=1660567612_TDZK0y47G6MezDecHvA36Ma1rnA5

Table 2-6 appx: Correlation coefficients and p-values outlining Spearman correlations between CPT3 outcomes and potential covariates to be used in EWAS models.

R (p)	PC2	PC3	PC4	PC5	CD8T	CD4T	NK	Bcell	Mono	Gran	Age
PC1	0.045 (0.304)	0.277 (9.444E-11)	-0.141 (0.001)	0.074 (0.092)	-0.007 (0.872)	-0.043 (0.320)	0.036 (0.412)	0.028 (0.523)	0.055 (0.206)	0.022 (0.617)	-0.097 (0.026)
PC2		-0.15 (5.381E-4)	-0.077 (0.077)	0.1 (0.021)	-0.082 (0.060)	0.089 (0.042)	0.102 (0.019)	0.169 (1.005E-4)	-0.096 (0.027)	-0.149 (5.924E-4)	0.114 (0.009)
PC3			0.117 (0.007)	0.11 (0.011)	0.024 (0.580)	0.042 (0.340)	0.03 (0.488)	0.044 (0.318)	0.092 (0.036)	-0.008 (0.862)	0.031 (0.479)
PC4				-0.075 (0.087)	-0.041 (0.351)	0.005 (0.911)	-0.238 (3.349E-8)	-0.118 (0.007)	-0.025 (0.574)	0.206 (1.815E-6)	-0.045 (0.304)
PC5					-0.043 (0.324)	0.037 (0.393)	0.026 (0.552)	0.055 (0.212)	-0.082 (0.060)	-0.062 (0.155)	0.035 (0.417)
CD8T						0.248 (7.758E-9)	-0.023 (0.603)	0.259 (1.562E-9)	-0.32 (5.640E-14)	-0.574 (0)	-0.091 (0.037)
CD4T							-0.12 (0.006)	0.458 (0.00E+00)	-0.425 (0)	-0.59 (0)	-0.094 (0.031)
NK								0.045 (0.300)	-0.184 (2.231E-5)	-0.425 (0)	-0.028 (0.528)
Bcell									-0.389 (0)	-0.612 (0)	-0.276 (1.115E-10)
Mono										0.42 (0)	0.19 (1.121E-5)
Gran											0.153 (4.224E-4)

(Table 2-6 appx. continued on next page)

Table 2-6 appx. continued...

R (p)	AMAI	Se	Folate	PUFAs	BMI	Response Style	Omission Errors	Perseverations	Block Change	ISI Change	Tanner
PC1	0.066 (0.131)	-0.041 (0.346)	-0.042 (0.34)	-0.039 (0.368)	-0.009 (0.842)	-0.011 (0.796)	0.015 (0.740)	-0.059 (0.179)	-0.034 (0.446)	-0.044 (0.316)	-0.001 (0.990)
PC2	-0.047 (0.286)	-0.052 (0.236)	-0.121 (0.005)	-0.08 (0.066)	0.055 (0.209)	0.024 (0.592)	-0.021 (0.636)	-0.037 (0.404)	0.012 (0.779)	-0.023 (0.608)	0.02 (0.656)
PC3	0.017 (0.699)	-0.01 (0.820)	-0.024 (0.583)	-0.095 (0.029)	0.016 (0.716)	-0.028 (0.523)	0.024 (0.590)	0.017 (0.700)	0.003 (0.953)	0.002 (0.961)	0.031 (0.492)
PC4	-0.119 (0.006)	-0.109 (0.013)	-0.152 (4.9E-4)	-0.194 (7.8E-6)	0.071 (0.106)	0.051 (0.246)	-0.004 (0.933)	0.01 (0.820)	0.21 (1.41E-6)	0.027 (0.534)	0.075 (0.093)
PC5	-0.023 (0.603)	-0.028 (0.522)	0.007 (0.877)	0.029 (0.507)	-0.03 (0.496)	-0.027 (0.538)	-0.073 (0.097)	-0.051 (0.251)	-0.046 (0.301)	-0.028 (0.531)	-0.017 (0.697)
CD8T	-0.085 (0.051)	0.02 (0.653)	0.038 (0.392)	0.026 (0.559)	-0.093 (0.034)	0.039 (0.382)	0.152 (5.31E-4)	0.104 (0.018)	-0.086 (0.051)	-0.008 (0.850)	-0.145 (0.001)
CD4T	-0.057 (0.193)	-0.044 (0.314)	0.01 (0.811)	-0.016 (0.714)	-0.169 (1E-4)	0.069 (0.116)	0.079 (0.072)	0.036 (0.414)	0.027 (0.540)	0.036 (0.409)	-0.126 (0.005)
NK	0.016 (0.720)	0.032 (0.471)	0.045 (0.309)	0.026 (0.546)	-0.009 (0.838)	-0.045 (0.309)	-0.014 (0.758)	-0.071 (0.108)	-0.11 (0.013)	-0.11 (0.013)	-0.083 (0.064)
Bcell	-0.017 (0.693)	0.011 (0.807)	0.008 (0.854)	-0.001 (0.974)	-0.105 (0.016)	0.054 (0.224)	0.157 (3.35E-4)	0.045 (0.307)	0.072 (0.103)	0.079 (0.072)	-0.189 (1.97E-5)
Mono	0.125 (0.004)	0.067 (0.127)	0.005 (0.912)	0.033 (0.447)	0.15 (5.87E-4)	-0.016 (0.715)	-0.057 (0.193)	0.019 (0.664)	-0.013 (0.763)	-0.029 (0.505)	0.086 (0.053)
Gran	0.018 (0.688)	-0.021 (0.626)	-0.057 (0.195)	-0.033 (0.447)	0.156 (3.51E-4)	0.007 (0.867)	-0.122 (0.005)	-0.034 (0.441)	0.073 (0.100)	0.032 (0.471)	0.242 (4.061E-8)
Age	0.069 (0.118)	0.029 (0.510)	0.005 (0.912)	0.046 (0.295)	0.244 (1.67E-8)	-0.048 (0.280)	-0.282 (6.4E-11)	-0.037 (0.405)	-0.116 (0.008)	-0.169 (1.1E-4)	0.602 (0)
AMAI		0.036 (0.414)	0.051 (0.240)	-0.015 (0.726)	-0.016 (0.712)	-0.028 (0.528)	-0.095 (0.030)	-0.034 (0.439)	-0.078 (0.078)	0.032 (0.472)	0.031 (0.492)
Se			0.662 (0)	0.542 (0)	0.002 (0.967)	0.013 (0.771)	-0.084 (0.056)	-0.027 (0.542)	-0.003 (0.944)	0.037 (0.398)	-0.008 (0.862)
Folate				0.641 (0)	-0.099 (0.024)	0.068 (0.122)	0.05 (0.256)	0.079 (0.072)	-0.006 (0.897)	0 (0.998)	-0.025 (0.583)
PUFAs					-0.149 (6.313E-4)	-0.018 (0.686)	-0.042 (0.343)	0.05 (0.254)	-0.036 (0.416)	-0.027 (0.547)	0.025 (0.573)
BMI						0.03 (0.499)	0.014 (0.757)	0.004 (0.936)	-0.056 (0.208)	-0.025 (0.575)	0.175 (7.9E-5)
Response Style							0.534 (0)	0.166 (1.528E-4)	0.171 (9.856E-5)	0.194 (8.704E-6)	-0.018 (0.693)
Omission Errors								0.515 (0)	0.123 (0.005)	0.212 (1.155E-6)	-0.194 (1.316E-5)
Perseveration									0.041 (0.357)	0.215 (7.993E-7)	-0.05 (0.271)
Block Change										0.203 (3.3E-6)	-0.055 (0.220)
ISI Change											-0.024 (0.601)

Table 2-7 appx: Summary of Significant findings for full EWAS across outcomes. *

CPT3 outcome	Model	Lambda	# with q-value (<0.05)	# with q-value (<0.2)	Probe IDs
Block Change	Main Model	1.405	1	1	cg22510337
Block Change	Sensitivity Model	0.910	1	1	cg22510337
ISI Change	Main Model	1.254	0	4	cg22510337, cg02280912, cg12603272, cg22601108
ISI Change	Sensitivity Model	1.142	0	0	NA
Perseveration	Main Model	1.096	0	0	NA
Perseveration	Sensitivity Model	0.899	1	2	cg22510337, cg23825347

* Main Model for Omissions Error EWAS was excluded from table, as there was evidence for p-value inflation in the results ($\lambda=1.99$).

Table 2-8 appx: Significant Results for Sensitivity EWAS Analyses.

ProbeID	Outcome	Estimate	SE	P-value	q-value	Gene Name	Chr.	Position	Relation to CpG Island	UCSC RefGene Annotation Group
cg02280912	ISI Change	0.732	0.146	7.11E-07	0.296	ZNF814	chr19	58400095	Island	Body
cg12603272	ISI Change	0.891	0.181	1.21E-06	0.296	ELF4	chrX	129221241	Open Sea	5'UTR
cg22510337	ISI Change	0.893	0.180	9.11E-07	0.296	ZNF814	chr19	58399967	Island	Body
cg22601108	ISI Change	1.880	0.387	1.55E-06	0.296	OR6K6	chr1	158723939	Open Sea	TSS1500
cg22510337	Block Change	0.990	0.167	6.09E-09	0.005	ZNF814	chr19	58399967	Island	Body
cg23825347	Perseveration	-0.023	0.004	2.25E-08	0.017		chr14	77381159	Open Sea	
cg22510337	Perseveration	0.017	0.003	2.34E-07	0.089	ZNF814	chr19	58399967	Island	Body

Note: Results are shown here from the sensitivity model of all outcomes if $q < 0.2$ in the sensitivity model or $q < 0.2$ in the main model for that outcome (see Table 3).

Table 2-9 appx: Significant results from gender stratified block change EWAS.

Model	ProbeID	Estimate	SE	t value	P value	Q value
Full	cg13097337	-0.412	0.22	-1.87	0.0615	0.67
Full	cg23024411	0.886	0.233	3.8	0.000162	0.67
Male	cg13097337	-0.070	0.24	-0.294	0.768894	1
Male	cg23024411	-0.209	0.432	-0.483	0.629436	1
Female	cg13097337	-3.014	0.582	-5.176	4.44E-07	0.17
Female	cg23024411	1.380	0.266	5.186	4.25E-07	0.17

Table 2-10 appx: Top KEGG terms enriched among results from the block change model.

KEGG term	Description	Block Change				
		N	DE	P-value	FDR q-value	Significant in sensitivity model, too?
path:hsa01521	EGFR tyrosine kinase inhibitor resistance	78	11	0.004	0.798	N
path:hsa05412	Arrhythmogenic right ventricular cardiomyopathy	77	10	0.009	0.798	Y
path:hsa05231	Choline metabolism in cancer	98	11	0.009	0.798	Y
path:hsa05214	Glioma	75	9	0.014	0.798	Y
path:hsa05163	Human cytomegalovirus infection	222	17	0.016	0.798	Y
path:hsa04929	GnRH secretion	64	8	0.018	0.798	N
path:hsa05230	Central carbon metabolism in cancer	70	8	0.019	0.798	Y
path:hsa04919	Thyroid hormone signaling pathway	121	12	0.019	0.798	N
path:hsa04724	Glutamatergic synapse	114	12	0.023	0.798	Y
path:hsa05213	Endometrial cancer	58	7	0.031	0.798	Y
path:hsa05223	Non-small cell lung cancer	72	8	0.036	0.798	N
path:hsa04915	Estrogen signaling pathway	137	11	0.038	0.798	N
path:hsa05168	Herpes simplex virus 1 infection	484	20	0.039	0.798	Y
path:hsa04151	PI3K-Akt signaling pathway	346	23	0.041	0.798	N
path:hsa05219	Bladder cancer	41	5	0.043	0.798	Y
path:hsa04260	Cardiac muscle contraction	83	7	0.043	0.798	N
path:hsa04010	MAPK signaling pathway	294	21	0.044	0.798	Y
path:hsa04630	JAK-STAT signaling pathway	152	10	0.046	0.798	N

Note: In table headings, N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest and the 25 terms with the lowest p-values that were also $p < 0.05$ were considered most significant and included in this table.

Table 2-11 appx: Top 25 GO terms enriched among results from the block change model.

Description	Term Identifier	ONTOLOGY	N	Block Change			
				DE	P-value	FDR q-value	Significant in Sensitivity model?
Alphav-beta3 integrin-PKCalpha complex	GO:0035866	CC	3	3	1.95E-04	1	N
CD4-positive, alpha-beta T cell differentiation	GO:0043367	BP	78	11	2.63E-04	1	N
T-helper cell differentiation	GO:0042093	BP	60	9	6.14E-04	1	N
CD4-positive, alpha-beta T cell differentiation involved in immune response	GO:0002294	BP	62	9	6.77E-04	1	N
Alpha-beta T cell activation involved in immune response	GO:0002287	BP	63	9	7.41E-04	1	N
Alpha-beta T cell differentiation involved in immune response	GO:0002293	BP	63	9	7.41E-04	1	N
T cell differentiation involved in immune response	GO:0002292	BP	71	9	1.13E-03	1	N
CD4-positive, alpha-beta T cell activation	GO:0035710	BP	96	11	1.19E-03	1	N
Regulation of interleukin-4 production	GO:0032673	BP	32	6	1.39E-03	1	N
Potassium:proton antiporter activity	GO:0015386	MF	11	4	1.40E-03	1	N
Sodium:proton antiporter activity	GO:0015385	MF	12	4	1.75E-03	1	N
Interleukin-4 production	GO:0032633	BP	35	6	1.85E-03	1	N
T-helper 2 cell cytokine production	GO:0035745	BP	15	4	1.93E-03	1	N
Integrin alphav-beta3 complex	GO:0034683	CC	2	2	1.95E-03	1	N
Alpha-beta T cell differentiation	GO:0046632	BP	106	12	1.97E-03	1	N
Type 2 immune response	GO:0042092	BP	39	6	2.35E-03	1	N
Cytokine production involved in immune response	GO:0002367	BP	102	10	2.59E-03	1	N
Monovalent cation:proton antiporter activity	GO:0005451	MF	13	4	2.62E-03	1	N
Positive regulation of interleukin-4 production	GO:0032753	BP	24	5	2.67E-03	1	N
Positive regulation of phospholipid catabolic process	GO:0060697	BP	3	2	2.77E-03	1	N
CD4-positive, CD25-positive, alpha-beta regulatory T cell lineage	GO:0002362	BP	2	2	3.04E-03	1	N

commitment							
Metal ion:proton antiporter activity	GO:0051139	MF	14	4	3.58E-03	1	N
Negative regulation of low-density lipoprotein particle receptor biosynthetic process	GO:0045715	BP	3	2	3.70E-03	1	N
Positive regulation of T-helper 2 cell differentiation	GO:0045630	BP	7	3	4.03E-03	1	N
L-methionine-(R)-S-oxide reductase activity	GO:0033745	MF	3	2	4.16E-03	1	N

Note: In table headings N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest and the 25 terms with the lowest p-values that were also $p < 0.05$ were considered most significant and included in this table.

Table 2-12 appx: Top KEGG terms enriched among results from the perseverance model.

KEGG term	Description	Perseverations				
		N	DE	P-value	FDR q-value	Significant in sensitivity model, too?
path:hsa00340	Histidine metabolism	22	4	0.004	1	Y
path:hsa04621	NOD-like receptor signaling pathway	175	12	0.013	1	N
path:hsa04961	Endocrine and other factor-regulated calcium reabsorption	53	7	0.016	1	Y
path:hsa04927	Cortisol synthesis and secretion	65	8	0.018	1	N
path:hsa04216	Ferroptosis	41	5	0.019	1	N
path:hsa04010	MAPK signaling pathway	294	22	0.022	1	N
path:hsa04974	Protein digestion and absorption	101	9	0.030	1	Y
path:hsa05140	Leishmaniasis	73	6	0.030	1	N
path:hsa04020	Calcium signaling pathway	238	18	0.033	1	N
path:hsa05168	Herpes simplex virus 1 infection	484	20	0.039	1	N
path:hsa05167	Kaposi sarcoma-associated herpesvirus infection	191	13	0.041	1	N
path:hsa04260	Cardiac muscle contraction	83	7	0.042	1	Y
path:hsa04022	cGMP-PKG signaling pathway	166	13	0.045	1	N
path:hsa05322	Systemic lupus erythematosus	118	6	0.047	1	N

Note: In table headings N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest and the 25 terms with the lowest p-values that were also $p < 0.05$ were considered most significant and included in this table.

Table 2-13 appx: Top 25 GO terms enriched among results from the perseverance model.

				Perseveration			
Description	Term Identifier	ONTOLOGY	N	DE	P-value	FDR q-value	Significant in Sensitivity model?
Negative regulation of chromatin binding	GO:0035562	BP	9	4	3.57E-04	1	Y
Interleukin-18 receptor activity	GO:0042008	MF	2	2	9.38E-04	1	N
Interleukin-18 receptor complex	GO:0045092	CC	2	2	9.38E-04	1	N
Calcium ion transmembrane transport	GO:0070588	BP	309	26	1.42E-03	1	Y
Regulation of chromatin binding	GO:0035561	BP	23	5	1.53E-03	1	N
Secondary metabolite biosynthetic process	GO:0044550	BP	24	5	1.62E-03	1	N
Rad51C-XRCC3 complex	GO:0033065	CC	2	2	1.98E-03	1	N
Protein K48-linked ubiquitination	GO:0070936	BP	57	8	2.01E-03	1	N
Calcium ion transport	GO:0006816	BP	423	31	2.02E-03	1	Y
Divalent metal ion transport	GO:0070838	BP	468	33	2.19E-03	1	N
Cellular response to salt stress	GO:0071472	BP	12	4	2.26E-03	1	N
Regulation of inositol 1,4,5-trisphosphate-sensitive calcium-release channel activity	GO:0031585	BP	7	3	2.36E-03	1	Y
Negative regulation of gene silencing by RNA	GO:0060967	BP	22	5	2.54E-03	1	N
Negative regulation of posttranscriptional gene silencing	GO:0060149	BP	22	5	2.54E-03	1	N
Cellular response to osmotic stress	GO:0071470	BP	42	7	2.58E-03	1	N
Divalent inorganic cation transport	GO:0072511	BP	475	33	2.63E-03	1	N
L-glutamate transmembrane transport	GO:0015813	BP	23	5	2.88E-03	1	Y
Cation-transporting ATPase complex	GO:0090533	CC	18	4	2.92E-03	1	N
Renal artery morphogenesis	GO:0061441	BP	2	2	3.01E-03	1	N
Response to osmotic stress	GO:0006970	BP	84	10	3.02E-03	1	N
Regulation of glutamate metabolic process	GO:2000211	BP	3	2	3.27E-03	1	Y
Lens morphogenesis in camera-type eye	GO:0002089	BP	22	5	3.67E-03	1	N
Four-way junction DNA binding	GO:0000400	MF	17	4	3.74E-03	1	Y
Negative regulation of protein sumoylation	GO:0033234	BP	11	3	3.86E-03	1	N
Protein nitrosylation	GO:0017014	BP	16	4	4.42E-03	1	N

Note: In table headings N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest and the 25 terms with the lowest p-values that were also $p < 0.05$ were considered most significant and included in this table.

Table 2-14 appx: Top KEGG terms enriched among results from the omissions error model.

KEGG term	Description	Omissions Error				
		N	DE	P-value	FDR q-value	Significant in sensitivity model, too?
path:hsa05135	Yersinia infection	135	13	0.010	1	N
path:hsa04710	Circadian rhythm	31	5	0.015	1	N
path:hsa05130	Pathogenic Escherichia coli infection	196	15	0.018	1	Y
path:hsa05161	Hepatitis B	159	13	0.018	1	Y
path:hsa05145	Toxoplasmosis	110	10	0.025	1	N
path:hsa05022	Pathways of neurodegeneration - multiple diseases	462	28	0.027	1	N
path:hsa05167	Kaposi sarcoma-associated herpesvirus infection	191	14	0.029	1	N
path:hsa00970	Aminoacyl-tRNA biosynthesis	43	5	0.035	1	N
path:hsa00410	beta-Alanine metabolism	31	4	0.044	1	Y

Note: In table headings N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest and the 25 terms with the lowest p-values that were also $p < 0.05$ were considered most significant and included in this table.

Table 2-15 appx: Top 25 GO terms enriched among results from the omissions error model.

Description	Term Identifier	ONTOLOGY	N	Omissions Error			
				DE	P-value	FDR q-value	Significant in Sensitivity model?
Regulation of transcription involved in cell fate commitment	GO:0060850	BP	16	5	1.01E-03	1	N
Type I pneumocyte differentiation	GO:0060509	BP	5	3	1.39E-03	1	N
Negative regulation of endoplasmic reticulum unfolded protein response	GO:1900102	BP	13	4	1.69E-03	1	N
Regulation of protein stability	GO:0031647	BP	292	23	2.28E-03	1	N
Rap protein signal transduction	GO:0032486	BP	14	4	3.02E-03	1	N
Protein stabilization	GO:0050821	BP	185	16	3.05E-03	1	N
Gamma-aminobutyric acid catabolic process	GO:0009450	BP	2	2	3.12E-03	1	N
Receptor metabolic process	GO:0043112	BP	197	18	3.40E-03	1	N
Fatty acid derivative binding	GO:1901567	MF	29	5	3.90E-03	1	N
Cellular response to interleukin-9	GO:0071355	BP	8	3	4.57E-03	1	N
Interleukin-9-mediated signaling pathway	GO:0038113	BP	8	3	4.57E-03	1	N
Regulation of intracellular cholesterol transport	GO:0032383	BP	10	3	4.60E-03	1	N
Regulation of intracellular lipid transport	GO:0032377	BP	10	3	4.60E-03	1	N
Regulation of intracellular sterol transport	GO:0032380	BP	10	3	4.60E-03	1	N
Cellular response to interleukin-21	GO:0098757	BP	8	3	4.81E-03	1	N
Interleukin-21-mediated signaling pathway	GO:0038114	BP	8	3	4.81E-03	1	N
Response to interleukin-21	GO:0098756	BP	8	3	4.81E-03	1	N
Negative regulation of cysteine-type endopeptidase activity involved in execution phase of apoptosis	GO:2001271	BP	3	2	4.91E-03	1	Y
Protein localization to endoplasmic reticulum	GO:0070972	BP	145	11	6.31E-03	1	N
Response to interleukin-9	GO:0071104	BP	9	3	6.55E-03	1	N
Receptor internalization	GO:0031623	BP	114	12	6.70E-03	1	N
Phenylalanyl-tRNA aminoacylation	GO:0006432	BP	3	2	7.00E-03	1	N
Metaphase plate congression	GO:0051310	BP	61	7	7.26E-03	1	N
Neuron-neuron synaptic transmission	GO:0007270	BP	8	3	8.01E-03	1	N
Cellular response to prostaglandin E stimulus	GO:0071380	BP	16	4	8.27E-03	1	N

Note: In table headings N = number of genes included in the GO or KEGG term; DE = number of genes that were differentially methylated. P-values were ranked from lowest to highest and the 25 terms with the lowest p-values that were also $p < 0.05$ were considered most significant and included in this table.

Table 2-16 appx: Significant associations between DNAm at cg12603272 and gene expression data.

Gene	Correlation Coefficient	P-value
<i>LINC00278</i>	-0.779	3.11E-15
<i>TTY14</i>	-0.779	3.11E-15
<i>PRKY</i>	-0.775	5.55E-15
<i>UTY</i>	-0.774	5.77E-15
<i>USP9Y</i>	-0.771	8.88E-15
<i>ZFY</i>	-0.767	1.55E-14
<i>DDX3Y</i>	-0.762	2.71E-14
<i>TXLNG2P</i>	-0.761	3.15E-14
<i>MAP7D2</i>	0.746	1.86E-13
<i>KDM5D</i>	-0.738	4.63E-13
<i>XIST</i>	0.729	1.18E-12
<i>TMSB4Y</i>	-0.712	6.91E-12
<i>TTY15</i>	-0.711	7.61E-12
<i>NCRNA00185</i>	-0.701	1.92E-11
<i>EIF1AY</i>	-0.691	5.07E-11
<i>BCORP1</i>	-0.677	1.66E-10
<i>ERCC6L</i>	0.622	1.14E-08
<i>KAL1</i>	-0.538	1.85E-06
<i>ZRSR2</i>	0.538	1.87E-06
<i>ARSD</i>	0.511	7.24E-06

Note: Significance determined using Bonferroni corrected critical value of 3.138-06 (0.05/15932).

Table 2-17 appx: Cross-study comparison: Results from current EWAS for Block Change, ISI Change, Perseverations, and Omissions Error scores for the candidate probes for ADHD in children identified by Meijer et al 2023.

Probe ID	Gene Name	Model results - Block Change - our study				Model results - ISI Change - our study				Model results - Perseverations - our study			
		Estimate	SE	pval	qval	Estimate	SE	pval	qval	Estimate	SE	pval	qval
cg04696980	GNG7	0.451	0.223	0.044	0.67	0.1083	0.237	0.647	0.945	-0.0072	0.0043	0.092	0.95
cg05045332	TNXB	0.111	0.16	0.49	0.873	0.2349	0.168	0.164	0.818	0.0009	0.0031	0.77	0.98
cg05475109	NA	-0.075	0.033	0.023	0.67	0.00917	0.035	0.79	0.971	0.00036	0.0006	0.57	0.97
cg07187957	CACNA1H	0.122	0.113	0.281	0.784	0.03658	0.119	0.759	0.966	0.00126	0.0022	0.561	0.97
cg11200794	ADAMTS2	0.38	0.411	0.355	0.819	0.12	0.434	0.782	0.97	0.0041	0.0079	0.605	0.97
cg11264098	TNXB	-0.015	0.207	0.941	0.99	0.04194	0.218	0.848	0.98	0.0034	0.004	0.393	0.96
cg12307237	ADAMTS2	0.047	0.211	0.824	0.967	-0.212	0.222	0.34	0.871	-0.0021	0.004	0.605	0.97
cg14514578	SDK2	0.122	0.16	0.446	0.857	0.3015	0.169	0.075	0.779	-8E-06	0.0031	1	1
cg14741143	SSR3	-0.127	0.066	0.054	0.67	0.0729	0.07	0.295	0.859	-0.0015	0.0013	0.248	0.95
cg14753408	SDK2	-0.016	0.289	0.956	0.993	0.2655	0.304	0.383	0.883	0.00897	0.0055	0.105	0.95
cg15829622	PFKL	-0.429	0.19	0.024	0.67	-0.5823	0.2	3.7E-3	0.728	-0.0024	0.0037	0.522	0.96
cg16933813	BRD2	-0.018	0.382	0.962	0.994	0.08632	0.404	0.831	0.977	0.01186	0.0073	0.107	0.95
cg24617723	ZSWIM2	0.133	0.137	0.334	0.809	-0.0585	0.145	0.686	0.953	-0.0016	0.0026	0.547	0.97
cg19992207	ACOXL	-0.087	0.16	0.584	0.905	-0.0846	0.168	0.615	0.939	-0.003	0.0031	0.323	0.95
cg09692492	ZDHHC8P	0.005	0.063	0.94	0.99	0.01972	0.066	0.767	0.967	0.00326	0.0012	6.9E-3	0.94
cg02136725	FLJ31306; ARID4A	-0.25	0.744	0.737	0.947	0.5532	0.784	0.481	0.908	0.00237	0.014	0.868	0.99

(Table 2-17 appx. continued on next page)

Table 2-17 appx. continued...

Probe ID	Gene Name	Model results -Omissions Error - our study				Model results - Meijer et al.		Model results - Mooney et al.		Model results - Neumann et al.		Model results - Goodman et al.	
		Estimate	SE	pval	q-val	Estimate	P-val	Estimate	P-val	Estimate	P-val	Estimate	p-val
cg04696980	GNG7	-0.0046	0.0034	0.177	0.544					0.622	5.7E-4		
cg05045332	TNXB	0.0012	0.0024	0.628	0.852			-2.725	3.5E-4				
cg05475109	NA	0.0006	0.0005	0.203	0.569	-0.124	1.7E-4					-1.343	0.0085
cg07187957	CACNA1H	0.0016	0.0017	0.358	0.694	0.0006	6.2E-4						
cg11200794	ADAMTS2	0.0005	0.0062	0.936	0.979					-2.322	3.3E-4		
cg11264098	TNXB	0.0039	0.0031	0.218	0.583	0.0128	9.4E-4						
cg12307237	ADAMTS2	0.0003	0.0032	0.913	0.971			-3.242	6.1E-4				
cg14514578	SDK2	-0.0006	0.0024	0.79	0.925	-0.014	6.0E-4						
cg14741143	SSR3	-0.0007	0.001	0.49	0.778							-1.331	8.3E-4
cg14753408	SDK2	0.0066	0.0043	0.132	0.495			-2.626	3.2E-4				
cg15829622	PFKL	-0.0004	0.0029	0.902	0.968							-0.96	4.6E-4
cg16933813	BRD2	0.0096	0.0058	0.096	0.449			-3.553	8.6E-4				
cg24617723	ZSWIM2	0.0009	0.0021	0.673	0.874							-1.356	8.7E-4
cg19992207	ACOXL	-0.0014	0.0024	0.566	0.82	0.042	8.5E-6						
cg09692492	ZDHHC8P	0.0025	0.0009	0.007	0.249							-1.245	7.3E-4
cg02136725	FLJ31306; ARID4A	0.0051	0.0112	0.649	0.862			-3.342	6.8E-4				

Table 2-17 References:

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Chapter 3

Aim 2: The Combined Associations of Mercury Exposure and DNA Methylation with Sustained Attention in Children in a Novel Analysis of Epigenetic Susceptibility

Abstract

BACKGROUND AND AIM: Due to its complex set of risk factors, the dynamic nature of the brain, and the unique combinations of various symptom profiles, the etiology of attention-deficit/hyperactivity disorder (ADHD) is not well-understood, despite it being one of the most common neurodevelopmental disorders worldwide. Past research suggests childhood methylmercury (meHg) exposure and DNA methylation (DNAm) levels are both important predictors in the development of ADHD, yet simultaneous assessment of these two variables is rare. Leveraging data from the ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants) cohort, this study aims to identify the combined associations of childhood mercury exposure and DNAm with sustained attention in adolescence, and how these associations differ by sex, in a novel analysis of *Epigenetic* - environment interaction. **METHODS:** Data generated from child participants from a longitudinal, multigenerational cohort (ELEMENT) was used from two separate study visits. When participants were aged 6-12 years, total mercury (Hg) was quantified in hair and urine, reflecting organic and inorganic sources of Hg respectively. An average of 5.6 years later, in the same sample of individuals, adolescent DNAm was quantified in DNA extracted from whole blood via the Infinium EPIC array. Also at this timepoint, the participants were administered the Conners Continuous Performance Test (CPT3), quantifying measures of inattention (CPT3 omissions errors), impulsivity (CPT3 perseverations), sustained attention (CPT3 hit reaction time (HRT) block change), and vigilance (CPT3 interstimulus interval (ISI) change). Using linear regression, we assessed associations between hair and urine Hg exposure and CPT3 scores (N=399), adjusting for covariates. We then added DNAm at loci associated with CPT3 scores into the model and tested the interaction between Hg and DNAm.

We performed these analyses in all children and stratified by gender. **RESULTS:** Significant associations were identified between Hg exposure and CPT3 performance. The magnitude and direction of these effects differed depending on the Hg biomarker and the CPT3 variable used for each model. For example, a significant relationship was documented between hair Hg and omissions error score ($\beta=-0.021$ (SE=0.0095), $p=0.027$), but no significant association was identified when urine Hg was used as the exposure instead ($\beta=-0.0045$ (SE=0.0078), $p=0.57$). These associations differed by gender. Urine Hg was associated with ISI change scores in males ($\beta=1.31$ (SE=0.65), $p=0.045$), but not in females ($\beta=-0.20$ (SE=0.81), $p=0.80$). Lastly, in the combined models containing both DNAm and Hg as predictors, significant interactions between Hg and DNAm were identified for omissions error (for two CpG sites) and ISI change outcomes (for one CpG site) in full population models. When stratified by gender, 157 significant interaction terms were identified in females for the omissions error models and 3 significant interactions were identified for the perseveration model in males. **CONCLUSIONS:** Both DNAm and mercury exposure, independently and together, are associated with adolescent CPT3 performance. DNAm and Hg may interact to affect their associations with the CPT3 outcomes, suggesting DNAm may influence one's susceptibility to developing ADHD or ADHD-like symptoms. The contribution of epigenetics to one's susceptibility to the toxic effects of exposures is an overlooked potential risk factor that should be considered in future analyses of toxicant-related disease.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a highly prevalent and complex neurodevelopmental disorder characterized by a dysregulation of normal executive function. In the US, 9.5% of children and 4.4% of adults are affected by this disorder. The precise statistics vary from country to country based on numerous aspects such as socioeconomic (SES) factors, environmental exposures, and access to healthcare systems, but ADHD along with autism are considered to be the two most common neurodevelopmental disorders worldwide [1]. Despite this, the etiology of ADHD is poorly understood.

Several studies have linked mercury (Hg) exposure to ADHD and ADHD-like behaviors in children and adolescents [2-6]. Mercury exists environmentally in multiple forms, including

organic, inorganic, and elemental, and can cycle between these types over time. The most prevalent form of organic Hg is methylmercury (meHg), which humans are predominantly exposed to through consumption of contaminated seafood. As testament to its broad prevalence, nearly all people in the world are believed to have at least some level of meHg in their bodies. This is concerning, because it is known to be highly neurotoxic in high doses, with less certainty on the effects of chronic low-dose exposures. Notably, the majority of studies relating meHg to adverse neurodevelopment focus on prenatal Hg exposures [2-4]. While this represents a neurodevelopmental period that is highly vulnerable to toxic insults, childhood and adolescence are also very important timeframes for brain development, especially for the frontal lobe. Because of this, exposure to meHg during this time may adversely impact the still developing frontal lobe of the brain, dysregulate normal executive functioning, and contribute to the etiology of ADHD [7-10]. Though less frequent than prenatal meHg exposure studies, associations between childhood and adolescent Hg levels and adverse neurobehavior and ADHD-related symptoms have been reported in different cohorts [5, 11]. Relevant to the present study, not all researchers have been able to identify adverse relationships between Hg exposure and neurocognitive and behavioral outcomes; some examples even imply a beneficial relationship [12-14]. This is counterintuitive to the current consensus that meHg is a potent neurotoxicant, implying that there may be important confounders missing or differences in susceptibility across populations. Selenium and polyunsaturated fatty acid (PUFA) exposures, for example, are neuroprotective seafood nutrients often co-exposed with meHg. Including these in statistical analyses has been shown to improve the ability to detect associations between meHg exposure and neurological effects [13, 15, 16].

In addition to chemical exposures, epigenetic influences are also believed to contribute to risk of ADHD, most notable to the present study is DNA methylation (DNAm) [17, 18]. This mitotically heritable epigenetic modification is stable, but reversible, and helps to regulate gene expression. DNAm generally acts to suppress gene expression and can arm researchers with valuable mechanistic insight regarding disease etiology and/or mechanisms of toxicity. In a previous publication produced by our lab group, DNAm was found to be associated with attention related symptoms in adolescent ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants) participants as quantified by the Conners Continuous Performance Test, 3rd edition (CPT3). This study was conducted in a population of Hispanic individuals

between the ages of 9 and 18 years, and the current research will build upon this finding in the same study population [19].

Despite the fact that both DNAm and Hg exposure are believed to influence ADHD etiology, only a handful of publications have explored the effects of both these variables on neurobehavioral outcomes within the same study, and of those examples, the analyses tended to focus on the potential mediating role of DNAm in Hg-related neurobehavioral impairments [18, 20]. The present study aims to examine the combined effects of DNAm and Hg exposure on ADHD-like symptoms not through mediation, but rather through a novel assessment that employs similar concepts that underly a standard gene - environment interaction (G x E) study. The basic principle behind a G x E study states that the effect of an environmental exposure on a person's health risk may vary depending on their genotype, meaning a person with a distinct genetic polymorphism in a gene may be genetically predisposed to toxicant-related disease, whereas people with another variant of that gene are less likely to develop the disease. Genetic predisposition or susceptibility to mercury-induced neurotoxicity has been proposed in past studies in human cohorts [5, 6, 21, 22]. We propose that if genetic susceptibility can exist, so, too, can epigenetic susceptibility. This present study suggests that the effect of Hg exposure on a person's risk of developing ADHD-like symptoms may vary depending on the methylation state of DNA at a given gene. To accomplish this, using child participants from the ELEMENT cohort, relationships between CPT3 performance and the two main predictors, DNAm and Hg exposure, need to first be identified independent of the other predictor. The relationship between DNAm and CPT3 scores was previously identified in our prior publication [19]. The current study aims to identify associations between child hair and urine Hg exposure and adolescent CPT3 performance in the same study population using adjusted linear regression models. Finally, in a novel approach to model *Epigenetic* – Environment (E x E) interaction, linear regression models nearly identical to the previous two were employed with the addition of an interaction term between the two main exposures of interest (Hg * DNAm). We hypothesize that childhood Hg exposure and adolescent DNAm will interact to affect their associations with adolescent CPT3 performance. This unique approach could lay foundational work and serve as a model for future researchers looking to explore the concept of epigenetic susceptibility to toxicant-associated health effects.

Methods

Cohort

Recruitment for the ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants) cohort took place between 1994 and 2004. During this period, 1012 mother/child dyads were recruited during pregnancy or at birth from hospitals and prenatal clinics from the Mexican Social Security Institute, serving low- to middle-income populations in Mexico City. Data were periodically collected from these women and their children at various timepoints from their recruitment through present day [23]. During one of these follow-up visits, referred to as the child visit (2008-2012), several different health related factors in children were recorded, including hair and urine Hg levels in 796 and 786 participants, respectively. Participants were between the ages of 6 and 12. In a subsequent follow-up in 2015, referred to as the adolescent timepoint, epigenome-wide DNAm was quantified in blood leukocytes for 526 (51% female and 49% male) participants between 9 and 18 years of age. The majority of the participants were also administered the Conners Continuous Performance Test, 3rd ed. (CPT3) to quantify ADHD-like tendencies [23]. While the authors acknowledge that puberty occurs at different rates for everyone, for coherency's sake the present study will broadly refer to this visit as the adolescence timepoint, despite varying stages of maturation of the participants. In an analysis published by our lab group in 2023, the relationship between adolescent DNAm and CPT3 performance (see Model 1 below) was assessed in approximately 517 participants for which both CPT3 performance and DNAm data were available. The present study focusses on a subset of 399 (see Model 2 below) participants for whom childhood Hg and adolescent CPT3 data were collected. When adding in epigenetics, a sample of 374 (see Model 3 below) of these participants with DNAm data at adolescence is included. Numerous other factors related to health, socioeconomic status, and general demographic information were collected for participants at both the childhood and adolescence visits and were used as covariates in statistical models as described below. The institutional review boards of the Mexico National Institute of Public Health and the University of Michigan approved all research protocols related to data and research relevant to both timepoints; both parental informed consent and child assent was obtained for all participants in this study.

Mercury biomarker

Hair and urine were collected from participants at the childhood study visit and total Hg was quantified in both biospecimens using a Direct Mercury Analyzer 80 (DMA-80, Milestone Inc., CT) as described in previous studies [24, 25]. In short, hair was cut from the proximal end in 2 cm segments, washed in acetone, rinsed three times with sterile water, dried overnight, and ~2-5mg of sample was placed into a nickel sampling boat. While the DMA-80 is unable to distinguish between the different types of Hg, it is known that hair Hg predominantly reflects exposure to organic Hg. Prior to Hg analysis in urine, which is generally a biomarker for its inorganic form, the sample was vortexed, a portion was transferred to a quartz sampling boat and specific gravity was measured via a refractometer (PAL-10S, Atago). The instrument was calibrated daily and procedural blanks and replicates were also utilized for quality control. CRM #13 for hair (National Institute for Environmental Studies, Japan) and QMEQAS for urine (Institut National de Santé Publique du Québec) were used as certified reference materials. Reference material recoveries were between 80 and 110% and the analytical limit of detection was less than 1 ng Hg per measure. Both hair and specific gravity adjusted Hg levels were log transformed in all statistical models discussed in this paper.

Epigenetic analysis

As outlined in a publication by Jansen *et al*, an epigenome-wide DNAm analysis was conducted using an EPIC array (Illumina Infinium Methylation EPIC BeadChip; CA, USA) for all ELEMENT participants who provided an adequate blood sample at the adolescent study visit [26]. The Bead Chip array is a probe-based assay quantifying methylation at >850,000 CpG sites across the human genome (comprising ~28 million CpG sites total) [27]. Despite the probes only covering a fraction of the total number of CpG sites in the human genome, it still represents a wide distribution of regions in and surrounding the genes. This includes enhancers, promoters, and intergenic regions and provides coverage at loci within all known gene [28]. Prior to analysis on the Bead Chip array, we extracted DNA from blood leukocytes of participants via Qiagen Flexigene kits (Hilden, Germany) and performed bisulfite conversion on the DNA samples.

Subsequently, bisulfite-converted samples were submitted to the University of Michigan Advanced Genomics Core where they were randomized across chips and positions and hybridized to BeadChips, and signals were read, subjected to initial processing, and underwent thorough quality control measures [26].

As previously described, data analysis was conducted in adherence to a standardized pipeline for this technology [26]. To briefly summarize, the raw image data files were read into R using the minfi package and RELIC was employed to correct for background noise and dye bias [29, 30]. On each chip, unwanted technical variability, estimated from control probes, was accounted for via quantile normalization [30, 31]. Probes with probable cross-reactivity, poor detection above background (in less than 5% of samples), and/or polymorphisms in the CpG or single base extension sites were excluded from analysis [32]. As added quality control measures, documented gender was compared to that which was estimated from the X/Y chromosome DNAm profiles, and we checked for low intensity across all probes and/or >5% probes failing. All 526 adolescent samples submitted for analysis passed the initial quality control check.

Considering the DNA used in this analysis was extracted from whole blood rather than from a homogeneous population of cells, to account for heterogeneity, cell type proportions were estimated using tissue-specific differentially methylated region (DMR) data included on the chips [33]. Intensity values from the non-negative control probes was utilized to generate variables representative of technical variation influencing the DNAm data via surrogate variable analysis [34]. This data was used in a previously published EWAS (epigenome-wide association study) which identified relationships between DNAm and ADHD-like behaviors and is presently being built upon by the current study [19]. From this foundational research, CpG sites having the 1000 lowest raw p-values for each outcome were selected for functional pathways enrichment analysis using both KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology). Significant enrichment for pathways related to ferroptosis, neurotransmission, inflammation, and immune response were documented [19]. Because these processes are believed to be involved in ADHD etiology and/or toxic response to heavy metals, we had reason to believe these CpG sites are functionally relevant and were thus selected for use in the present analyses.

Cognitive performance test

The CPT3 is a computerized test designed to assess four distinct aspects of attention: inattentiveness, impulsivity/hyperactivity, sustained attention, and vigilance (see Table 1-1, Chapter 1). During the examination, children are presented with a series of stimuli to which they must either respond or not by hitting a button. Continuous variables are generated from the response data to reflect the aforementioned aspects of attention [35, 36]. The CPT3 was administered by trained research staff in Mexico during the adolescence study visit. For the present study, Omissions Errors, Perseverations, Interstimulus Interval (ISI) Change, and Hit Reaction Time (HRT) Block Change variables were selected as outcomes based on ease of interpretation and prevalence of use in the literature. They respectively reflect inattentiveness, impulsivity/hyperactivity, vigilance, and sustained attention. Response style is another relevant variable generated by the CPT3 test that alone does not accurately represent any aspect of attention but rather accounts for individualistic style of responding and may act as a confounding factor [35-37].

Covariates

In addition to the main exposure and outcome variables already described, numerous other variables were reported at both the childhood and adolescence study visits. In a preliminary examination leading to the aims of this study, covariates were carefully selected on basis of correlation with the outcomes and/or biological relevance. A more complete assessment of covariate selection is described by Ehlinger *et al* 2023, but an overview of the covariates as they pertain to this present study is briefly described below. Among these factors, child age, gender, socioeconomic status, and polyunsaturated fatty acid (PUFA) levels estimated from FFQs (food frequency questionnaires) were documented at both the childhood and adolescence timepoint. The CPT3 Response Style, cell type proportions in whole blood (CD8⁺ T cells, B cells and granulocytes), and principal component 4 (representing batch effects from the EPIC data) were generated at the adolescence study visit only and were also considered as important confounders in the analyses that included DNA methylation data. The Mexican Association of Marketing Research and Public Opinion Agencies (AMAI) scaling system was specifically designed for Mexican populations and was selected for use in this study as a metric to assess SES [38, 39].

Notably, an estimation of pubertal status (Tanner staging) was determined by trained physicians at the study visits. This developmental measure was also taken into consideration but ultimately not included in the models. Tanner staging neither improved the fit when included in the model nor did it associate with the outcome variables [19]. A more concise breakdown of covariates used in each model is included in the “Statistical methods” section below.

Statistical methods

After performing descriptive statistics examining distribution, sample size, mean, and standard deviation of variables, we answered the two research questions using linear regression in R (version 4.0.3). The study schematic is depicted in Figure 3-1. The first model aims to identify the associations between Hg exposure and CPT3 performance without consideration of DNAm. The second model addresses the research question of whether DNAm at CPT3-associated loci modifies the relationship between Hg and outcomes, modeled through an interaction term between the two predictors. The models can be respectively described by the equations below.

Equations

- **Model 1**: $\text{CPT3 score} = \beta_{\text{Hg}}X_{\text{Hg}} + \beta_iX_i \dots + \beta_0 + \varepsilon$
- **Model 2**: $\text{CPT3 score} = \beta_{\text{int}}X_{\text{Hg}}*X_{\text{DNAm}} + \beta_{\text{Hg}}X_{\text{Hg}} + \beta_{\text{DNAm}}X_{\text{DNAm}} + \beta_iX_i \dots + \beta_0 + \varepsilon$
 - **Annotations**: CPT3 score = one of four dependent variables generated by the CPT3; β_{DNAm} =slope of the DNAm coefficient for 1-1000 distinct CpG sites in the genome; β_{Hg} = slope of the Hg variable (both log hair Hg or log specific gravity-adjusted urine mercury was used in otherwise identical models); β_{int} = slope of the interaction between Hg and DNAm; β = slope of the coefficient; X = value of covariate ; i =covariates controlled for in each model; β_0 = intercept; ε = residual error

Associations of Hg exposure with CPT3 Performance (Model 1)

Multiple linear regression models controlling for age, sex, SES, and CPT3 response style were employed to examine the relationships between hair and urine Hg levels and four CPT3 outcomes representative of inattention (Omissions Errors), impulsivity (Perseverations),

vigilance (HRT ISI Change), and sustained attention (HRT Block Change). This was conducted in total population models (N=399) and sex stratified models (N = 203 females; N= 196 males). PUFA levels (calculated from food frequency questionnaires administered at childhood) were also included as an additional covariate in a sensitivity analysis (N=262 based on availability of childhood PUFA data) to account for potential protective effects of beneficial seafood nutrients co-exposed with Hg during childhood.

Combined associations of Hg exposure and DNAm with CPT3 Performance (Model 2)

We conducted an analysis that considered Hg and DNAm together. To accomplish this, we designed a model that would consider how one predictor might modify the relationship of the other with the outcome, by adding an interaction term between DNAm and Hg exposure to linear regression models assessing the relationships between the CPT3 outcome and the exposures. The analysis was run using each of the four CPT3 variables previously described, with all models controlling for age, sex, SES, CPT3 response style, and adolescent PUFA levels. For each outcome only the CpG sites corresponding to the 1000 lowest raw p-values identified in our previous publication as associated with CPT3 outcomes were used [19]. Benjamini-Hochberg test was used to adjust for multiple comparisons. Gender stratified models were also conducted.

Results

The study population was comprised of a relatively even mix of both males (49%) and females (51%). Average hair and specific gravity adjusted urine Hg measured at the childhood visit were respectively 0.62 ± 0.03 $\mu\text{g/g}$ and 1.09 ± 0.14 $\mu\text{g/L}$ (Figure 3-2; for Hg by gender see Appendix Figures 3-4 and 3-5 appx). ELEMENT participants comprised varying levels of

socioeconomic status, with the majority of participants representing low- to middle-income families (Figure 3-3). Adolescent CPT3 scores, recorded after testing in the same participants an average of 5.6 years later, were on average between 50 and 52 for each outcome, exhibiting broad ranges of both high and low results (Table 3-1; for descriptive statistics stratified by gender see Appendix Table 3-12 appx). In a previous publication produced by our lab, significant associations between adolescent DNAm and CPT3 performance were identified in this population [19].

Associations of Hg exposure with CPT3 Performance

Childhood hair and specific gravity adjusted urine Hg levels were associated with CPT3 performance at adolescence in full population and gender stratified models. The nature of these relationships was dependent upon the Hg biomarker and CPT3 variable used for each analysis. In the regression models using omissions errors as the outcome, for example, hair Hg was associated with reduced inattentiveness ($\beta=-0.021(0.0095)$, $p=0.027$). However, when urine Hg was used instead, no significant associations were identified, but the direction of effect remained the same ($\beta=-0.0045(0.0078)$, $p=0.57$). In many instances, gender also modified associations between Hg exposure and outcome. For example, specific gravity adjusted urine Hg exposure was significantly related to loss of vigilance in males ($\beta=1.31(0.65)$, $p=0.045$), but not in females ($\beta=-0.20(0.81)$, $p=0.80$) (Table 3-2). Lastly, the significance level and direction and magnitude of effect was similar for nearly all these associations with the addition of the childhood PUFA variable to each model for the sensitivity analysis (Tables 3-2 and 3-3).

Combined associations of Hg exposure and DNAm with CPT3 Performance

In the *Epigenetic* x Environment interaction analysis, based on a significance threshold of $q < 0.10$, in full population models, hair Hg significantly interacted with DNAm at one CpG site cg05093254 (annotated to the *ABCA3* gene) ($\beta_{\text{int}}=5.01$, $SE=1.24$, $q=0.067$) in its association with HRT ISI change score (Tables 3-4 and 3-5). For the full population omissions errors models, urine Hg significantly interacted with DNAm at two CpG sites: cg10396484 (annotated to *LBX1*) ($\beta_{\text{int}}=0.048$, $SE=0.012$, $q=0.057$) and cg21442182 ($\beta_{\text{int}}=-0.013$, $SE=0.0033$, $q=0.075$) (Tables 3-4 and 3-6). No significant interactions were identified for the perseverations or HRT block change outcomes in the full population models. For the perseveration outcome when stratified by gender, however, urine Hg in males significantly interacted with 3 CpG sites, cg03760895 (*RSL1D1*), cg17227358 (*DRGX*), and cg19505184. (Tables 3-7 and 3-8). In females, significant interactions were identified between DNAm at 157 CpG sites and hair Hg for the omissions error outcomes (Tables 3-7 and 3-9). Select observations are presented in Table 3-9. See Appendix Table 3-11 appx for all 157 significant interaction results, plus the results from the other Hg biomarker for comparison. It is important to note that the interaction terms only represent part of the picture. When interpreting results with respect to the outcome, one must consider the main effects of Hg and DNAm, as well.

Discussion

Previous publications suggest aberrant childhood DNAm and Hg exposure are both important predictors of developing ADHD and ADHD-like symptoms, but few studies consider the combined effects of these two key variables as is done in the present study through a novel analysis of epigenetic – environment interaction. In a population of Hispanic children (6 – 12 years of age) followed up through adolescence (9 – 18 years of age) in the ELEMENT cohort,

significant associations between adolescent DNAm and CPT3 performance were previously identified [19]. In this study, we identify associations between childhood Hg exposure and adolescent CPT3 performance (model 1). After first identifying these initial associations, in a combined analysis adding an interaction term between Hg and DNAm to the adjusted linear regression models (model 2), significant interaction terms were identified at one CpG site for hair Hg and HRT ISI change models and at two CpG sites for urine Hg and omissions errors models. Notably, this last model provides a unique method for probing the question of epigenetic susceptibility to toxicant-associated disease. In all three sets of models there were examples where the main associations between the exposure and the outcome differed by gender.

Gender differences appeared to be especially important in the interaction models, because gender stratified assessments revealed 157 significant interactions between DNAm and hair Hg in females for the omissions error outcome and 3 significant interactions between DNAm and urine Hg in males for the perseverations outcome (Tables 3-7 and 3-9). These specific outcomes for which the significant interactions were identified are particularly interesting and may help highlight some key differences in how ADHD symptoms tend to present themselves in males versus females. The omissions error outcome, for example, is meant to reflect inattentiveness, the predominant symptom profile exhibited by females. Similarly, the perseverations outcome represents impulsivity, which tends to be more common in males. The number of significant findings in these gender-stratified interaction models exceeds those identified in the full population and other gender-stratified analyses in any of the sets of models described here and in our previous publication [19]. The present findings suggest that both gender and DNAm may be important modifiers in the relationship between Hg exposure and CPT3 performance and that females may be more susceptible to effect modification by DNAm than males. Future research

examining the precise mechanisms underlying these proposed gender differences is warranted to elucidate any potential implications of these results.

DNAm has been shown to be associated with neurodevelopmental outcomes both in this cohort and in other studies. In Ehlinger *et al* 2023, we described significant associations in ELEMENT adolescents between poorer ISI change performance and increased DNAm (hypermethylation) at four probes annotated to three genes, *ZNF814*, *ELF4*, and *OR6K6*. Two of these probes were annotated to *ZNF814*, encoding Zinc Finger Protein 814, and one of which was also significantly related to the block change outcome. In all examples, hypermethylation was associated with higher CPT3 scores, or poorer test performance. Notably, when models were stratified by gender, DNAm at two additional probes were significant in females for their associations with HRT block change scores. One of the probes, annotated to the *DSCR9* (Down Syndrome Critical Region 9) gene, exhibited the same pattern as the full population model results, with hypermethylation relating to poorer test performance. The second probe (annotated to the *GLI3* gene), on the other hand, exhibited the opposite relationship, with lower DNAm levels related to poorer CPT3 scores. Interestingly, this gene encodes GLI Family Zinc Finger 3, which belongs to the C2H2-type zinc finger protein family similar to Zinc Finger Protein 814.

In addition to our study, previous publications have identified associations between DNAm and ADHD or ADHD-like symptoms in human cohorts. Mooney *et al* 2020, for example, which was one of the first ADHD epigenome-wide association studies (EWAS) of its kind, identified potential novel epigenetic biomarkers for this disorder. This was accomplished through modeling associations between DNAm and both ADHD diagnosis and polygenic risk burden via adjusted linear regression analyses [40]. In a more recent analysis conducted by Sun *et al* (2023), researchers identified significant relationships between DNAm and externalizing

behaviors, gray matter volumes (reflecting structural brain networks), and behavioral problems [41]. The results of this and other studies indicate differentially methylated regions (DMRs) may serve vital roles in the development of the brain and can contribute to the etiology of neurodevelopmental disorders like ADHD [18, 41, 42]. Furthermore, specific EWAS results can provide mechanistic insights regarding biological processes that might underly disease onset and inform future research that takes a more targeted approach to the research question. Li *et al*, for example, identified a list of potentially relevant differentially methylated genes based on a small EWAS analysis (9 controls and 12 ADHD cases) using whole blood DNA of Taiwanese children. Subsequently, pyrosequencing was used to quantify DNAm levels at candidate genes, for which significant associations were identified between hypermethylation at *LIME1* and hypomethylation at *SPTBN2* and poorer CPT performance [42]. Aberrant methylation at both genes may contribute to ADHD etiology through neuroinflammation and dysregulation of glutamate signaling pathways for *LIME1* and *SPTBN2* respectively.

With regard to Hg exposure, the second main predictor assessed in this study, it is not at all surprising that we see a combination of both positive and negative associations with CPT3 performance; split findings like these are common amongst epidemiological studies previously published on this topic. Considering Hg is a known neurotoxicant, we would intuitively expect a positive association, with higher levels of Hg associated with poorer test performance (higher CPT3 scores). While we see examples of positive associations in the full population analyses, none were statistically significant in either the main model or the sensitivity analysis. There was, however, an example of a significant negative association between hair Hg and omissions error ($\beta=-0.021(0.0095)$, $p=0.027$), which remained significant with the addition of the PUFA variable in the sensitivity analysis ($-0.030(0.012)$, 0.012). PUFAs are beneficial seafood nutrients that

promote healthy neurodevelopment and may play a role in Hg detoxification. Past studies have shown that controlling for them in epidemiological analyses focused on meHg improves the statistical model, as it is a very important confounding factor that is highly co-exposed with meHg through seafood consumption. In our study, adjusting for PUFA did not substantially change results. However, unlike the Hg variables which were measured in biological materials, PUFA levels were calculated from food frequency questionnaires (FFQs). This calculation is inherently subject to error due to factors such as innate biological differences between participants in how they process the nutrients and potential recall bias in the FFQ responses. Another consideration is the reduction of the sample size from 399 in the main model to 262 in the sensitivity analyses; not all participants had data recorded for the PUFA variable, thus reducing the statistical power. Significant associations were also identified in the gender stratified models. Despite the inevitable reduction in sample size that also accompanies gender stratification, hair Hg levels in females was associated with fewer omissions errors and perseverations. The direction and strength of associations were similar when urine was used as the Hg biomarker instead of hair for female omissions errors scores and perseverations scores. No associations were significant in males for either of these CPT3 outcomes regardless of the Hg biomarker used. For the HRT ISI change outcome, however, urine Hg exposure was associated with poorer CPT3 scores in males, but not in females. In models that utilized hair Hg instead, the direction of effect was comparable to the urine Hg models in males and females, but were not statistically significant. This difference in significance could be due to the fact that hair Hg is better representative of organic Hg (meHg) and urine Hg better reflects inorganic Hg; the two could have different effects on neurodevelopment and behavior. Notably, the two exposure biomarkers are highly correlated (Spearman) to one another ($\rho = 0.39$, $p = 2.2 \times 10^{-16}$) and the fact

that the direction of association was similar for both biomarkers may be an artifact of that correlation between them.

There is a lack of consensus in the literature linking chronic exposure to low levels of Hg during childhood to adverse neurodevelopment in human cohorts. One study by Lozano *et al*, for example, identified significant adverse relationships between childhood hair Hg levels and neurobehavior as quantified via the Child Behavior Checklist test (CBCL), the Conners Parent Rating Scales-Revised: Short Form (CPRS-R:S), and the Attention Network Test (ANT) [5, 43-45]. This study was conducted in a birth cohort based in Spain, using data generated from participants at study visits when participants were 9 (hair Hg, CBCL, and CPRS-R:S) and 11 years of age (CBCL, CPRS-R:S, and ANT). Adjustment for seafood intake had little impact on the relationships observed for any model, similar to the present study's sensitivity analysis. In the Lozano *et al* study, gender significantly modified associations for both the CBCL externalizing behavior and total problems scales. Authors noted that the associations were negative for females and positive for males in models for both outcomes, though not statistically significant. This is possibly due to the reduced samples size. The same phenomena of effect modification by gender was observed for models using the ADHD index scores of the CPRS-R:S test. These findings are particularly interesting when compared to the results of the present study, because in gender stratified models, significant negative associations were identified between Hg exposure and the CPT3 scores reflecting inattention and impulsivity in females, but not for males. Additionally, when associations were modeled using the HRT ISI change outcome (reflecting vigilance), higher urine Hg levels were significantly related to increased CPT3 scores in males, but not in females. Though not statistically significant for all models, associations were positive in males and negative in females regardless of the Hg biomarker used for this outcome (Table 3-2). Taken

together with the Lozano *et al* paper, results suggest that males may be more susceptible to the neurotoxic effects of meHg than females and/or less responsive to the beneficial effects of PUFAs or other seafood nutrients often co-exposed with meHg. Studies in several other cohorts, such as those based in Canada and the Faroe Islands, have also reported significant adverse relationships between Hg exposure and neurobehavior / development in children, and also suggest gender impacts these associations [2, 3].

In other publications, however, adverse relationships could not be identified between Hg exposure and neuro-outcomes in children, with several studies implicating a positive impact of Hg on neurodevelopmental and behavioral outcomes [12-14]. This might be the result of poor modeling, likely related to an inability to control for unmeasured confounding variables and/or a lack of consideration of epigenetic susceptibility to the toxic effects of Hg. These inconsistencies in results are exemplified in a publication by Davidson *et al* conducted in a seminal cohort, the Seychelles Child Development Study (SCDS), that was specifically designed to study the effects of prenatal Hg exposure on neurological outcomes. To date, it is the longest longitudinal cohort of its kind. Davidson *et al* did not find consistent adverse patterns in the associations between prenatal Hg exposure and any of the 27 neurological outcomes tested, but some models suggested neuroprotective effects of Hg [12]. This is consistent with findings of earlier SCDS publications, utilizing data from younger participants [46, 47]. Davidson *et al* noted that no PUFAs or other measures related to seafood intake were included in these models and that the beneficial relationships observed are likely due to confounding by these variables [12]. Another important factor to consider is the timing of the exposure, which was *in utero* for the Davidson *et al* study. In a subsequent SCDS study, Thurston *et al* describe several significant adverse associations between time-weighted *postnatal* hair Hg measures (from childhood to early

adulthood) and outcomes related to attention, executive, and achievement domains observed in participants at 9, 17, 22, and 24 years of age. This study also did not control for seafood nutrient intake [11]. This might suggest that postnatal exposures are more deleterious than prenatal exposures to meHg, but this is unlikely. Notably, the prenatal Hg levels described by Davidson *et al* were measured in maternal hair during pregnancy. Regardless, human cohort studies are highly nuanced and subject to great variability, due to the infinite number of variables that could potentially be influencing results.

While no model can account for all environmental and biological factors, childhood Hg exposure and DNAm are two variables that may be important in ADHD etiology, which is why the present study models them under the same analysis in what we believe to be the first study of its kind. We conjectured that by accounting for epigenetic susceptibility, as modeled through interaction, we would optimize our analysis, allowing us to uncover true relationships between Hg and ADHD-like behaviors. In full population models we identified significant interactions between childhood Hg exposure and DNAm at three distinct CpG sites. Two of these sites (cg10396484 and cg21442182) were significant in models using urine Hg and CPT3 omissions errors scores, and the last (cg05093254) was significant in models using hair Hg and ISI change scores. Because of the dynamic nature of these relationships, it can be difficult to conceptualize what they mean in the context of CPT3 performance. In a simplified interpretation that assumes all other variables including Hg levels remain constant, higher levels of DNAm at the significant locus would result in the effects of Hg on CPT3 performance being augmented when the interaction term is positive. In other words, if the main effect of Hg were positive, then we would predict an even larger increase in CPT3 scores with more DNAm, but if β_{Hg} were negative, a more pronounced reduction in CPT3 score would be expected (Table 3-10). Interpreting the

effects of negative interaction terms on the outcome is less straight forward. It suggests that with higher levels of DNAm, assuming Hg and other covariates are held constant, the effect of Hg on CPT3 performance is reduced. Meaning that if the main effect of Hg was positive, it would result in a less dramatic increase in CPT3 scores. Conversely, if the main effect of Hg was negative, it would likely result in a less pronounced decrease in CPT3 scores. Notably, these scenarios are situationally dependent, as they do not account for the magnitudes of effect for the DNAm x Hg interaction term, or the main effects of Hg and DNAm. Depending upon how these magnitudes of effects compare to one another, there is the possibility that the effect of the negative interaction could cancel out that of Hg's main effect, resulting in no change in CPT3 performance or even the opposite direction of effect on CPT3 scores. Regardless of whether the interaction term is negative or positive, the interpretations described here and in Table 3-10 are overly simplified because they assume all variables including sex, age, SES, response style, and Hg levels are constant, which is fundamentally unrealistic.

While some significant interactions were able to be identified in the full population models, the majority of findings were observed in the gender stratified models, with 157 CpG sites significantly interacting with hair Hg levels in females and 3 CpG sites significantly interacting with urine Hg in males for the omissions errors and perseverations outcomes respectively (Table 3-7). This is not surprising given that gender modified associations with CPT3 performance in assessments where DNAm and Hg were modeled separately using ELEMENT data (models 1 and 2). These findings suggest that meHg interacts with DNAm in their associations with symptoms of inattention in females, but not necessarily in males. Gender appears to be highly relevant to both DNAm and Hg exposure in their associations with ADHD-

like symptoms separately, but also in how the two predictors interact with one another in these associations.

While epigenetic susceptibility has not yet been modeled in past research, several studies suggest genetic factors predispose children to neurotoxic effects of meHg exposure. Lozano *et al*, for example, in the same study previously described, reported significant interaction effects for single nucleotide polymorphisms (SNPs) in Hardy-Weinberg equilibrium in three genes: the *GSTP1*, *BDNF*, and *APOE* [5]. In another study, a population of Portuguese children, comparable in age to those included in the present study, were subjected to several neuropsychological batteries, urinary Hg analysis, and genotyping for variants in 13 neurodevelopmentally relevant genes. Several associations between Hg exposure and neurobehavior were significantly modified by genetic variation, but mostly in boys [48]. In addition to genetic variants, epigenetic factors have been implicated in the relationship between aberrant neurodevelopment and Hg exposure, but are seldom modeled together much less through interaction [20, 49]. One study conducted by Cardenas *et al*, for instance, performed an EWAS to identify genes differentially methylated by prenatal Hg exposure (*PON1*, *WBP11P1*, and *TOR4A* were significant) and a subsequent analysis linking DNAm at these sites to cognitive performance in early childhood (modified by sex). While measures of Hg exposure, DNAm, and behavioral outcomes were all recorded, the authors did not assess the variables simultaneously [49]. Similarly, the SCDS has produced research relating Hg exposure to neurodevelopmental outcomes and DNAm, but to our knowledge no publication from these Seychellois cohorts considers these variables jointly [11, 12, 50].

The present study exhibits many strengths, as it addresses numerous gaps in the literature and does so in an underrepresented Hispanic population. Furthermore, this research focuses on

understudied childhood and adolescent exposures, which are important neurodevelopmental timepoints that are vulnerable to the influence of toxic exposures and aberrant DNAm. By modeling interaction between the two main predictors of interest, DNAm and Hg exposure, we assess a novel concept we termed epigenetic susceptibility. This has the potential to be used as a model for future research, potentially utilizing other toxicants and disease outcomes. Admittedly, standard $G \times E$ interaction studies are easier to interpret than $E \times E$ interaction studies, such as ours, but by assessing interaction between two continuous variables researchers may be able to detect more subtle effects than what can be observed through effect modification by binary SNPs. Significant DNAm-Hg interactions, as were identified in the present study, may serve as novel biomarkers of disease and/or provide mechanistic insight that may be overlooked in assessments of either variable alone. Also, DNAm is more malleable and dynamic in nature than its genetic counterpart, which may have mechanistic implications that can inform future therapeutic research. One notable weakness is the fact that DNAm was measured in blood, rather than brain, the target tissue of interest. While DNAm in biologically available blood is often correlated to DNAm in the brain, caution must be taken in interpretation. Mechanistic implications are better validated in *in vitro* or *in vivo* experiments.

Another strength of this study is the large sample size and high number of variables generated by the longitudinal ELEMENT cohort. It allowed us to control for important factors like PUFAs, without a dramatic reduction in sample size. One potential weakness of the study is the fact that PUFAs were estimated through dietary survey, rather than measured, and fewer participants responded to the survey at the childhood timepoint compared to the adolescent visit. For the interaction models (model 2) we used PUFA levels assessed during the adolescent visits (supporting healthy brain development and function) rather than PUFA levels co-exposed with

Hg at the childhood timepoint (potentially affecting Hg levels through detoxifying pathways) to increase the sample size of the models. While controlling for PUFAs at the earlier timepoint may be considered preferable and more biologically relevant to some, using the later timepoint improved the power to detect smaller effects. The fact that childhood and adolescent PUFAs were correlated with one another ($\rho=0.203$, $p=7.75e-04$) reassured us in this decision. Regardless, no model is perfect, and caution must always be taken in interpreting results, especially in such complex models. Future studies can consider other covariates and/or co-exposures with other chemicals using mixtures analysis.

Another point of consideration when interpreting results is how this population is both similar and different to other studies with similar research questions. While the average hair and urine Hg levels in the children used this study were reported at levels between three and five times those of similarly aged populations in the US, they are not nearly as high as Hg levels in children in areas where high seafood consumption is more standard, i.e. in the Faroe Islands or in Seychelles [3, 25, 46, 50]. Still, the participants in this study exhibited a broad range of both low and high hair and urine Hg levels, representative of populations with varying levels of exposure across the world. Additionally, the ELEMENT participants' average CPT3 performance was comparable to that of populations of children of similar ages in different parts of the world. Broad ranges of good (low) and bad (high) scores were also documented for participants, making it easier for the results of this research to be compared across different studies (Table 3-1).

Conclusion

This research used an innovative approach to examine relationships between childhood Hg exposure, DNAm, and adolescent CPT3 scores. In a cohort of Mexican children, we demonstrate associations between hair / urine Hg levels and the same outcomes, meant to assess

four distinct aspects of attention: inattention, impulsivity, vigilance, and sustained attention.

Lastly, we combine the principles of the first two models and considered the combined effects of DNAm and Hg exposure on the CPT3 outcomes. Through this we identified numerous significant interactions between DNAm and Hg exposure in full population and gender stratified models, suggesting epigenetic susceptibility to Hg-related ADHD-like behaviors. This innovative model of *Epigenetic* x Environment interaction is the first study of its kind and can be modified by future researchers interested in epigenetic susceptibility to toxicant-induced disease.

Figures and Tables

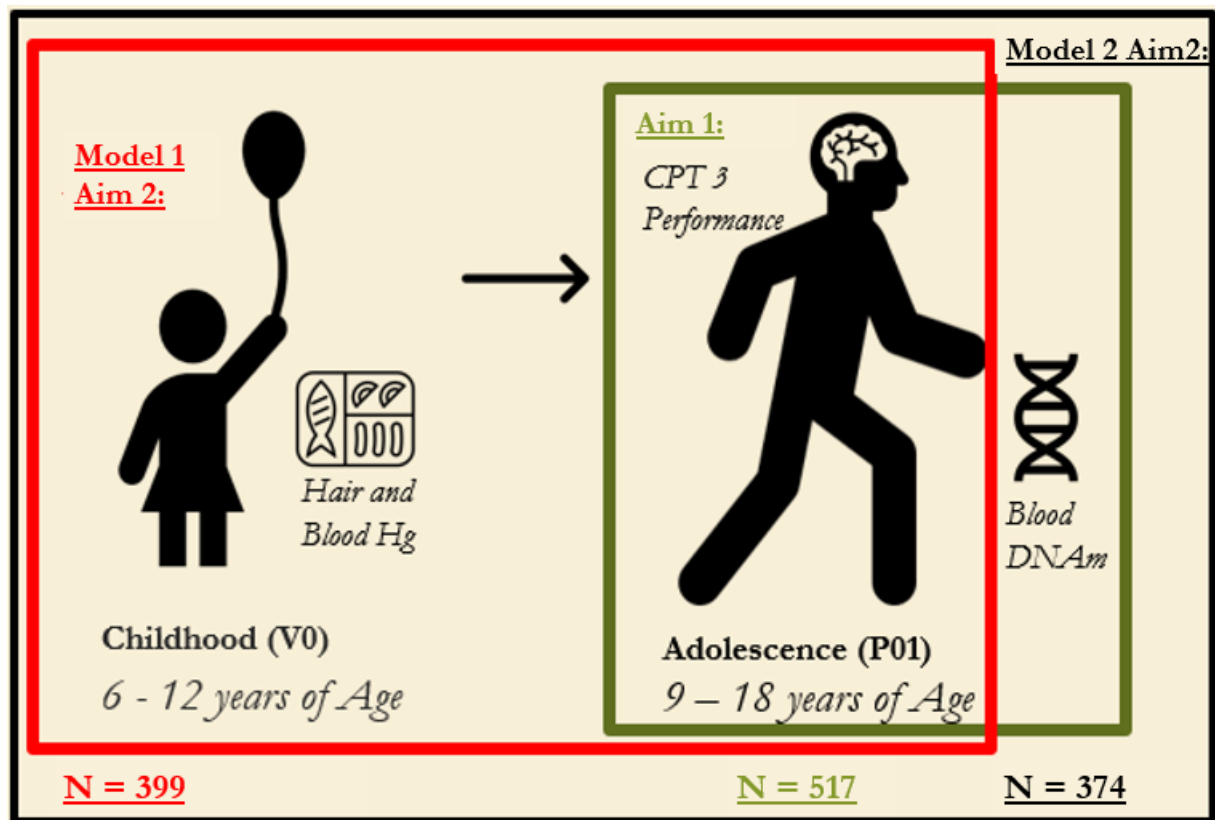


Figure 3-1: Schematic of the timing of key predictors and outcomes in ELEMENT that are the focus of this research.

The green box outlines the timepoints, variables, and sample size relevant to Aim 1, the red box outlines those relevant to Aim 2 model 1, and the black box those pertinent to Aim 2 model 2.

Table 3-1: Descriptive statistics for key variables.

Variable	N	Mean	Std.Dev.	Min	Pctl. 25	<u>Median.</u> 50	Pctl. 75	Max
Urine Hg (specific gravity adjusted, µg/L)	399	1.085	2.855	0.087	0.319	0.54	1.057	49.672
Hair Hg (µg/g)	395	0.625	0.575	0.062	0.277	0.46	0.799	6.217
Omissions Errors (T score)	399	50.376	10.457	41	44	47	51.5	90
Perseverations (T score)	399	50.915	11.079	44	45	46	51	90
HRT ISI Change (T score)	399	50.684	9.677	27	44	50	56	87
HRT Block Change (T score)	398	51.008	8.955	13	46	51	56	88
Response Style (T score)	399	52.1	9.193	31	46	52	57	90
V0 Age (years)	378	8.9	1.3	5.7	7.5	9.1	9.9	12
P01 Age (years)	399	14.278	2.091	9	12.5	15	16	18
Years between Visits	378	5.6	0.99	2.9	4.8	5.8	6.3	7.5
V0 PUFA (g/day)	262	10.62	5.08	2.784	6.58	10.05	13.254	28.893
P01 PUFA (g/day)	399	14	6.9	1.7	8.6	12	18	49
Gender	399							
... Female	203	50.90%						
... Male	196	49.10%						
AMAI	399							
... E	1	0.30%						
... D	99	24.80%						
... D+	90	22.60%						
... C-	103	25.80%						
... C	76	19%						
... C+	27	6.80%						
... A/B	3	0.80%						

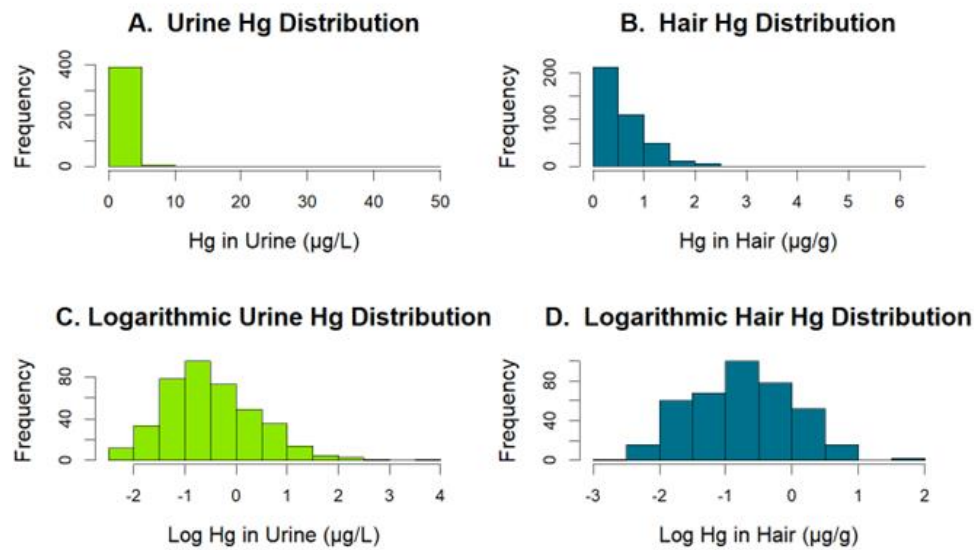


Figure 3-2: Distribution of urine and hair Hg variables.
(A) urine Hg (B) hair hg (C) log transformed urine Hg (D) log transformed hair Hg.

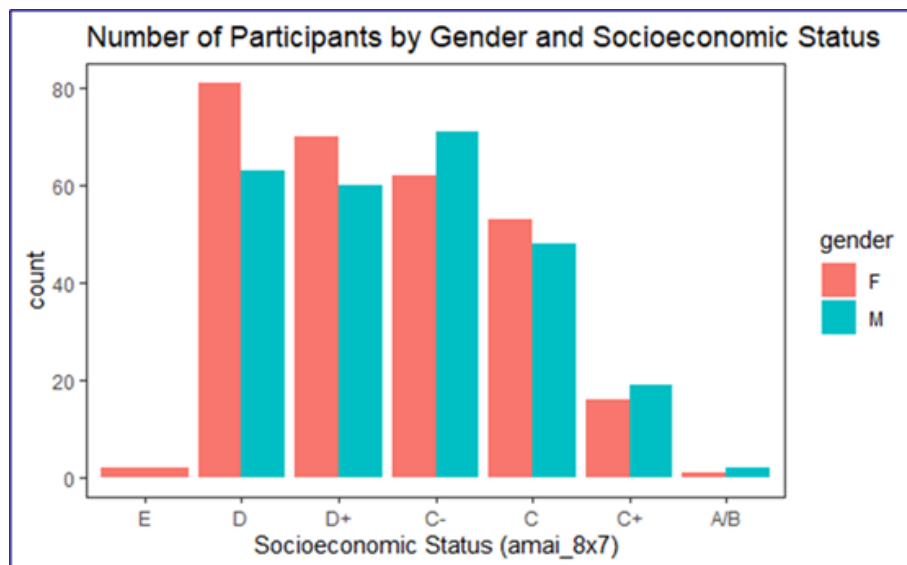


Figure 3-3: Distribution of Male and Female ELEMENT participants across varying levels of socioeconomic status.
Category “E” is indicative of a “poorer” SES level and category “A/B” is reflective of a higher SES level, according to the AMAI scoring system.

Table 3-2: Summary of associations between mercury exposure biomarkers and CPT3 outcomes in adjusted linear regression models in all children and stratified by sex.

CPT 3 variable:	Omissions Errors β (S.E.) p-value		Perseverations β (S.E.) p-value		HRT ISI Change β (S.E.) p-value		HRT Block Change β (S.E.) p-value	
Aspect of Attention	Inattention		Impulsivity		Vigilance		Sustained Attention	
Hair Hg	-0.021 (0.0095) 0.027*	M= -0.018 (0.014) 0.194	-0.017 (0.012) 0.162	M= -0.001 (0.017) 0.95	-0.22 (0.64) 0.74	M= 0.59 (0.82) 0.47	0.45 (0.58) 0.44	M= 0.85 (0.83) 0.31
		F= -0.026 (0.013) 0.045*		F= -0.034 (0.017) 0.048*		F= -1.13 (0.99) 0.26		F= 0.104 (0.80) 0.897
Urine Hg	-0.0045 (0.0078) 0.56	M= 0.0086 (0.011) 0.45	-0.012 (0.0097) 0.23	M= 0.0074 (0.013) 0.59	0.61 (0.52) 0.25	M= 1.31 (0.65) 0.045*	-0.095 (0.47) 0.84	M= 0.28 (0.68) 0.69
		F= -0.018 (0.011) 0.085▪		F= -0.030 (0.014) 0.027*		F= -0.20 (0.81) 0.80		F= -0.48 (0.65) 0.46

Table 3-3: Summary of sensitivity analysis of associations between mercury exposure and CPT3 outcomes in adjusted linear regression models in all children and stratified by sex.

CPT 3 variable:	Omissions Errors β (S.E.) p-value		Perseverations β (S.E.) p-value		HRT ISI Change β (S.E.) p-value		HRT Block Change β (S.E.) p-value	
Aspect of Attention	Inattention		Impulsivity		Vigilance		Sustained Attention	
Hair Hg	-0.030 (0.012) 0.012*	M= -0.023 (0.018) 0.192	-0.013 (0.015) 0.413	M= 0.011 (0.022) 0.61	-0.24 (0.80) 0.77	M=0.62 (1.00) 0.54	0.052 (0.71) 0.94	M=0.82 (1.05) 0.44
		F= -0.035 (0.016) 0.026*		F= -0.034 (0.021) 0.113		F= -1.24 (1.25) 0.32		F= -0.589 (0.94) 0.532
Urine Hg	-0.0047 (0.0094) 0.62	M= 0.016 (0.014) 0.27	-0.013 (0.012) 0.28	M= 0.0070 (0.017) 0.68	0.99 (0.62) 0.11	M= 2.03 (0.80) 0.013*	-0.70 (0.56) 0.21	M=-0.403 (0.87) 0.64
		F= -0.020 (0.012) 0.094▪		F= -0.032 (0.016) 0.047*		F= 0.20 (0.94) 0.83		F= -1.19 (0.71) 0.10▪

Notation for Tables 3-2 & 3-3: higher scores for CPT3 variables reflect poorer performance. Omissions errors and perseverations were log transformed. * reflects a significance value ≤ 0.05 and ▪ reflects a significance value ≤ 0.10 . M=males only models. F=females only models. Complete models, if not otherwise specified. All models adjusted for age, sex, SES, and response style. Sensitivity models (Table 3) also control for childhood PUFA levels.

Table 3-4: Number of CpG sites with Significant Interactions (q<0.1) Between DNAm and Hg Exposure in the full population models.

	Omissions Errors	Perseverations	HRT ISI Change	HRT Block Change
Hair Hg	0	0	1	0
Urine Hg	2	0	0	0

Table 3-5: Assessing interactions between DNAm and Hg exposure in their association with CPT3 ISI Change Scores in the full population models.

Hg biomarker	DNAm at ABCA3 (cg05093254) β (S.E.)q-value	Hg β (S.E.)q-value	Hg x DNAm β (S.E.)q-value
Urine Hg	-1.12 (0.64) 0.097	-334.15 (132.97) 1.0	3.39 (1.35) 1.0
Hair Hg	-1.60 (0.52) 0.016	-494.99 (122.64) 0.066	5.01 (1.24) 0.067

Note: Significance Threshold q<0.10

Table 3-6: Assessing interactions between DNAm and Hg exposure in their association with CPT3 Omissions Error Scores in the full population models.

Hg Biomarker	Gene (CpG site)	Main effect of DNAm β (S.E.)q-value	Main effect of Hg β (S.E.) q-value	Hg x DNAm β (S.E.)q-value
Urine Hg	<i>LBX1</i> (cg10396484)	0.049 (0.010) 9.60E-05	-0.11 (0.027) 0.040	0.048 (0.012)0.057
	NA (cg21442182)	-0.020 (0.0036) 2.71E-05	1.06 (0.28) 0.080	-0.013 (0.0033)0.075
Hair Hg	<i>LBX1</i> (cg10396484)	0.045 (0.015) 0.040	-0.050 (0.032) 0.66	0.013 (0.013) 0.92
	NA (cg21442182)	-0.013 (0.0043) 0.034	0.19 (0.33) 0.86	-0.0025 (0.0039) 0.92

Note: Significance Threshold q<0.10

Table 3-7: Number of CpG sites with Significant Interactions Between DNAm and Hg Exposure in the gender stratified models.

	Omissions Errors	Perseverations	HRT ISI Change	HRT Block Change
Hair Hg	M= 0 F= 157	M= 0 F= 0	M= 0 F= 0	M= 0 F= 0
Urine Hg	M= 0 F= 0	M= 3 F= 0	M= 0 F= 0	M= 0 F= 0

Note: Significance Threshold $q < 0.10$; F= female only models; M = male only models

Table 3-8: Assessing interactions between DNAm and Hg exposure in their associations with CPT3 Perseverations Scores in Males.

Hg biomarker	Gene Name	ProbeID	DNAm β (S.E.) q-value	Hg β (S.E.) q-value	Hg x DNAm β (S.E.) q-value
Urine	<i>RSL1D1</i>	cg03760895	4.09e-03 (0.02) 0.86	-6.7 (1.5) 0.02	0.07 (0.02) 0.02
Hair	<i>RSL1D1</i>	cg03760895	-0.03 (0.03) 0.48	-1.92 (2.49) 0.98	0.02 (0.03) 0.98
Urine	<i>DRGX</i>	cg17227358	-0.01 (0.01) 0.13	-2.05 (0.5) 0.03	0.02 (0.01) 0.03
Hair	<i>DRGX</i>	cg17227358	1.67e-03 (0.01) 0.91	-2.04 (1.2) 0.98	0.02 (0.01) 0.98
Urine		cg19505184	0.02 (0.02) 0.38	-5.71 (1.49) 0.06	0.06 (0.02) 0.06
Hair		cg19505184	0.02 (0.02) 0.5	-3.7 (2.01) 0.98	0.04 (0.02) 0.98

Table 3-9: Assessing interactions between DNAm and Hg exposure in their associations with CPT3 Omissions Error Scores in Females (select results from 157 total significant observations).

Hg biomarker	Gene Name	ProbeID	DNAm β (S.E.) q-value	Hg β (S.E.) q-value	Hg x DNAm β (S.E.) q-value
Hair	<i>GABRG3</i>	cg27553667	0.02 (0.02) 0.96	0.09 (0.04) 0.15	-0.04 (0.02) 0.09
Urine	<i>GABRG3</i>	cg27553667	0.06 (0.01) 4.57e-04	0.01 (0.03) 1	-0.01 (0.01) 1
Hair	<i>POLD4</i>	cg16509978	-1.85e-03 (0.01) 0.98	-0.43 (0.17) 0.07	0.01 (4.99e-03) 0.09
Urine	<i>POLD4</i>	cg16509978	-0.01 (4.71e-03) 0.04	-0.04 (0.16) 1	7.40e-04 (4.91e-03) 1
Hair	<i>DAAM2</i>	cg06159534	-3.80e-03 (0.01) 0.96	2.01 (0.75) 0.07	-0.02 (0.01) 0.08
Urine	<i>DAAM2</i>	cg06159534	0.01 (0.01) 0.37	0.65 (0.65) 1	-0.01 (0.01) 1
Hair	<i>TNFRSF10A</i>	cg23303108	-4.88e-03 (0.01) 0.96	-2.56 (0.77) 0.04	0.03 (0.01) 0.05
Urine	<i>TNFRSF10A</i>	cg23303108	-0.02 (0.01) 0.04	-0.25 (0.73) 1	2.35e-03 (0.01) 1
Hair	<i>STAT1</i>	cg07052015	4.41e-03 (0.02) 0.98	-0.24 (0.07) 0.04	0.06 (0.02) 0.06
Urine	<i>STAT1</i>	cg07052015	-0.03 (0.01) 0.05	-0.01 (0.07) 1	-1.57e-03 (0.02) 1
					... 304 more rows

Table 3-10: Simplified interpretation of the positive interaction between Hg and DNAm: effect of constant Hg levels on CPT3 performance at different levels of DNAm.

β_{Hg} : Direction of Main Effect	Direction of change in DNAm levels	Effect on CPT3 scores
+	↑	↑
+	↓	↓
-	↑	↓
-	↓	↑

Note: Large black arrows indicate an increase (Up) or decrease (down) in DNAm levels or CPT3 scores. These interpretations are overly simplified, as they do not consider the main effect of DNAm on CPT3 performance and assumes all other variables are held constant.

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Appendix

Table 3-11 appx: Assessing interactions between DNAm and Hg exposure in their associations with CPT3 Omissions Error Scores in Females (complete results from 157 significant observations in [Table 3-9](#)).

ProbeID	DNAm β (S.E.) q-value	Hg β (S.E.) q-value	Hg x DNAm β (S.E.) q-value	Hg biomarker	UCSC_RefGene_Name	chr	pos
cg27232494	0.01 (0.01) 0.96	-2.55 (0.6) 0.01	0.03 (0.01) 0.02	Hair	TOM1L2;TOM1L2	chr17	17832220
cg05591749	-0.01 (0.01) 0.96	3.84 (0.98) 0.03	-0.04 (0.01) 0.02	Hair		chr13	66481938
cg15612131	6.62e-04 (0.01) 0.99	-0.25 (0.06) 0.01	0.03 (0.01) 0.02	Hair	CCDC86	chr11	60610626
cg17000356	-0.01 (0.01) 0.96	0.09 (0.03) 0.07	-0.04 (0.01) 0.02	Hair	PTPRB;PTPRB;PTPRB;PTPRB	chr12	71003679
cg02413141	2.75e-03 (3.96e-03) 0.96	-0.99 (0.27) 0.04	0.01 (3.88e-03) 0.04	Hair		chr6	88888098
cg02908609	-9.81e-04 (4.97e-03) 0.98	-1.58 (0.45) 0.04	0.02 (4.85e-03) 0.04	Hair	DNAJC9;DNAJC9-AS1;DNAJC9-AS1	chr10	75008215
cg06831408	-0.01 (0.02) 0.96	5.21 (1.53) 0.04	-0.06 (0.02) 0.04	Hair	MGMT	chr10	131326938
cg11441879	-2.96e-03 (0.01) 0.96	1.9 (0.54) 0.04	-0.02 (0.01) 0.04	Hair		chr1	169074295
cg12727407	1.95e-03 (2.93e-03) 0.96	-0.58 (0.16) 0.04	0.01 (2.92e-03) 0.04	Hair	CNTN4;CNTN4	chr3	2568709
cg14842777	-0.01 (0.01) 0.96	0.06 (0.03) 0.12	-0.03 (0.01) 0.04	Hair	SOX1;SOX1	chr13	112723226
cg17413604	-0.01 (0.01) 0.96	2.11 (0.61) 0.04	-0.03 (0.01) 0.04	Hair		chr3	72366496
cg17447008	-0.03 (0.02) 0.96	7.33 (2.13) 0.04	-0.08 (0.02) 0.04	Hair		chr9	99260562
cg18092047	4.50e-03 (0.02) 0.98	5.17 (1.47) 0.04	-0.05 (0.02) 0.04	Hair	EPB41L5;EPB41L5	chr2	120934017
cg21459796	-4.60e-03 (0.01) 0.96	2.58 (0.73) 0.04	-0.03 (0.01) 0.04	Hair		chr2	11565525
cg25396229	1.41e-03 (0.01) 0.98	-0.35 (0.1) 0.04	0.02 (0.01) 0.04	Hair	YRDC;C1orf122;C1orf122	chr1	38273072
cg25424205	4.52e-03 (3.88e-03) 0.96	-1.05 (0.3) 0.04	0.01 (3.71e-03) 0.04	Hair	CPM;CPM;CPM	chr12	69264281
cg26632656	-1.91e-03 (0.02) 0.99	-0.28 (0.07) 0.03	0.06 (0.02) 0.04	Hair	COL9A2	chr1	40781389
cg26800915	-5.87e-04 (4.01e-03) 0.99	-1.11 (0.32) 0.04	0.01 (3.76e-03) 0.04	Hair	OR51B4	chr11	5324199
cg07848601	-0.04 (0.03) 0.96	0.07 (0.03) 0.1	-0.1 (0.03) 0.05	Hair	RANBP17	chr5	170289430
cg18595911	-2.99e-03 (0.02) 0.99	5.38 (1.62) 0.04	-0.06 (0.02) 0.05	Hair		chr21	40722699
cg19515484	4.74e-03 (4.76e-03) 0.96	-0.45 (0.13) 0.04	0.01 (4.46e-03) 0.05	Hair		chr6	1606877
cg06942637	0.01 (0.01) 0.96	-2.22 (0.67) 0.04	0.02 (0.01) 0.05	Hair	SNAPIN	chr1	153633895
cg19355919	-4.35e-03 (0.01) 0.96	-0.23 (0.07) 0.04	0.02 (0.01) 0.05	Hair	MRO;MRO;MRO;MRO	chr18	48346593

cg23303108	-4.88e-03 (0.01) 0.96	-2.56 (0.77) 0.04	0.03 (0.01) 0.05	Hair	TNFRSF10A	chr8	23083578
cg03867963	1.54e-03 (0.01) 0.99	2.86 (0.89) 0.04	-0.03 (0.01) 0.05	Hair	CCDC85A	chr2	56556345
cg13632646	-0.01 (0.01) 0.96	2.02 (0.64) 0.04	-0.02 (0.01) 0.06	Hair		chr17	37908170
cg14084347	-2.30e-03 (4.42e-03) 0.96	-1.02 (0.31) 0.04	0.01 (3.83e-03) 0.06	Hair		chr6	132381836
cg20250015	2.60e-03 (0.01) 0.96	-1.38 (0.43) 0.04	0.02 (0.01) 0.06	Hair	IGFBP7;IGFBP7	chr4	57941240
cg23869228	8.94e-04 (4.00e-03) 0.98	-0.97 (0.3) 0.04	0.01 (3.57e-03) 0.06	Hair		chr5	124316882
cg01381288	-1.88e-04 (0.01) 1	-0.3 (0.09) 0.04	0.03 (0.01) 0.06	Hair	FHOD1	chr16	67278614
cg02897974	1.40e-03 (3.64e-03) 0.96	-0.94 (0.29) 0.04	0.01 (3.77e-03) 0.06	Hair		chr13	112673923
cg03317402	-3.69e-03 (0.01) 0.96	-0.26 (0.07) 0.04	0.03 (0.01) 0.06	Hair		chr3	155462502
cg08206071	0.01 (0.01) 0.96	-0.33 (0.1) 0.04	0.02 (0.01) 0.06	Hair	POLDIP2;POLDI P2;TMEM199	chr17	26683926
cg09848079	-8.54e-04 (4.68e-03) 0.99	-0.89 (0.28) 0.04	0.01 (4.22e-03) 0.06	Hair	IKBKB;IKBKB;IKBKB;IKBKB;IKBKB	chr8	42142289
cg11312896	-3.41e-03 (0.01) 0.96	0.1 (0.04) 0.09	-0.02 (0.01) 0.06	Hair	ZSCAN1	chr19	58545122
cg11590602	2.51e-03 (3.59e-03) 0.96	-0.77 (0.24) 0.04	0.01 (3.66e-03) 0.06	Hair		chr8	52169322
cg15975574	-0.01 (0.01) 0.96	0.13 (0.05) 0.08	-0.03 (0.01) 0.06	Hair	RUNX1T1	chr8	93107629
cg26413672	2.55e-03 (4.83e-03) 0.96	-0.82 (0.25) 0.04	0.01 (4.14e-03) 0.06	Hair		chr15	102084204
cg01035260	-0.01 (0.01) 0.96	-1.58 (0.52) 0.05	0.02 (0.01) 0.06	Hair		chr15	40721473
cg06003296	-0.01 (0.01) 0.96	0.06 (0.03) 0.16	-0.03 (0.01) 0.06	Hair	PTPRB;PTPRB	chr12	71003681
cg06243458	0.01 (0.01) 0.96	-0.7 (0.22) 0.04	0.02 (0.01) 0.06	Hair	BANF2;BANF2;BANF2	chr20	17679422
cg07052015	4.41e-03 (0.02) 0.98	-0.24 (0.07) 0.04	0.06 (0.02) 0.06	Hair	STAT1;STAT1	chr2	191879114
cg07461682	0.01 (0.01) 0.96	-4.19 (1.4) 0.05	0.04 (0.01) 0.06	Hair		chr3	187800194
cg08743776	-3.71e-03 (0.01) 0.96	-0.23 (0.07) 0.04	0.02 (0.01) 0.06	Hair	LOC645332	chr11	67572988
cg08880154	1.31e-03 (0.01) 0.98	-0.85 (0.27) 0.04	0.02 (0.01) 0.06	Hair		chr1	86926798
cg09661126	1.20e-03 (3.73e-03) 0.98	-0.37 (0.12) 0.04	0.01 (3.30e-03) 0.06	Hair	CTIF;CTIF	chr18	46238526
cg10472283	-0.02 (0.04) 0.96	-0.12 (0.03) 0.04	0.11 (0.04) 0.06	Hair	EIF4G2;EIF4G2;LOC101928053;EIF4G2	chr11	10830003
cg11969556	-0.01 (0.02) 0.96	0.11 (0.05) 0.1	-0.05 (0.02) 0.06	Hair	HOXD8;HOXD8	chr2	176994498
cg12088387	-2.11e-03 (4.34e-03) 0.96	0.1 (0.04) 0.1	-0.01 (4.23e-03) 0.06	Hair	CXCR7	chr2	237476950
cg150061	1.49e-03 (4.67e-	-1.09 (0.35)	0.01 (4.57e-03)	Hair		chr14	107035310

13	03) 0.98	0.04	0.06				
cg156476 99	-1.03e-03 (0.01) 0.99	-0.23 (0.07) 0.04	0.02 (0.01) 0.06	Hair	SDCBP;SDCBP;S DCBP;SDCBP;S DCBP	chr8	59465342
cg156916 59	3.93e-03 (4.94e- 03) 0.96	-0.69 (0.22) 0.04	0.01 (4.57e-03) 0.06	Hair		chr20	44603978
cg159003 20	0.01 (0.02) 0.96	-0.23 (0.07) 0.04	0.05 (0.02) 0.06	Hair	LOC728743	chr7	150102724
cg162263 80	-1.18e-03 (4.42e- 03) 0.98	-0.89 (0.29) 0.05	0.01 (3.48e-03) 0.06	Hair	TXNDC11;TXN DC11	chr16	11776324
cg213237 20	4.53e-04 (2.97e- 03) 0.99	-0.31 (0.09) 0.04	0.01 (2.76e-03) 0.06	Hair		chr4	114357601
cg229634 14	-3.56e-03 (0.01) 0.96	-0.16 (0.05) 0.04	0.03 (0.01) 0.06	Hair	ZFYVE1	chr14	73493969
cg241569 49	-2.47e-04 (0.01) 1	1.23 (0.41) 0.05	-0.02 (0.01) 0.06	Hair	LDLRAP1	chr1	25874418
cg251213 65	3.16e-03 (3.14e- 03) 0.96	-0.54 (0.17) 0.04	0.01 (2.96e-03) 0.06	Hair	CYFIP2;CYFIP2; CYFIP2;CYFIP2	chr5	156695069
cg269837 10	-0.01 (0.01) 0.96	0.05 (0.03) 0.16	-0.02 (0.01) 0.06	Hair	GALNT13	chr2	154728202
cg052125 10	-1.01e-05 (2.69e- 03) 1	0.61 (0.22) 0.06	-0.01 (2.33e-03) 0.06	Hair	KCNG2	chr18	77623544
cg260221 59	-5.92e-04 (0.01) 0.99	-1.34 (0.44) 0.05	0.02 (0.01) 0.06	Hair	UBE2E2;MIR548 AC	chr3	23484930
cg015836 46	1.55e-03 (3.87e- 03) 0.96	-0.41 (0.13) 0.04	0.01 (4.44e-03) 0.06	Hair	TGM7	chr15	43576419
cg105890 94	8.59e-04 (4.37e- 03) 0.98	-0.96 (0.32) 0.05	0.01 (4.21e-03) 0.06	Hair	TNFRSF1A	chr12	6452596
cg167837 44	-3.03e-03 (0.01) 0.96	0.07 (0.04) 0.13	-0.02 (0.01) 0.06	Hair	DPYS	chr8	105478855
cg250781 87	-2.41e-03 (3.05e- 03) 0.96	-0.67 (0.22) 0.05	0.01 (2.63e-03) 0.06	Hair		chr7	142447339
cg039444 74	-1.55e-04 (4.50e- 03) 1	-0.96 (0.32) 0.05	0.01 (4.31e-03) 0.06	Hair	ADARB2	chr10	1532238
cg052139 35	4.25e-03 (4.10e- 03) 0.96	-0.81 (0.27) 0.05	0.01 (3.34e-03) 0.06	Hair		chr2	89161256
cg029913 38	-4.87e-03 (0.01) 0.96	0.05 (0.03) 0.2	-0.02 (0.01) 0.06	Hair	FOXG1	chr14	29236017
cg031128 87	3.43e-03 (0.01) 0.96	-0.94 (0.32) 0.05	0.02 (0.01) 0.06	Hair	UBFD1;EARS2;E ARS2;EARS2	chr16	23568076
cg220592 11	1.25e-03 (4.93e- 03) 0.98	-1.24 (0.43) 0.05	0.01 (4.68e-03) 0.06	Hair		chr8	735312
cg259506 25	-1.08e-03 (2.27e- 03) 0.96	0.07 (0.03) 0.14	-0.01 (1.96e-03) 0.06	Hair	PLCXD3	chr5	41510116
cg274885 03	5.21e-04 (4.12e- 03) 0.99	-0.26 (0.08) 0.04	0.01 (3.87e-03) 0.06	Hair	GRK4;GRK4;GR K4;NOP14	chr4	2964223
cg000070 76	3.01e-03 (4.47e- 03) 0.96	-0.61 (0.2) 0.05	0.01 (4.45e-03) 0.07	Hair	RRS1;RRS1	chr8	67342600
cg186249 00	-0.01 (0.02) 0.96	0.04 (0.02) 0.23	-0.05 (0.02) 0.07	Hair	SLC16A12	chr10	91295643
cg243307 84	-4.61e-03 (4.25e- 03) 0.96	-0.51 (0.17) 0.05	0.01 (4.09e-03) 0.07	Hair	ZSCAN2;ZSCAN 2;ZSCAN2	chr15	85149026
cg190791 94	9.04e-04 (0.01) 0.99	0.08 (0.04) 0.13	-0.04 (0.01) 0.07	Hair	ERBB4;ERBB4;E RBB4;ERBB4	chr2	213403321
cg240867	-1.07e-03 (3.10e-	-0.3 (0.1)	0.01 (3.23e-03)	Hair	GLIS1	chr1	54154485

17	03) 0.97	0.05	0.07				
cg00048746	-2.57e-03 (0.01) 0.96	-0.18 (0.06) 0.04	0.02 (0.01) 0.07	Hair	CCDC103;EFTUD2;EFTUD2;EFTUD2	chr17	42976844
cg09251557	4.41e-03 (4.15e-03) 0.96	-0.54 (0.18) 0.05	0.01 (4.11e-03) 0.07	Hair	ALDH5A1;ALDH5A1	chr6	24521516
cg14691884	-1.19e-03 (0.01) 0.99	-0.19 (0.06) 0.04	0.02 (0.01) 0.07	Hair		chr6	159528654
cg16415476	-1.28e-03 (0.02) 1	-0.18 (0.06) 0.04	0.05 (0.02) 0.07	Hair	UMODL1;UMODL1	chr21	43482628
cg06591728	-0.01 (4.56e-03) 0.96	-1.01 (0.35) 0.06	0.01 (4.00e-03) 0.07	Hair	TTLL11	chr9	124708685
cg10823473	-3.10e-03 (0.01) 0.98	0.04 (0.03) 0.21	-0.03 (0.01) 0.07	Hair	ISL2	chr15	76630514
cg16427752	-1.80e-04 (0.01) 1	-0.88 (0.31) 0.06	0.01 (0.01) 0.07	Hair	MSH4	chr1	76262302
cg10717991	7.46e-04 (0.01) 0.99	-0.59 (0.2) 0.05	0.01 (4.87e-03) 0.07	Hair		chr2	47951724
cg13035766	4.46e-03 (0.01) 0.96	-1.05 (0.37) 0.06	0.02 (0.01) 0.07	Hair		chr17	42412326
cg15648188	-0.01 (0.01) 0.96	3.07 (1.12) 0.07	-0.03 (0.01) 0.07	Hair		chr1	147531342
cg19416795	-2.81e-03 (0.02) 0.99	3.66 (1.34) 0.07	-0.04 (0.01) 0.07	Hair		chr18	76270967
cg20765420	1.14e-03 (0.01) 0.98	-0.31 (0.1) 0.05	0.02 (0.01) 0.07	Hair		chr12	52269081
cg08881202	-2.48e-05 (0.03) 1	-0.2 (0.07) 0.04	0.07 (0.02) 0.07	Hair	CCNB1IP1;CCNB1IP1;CCNB1IP1	chr14	20801617
cg24698925	0.01 (0.01) 0.96	2.04 (0.75) 0.07	-0.02 (0.01) 0.07	Hair		chr15	57873089
cg25813357	0.01 (0.02) 0.96	5.22 (1.91) 0.07	-0.06 (0.02) 0.07	Hair	CYFIP2;CYFIP2;CYFIP2;CYFIP2	chr5	156752564
cg04697056	-1.21e-03 (3.15e-03) 0.96	-0.61 (0.22) 0.06	0.01 (3.21e-03) 0.07	Hair	FAT1	chr4	187565389
cg08568706	3.20e-03 (4.68e-03) 0.96	-0.41 (0.14) 0.06	0.01 (0.01) 0.07	Hair		chr20	29591086
cg10561323	-2.17e-03 (0.01) 0.98	2.28 (0.85) 0.07	-0.03 (0.01) 0.08	Hair	RASL10B	chr17	34062395
cg01474953	0.01 (0.01) 0.96	1.81 (0.68) 0.07	-0.02 (0.01) 0.08	Hair	GBX1	chr7	150862828
cg04800771	-3.70e-03 (0.01) 0.96	1.74 (0.65) 0.07	-0.02 (0.01) 0.08	Hair	KRT79	chr12	53215823
cg06159534	-3.80e-03 (0.01) 0.96	2.01 (0.75) 0.07	-0.02 (0.01) 0.08	Hair	DAAM2;DAAM2	chr6	39838887
cg08609155	2.20e-03 (4.91e-03) 0.96	-0.96 (0.35) 0.07	0.01 (4.81e-03) 0.08	Hair		chr14	34472924
cg19213569	1.34e-03 (0.01) 0.99	-0.17 (0.06) 0.05	0.03 (0.01) 0.08	Hair	FGGY;FGGY;FGGY	chr1	59762605
cg19761848	-1.97e-03 (4.16e-03) 0.96	0.03 (0.02) 0.3	-0.01 (3.99e-03) 0.08	Hair	GBX2	chr2	237076815
cg26115633	-3.40e-03 (0.01) 0.96	0.03 (0.03) 0.31	-0.02 (0.01) 0.08	Hair	FOXI2	chr10	129535893
cg26220203	-5.19e-04 (0.01) 0.99	1.41 (0.53) 0.07	-0.02 (0.01) 0.08	Hair		chr1	201100716
cg096487	-2.40e-04 (4.35e-	-0.63 (0.23)	0.01 (3.69e-03)	Hair		chr6	116408696

20	03) 1	0.07	0.08				
cg18101707	2.01e-03 (0.01) 0.96	-0.25 (0.09) 0.05	0.01 (4.75e-03) 0.08	Hair	DLGAP1;DLGAP1;DLGAP1;DLGAP1;DLGAP1	chr18	3874222
cg11776334	-1.26e-03 (4.12e-03) 0.98	-0.73 (0.27) 0.07	0.01 (3.89e-03) 0.08	Hair	PLCE1-AS1;PLCE1;PLCE1;PLCE1	chr10	96046836
cg16643706	2.28e-04 (4.41e-03) 1	-0.88 (0.32) 0.07	0.01 (3.94e-03) 0.08	Hair	C5orf13;C5orf13	chr5	111289503
cg23092003	3.89e-03 (0.01) 0.96	-1.26 (0.46) 0.07	0.01 (0.01) 0.08	Hair	CLYBL-AS2;CLYBL;CLYBL	chr13	100343915
cg27265630	1.67e-03 (0.01) 0.98	1.49 (0.57) 0.07	-0.02 (0.01) 0.08	Hair	PCDH21	chr10	85958429
cg22257107	-1.93e-03 (4.41e-03) 0.96	-0.42 (0.15) 0.07	0.01 (4.36e-03) 0.08	Hair	KIAA1755	chr20	36851725
cg24228145	-1.58e-03 (0.01) 0.98	-1.32 (0.49) 0.07	0.01 (0.01) 0.08	Hair	USP28;USP28	chr11	113702127
cg05584070	-4.18e-03 (2.97e-03) 0.96	-0.6 (0.22) 0.07	0.01 (2.75e-03) 0.08	Hair	CARS2	chr13	111329221
cg10915728	-5.53e-04 (4.91e-03) 0.99	-0.54 (0.2) 0.07	0.01 (4.49e-03) 0.08	Hair		chr19	55118836
cg12270707	1.50e-03 (0.01) 0.98	-1.13 (0.42) 0.07	0.01 (4.99e-03) 0.08	Hair	TMPRSS7;TMPRSS7	chr3	111772306
cg16727774	-1.52e-03 (3.38e-03) 0.96	-0.6 (0.22) 0.07	0.01 (2.79e-03) 0.08	Hair	CA5A	chr16	87958281
cg18967835	1.33e-03 (3.93e-03) 0.98	-0.76 (0.28) 0.07	0.01 (3.51e-03) 0.08	Hair	GPR18;GPR18;UBAC2;UBAC2;UBAC2	chr13	99907350
cg11168904	1.43e-03 (0.01) 0.99	-2.88 (1.09) 0.07	0.03 (0.01) 0.08	Hair	ATP8A1;ATP8A1	chr4	42641925
cg00063279	8.64e-04 (3.78e-03) 0.98	-0.19 (0.07) 0.05	0.01 (3.56e-03) 0.08	Hair	MAP2K2	chr19	4123248
cg01128566	-1.54e-03 (0.01) 0.99	0.09 (0.05) 0.15	-0.04 (0.01) 0.08	Hair	SVIL	chr10	30026028
cg04477502	0.01 (0.02) 0.96	4.15 (1.61) 0.08	-0.04 (0.02) 0.08	Hair	EDN3;EDN3;EDN3;EDN3;EDN3	chr20	57890837
cg14997711	2.82e-03 (4.58e-03) 0.96	-0.81 (0.3) 0.07	0.01 (3.97e-03) 0.08	Hair	PTPRJ;PTPRJ	chr11	48065851
cg26592175	1.37e-03 (4.37e-03) 0.98	-0.94 (0.35) 0.07	0.01 (4.29e-03) 0.08	Hair	CCDC167	chr6	37463570
cg15195861	2.45e-03 (0.01) 0.96	-1.24 (0.47) 0.07	0.01 (0.01) 0.09	Hair	CRIM1	chr2	36694850
cg10508993	-1.15e-04 (0.01) 1	0.05 (0.03) 0.22	-0.03 (0.01) 0.09	Hair	SCGB3A1	chr5	180018495
cg06745030	-2.05e-04 (2.13e-03) 0.99	0.09 (0.05) 0.15	-0.01 (2.15e-03) 0.09	Hair	B3GNT7	chr2	232260139
cg11532578	4.00e-03 (0.02) 0.98	4.61 (1.8) 0.08	-0.05 (0.02) 0.09	Hair	KIF1B	chr1	10387937
cg06568145	-0.01 (0.02) 0.98	-0.13 (0.04) 0.05	0.04 (0.02) 0.09	Hair		chr7	25138216
cg13438673	-0.01 (0.01) 0.96	-0.2 (0.07) 0.07	0.03 (0.01) 0.09	Hair	ZNF526	chr19	42724717
cg240300	1.17e-03 (0.01)	-1.05 (0.4)	0.01 (4.53e-03)	Hair		chr5	67433852

07	0.98	0.08	0.09				
cg26911830	-2.96e-04 (3.19e-03) 0.99	-0.55 (0.21) 0.07	0.01 (3.16e-03) 0.09	Hair		chr1	157219222
cg01516645	2.27e-03 (3.00e-03) 0.96	-0.52 (0.2) 0.07	0.01 (2.90e-03) 0.09	Hair		chr1	28471495
cg05772125	8.46e-04 (3.57e-03) 0.98	-0.62 (0.23) 0.07	0.01 (3.36e-03) 0.09	Hair	AHSP	chr16	31539169
cg06525651	-5.38e-04 (0.01) 1	0.05 (0.03) 0.23	-0.03 (0.01) 0.09	Hair	FAM196A;FAM196A;DOCK1	chr10	128994297
cg12760479	-1.44e-03 (4.21e-03) 0.98	-0.22 (0.08) 0.07	0.01 (4.10e-03) 0.09	Hair		chr22	50323140
cg14633012	5.66e-04 (4.11e-03) 0.99	-0.74 (0.28) 0.07	0.01 (4.29e-03) 0.09	Hair		chr21	39329472
cg19874862	4.65e-04 (4.69e-03) 0.99	-0.95 (0.37) 0.08	0.01 (4.93e-03) 0.09	Hair		chr1	18297461
cg26207423	6.03e-04 (3.81e-03) 0.99	-0.73 (0.28) 0.07	0.01 (3.51e-03) 0.09	Hair	HMBOX1;HMB OX1	chr8	28787952
cg05150569	3.09e-03 (4.60e-03) 0.96	-0.31 (0.11) 0.07	0.01 (4.33e-03) 0.09	Hair	RPS13	chr11	17098362
cg05411932	3.80e-03 (0.01) 0.96	-1.06 (0.41) 0.08	0.01 (4.77e-03) 0.09	Hair	METTL9;METTL9;LOC101927814	chr16	21608429
cg07293152	8.25e-04 (0.01) 0.99	-0.9 (0.35) 0.08	0.01 (4.28e-03) 0.09	Hair		chrX	118747464
cg08538385	-0.01 (0.01) 0.96	1.76 (0.71) 0.09	-0.02 (0.01) 0.09	Hair	ZFAND3	chr6	38110088
cg14532827	-4.09e-04 (3.01e-03) 0.99	0.05 (0.03) 0.22	-0.01 (2.96e-03) 0.09	Hair	GBX2;GBX2	chr2	237076820
cg17384889	-2.15e-03 (3.86e-03) 0.96	0.05 (0.03) 0.21	-0.01 (3.46e-03) 0.09	Hair	NKAPL	chr6	28227068
cg17910564	-4.39e-03 (4.22e-03) 0.96	-0.84 (0.32) 0.08	0.01 (4.08e-03) 0.09	Hair	VDAC3;VDAC3	chr8	42248702
cg20132590	-3.63e-04 (3.76e-03) 0.99	0.05 (0.03) 0.22	-0.01 (4.01e-03) 0.09	Hair	PDZRN3	chr3	73673893
cg20685554	-0.01 (0.01) 0.96	0.13 (0.06) 0.13	-0.03 (0.01) 0.09	Hair	LRP11	chr6	150185632
cg22989103	2.65e-03 (0.01) 0.98	0.06 (0.04) 0.19	-0.03 (0.01) 0.09	Hair	FAM19A5;FAM19A5	chr22	48972331
cg00189887	-9.45e-04 (0.01) 0.99	-0.63 (0.24) 0.08	0.02 (0.01) 0.09	Hair	CUEDC1;CUEDC1	chr17	55951557
cg14287809	-4.80e-03 (0.01) 0.96	-0.2 (0.07) 0.07	0.03 (0.01) 0.09	Hair	LETM2;LETM2;LETM2;LETM2;LETM2;LETM2	chr8	38244679
cg07816665	-1.27e-03 (4.76e-03) 0.98	-0.28 (0.11) 0.07	0.01 (4.48e-03) 0.09	Hair		chr11	119381053
cg27553667	0.02 (0.02) 0.96	0.09 (0.04) 0.15	-0.04 (0.02) 0.09	Hair	GABRG3	chr15	27216083
cg10964757	0.01 (0.01) 0.96	-0.16 (0.06) 0.06	0.02 (0.01) 0.09	Hair	NUDC	chr1	27248607
cg16509978	-1.85e-03 (0.01) 0.98	-0.43 (0.17) 0.07	0.01 (4.99e-03) 0.09	Hair	LOC100130987;P OLD4	chr11	67121607
cg17084204	-4.90e-03 (0.02) 0.98	4.09 (1.66) 0.09	-0.04 (0.02) 0.09	Hair	IKBKB;IKBKB;I KBKB;IKBKB;I KBKB;IKBKB	chr8	42182071
cg164160	-1.30e-03 (3.55e-03)	-0.58 (0.23)	0.01 (3.42e-03)	Hair		chr3	110271653

14	03) 0.96	0.08	0.09				
cg21442182	-0.01 (0.01) 0.96	-0.63 (0.5) 0.32	0.01 (0.01) 0.35	Hair		chr13	31446420
cg10396484	0.04 (0.03) 0.96	-0.02 (0.05) 0.73	-1.17e-03 (0.02) 0.97	Hair	LBX1;FLJ41350	chr10	102988831
cg00007076	-4.59e-03 (3.62e-03) 0.23	-0.07 (0.16) 1	1.15e-03 (3.52e-03) 1	Urine	RRS1;RRS1	chr8	67342600
cg00048746	-0.02 (0.01) 0.05	8.44e-04 (0.07) 1	-2.99e-03 (0.01) 1	Urine	CCDC103;EFTUD2;EFTUD2;EFTUD2	chr17	42976844
cg00063279	-3.58e-03 (2.99e-03) 0.25	-0.11 (0.06) 1	0.01 (3.11e-03) 1	Urine	MAP2K2	chr19	4123248
cg00189887	-0.01 (0.01) 0.08	-0.11 (0.22) 1	2.43e-03 (0.01) 1	Urine	CUEDC1;CUEDC1	chr17	55951557
cg01035260	-0.02 (4.83e-03) 2.79e-03	-0.25 (0.48) 1	2.62e-03 (0.01) 1	Urine		chr15	40721473
cg01128566	0.03 (0.01) 3.31e-03	-0.01 (0.03) 1	-3.62e-03 (0.01) 1	Urine	SVIL	chr10	30026028
cg01381288	-0.02 (0.01) 0.03	-1.79e-03 (0.07) 1	-1.78e-03 (0.01) 1	Urine	FHOD1	chr16	67278614
cg01474953	0.02 (0.01) 0.01	0.6 (0.57) 1	-0.01 (0.01) 1	Urine	GBX1	chr7	150862828
cg01516645	-0.01 (2.50e-03) 0.05	0.21 (0.19) 1	-3.42e-03 (2.75e-03) 1	Urine		chr1	28471495
cg01583646	-0.01 (3.14e-03) 0.08	-0.07 (0.1) 1	1.84e-03 (3.55e-03) 1	Urine	TGM7	chr15	43576419
cg02413141	-0.01 (3.14e-03) 0.14	-0.27 (0.19) 1	3.66e-03 (2.68e-03) 1	Urine		chr6	88888098
cg02897974	-0.01 (3.15e-03) 0.05	0.07 (0.24) 1	-1.12e-03 (3.08e-03) 1	Urine		chr13	112673923
cg02908609	-0.01 (4.24e-03) 0.04	-0.2 (0.43) 1	1.99e-03 (4.65e-03) 1	Urine	DNAJC9;DNAJC9-AS1;DNAJC9-AS1	chr10	75008215
cg02991338	0.01 (3.83e-03) 0.04	-0.04 (0.02) 1	2.50e-03 (3.87e-03) 1	Urine	FOXG1	chr14	29236017
cg03112887	-0.01 (4.12e-03) 0.09	-0.15 (0.23) 1	2.21e-03 (3.98e-03) 1	Urine	UBFD1;EARS2;EARS2;EARS2	chr16	23568076
cg03317402	-0.02 (0.01) 0.07	-0.05 (0.08) 1	4.52e-03 (0.01) 1	Urine		chr3	155462502
cg03867963	0.02 (0.01) 0.02	0.28 (0.75) 1	-3.22e-03 (0.01) 1	Urine	CCDC85A	chr2	56556345
cg03944474	-0.01 (3.69e-03) 0.06	-0.29 (0.27) 1	3.65e-03 (3.68e-03) 1	Urine	ADARB2	chr10	1532238
cg04477502	0.05 (0.02) 0.02	-0.13 (1.49) 1	1.16e-03 (0.02) 1	Urine	EDN3;EDN3;EDN3;EDN3;EDN3	chr20	57890837
cg04697056	-0.01 (2.50e-03) 0.01	0.09 (0.16) 1	-1.58e-03 (2.38e-03) 1	Urine	FAT1	chr4	187565389
cg04800771	0.01 (0.01) 0.11	-0.06 (0.6) 1	4.66e-04 (0.01) 1	Urine	KRT79	chr12	53215823
cg05150569	-0.01 (3.81e-03) 0.11	-0.01 (0.09) 1	-4.60e-04 (3.61e-03) 1	Urine	RPS13	chr11	17098362
cg05212510	0.01 (2.23e-03) 0.05	0.12 (0.2) 1	-1.53e-03 (2.17e-03) 1	Urine	KCNG2	chr18	77623544
cg05213935	-2.87e-03 (3.36e-03) 0.41	-0.35 (0.27) 1	4.15e-03 (3.31e-03) 1	Urine		chr2	89161256

cg05411932	-3.30e-03 (4.61e-03) 0.48	-0.31 (0.36) 1	3.35e-03 (4.09e-03) 1	Urine	METTL9;METTL9;LOC101927814	chr16	21608429
cg05584070	-0.01 (2.57e-03) 0.01	-0.18 (0.21) 1	1.97e-03 (2.70e-03) 1	Urine	CARS2	chr13	111329221
cg05591749	0.01 (0.01) 0.08	1.02 (0.87) 1	-0.01 (0.01) 1	Urine		chr13	66481938
cg05772125	-0.01 (2.77e-03) 0.03	0.17 (0.21) 1	-2.85e-03 (3.03e-03) 1	Urine	AHSP	chr16	31539169
cg06003296	0.02 (0.01) 0.08	-0.03 (0.03) 1	4.67e-03 (0.01) 1	Urine	PTPRB;PTPRB	chr12	71003681
cg06159534	0.01 (0.01) 0.37	0.65 (0.65) 1	-0.01 (0.01) 1	Urine	DAAM2;DAAM2	chr6	39838887
cg06243458	-0.01 (4.76e-03) 0.11	-0.02 (0.19) 1	4.47e-06 (4.55e-03) 1	Urine	BANF2;BANF2;BANF2	chr20	17679422
cg06525651	0.02 (0.01) 0.01	-0.03 (0.02) 1	6.34e-04 (0.01) 1	Urine	FAM196A;FAM196A;DOCK1	chr10	128994297
cg06568145	-0.04 (0.01) 0.02	-0.02 (0.04) 1	2.27e-03 (0.02) 1	Urine		chr7	25138216
cg06591728	-0.01 (3.85e-03) 0.01	-0.36 (0.35) 1	3.92e-03 (3.98e-03) 1	Urine	TTLL11	chr9	124708685
cg06745030	4.14e-03 (1.64e-03) 0.04	-0.04 (0.04) 1	6.75e-04 (1.78e-03) 1	Urine	B3GNT7	chr2	232260139
cg06831408	0.03 (0.01) 0.09	0.52 (1.33) 1	-0.01 (0.01) 1	Urine	MGMT	chr10	131326938
cg06942637	-1.23e-03 (0.01) 0.87	-1.12 (0.85) 1	0.01 (0.01) 1	Urine	SNAPIN	chr1	153633895
cg07052015	-0.03 (0.01) 0.05	-0.01 (0.07) 1	-1.57e-03 (0.02) 1	Urine	STAT1;STAT1	chr2	191879114
cg07293152	-0.01 (4.27e-03) 0.09	0.08 (0.35) 1	-1.24e-03 (4.27e-03) 1	Urine		chrX	118747464
cg07461682	-0.01 (0.01) 0.59	-1.35 (1.14) 1	0.01 (0.01) 1	Urine		chr3	187800194
cg07816665	-0.01 (3.64e-03) 0.01	0.12 (0.09) 1	-0.01 (3.84e-03) 1	Urine		chr11	119381053
cg07848601	0.05 (0.01) 0.01	-0.02 (0.02) 1	-3.99e-03 (0.02) 1	Urine	RANBP17	chr5	170289430
cg08206071	-0.01 (4.21e-03) 0.23	-0.05 (0.07) 1	1.65e-03 (4.11e-03) 1	Urine	POLDIP2;POLDIP2;TMEM199	chr17	26683926
cg08538385	0.01 (0.01) 0.24	0.13 (0.57) 1	-1.63e-03 (0.01) 1	Urine	ZFAND3	chr6	38110088
cg08568706	-0.01 (3.76e-03) 0.08	0.03 (0.11) 1	-1.69e-03 (3.84e-03) 1	Urine		chr20	29591086
cg08609155	-0.01 (3.79e-03) 0.07	0.03 (0.26) 1	-6.57e-04 (3.68e-03) 1	Urine		chr14	34472924
cg08743776	-0.02 (0.01) 0.03	-0.03 (0.08) 1	1.34e-03 (0.01) 1	Urine	LOC645332	chr11	67572988
cg08880154	-0.01 (4.59e-03) 0.04	0.01 (0.22) 1	-5.86e-04 (4.59e-03) 1	Urine		chr1	86926798
cg08881202	-0.03 (0.02) 0.14	-0.1 (0.06) 1	0.03 (0.02) 1	Urine	CCNB1IP1;CCNB1IP1;CCNB1IP1	chr14	20801617
cg09251557	-0.01 (3.54e-03) 0.12	0.12 (0.17) 1	-3.19e-03 (3.92e-03) 1	Urine	ALDH5A1;ALDH5A1	chr6	24521516
cg09648720	-0.01 (3.62e-03) 0.02	0.14 (0.23) 1	-2.52e-03 (3.76e-03) 1	Urine		chr6	116408696
cg096611	-0.01 (2.92e-03)	-0.02 (0.11)	2.07e-04 (3.18e-	Urine	CTIF;CTIF	chr18	46238526

26	0.05	1	03) 1				
cg09848079	-0.01 (3.95e-03) 0.03	-0.08 (0.27) 1	8.97e-04 (4.09e-03) 1	Urine	IKBKB;IKBKB;IKBKB;IKBKB;IKBKB	chr8	42142289
cg10396484	0.04 (0.01) 0.01	-0.1 (0.04) 1	0.04 (0.02) 1	Urine	LBX1;FLJ41350	chr10	102988831
cg10472283	-0.08 (0.03) 0.04	-0.07 (0.03) 1	0.06 (0.04) 1	Urine	EIF4G2;EIF4G2;LOC101928053;EIF4G2	chr11	10830003
cg10508993	0.03 (0.01) 0.01	-0.02 (0.03) 1	-9.30e-04 (0.01) 1	Urine	SCGB3A1	chr5	180018495
cg10561323	0.02 (0.01) 0.06	0.15 (0.56) 1	-1.93e-03 (0.01) 1	Urine	RASL10B	chr17	34062395
cg10589094	-0.01 (3.64e-03) 0.13	-0.46 (0.3) 1	0.01 (3.91e-03) 1	Urine	TNFRSF1A	chr12	6452596
cg10717991	-0.01 (4.12e-03) 0.08	-0.16 (0.17) 1	3.31e-03 (4.06e-03) 1	Urine		chr2	47951724
cg10823473	0.03 (0.01) 8.00e-04	-1.71e-03 (0.02) 1	-0.01 (0.01) 1	Urine	ISL2	chr15	76630514
cg10915728	-0.01 (4.06e-03) 0.05	-1.96e-03 (0.17) 1	-3.81e-04 (3.89e-03) 1	Urine		chr19	55118836
cg10964757	-0.02 (0.01) 0.1	0.04 (0.05) 1	-0.01 (0.01) 1	Urine	NUDC	chr1	27248607
cg11168904	-0.02 (0.01) 0.08	0.04 (1.12) 1	-6.85e-04 (0.01) 1	Urine	ATP8A1;ATP8A1	chr4	42641925
cg11312896	0.01 (4.05e-03) 0.03	-0.05 (0.03) 1	3.25e-03 (3.79e-03) 1	Urine	ZSCAN1	chr19	58545122
cg11441879	0.01 (0.01) 0.05	0.06 (0.4) 1	-9.57e-04 (0.01) 1	Urine		chr1	169074295
cg11532578	0.04 (0.01) 0.03	1.99 (1.2) 1	-0.02 (0.01) 1	Urine	KIF1B	chr1	10387937
cg11590602	-0.01 (2.85e-03) 0.04	0.07 (0.18) 1	-1.45e-03 (2.82e-03) 1	Urine		chr8	52169322
cg11776334	-0.01 (2.79e-03) 4.10e-03	0.22 (0.24) 1	-3.53e-03 (3.46e-03) 1	Urine	PLCE1-AS1;PLCE1;PLCE1;PLCE1	chr10	96046836
cg11969556	0.05 (0.02) 0.02	-0.05 (0.04) 1	0.01 (0.02) 1	Urine	HOXD8;HOXD8	chr2	176994498
cg12088387	0.01 (2.65e-03) 0.01	-0.03 (0.03) 1	7.63e-04 (2.91e-03) 1	Urine	CXCR7	chr2	237476950
cg12270707	-0.01 (4.00e-03) 0.01	0.57 (0.33) 1	-0.01 (3.94e-03) 1	Urine	TMPRSS7;TMPRSS7	chr3	111772306
cg12727407	-0.01 (2.30e-03) 0.05	1.22e-03 (0.1) 1	-4.17e-04 (1.84e-03) 1	Urine	CNTN4;CNTN4	chr3	2568709
cg12760479	-0.01 (3.35e-03) 0.02	0.01 (0.07) 1	-1.59e-03 (3.66e-03) 1	Urine		chr22	50323140
cg13035766	-0.01 (0.01) 0.09	0.24 (0.36) 1	-4.26e-03 (0.01) 1	Urine		chr17	42412326
cg13438673	-0.03 (0.01) 0.01	0.01 (0.07) 1	-0.01 (0.01) 1	Urine	ZNF526	chr19	42724717
cg13632646	0.01 (0.01) 0.13	0.15 (0.49) 1	-1.90e-03 (0.01) 1	Urine		chr17	37908170
cg14084347	-0.01 (3.54e-03) 0.01	-0.01 (0.32) 1	-1.27e-04 (3.88e-03) 1	Urine		chr6	132381836
cg142878	-0.02 (0.01) 0.08	-0.06 (0.06)	0.01 (0.01) 1	Urine	LETM2;LETM2;	chr8	38244679

09		1			LETM2;LETM2; LETM2;LETM2; LETM2		
cg145328 27	0.01 (2.46e-03) 0.05	-0.03 (0.03) 1	1.16e-03 (2.53e- 03) 1	Urine	GBX2;GBX2	chr2	237076820
cg146330 12	-0.01 (3.53e-03) 0.05	0.13 (0.23) 1	-2.22e-03 (3.52e-03) 1	Urine		chr21	39329472
cg146918 84	-0.01 (0.01) 0.05	-0.06 (0.05) 1	4.87e-03 (0.01) 1	Urine		chr6	159528654
cg148427 77	0.02 (0.01) 0.01	-0.03 (0.02) 1	2.81e-04 (4.67e- 03) 1	Urine	SOX1;SOX1	chr13	112723226
cg149977 11	-1.53e-03 (3.99e- 03) 0.71	-0.54 (0.31) 1	0.01 (4.09e-03) 1	Urine	PTPRJ;PTPRJ	chr11	48065851
cg150061 13	-4.64e-03 (3.72e- 03) 0.24	-0.55 (0.28) 1	0.01 (3.71e-03) 1	Urine		chr14	107035310
cg151958 61	-0.01 (4.97e-03) 0.08	0.05 (0.44) 1	-8.73e-04 (0.01) 1	Urine	CRIM1	chr2	36694850
cg156121 31	-0.01 (0.01) 0.13	-0.04 (0.05) 1	3.78e-03 (0.01) 1	Urine	CCDC86	chr11	60610626
cg156476 99	-0.01 (0.01) 0.04	-0.03 (0.07) 1	1.65e-03 (0.01) 1	Urine	SDCBP;SDCBP;S DCBP;SDCBP;S DCBP	chr8	59465342
cg156481 88	0.02 (0.01) 0.06	-0.8 (0.98) 1	0.01 (0.01) 1	Urine		chr1	147531342
cg156916 59	-0.01 (4.23e-03) 0.05	0.1 (0.21) 1	-2.50e-03 (4.34e-03) 1	Urine		chr20	44603978
cg159003 20	-0.03 (0.01) 0.08	-0.01 (0.07) 1	-1.87e-03 (0.02) 1	Urine	LOC728743	chr7	150102724
cg159755 74	0.01 (0.01) 0.18	0.03 (0.04) 1	-0.01 (0.01) 1	Urine	RUNX1T1	chr8	93107629
cg162263 80	-0.01 (3.65e-03) 0.05	-0.3 (0.27) 1	3.28e-03 (3.23e- 03) 1	Urine	TXNDC11;TXN DC11	chr16	11776324
cg164154 76	-0.02 (0.02) 0.28	-0.1 (0.06) 1	0.03 (0.02) 1	Urine	UMODL1;UMO DL1	chr21	43482628
cg164160 14	-0.01 (3.18e-03) 0.02	0.21 (0.19) 1	-3.46e-03 (2.97e-03) 1	Urine		chr3	110271653
cg164277 52	-0.01 (4.58e-03) 0.07	-0.09 (0.29) 1	1.14e-03 (4.78e- 03) 1	Urine	MSH4	chr1	76262302
cg165099 78	-0.01 (4.71e-03) 0.04	-0.04 (0.16) 1	7.40e-04 (4.91e- 03) 1	Urine	LOC100130987;P OLD4	chr11	67121607
cg166437 06	-0.01 (4.08e-03) 0.09	0.01 (0.32) 1	-4.04e-04 (3.95e-03) 1	Urine	C5orf13;C5orf13	chr5	111289503
cg167277 74	-0.01 (2.84e-03) 0.07	-0.33 (0.2) 1	3.96e-03 (2.55e- 03) 1	Urine	CA5A	chr16	87958281
cg167837 44	0.01 (4.75e-03) 0.03	-0.04 (0.03) 1	4.27e-03 (0.01) 1	Urine	DPYS	chr8	105478855
cg170003 56	0.01 (0.01) 0.07	-0.03 (0.03) 1	1.68e-03 (0.01) 1	Urine	PTPRB;PTPRB;P TPRB;PTPRB	chr12	71003679
cg170842 04	0.03 (0.01) 0.03	-0.6 (1.43) 1	0.01 (0.02) 1	Urine	IKBKB;IKBKB;I KBKB;IKBKB	chr8	42182071
cg173848 89	3.04e-03 (3.25e- 03) 0.36	0.01 (0.03) 1	-3.82e-03 (3.32e-03) 1	Urine	NKAPL	chr6	28227068
cg174136 04	0.01 (0.01) 0.08	-0.33 (0.43) 1	3.74e-03 (0.01) 1	Urine		chr3	72366496
cg174470	0.03 (0.02) 0.11	-0.02 (1.75)	-3.15e-05 (0.02)	Urine		chr9	99260562

08		1	1				
cg17910564	-0.01 (3.48e-03) 0.03	-0.33 (0.28) 1	3.96e-03 (3.46e-03) 1	Urine	VDAC3;VDAC3	chr8	42248702
cg18092047	0.04 (0.02) 0.05	1.64 (1.7) 1	-0.02 (0.02) 1	Urine	EPB41L5;EPB41L5	chr2	120934017
cg18101707	-0.01 (4.20e-03) 0.06	0.02 (0.08) 1	-2.28e-03 (4.34e-03) 1	Urine	DLGAP1;DLGAP1;DLGAP1	chr18	3874222
cg18595911	0.04 (0.01) 0.04	-0.09 (1.46) 1	7.54e-04 (0.02) 1	Urine		chr21	40722699
cg18624900	0.04 (0.01) 4.57e-04	-0.01 (0.02) 1	-0.01 (0.01) 1	Urine	SLC16A12	chr10	91295643
cg18967835	-2.81e-03 (3.29e-03) 0.41	-0.52 (0.24) 1	0.01 (3.06e-03) 1	Urine	GPR18;GPR18;UBAC2;UBAC2;UBAC2	chr13	99907350
cg19079194	0.03 (0.01) 0.02	-0.03 (0.03) 1	2.56e-03 (0.01) 1	Urine	ERBB4;ERBB4;ERBB4;ERBB4	chr2	213403321
cg19213569	-0.01 (0.01) 0.23	-0.02 (0.06) 1	-9.90e-05 (0.01) 1	Urine	FGGY;FGGY;FGGY	chr1	59762605
cg19355919	-0.01 (0.01) 0.06	-0.09 (0.07) 1	0.01 (0.01) 1	Urine	MRO;MRO;MRO;MRO	chr18	48346593
cg19416795	0.02 (0.01) 0.16	1.33 (1.3) 1	-0.01 (0.01) 1	Urine		chr18	76270967
cg19515484	-0.01 (4.16e-03) 0.1	0.01 (0.12) 1	-1.23e-03 (4.34e-03) 1	Urine		chr6	1606877
cg19761848	0.01 (3.02e-03) 0.04	-0.03 (0.02) 1	1.11e-03 (2.73e-03) 1	Urine	GBX2	chr2	237076815
cg19874862	-0.01 (4.57e-03) 0.1	0.03 (0.39) 1	-6.62e-04 (0.01) 1	Urine		chr1	18297461
cg20132590	0.01 (2.46e-03) 0.01	-0.04 (0.02) 1	2.19e-03 (2.38e-03) 1	Urine	PDZRN3	chr3	73673893
cg20250015	-0.01 (4.34e-03) 0.06	-0.04 (0.34) 1	3.26e-04 (4.42e-03) 1	Urine	IGFBP7;IGFBP7	chr4	57941240
cg20685554	0.01 (0.01) 0.11	-0.1 (0.05) 1	0.01 (0.01) 1	Urine	LRP11	chr6	150185632
cg20765420	-0.01 (4.43e-03) 0.03	0.01 (0.09) 1	-1.70e-03 (4.83e-03) 1	Urine		chr12	52269081
cg21323720	-0.01 (2.21e-03) 0.02	-0.01 (0.06) 1	-4.76e-04 (1.89e-03) 1	Urine		chr4	114357601
cg21442182	-0.02 (0.01) 0.01	0.67 (0.41) 1	-0.01 (4.77e-03) 1	Urine		chr13	31446420
cg21459796	0.02 (0.01) 0.03	0.63 (0.55) 1	-0.01 (0.01) 1	Urine		chr2	11565525
cg22059211	-0.01 (3.99e-03) 0.11	-0.25 (0.35) 1	2.55e-03 (3.90e-03) 1	Urine		chr8	735312
cg22257107	-0.01 (3.46e-03) 0.02	-1.47e-04 (0.11) 1	-5.06e-04 (3.15e-03) 1	Urine	KIAA1755	chr20	36851725
cg22963414	-0.01 (0.01) 0.13	-0.04 (0.06) 1	0.01 (0.01) 1	Urine	ZFYVE1	chr14	73493969
cg22989103	0.03 (0.01) 1.79e-03	-0.02 (0.03) 1	-1.40e-03 (0.01) 1	Urine	FAM19A5;FAM19A5	chr22	48972331
cg23092003	-0.01 (0.01) 0.16	0.05 (0.41) 1	-9.10e-04 (0.01) 1	Urine	CLYBL-AS2;CLYBL;CLYBL	chr13	100343915
cg23303108	-0.02 (0.01) 0.04	-0.25 (0.73) 1	2.35e-03 (0.01) 1	Urine	TNFRSF10A	chr8	23083578

cg238692 28	-0.01 (3.15e-03) 0.05	-0.15 (0.26) 1	1.60e-03 (3.12e- 03) 1	Urine		chr5	124316882
cg240300 07	-0.01 (4.58e-03) 0.07	0.05 (0.41) 1	-8.19e-04 (4.66e-03) 1	Urine		chr5	67433852
cg240867 17	-0.01 (2.77e-03) 0.08	-0.12 (0.09) 1	3.38e-03 (3.10e- 03) 1	Urine	GLIS1	chr1	54154485
cg241569 49	0.01 (0.01) 0.17	0.66 (0.46) 1	-0.01 (0.01) 1	Urine	LDLRAP1	chr1	25874418
cg242281 45	-0.01 (0.01) 0.12	-0.5 (0.48) 1	0.01 (0.01) 1	Urine	USP28;USP28	chr11	113702127
cg243307 84	-0.01 (3.68e-03) 4.10e-03	0.02 (0.16) 1	-8.71e-04 (3.85e-03) 1	Urine	ZSCAN2;ZSCAN 2;ZSCAN2	chr15	85149026
cg246989 25	0.02 (0.01) 0.02	0.41 (0.77) 1	-4.80e-03 (0.01) 1	Urine		chr15	57873089
cg250781 87	-0.01 (2.80e-03) 0.05	-0.2 (0.23) 1	2.21e-03 (2.79e- 03) 1	Urine		chr7	142447339
cg251213 65	-2.60e-03 (2.52e- 03) 0.32	-0.19 (0.15) 1	2.93e-03 (2.53e- 03) 1	Urine	CYFIP2;CYFIP2; CYFIP2;CYFIP2	chr5	156695069
cg253962 29	-0.02 (0.01) 0.02	-0.05 (0.07) 1	2.24e-03 (4.52e- 03) 1	Urine	YRDC;C1orf122; C1orf122	chr1	38273072
cg254242 05	-0.01 (3.34e-03) 0.07	0.1 (0.29) 1	-1.45e-03 (3.64e-03) 1	Urine	CPM;CPM;CPM	chr12	69264281
cg258133 57	0.05 (0.02) 0.02	-0.07 (1.8) 1	5.45e-04 (0.02) 1	Urine	CYFIP2;CYFIP2; CYFIP2;CYFIP2	chr5	156752564
cg259506 25	2.21e-03 (1.85e- 03) 0.25	0.04 (0.03) 1	-3.32e-03 (1.88e-03) 1	Urine	PLCXD3	chr5	41510116
cg260221 59	-0.01 (4.07e-03) 0.02	-0.16 (0.35) 1	1.64e-03 (4.22e- 03) 1	Urine	UBE2E2;MIR548 AC	chr3	23484930
cg261156 33	0.01 (0.01) 0.1	-0.01 (0.02) 1	-3.54e-03 (0.01) 1	Urine	FOX12	chr10	129535893
cg262074 23	-2.67e-03 (3.42e- 03) 0.45	-0.52 (0.3) 1	0.01 (3.79e-03) 1	Urine	HMBBOX1;HMB OX1	chr8	28787952
cg262202 03	0.01 (4.98e-03) 0.05	0.18 (0.43) 1	-2.27e-03 (0.01) 1	Urine		chr1	201100716
cg264136 72	-0.01 (3.92e-03) 0.07	-0.11 (0.25) 1	1.50e-03 (3.93e- 03) 1	Urine		chr15	102084204
cg265921 75	-0.01 (3.55e-03) 0.12	-0.23 (0.28) 1	2.63e-03 (3.48e- 03) 1	Urine	CCDC167	chr6	37463570
cg266326 56	-0.04 (0.01) 0.03	-0.06 (0.06) 1	0.01 (0.01) 1	Urine	COL9A2	chr1	40781389
cg268009 15	-0.01 (3.54e-03) 0.02	0.03 (0.31) 1	-6.20e-04 (3.74e-03) 1	Urine	OR51B4	chr11	5324199
cg269118 30	-0.01 (2.83e-03) 0.05	0.08 (0.2) 1	-1.55e-03 (3.13e-03) 1	Urine		chr1	157219222
cg269837 10	0.01 (4.28e-03) 0.03	-0.04 (0.02) 1	4.64e-03 (4.50e- 03) 1	Urine	GALNT13	chr2	154728202
cg272324 94	-0.02 (0.01) 0.02	0.3 (0.6) 1	-3.76e-03 (0.01) 1	Urine	TOM1L2	chr17	17832220
cg272656 30	0.01 (0.01) 0.03	-0.03 (0.46) 1	7.16e-05 (0.01) 1	Urine	PCDH21	chr10	85958429
cg274885 03	-0.01 (3.50e-03) 0.11	-0.08 (0.08) 1	3.05e-03 (3.75e- 03) 1	Urine	GRK4;GRK4;GR K4;NOP14	chr4	2964223
cg275536 67	0.06 (0.01) 4.57e-04	0.01 (0.03) 1	-0.01 (0.01) 1	Urine	GABRG3	chr15	27216083

Table 3-12 appx: Descriptive statistics for key variables in males and females separately.

Gender	Variable	N	Mean	Std. Dev.	Min	Pctl. 25	Pctl. 75	Max
Males	Urine Hg (specific gravity adjusted, ug/L)	196	0.94	1.7	0.087	0.31	1	20
Males	Hair Hg (ug/g)	193	0.61	0.66	0.091	0.27	0.73	6.2
Males	Omissions Errors (T score)	196	51	11	41	43	54	90
Males	Perseverations (T score)	196	51	11	44	45	52	90
Males	HRT ISI Change (T score)	196	50	8.6	32	45	54	75
Males	HRT Block Change (T score)	195	49	8.6	13	43	54	79
Males	Response Style (T score)	196	52	8.8	32	46	57	90
Males	Age	196	14	2.1	9	13	16	17
Males	PUFA	129	11	4.8	2.9	6.8	13	29
Males	AMAI	196						
Males	... E	0	0%					
Males	... D	43	22%					
Males	... D+	42	21%					
Males	... C-	57	29%					
Males	... C	36	18%					
Males	... C+	16	8%					
Males	... A/B	2	1%					
Females	Urine Hg (specific gravity adjusted, ug/L)	203	1.2	3.7	0.091	0.35	1.1	50
Females	Hair Hg (ug/g)	200	0.64	0.48	0.062	0.28	0.83	2.4
Females	Omissions Errors (T score)	203	50	10	43	44	50	90
Females	Perseverations (T score)	203	51	11	44	45	50	90
Females	HRT ISI Change (T score)	203	51	11	27	44	58	87
Females	HRT Block Change (T score)	203	53	8.8	34	47	59	88
Females	Response Style (T score)	203	52	9.6	31	47	58	82
Females	Age	203	14	2.1	10	12	16	18
Females	PUFA	133	10	5.3	2.8	6.4	14	29
Females	AMAI	203						
Females	... E	1	0%					
Females	... D	56	28%					
Females	... D+	48	24%					
Females	... C-	46	23%					
Females	... C	40	20%					
Females	... C+	11	5%					
Females	... A/B	1	0%					

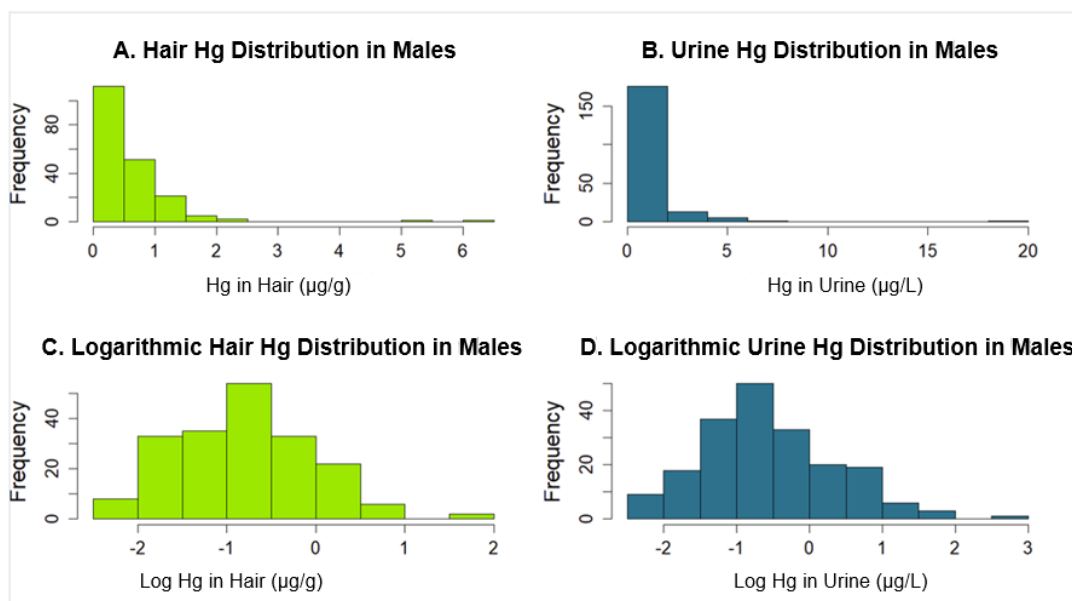


Figure 3-4 appx: Distribution of urine and hair Hg variables in MALES. (A) hair Hg (B) urine hg (C) log transformed hair Hg (D) log transformed urine Hg.

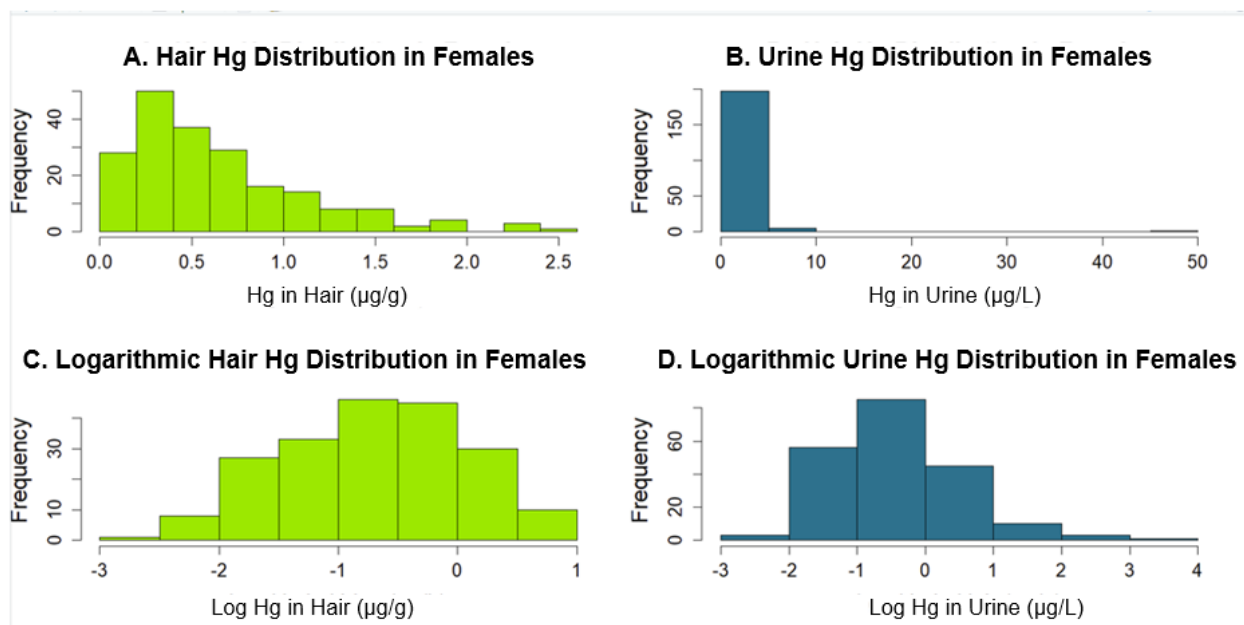


Figure 3-5 appx: Distribution of urine and hair Hg variables in FEMALES. (A) hair Hg (B) urine Hg (C) log transformed hair Hg (D) log transformed urine Hg.

Chapter 4

Aim 3: The Effects of Biologically Relevant Doses of Methylmercury Exposure on Gene Expression and DNA Methylation in SH-SY5Y Cells Differentiated to Dopaminergic-like Neurons

Abstract

Both DNA methylation and exposure to methylmercury (meHg) are believed to adversely affect neurodevelopment during childhood and adolescence. Despite this and the facts that meHg is greatly environmentally prevalent and known to be highly neurotoxic, the effects of low, environmentally relevant doses on mature neurons are not well understood. Mechanistic studies of these types of meHg exposures, such as transcriptomic (AIM 3A) and epigenetic (AIM 3B) research, are warranted. Through the pairing of these methodologies, as is done in this present study, we gain a more complete mechanistic understanding of meHg neurotoxicity than either expression or methylation data alone. In SH-SY5Y cells exposed to low, biologically relevant and neurotoxic doses of meHg after being differentiated to dopaminergic-like neurons, significant differentially expressed genes and numerous gene set enrichment pathways were enriched in each of the three treatment groups. Some key GO pathways enriched among all groups included those related to chromatin architecture, inflammation, immune response, and cell migration. In a targeted analysis of DNA methylation, for biologically relevant doses of meHg, differential methylation (averaged across CpG sites within each amplicon) was identified for *STAT1*, *ENSG00000242479*, and *ENSG00000258830* genes. Differential methylation of specific CpG sites for *GABRG3* and *DAAM2* were also identified in response to low-dose

exposures to meHg. Many associations were dependent upon the distinguishment between 5-methylcytosine and 5-hydroxymethylcytosine from traditional total methylation analyses. Future research is warranted to explore any mechanistic implications of this current study.

Introduction

According to the United Nations Environment Programme (UNEP), mercury exists environmentally at quantities 450% higher than natural levels due to anthropogenic activities [1]. It is ranked by the World Health Organization among the top ten chemicals of major public health concern [2]. Mercury exists naturally in different forms, of which organic mercury is the most toxic. Methylmercury (meHg) is the most environmentally prevalent species of organic mercury and is known to be neurotoxic. The primary exposure to meHg is through consumption of contaminated seafood. Numerous case studies published in the wake of environmental disasters and epidemiologic research conducted in diverse populations across the world have confirmed this exposure as a potent neurotoxicant [3-6]. In various cohorts around the world meHg exposure has been associated with adverse neurological outcomes, such as affective mood disorders, deficits in attention and executive function, and neurobehavioral/developmental delays [7-11]. Most of these examples, however, exemplify the effects of high-dose meHg exposure on neurological outcomes, with most cohorts representing populations whose average whole blood or cord blood Hg levels exceeded the current reference dose outlined by the US Environmental Protection Agency (EPA) (5.8 µg/L or ~0.03 µM) and/or whose Hg consumption exceeded the WHO Provisional Tolerable Weekly Intake (1.6 µg/kg of body weight per week). Interestingly, there is a lack of consistency in the literature linking low-dose exposures to adverse neurodevelopment and function [11-13]. While high-dose exposures to meHg are known to be

severely neurotoxic in humans, the effects of low-dose exposures on neurodevelopment, as explored by this study, remain poorly understood.

In recent years, much attention has been placed on studying meHg due to its known associations between higher dose exposures and adverse neurological health effects in humans. In efforts to better understand the toxic and detoxifying mechanisms of meHg in the brain, several meaningful *in vitro* and *in vivo* experiments have been conducted. By using cell models, for instance, we now know that meHg induces oxidative stress and excitotoxicity in neurons. Dysregulation of intracellular calcium and glutamate homeostasis have also been documented in cultured neurons exposed to meHg. The toxicant can oxidize and bind with high affinity to proteins and non-protein molecules via their thiol and selenol groups to exert a toxic effect [14-17]. Numerous other pathways and processes, including neuroinflammation, increased production of cytokines, mitochondrial dysfunction, and induction of various cell death pathways, have been implicated in the biological response to meHg exposure based on *in vitro* and *in vivo* mechanistic studies [18-20]. Another proposed mechanism of meHg-induced toxicity of particular interest to this study is epigenetic regulation of gene expression [21]. Epigenetic mechanisms describe biological processes or modifications occurring on top of genes to influence gene expression, but do not alter the genetic code itself. Some examples include DNA methylation (DNAm), chromatin architecture, and small RNA interference, all of which have been implicated in meHg-induced neurotoxicity [21-26].

Despite this font of literature, the majority of these studies focus on higher dose exposures to meHg usually reflecting ranges between 1 and 30 μ M (exceeding the US EPA reference blood levels) and/or model early neurodevelopmental periods comparable to *in utero* exposures. In a study by Bose *et al*, for example, low levels of meHg were associated with

altered gene expression and decrease in global DNAm in primary cultures of rat embryonic cortical neural stem cells (NSCs). This effect was also observed in the daughter cells of the exposed NSCs, despite the removal of the initial exposure [27]. Notably, this study along with most prior publications in this research area, failed to distinguish between 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) marks, in favor of total methylation analyses that inappropriately quantify these distinct marks jointly. In another study by Neely *et al*, low doses of meHg were associated with alterations in gene expression and functional pathway enrichment in human induced pluripotent stem cells (hiPSCs). Exposure took place during the differentiation of the hiPSCs into cortical glutamatergic neurons but stopped before cells were fully differentiated [28]. This is a common and justifiable exposure paradigm, because it better reflects the effects of *in utero* exposures which is a highly vulnerable window of susceptibility. Pregnancy is not the only period vulnerable to the effects of meHg, however. The brain is still developing during childhood and adolescence and is thus susceptible to toxic insult by meHg [29-32]. The hippocampus, for example, is proposed to be exceptionally vulnerable to methylmercury (meHg) toxicity in childhood extending into prepubescence [29]. Dopamine signaling in the hippocampal region is critical in regulating spatial learning, memory, attention, and synaptic plasticity. In fact, methylphenidate, one of the most commonly prescribed ADHD (attention deficit disorder with hyperactivity) medications, works by increasing synaptic dopamine availability in the hippocampus and prefrontal cortex [33]. Taken together, this data suggests that meHg exposure in childhood may help dysregulate dopamine signaling in the brain and subsequently influence etiology of certain neurodevelopmental disorders such as ADHD, depression, or affective mood disorders. Despite this knowledge, the effects of meHg toxicity in the brain remains under explored at this timepoint.

In the present study, the effects of low levels of meHg on gene expression and DNAm in human cells differentiated to dopaminergic neurons is examined. Studying the effects of low-levels of meHg is highly relevant, because various populations throughout the world are chronically exposed to low levels of meHg with little to no understanding of how this exposure may contribute to various health risks [34]. This study employs the use of a more homogeneous population of fully differentiated dopaminergic-like neurons, because it is better suited to study a more mature brain than exposing NSCs or iPSCs during differentiation. It also allows a more simplistic interpretation of the effects in a single cell type, rather than dealing with the complexities accompanying heterogeneous models. This provides the necessary foundation for essential future research in more complex systems. The first dose selected for use in this study (0.01 μM) is a biologically relevant dose, comparable to exposure levels from populations in various parts of the world and calculated from the average blood mercury levels of a cohort of children (average age 10.3 ± 2.6 years) residing in Mexico City [34-37]. The second dose (0.25 μM) used in this research is a slightly higher biologically relevant dose that is more comparable to blood mercury levels in populations with higher fish consumption [8, 38-40]. The final dose (6.25 μM) was selected, due to its more cytotoxic nature. By exploring the effects of meHg on transcriptomics and epigenetics in more mature human neurons, we help build a better understanding of the mechanistic implications of biologically relevant doses of meHg using a model that mimics an understudied and vulnerable neurodevelopmental timepoint.

Methods

Cell Culture

The SH-SY5Y cell line (ATCC, CRL-2266), originally derived from a female neuroblastoma patient, was selected for use in this study for its neuron-like phenotype, ease of

use for neurotoxicity *in vitro* research, and ability to be differentiated to a more mature neuron-like phenotype [41, 42]. Undifferentiated cells were maintained in culture using complete culture media, which comprises a 1:1 mixture of Ham F-12 (1x) Nutrient Mixture (Gibco™ 11765-054) and Eagle's Minimum Essential Medium (ATCC 30-2003), 10% heat inactivated fetal bovine serum (Thermo, A3840001), and 1% penicillin-streptomycin (Thermo, 15140-122). The SH-SY5Y cells were plated (passage number between 37 and 48) in 35 mm culture dishes, grown to 80 % confluency, and differentiated to dopaminergic-like neurons following a previously described and well-established protocol, described in depth by Shipley *et al* 2014. In short, over the course of 18 days it utilizes a gradual serum deprivation partnered with the introduction of extracellular matrix proteins and neurotrophic factors. It also includes two 1:1 split cycles, where cells are trypsinized and transferred to new plates. This process selects against the epithelial-like phenotype and toward the neuron-like phenotype exhibited by the SH-SY5Y line, generating a more homogeneous population of cells. At day 18, cells are considered fully differentiated and ready for use in experiments. Both undifferentiated and differentiated cells were maintained in an incubator at 37°C and 5% CO₂ [43].

Proof of Differentiation

As a quality check, we employed immunocytofluorescence microscopy in three distinct sets of differentiation and three separate passages of undifferentiated cells (between passage 37 and 40) to visualize and compare fluorescent intensities of three main markers of neurodifferentiation—microtubule-associated protein 2 (MAP2), growth-associated protein 43 (GAP43), and beta- tubulin III, as well as DNA. Cells were fixed in 4% paraformaldehyde, permeabilized with Triton X, and incubated in Hoechst 33342 (Thermo, H3570) to visualize nuclei, and beta tubulin III conjugate (Thermo, 50-4510-82), GAP43 primary (Abcam, ab75810),

and MAP2 primary (Thermo, PA1-10005) antibodies. For the two antibodies lacking a conjugated fluorophore—GAP43 (Abcam, ab150077) and MAP2 (Thermo, A11041)—a subsequent incubation in secondary antibodies was required. Cells were then visualized using four different fluorescent channels on an EVOS M7000 Imaging System and representative images were taken for each representing several hundred cells. The images were then overlaid to produce a merged image reflecting all four fluorescent markers in the cells simultaneously. This procedure was independently performed three times in both differentiated and undifferentiated cells. Fluorescent intensities for each marker was quantified using a previously established pipeline on CellProfiler and compared to that of the undifferentiated cells.

Preparation of meHg

Stock solutions of meHg were prepared by dissolving meHg chloride (meHgCl₂) powder in sterile nuclease free water, which was added to differentiation media three to attain 0.01, 0.05, 0.25, 1.25, and 6.25 μ M meHg concentrations. For consistence, the same quantity of vehicle was added to each treatment media. All treatment groups were used in the Presto Blue assay, but only three were used in RNA sequencing transcriptomic analysis.

Presto Blue Assay

In a series of five distinct sets of differentiation, using SH-SY5Y cells between passage 37 and 48, cells were differentiated following the aforementioned protocol. During the final split cycle of the procedure, cells were plated in 96-well plates and allowed to grow to confluency through day 18. The differentiated cells were then exposed to 0.01, 0.05, 0.25, 1.25, or 6.25 μ M meHg treatment media for 48 hours. Negative (vehicle-treated) and positive (exposed to cytotoxic levels of DMSO) controls were included, as well. All seven treatment groups were

plated in triplicate and a total of five independent experiments were conducted. For each experiment, the plate was read on a plate reader and fluorescent intensity corresponding to the amount of metabolic activity for each well was quantified. An average was taken of the fluorescent intensities for the three technical replicates within each treatment group. This was then normalized to that of the negative control, calculating RFU (relative fluorescent units). A generalized linear model (GLM) controlling for experimental batch was employed to assess the relationship between fluorescent intensity and meHg doses. This assay provided valuable insight regarding the effect of meHg on cell metabolism, which is usually correlated with cell viability. Results from this assay were used in part as the rationale behind the doses selected for transcriptomic analysis.

RNA Sequencing (AIM 3A)

On day 15 of the Shipley *et al* protocol, cells were transferred to ECM-coated 6-well dishes and allowed to continue differentiating for the remaining 4 days of the protocol. At this time, cells were exposed to 0, 0.01, 0.25, and 6.25 μ M meHg treatment media for 48 hours. These doses were selected based on their effects on cellular metabolism as outlined in the Presto Blue assay, as well as on the basis of potential biological relevance. This was replicated in three independent experiments. RNA and DNA were extracted using the AllPrep DNA/RNA/Protein Mini kit (Qiagen, 80004) for use in transcriptomic and targeted DNAm analyses, respectively. This was conducted in three independent experiments using cells between passage number 38 and 41.

RNA was submitted to the University of Michigan Advanced Genomics Sequencing Core for ribosomal RNA depletion library prep (NEBNext rRNA Depletion Kit (Human) v2) and 151bp paired end sequencing using the Illumina NovaSeq X Plus Sequencing Platform. Read trimming

was conducted using Cutadapt v2.3 [44]. FastQC v0.11.8 and Fastq Screen v were used for quality control and to screen for multiple types of contamination [45, 46]. Following the ENCODE standards for RNA sequencing, reads were then mapped to a human reference genome (ENSEMBL, GRCh38) using STAR alignment v2.7.8a, and RSEM v1.3.3 was utilized to assign the count estimates to specific genes [47, 48]. Between 54,573,770 and 73,742,154 reads per sample mapped to the reference genome, of which between 75.04 % and 86.21 % of reads per sample were uniquely mapped. A comprehensive report compiling quality control data from these various tools was created using Multiqc v1.7 [49].

Following initial quality control, statistical analyses were conducted using R statistical software version 4.3.2 (2023-10-31 ucrt). Differential expression comparing each treatment group to the vehicle control was analyzed using DESeq2, including only genes where 80% samples or more had at least 5 reads mapped. This resulted in a total of 20,251 genes for use in this transcriptomic analysis. Differentially enriched pathways were also identified through gene set enrichment analysis comparing each treatment group to the negative control using a significance threshold of $q < 0.05$. Both differential expression and functional pathways analyses were followed by pairwise Benjamini-Hochberg test for multiple comparisons.

DNA Methylation Analysis (AIM 3B)

Seven candidate genes were used for quantification of total, 5-methylcytosine (5mC), and 5-hydroxymethylcytosine (5hmC) methylation levels across multiple CpG sites within each gene. To be considered for this analysis, genes must have met one of two sets of selection criteria. The first set of criteria states that a gene must have significant differential expression in either the medium (0.25 μ M) or the low (0.01 μ M) dose meHg treatment groups as identified in the RNA-seq analysis. Genes were considered significant if they had both an adjusted p-value

less than 0.05 and the absolute value of the log2FoldChange was greater than one. To meet the second set of criteria, a gene must have complied with all the following four rules. First, it must contain a CpG site that has previously been shown to interact with meHg in its association with a neurodevelopmental outcome in females as described in aim 2 of this dissertation, with q-value for the interaction term less than 0.10. The rationale behind using female only models is that the SH-SY5Y cell line was derived from a female donor. Second, it must be present in multiple GO pathways enriched in at least two treatment groups. It must also have significant differential expression in the high dose meHg treatment group from the RNA-seq analysis (p-value < 0.05). Lastly, it must have functional implications in heavy metal toxicity and/or neurodevelopment. Forward and reverse primers were designed for bisulfite converted DNA using the publicly available web-based tool BiSearch Primer Design and Search Tool [50, 51]. When applicable amplicons were designed to include a CpG site of interest. If no particular CpG site was previously identified, the amplicon was designed around regions of DNA that exhibited H3K27Ac enrichment either within the gene itself or approximately 1500 base pairs upstream of the transcription start site, as observed using University of California, Santa Cruz (UCSC) Genome Browser. This histone mark is often associated with regulatory regions of DNA.

Targeted methylation analysis of *GABRG3*, *POLD4*, *DAAM2*, *TNFRSF10A*, *STAT1*, *ENSG00000242479*, and *ENSG00000258830* genes was conducted using Oxford NanoPore Technologies (ONT) MinION Mk1C sequencing device to quantify both 5hmC and 5mC levels. To distinguish between the two types of methylation marks on the NanoPore, DNA was first converted using bisulfite conversion and oxidative bisulfite conversion in parallel reactions following the TrueMethyl oxBS Module protocol (Nugen, 0414-32). Standard bisulfite conversion helps capture total methylation, which comprises both 5hmC and 5mC. Conversely,

oxidative bisulfite conversion enables quantification of solely the 5mC mark. Using simple subtraction, 5hmC levels were calculated by finding the difference between the results of the bisulfite-converted samples (5mC+5hmC) and the oxidative bisulfite-converted samples (5mC). Notably, 5hmC is based on a calculation rather than a measurement, meaning that, as an artifact of random noise, it can sometimes result in a negative number in places where methylation is sufficiently low [52, 53]. Negative numbers were omitted from analysis. Prior to sequencing, polymerase chain reaction was used to amplify the seven candidate regions of bisulfite converted and oxidative bisulfite converted DNA. All PCR products generated for each of the twelve samples were pooled and barcoded using the Rapid Barcoding Kit 24 V14 to make each sample distinguishable from one another (ONT, SQK-RBK114.24). Adapters were also ligated to the DNA during the Rapid Barcoding protocol to help facilitate the amplicon capture and its threading through the pore during sequencing. After the library preparation either bisulfite converted samples or oxidative bisulfite converted samples were loaded onto a Flongle flow cell (ONT, FLO-FLG114) and sequenced on separate runs using MinKNOW core 1.11.5 version software. Run parameters for both sets of samples were consistent, employing Guppy super accurate base calling (SUP) model for both [54]. ONT sequencing uses an electric current to facilitate the movement of single strands of DNA across a pore on the membrane of a flow cell. Different nucleotides passing through the channel are associated with different electric signatures, which are transcribed by the software. The recorded sequences were then mapped to reference sequences for each amplicon and percent methylation for 5mC and 5hmC were calculated for each treatment group and compared to the untreated control.

Statistical Analysis

All statistical analyses were performed using R statistical software (version 4.0.3). RFU generated from the PrestoBlue assay was used in a generalized linear model (GLM) controlling for experimental batch to assess the metabolic activity of cells (via RFU) from each of the meHg treatment groups compared to that of the untreated control group. Differences were considered statistically significant based on a significance threshold of $p\text{-value} < 0.05$. In the RNA-sequencing experiment, 20,251 genes remained after quality control and filtering of those where less than five of their transcripts mapped to the genome in 20% or more of the samples. For these genes, differential expression between each meHg treatment group and the control group was assessed using DESeq2. Benjamini-Hochberg correction was used to control for multiple comparisons and Gene Set Enrichment Analysis was conducted using Gene Ontology (GO). For both expression and pathways analyses significance was defined at $p_{\text{adj}} < 0.05$. Lastly, differential total, 5mC, and 5hmC methylation analyses were conducted for each candidate gene using GLM controlling for experimental batch. Methylation was expressed as an average of each CpG site for each amplicon and for specific positions of interest when applicable. Positions were removed from analysis if less than 10 reads mapped to the reference genome. Differential methylation was considered statistically significant at $p\text{-value} < 0.10$.

Results

Fluorescence Data

Cells were successfully differentiated following the Shipley *et al.* protocol (Figure 4-1). Dopaminergic-like neurons that were generated were then treated with five different doses of meHg (0 – 6.25 μM) for 48 hours. The PrestoBlue Assay was used to assess metabolic activity (positively correlated with fluorescent intensity) within each treatment group. The average percent difference in fluorescence relative to the negative control (and standard deviation) for the

positive control and the 0.01, 0.25, and 6.25 μM meHg treatment groups were 3.01 (3.27) %, 99.21 (38.61) %, 178.52 (79.20) %, and 38.73 (30.73) %, respectively (Table 4-1). In a generalized linear model adjusting for experimental batch, the 0.25 μM meHg treatment group was associated with a 0.80 ($p=3.84\text{E-}08$) increase in relative fluorescent units (RFU) compared to the negative control group. The 6.25 μM meHg dose, on the other hand, was associated with a 0.60 ($p=2.20\text{E-}05$) decrease in RFU. In the same model, however, a dose of 0.01 μM meHg was not associated with altered viability ($\beta=0.01$; $p=0.96$) (Table 4-1 and Figure 4-2). In addition to a vehicle control, meHg doses of 0.01 μM , 0.25 μM , and 6.25 μM were selected for RNA-seq and DNAm analysis (Table 4-1).

RNA Sequencing Data (AIM 3A)

Using RNA extracted from differentiated SH-SY5Y cells exposed to 0 μM , 0.01 μM , 0.25 μM , and 6.25 μM meHg, we performed DESeq2 differential expression analysis using expected counts data. Benjamini-Hochberg correction for multiple comparisons was used to calculate adjusted p-values (p_{adj}). Based on a significance threshold of $p_{\text{adj}} < 0.05$, differentially expressed genes (DEG) were identified in all three treatment groups compared to control. Two of these genes were significant in more than one group (Figure 4-3 and Table 4-2). A total of one, two, and 5,564 DEGs were identified for the 0.01, 0.25, and 6.25 μM meHg treatment groups, respectively (Table 4-2). In the 0.01 μM treatment group, ENSG00000258830 had 3.05 ($\text{SE}=0.52$) log2Fold change decrease in expression compared to the untreated control and p_{adj} of $8.21\text{E-}05$. This gene was also significant in the 6.25 μM group, but with an opposite direction of effect, 1.17 (0.36), p_{adj} of $7.03\text{E-}03$. ENSG00000242479 and *RGPD5* expression were significantly different from the control with respective statistical findings of -6.7 (1.3), $q=4.68\text{E-}03$ and 0.94 (0.2), $q=0.019$ for each DEG. *RGPD5* was also among the DEGs that were

significant in the 6.25 μ M treatment group—1.3 (0.2), $q=2.61E-09$ (Table 4-3). Thousands of other significant DEGs were identified in the 6.25 μ M meHg group (Tables 4-2 and 4-3). See Table 4-3 below for significant differential expression results from the lowest two doses, significant DEGs across multiple treatment groups, and a partial list of the twenty most significant DEGs in the 6.25 μ M meHg group. The complete Table 4-3 with all 5,567 significant findings can be found in Aim 3 appendix.

After gene set enrichment (GSE) analysis, several GO (Gene Ontology) pathways were enriched in all treatment groups. A total of 226, 281, and 1119 pathways were enriched in the respective 0.01, 0.25, and 6.25 μ M meHg treatment groups (Tables 4-2 and 4-4; Aim 3 Appendix: Tables 4-7 – 4-9 appx). There was also substantial overlap in pathways enriched in multiple treatment groups, with 86 pathways being significantly enriched among all three groups (Table 4-2 and Figure 4-4). Some common pathways enriched across all three treatments include inflammatory response, immune receptor activity, structural constituent of chromatin, protein-DNA complex assembly, peptidyl-tyrosine phosphorylation, positive regulation of MAPK cascade, and those related to cell signaling and migration (Aim 3 Appendix: Table 4-10 appx).

DNA Methylation Data (AIM 3B)

PCR primers were designed for bisulfite sequences annotated to *GABRG3*, *POLD4*, *DAAM2*, *TNFRSF10A*, *STAT1*, *ENSG00000242479*, and *ENSG00000258830* genes (Aim 3 Appendix: Table 4-11 appx). *POLD4* was dropped due to low read count following ONT sequencing. Average total methylation at *STAT1* was significantly different at the 0.01 μ M meHg treatment group ($\beta=0.55$ (SE=0.19), 0.029) relative to the untreated control, but no significant differences were identified in either the 5mC or 5hmC GLM models for this gene. Conversely,

no significant changes in average total methylation were identified in the *ENSG00000242479* gene, but after distinguishing between 5mC and 5hmC significant differential methylation was observed for both marks. Treatment with 0.01 and 0.25 μ M meHg resulted in reduced 5mC methylation levels: -2.56 (1.25), $p=0.086$ and -7.56 (1.25), $p=0.000917$ for each group respectively. For the 5hmC models, however, all treatment groups were associated with higher hydroxymethylation (5hmC) levels. Similar to the 5mC models, the 0.25 μ M group exhibited the greatest magnitude of effect: 10.42 (3.56), $p = 0.0327$. The 0.01 and 6.25 μ M meHg treatment group had slightly smaller effect sizes: 8.75 (3.56), $p = 0.0572$ and 8.08 (3.56) $p = 0.0722$, respectively. For the *ENSG00000258830* gene, average 5hmC methylation was higher in the 0.25 μ M meHg treatment group relative to control: 21.86 (10.63), $p = 0.095$ (Table 4-5).

Position-specific methylation differences were also identified for the *GABRG3* (p44) and *DAAM2* (p171). For *GABRG3*, DNAm at this CpG site was related to a 2.67 (1.35) % increase in total methylation for the 0.01 μ M group ($p = 0.096$) and a 6.33 (2.75) % increase in 5mC levels for the 0.25 μ M meHg group ($p = 0.0605$). Lastly, for *DAAM2* (p171), total methylation was associated with a 4.33 (1.79) % decrease in the 0.25 μ M meHg treatment group ($p = 0.052$) compared to the control (Table 4-6).

Discussion

In human SH-SY5Y cells exposed to meHg for 48 hours post-differentiation to dopaminergic-like neurons, significant findings were identified for both biologically relevant and cytotoxic doses for all analyses performed in this study. Fluorescence data generated from the Presto Blue assay demonstrates a non-monotonic metabolic response to low-levels of meHg. The data suggests a significant decrease in cell viability in the 6.25 μ M meHg group and a significant increase in cell viability in the 0.05 μ M and 0.25 μ M groups (Table 4-1 and Figure 4-2). Though

Presto Blue is regarded as a "viability assay", what it quantifies is metabolic activity of cells that is often correlated with cell viability [55-57]. While it is possible that this increase in fluorescent signal identified at the 0.05 and 0.25 μM meHg doses is the result of an increase in viability, it is highly unlikely. Exposure did not occur until after cells had undergone the full Shipley *et al* differentiation protocol and post-mitotic neurons do not readily proliferate. A more likely interpretation of these results could be attributed to a spike in metabolic activity in response to the initial toxic insult by meHg rather than a true increase in cell viability. Notably, a 48-hour exposure like the one used in this study is not representative of a chronic exposure likely to be experienced by human populations in a real-world scenario. If the exposure period were to continue past the allotted 48-hours, it is likely that many of the cells would die off in response to chronic excitotoxicity which is a commonly cited neuronal response to meHg exposure [15, 16]. Because the present study did not assess this outcome, the results of the Presto Blue assay should be taken at face value and used for its intended purpose as a quality check prior to dose selection. Metabolic activity of the 0.01 μM meHg dose was not significantly different from the negative control. This, along with the 0.25 μM dose, was selected for subsequent RNA sequencing, because similar concentrations of mercury have been documented in human blood in different cohorts of children across the world. Conversely, the 6.25 μM meHg group was selected for comparison to a more cytotoxic dose.

Using a significance threshold of $p\text{-adj} < 0.05$, the transcriptomic data identified significant differences in both gene expression and pathway enrichment in all treatment groups compared to the untreated control. The *ENSG00000258830* gene, for example, is associated with a decrease in gene expression in the lowest meHg treatment group and an increase in expression in the highest treatment group. This gene encodes a novel protein containing a SUZ domain,

which is a conserved RNA-binding motif found in eukaryotes. Appropriately, one GO term associated with this gene is *nucleic acid binding* [58, 59].

In the 0.25 μ M meHg dose, there was a significant decrease in expression of *ENSG00000242479*. No significant associations were identified for this gene in either the lowest or highest dose treatment groups. *ENSG00000242479*, which encodes EIF2AK1P1 (Eukaryotic Translation Initiation Factor 2 Alpha Kinase 1 Pseudogene), may be helping to drive the different response seen at this dose [58, 59]. Due to its classification as a pseudogene, EIF2AK1P1 remains poorly researched. In the past, pseudogenes have been regarded as “junk DNA”, or non-functional artifacts of the parent gene from which it was derived. There is a growing body of research, however, supporting the idea that “junk DNA”, including pseudogenes and associated noncoding RNAs, are more functional than previously believed. Transcripts of certain pseudogenes, for instance, are now known to be dynamic and tissue-specific. Additionally, about 50% of pseudogenes in primates are evolutionarily conserved over the course of many millennia, implicating their potential biological relevance. Some noncoding RNAs have even been experimentally shown to provide important regulatory roles in various cellular processes, including gene expression, function, transcript stability, and translational efficiency [60]. More research is necessary to draw any conclusions about the potential functional or regulatory significance of EIF2AK1P1 in response to meHg exposure. The second significant DEG identified in the 0.25 μ M meHg treatment group was *RGPD5*, which encodes RANBP2 Like and GRIP Domain Containing 5. This protein is a member of the RAS superfamily which are GTP-binding proteins associated with the nuclear membrane and are believed to help regulate various cellular functions through dynamic interactions with other proteins [58, 59].

In contrast to the lower two treatment groups, there were thousands of significant DEGs identified in the 6.25 μ M meHg group (Tables 4-2 and 4-3). Among them was *RGPD5*, which was also significant in the 0.25 μ M treatment group (Table 4-3). In both cases meHg was associated with an increase in gene expression. With 5,564 significant DEGs in this group, condensing these findings into a meaningful interpretation can be particularly daunting, which is why the GSE pathways analysis proved greatly helpful. Considering 6.25 μ M meHg is a substantially higher dose than the other two treatment groups, it is not surprising that so many more significant hits were documented, even without a more chronic exposure. While there was a lot of overlap in enriched pathways across treatment groups there were numerous significant GSE pathways that were specific to this group. Several GO pathways explicitly related to cytotoxicity and necrosis, for example, were enriched in this higher-dose treatment group. None of these were identified in either of the lower-dose groups (Aim 3 Appendix: Tables 4-7 – 4-9 appx).

Not only were several GO pathways enriched in each treatment group individually after conducting our GSE analysis, but there were also 86 overlapping pathways enriched in all three meHg treatment groups (Figure 4-4; Tables 4-2 and 4-4; Aim 3 Appendix: Tables 4-7 – 4-10 appx). Inflammatory response and immune receptor activity are among these common pathways. These are of particular interest, because neuroinflammation is a proposed mechanism of meHg-induced neurotoxicity and is believed to be a risk factor for developmental disorders like ADHD [18, 61, 62]. Notably, the results of several epidemiological analyses across the world suggest meHg exposure may be a risk factor for both ADHD and autism spectrum disorder (ASD) [8-11, 63, 64]. In a study conducted by Song *et al.*, a commonly used *in vitro* model for ADHD was employed to test the effect of an anti-inflammatory drug on symptoms of hyperactivity and

impulsivity. The drug was administered orally to juvenile spontaneously hypertensive rats (SHRs) over a period of 4 weeks. In SHRs who received the treatment, there was a significant reduction in hyperactive and impulsive behaviors, as well as in the activation of microglia, astrocytes and mast cells in the prefrontal cortex (PFC) and hippocampus. Additionally, researchers also observed a significant decrease in the levels of pro-inflammatory factors interleukin (IL)-1 β , IL-4, IL-6, tumor necrosis factor (TNF)- α and monocyte chemoattractant protein (MCP)-1, and a significant increase in anti-inflammatory factor IL-10 in SHRs administered the anti-inflammatory agent [62]. The results of our present study suggest that exposure to meHg even at low levels is associated with a heightened inflammatory and immune response in the brain. These effects may or may not translate to adverse neurodevelopmental outcomes in children but taken with the Song *et al* study there is potential for intervention through anti-inflammatory medications. Another notable GO pathway that was significantly enriched in all three of our meHg treatment groups was the positive regulation of MAPK (mitogen-activated protein kinase) cascade, which comprises several intracellular signaling pathways vital in the regulation of neurodevelopment among other essential physiological processes (Aim 3 Appendix: Table 4-10 appx). Dysregulation of MAPK signaling has already been implicated in mercury-induced neurotoxicity and is suspected to play a role in the etiology of some neurodevelopmental disorders such as ASD [63]. Interestingly, the MAPK signaling cascade was also perturbed in the SHRs from the Song *et al* study, but the results suggested that another mechanism of action of the anti-inflammatory drug was to protect the blood-brain barrier by suppressing MAPK and nuclear factor (NF)- κ B signaling pathways [62, 63, 65]. While the findings from our study fit well with the current body of literature, more research is needed to elucidate any mechanistic implications.

As previously mentioned, epigenetic mechanisms such as DNA methylation and histone modification structure are believed to play a role in the etiology of neurodevelopmental disorders and meHg-induced neurotoxicity [21-24, 66]. While the transcriptomic analysis conducted in this study does not directly assess epigenetic mechanisms the results of the GSE pathways analysis suggest some degree of epigenetic involvement. Structural constituent of chromatin and protein-DNA complex assembly are two significant GO terms that may support this concept. Both terms are among the 86 pathways enriched in all 3 treatment groups, which implies aberrant epigenetic response to meHg at both low and higher doses (Aim 3 Appendix: Table 4-10 appx). One of the main underlying principles of epigenetic regulation of gene expression is through modulation of chromatin dynamics. Chromatin consists of proteins, such as histones, RNA, and genomic DNA. How and which genes are expressed relies heavily on how these different components interact with one another affecting how the chromatin is packaged and behaves within the cell. While this study does not directly assess DNA-protein interactions, it does directly assess a different epigenetic mechanism, DNAm.

DNAm patterns are usually very stable in terminally differentiated cells, but recent research suggests that DNAm dynamics in the brain may play an important mechanistic role in regulating behavioral plasticity in animals [67]. The present research suggests that DNAm in neurons may also be affected by exposure to meHg. For example, differential total, 5mc, and 5hmC methylation analyses of specific genes as outlined in the present study suggest epigenetic mechanisms may be modulating how the brain responds to toxic insult by meHg. Average total methylation of *STAT1*, for example, was significantly higher in the 0.01 μ M meHg treatment group relative to the untreated control. From the RNA sequencing results, *STAT1* was not associated with differential expression and was also not present in any of the enriched GO

pathways for this treatment group. In the 0.25 and 6.25 μM meHg treatment groups, however, *STAT1* was present in several of the significant GSE pathways. The *STAT1* (Signal transducer and activator of transcription 1) protein is a transcription factor that is involved in multiple immune-related pathways and is believed to play a role in neuroplasticity, learning, and memory. Notably, neurons expressing the NeuN protein (a marker of mature neurons) are associated with lower *STAT1* DNAm and higher *STAT1* expression levels, suggesting that DNAm may play a role in synaptic plasticity [67]. Total DNAm at a specific CpG site (position 171 in amplicon; chromosome 6 position 39,838,887 in human reference genome; TSS200; CpG island) annotated to the *DAAM2* gene was significantly lower in the 0.25 μM meHg treatment group than in untreated cells. The Daam2 protein (Dishevelled associated activator of morphogenesis 2) is important in regulating myelin compaction throughout neurodevelopment, motor learning during adulthood, and the differentiation of oligodendrocytes [68]. Future mechanistic research is warranted to determine whether changes in DNAm patterning related to low-level exposure to meHg may lead to behavioral modifications in animals.

Notably, many of the significant differential methylation results in this study were not identified until a distinction was made between 5mC and 5hmC. These two distinct covalent modifications often perform different functions but due to their structural similarities many standard methodologies quantify both marks jointly in a measure of total methylation. Not unsurprisingly, 5mC and 5hmC methylation levels for the *ENSG00000242479* gene exhibited the opposite direction of effect in their association with meHg, which is likely why no significant effects were identified in the total DNAm analysis. Treatment with meHg was related to a decrease in 5mC and an increase in 5hmC levels but did not follow a traditional monotonic dose-response. For both marks the magnitude of effect was largest in the 0.25 μM meHg treatment

group, but smaller in the lowest and highest treatments. Notably, 5mC, especially in gene promoter regions, is usually inversely related to expression levels of that gene. The expression and methylation patterns observed in the present experiment, however, suggest the opposite. The 0.25 μ M meHg treatment group was also associated with higher 5hmC levels at the *ENSG00000258830* gene, but no significant alterations in expression for that gene. While 5hmC does not appear to be modulating transcription of *ENSG00000258830* it could be playing a role in transcriptional regulation of other genes which is a common function of 5hmC. Lastly, higher total and 5mC DNAm levels at one CpG site in the *GABRG3* gene (position 44 in amplicon; chromosome 15 position 27,216,083 in human reference genome; TSS1500) were associated with biologically relevant doses of meHg. *GABRG3* (Gamma-Aminobutyric Acid (GABA) Type A Receptor Subunit Gamma3) encodes a GABA-A receptor subunit. In mammalian brains GABA (GABA-A receptor ligands) is the major inhibitory neurotransmitter, suggesting dysregulation of this process in the brain could result in excitotoxicity. Past research has shown that meHg can directly interact with GABA-A receptors via alkylation of sulfhydryl groups of their cysteinyl residues, induce a conformational modification, and potentially influence the binding affinity of GABA-A receptor ligands and agonists [69]. This is one potential mechanism in which low levels of meHg may impact the brain. In another study, using bisulfite sequencing and chromatin immunoprecipitation assays the MeCP2 (methyl CpG binding protein 2) protein was shown to bind methylated CpG sites within *GABRB3* in unexposed human brain cells [70]. MeCP2 is a chromosomal protein that interacts with methylated CpG sites to influence gene expression through dynamic interactions between DNA and proteins. It binds 5mC and 5hmC marks with similar affinities in neurons, but may promote the opposite effect on gene transcription depending on which mark is bound [71]. MeCP2 is one of the key regulators of heterochromatin

and plays a role in microRNA processing and mRNA splicing in the brain, much of this is believed to be mediated by its interaction with methylated CpG sites [72]. In the present study, the potential interaction between this methylated *GABRG3* CpG site and MeCP2 was not tested, but our data suggests it may be another mechanism by which human neurons respond to meHg exposures. Future analysis of protein-DNA interaction is warranted.

The present study combines several distinct techniques to address some vital gaps in the literature, but as with any study it is met with both strengths and weaknesses. One strength is that it combines transcriptomic and epigenetic methodologies, which provides an added mechanistic layer of understanding to how cells respond to toxic insult by meHg. While presence of DNAm usually is indicative of gene suppression, this is not always the case, which could be attributed to the fact that most DNAm studies do not distinguish between 5hmC and 5mC marks. Because of this, using parallel oxidative and standard bisulfite conversion reactions for DNA prior to sequencing via Oxford NanoPore Technologies was a major strength to this study. Additionally, partnering it with transcriptomic data is essential in connecting some of these dots. It combines genome-wide data with site-specific methodologies, which allows for the potential detection of changes in the expression of genes across the entire genome that may otherwise be overlooked. Using a site-specific approach for methylation analysis on the other hand has the opposite benefits, in that it may be able to detect small differences that higher throughput data may lack the power to detect. By relating expression results back to DNA methylation in a subsequent analysis, it will allow us to infer whether DNAm is facilitating the changes in gene expression seen in the RNA-seq. In order to truly demonstrate, however, that changes in DNA methylation are causing the expression changes for a gene or related molecular pathway, one would need to impede a cell's ability to methylate or demethylate DNA. This could be considered in future

analyses, that knock down DNA methyltransferases (DNMT) and ten-eleven translocation methylcytosine dioxygenases (TET) that facilitate these two processes respectively. Another notable aspect of this study that should be considered in the interpretation of results is the use of retinoic acid (RA) in the differentiation protocol. RA has been shown to activate survival pathways (PI3K/AKT signaling) in SH-SY5Ys and impact the cellular response to neurotoxic heavy metals. Mitochondria and energy metabolism in these cells may be altered by RA, leading to greater resistance to oxidative stress [73]. These factors may be contributing to the reported increase in metabolic activity in the 0.05 and 0.25 μ M meHg observed in the Presto Blue assay of this study. This could likely affect the cellular response to meHg and complicate interpretation of our results. However, RA provides a reliable and relatively simple differentiation procedure that is commonly used where results can more readily be compared across studies.

Other limitations to this study include the consideration of only one type of neuron and lack of interactions between other cells, tissues, and organs. Not only are there several other types of neurons apart from dopaminergic neurons but there are also various non-neural cells in the brain that influence neurotoxicity. Astrocytes, for example, play essential roles in antioxidant defense in the brain, as it supplies essential GSH precursors to neurons. This process is vital to meHg detoxification and is likely dysregulated in meHg exposed astrocytes. They also play vital roles in the metabolism and uptake of neurotransmitters, which can be perturbed by meHg and lead to excitotoxicity [74]. Another highly relevant cell type of potential consideration in the central nervous system is microglia. They have been shown to be involved in numerous neuro-relevant processes and events, including regulation of brain homeostasis, cognitive processes, neurodegenerative diseases, inflammation, and stroke [74-76].

Understanding how these cell types individually respond to meHg is an important first experimental stage, but building upon this foundational research in more complex models and studies is necessary to understand the complete picture of meHg-induced neurotoxicity. No cell, tissue, or system exists in isolation. In the absence of interactions between such diverse and dynamic networks, systems, and signaling cascades caution must be taken in interpretation of experimental results such as those from the present study. By limiting our model to just one type of cell, we may not be getting a full picture of neurotoxic effects. We will however test the direct effect of the toxicant on one target cell type which cannot be done in either epidemiological or *in vivo* studies. These types of future studies may also be better suited to address another limitation of this research, the duration of the exposure. While 48-hours is a longer exposure window than many prior *in vitro* studies of meHg exposure, it still does not accurately reflect a chronic exposure which is more likely in a real world-situation.

While this study has limitations, it combines several innovative resources and technologies and investigates lower levels of meHg exposure that are relevant to human populations. Overall, this study addresses knowledge gaps by stressing childhood as a critical neurodevelopmental period that is vulnerable to toxic insult by meHg. Where prior studies predominantly explore effects of prenatal exposures, the timepoint of the exposure selected in this study remain underexplored, despite its susceptibility to toxicant and epigenetic influence. The selected doses are also a major strength to the study as they are comparable to biologically relevant doses rather than strictly neurotoxic doses. It also enables the direct assessment of a specific tissue type of interest, ie. dopaminergic neurons, which is impossible in more complex epidemiological models. Lastly, it combines transcriptomic and epigenetic assays and can even distinguish between 5mC and 5hmC, which most studies of DNAm fail to do. This work aims to

inform future research and interventions in human mercury toxicity and the etiology of neurodevelopmental disorders. While this study nicely builds upon existing literature, more research is warranted to better elucidate the complex effects of chronic, low-dose meHg exposure on neurodevelopment.

Figures and Tables

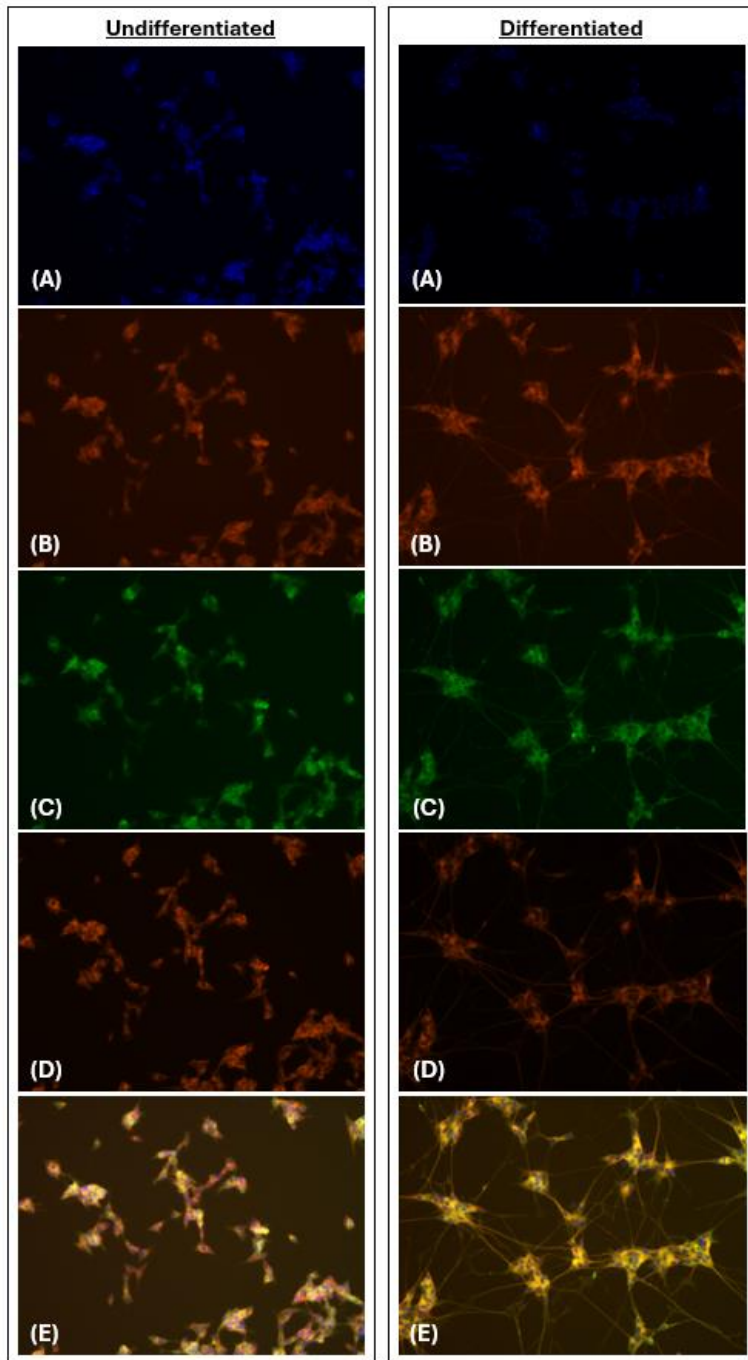


Figure 4-1: Representative immunofluorescent micrographs visualizing three different markers of differentiation in differentiated and undifferentiated SH-SY5Y cells.

Left and right panels denote undifferentiated and differentiated cells, respectively. Each panel shows cells in one fixed position using four distinct fluorescent channels corresponding to (A) DNA (Hoechst), (B) MAP2, (C) GAP43, and (D) β tubulin III. (E) Merged image of all four channels. Each image taken at 20x objective.

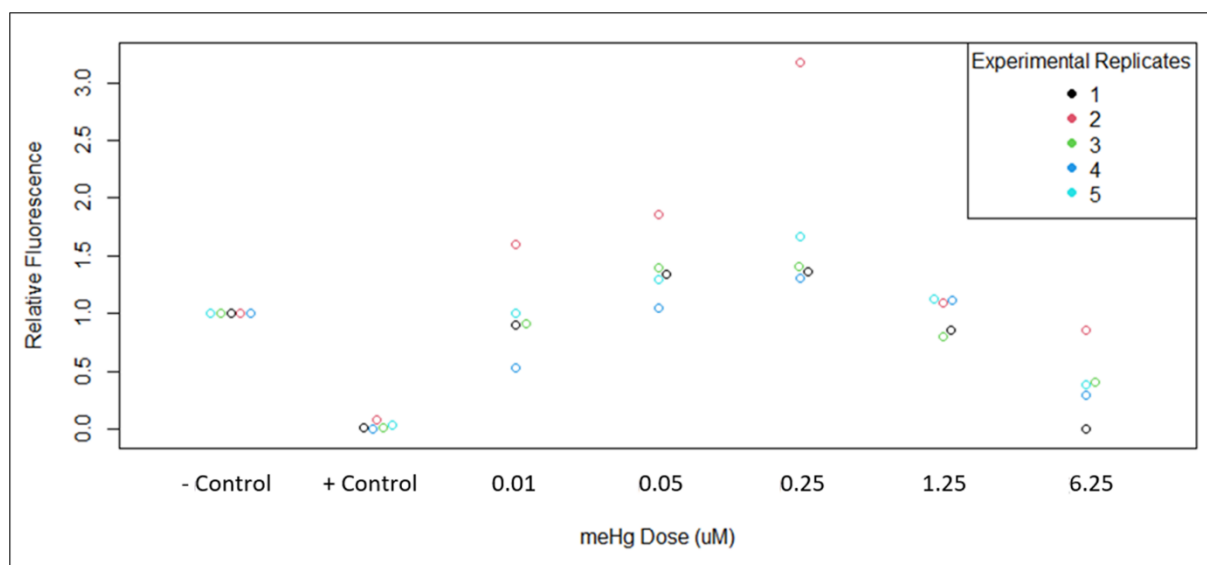


Figure 4-2: Bee swarm plot of fluorescence relative to negative control in differentiated SH-SH5Y cells after 48- hour incubation with varying doses of meHg from Presto Blue assay.

Each point represents the average RFU of three technical replicates normalized to the negative control. There are five points per treatment group, indicative of the five distinct experiments. + Control cells were treated with cytotoxic levels (40%) of DMSO media.

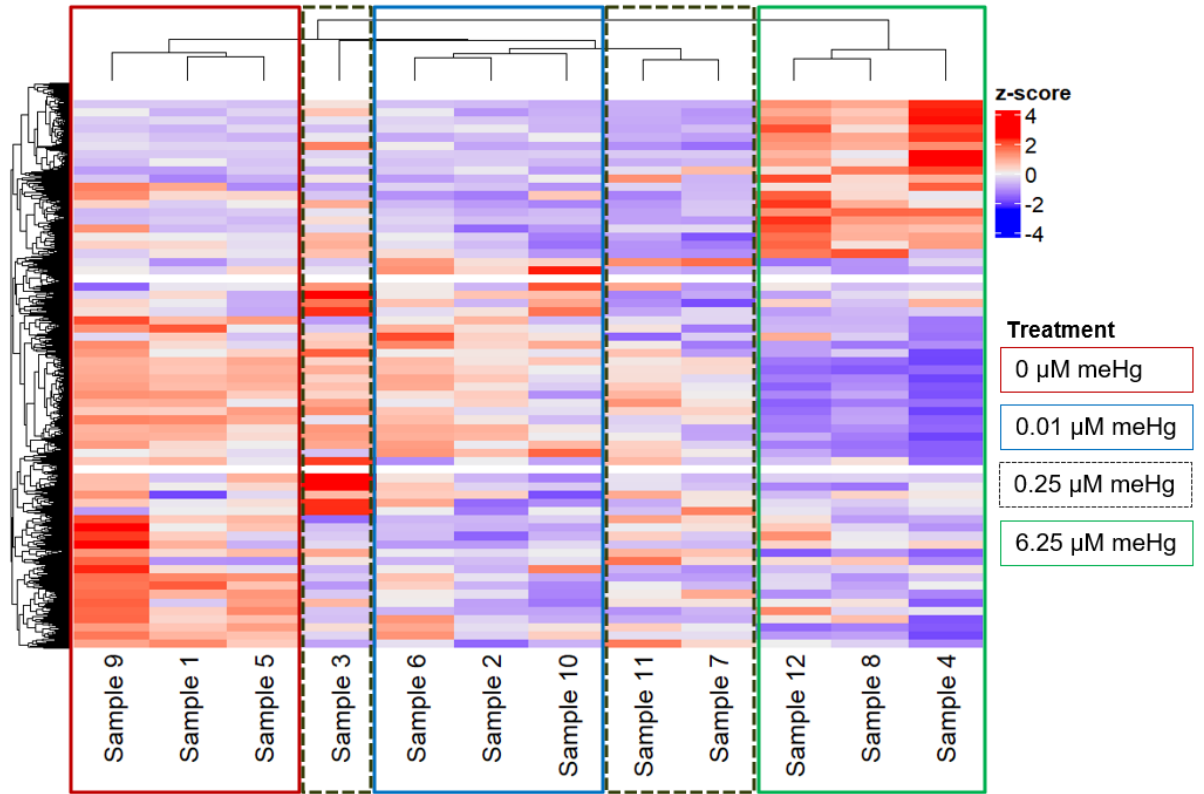


Figure 4-3: Heatmap of gene expression for all samples across all meHg treatment groups.

Samples from the 0 μM , 0.01 μM , 0.25 μM , and 6.25 μM meHg groups are denoted by solid red, solid blue, dashed black and solid green lines, respectively. 2717 genes are visualized in this figure.

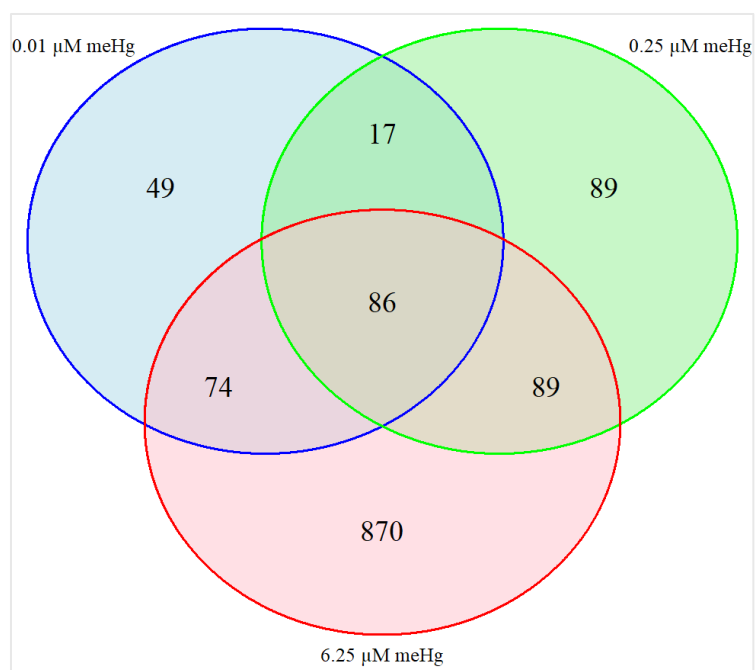


Figure 4-4: Venn diagram of enriched GO pathways by treatment group after GSE analyses. Numbers represent number of pathways enriched based on a significance threshold of $p_{adj} < 0.05$.

Table 4-1: Summary of fluorescence data generated in the Presto Blue assay and GLM results comparing RFU from each treatment group to the negative control.

Treatment Group (μM meHg)	Average (S.D.) Percent Difference in RFU	GLM β (S.E.) p-value	Notes
0 (- Control)	NA	NA	Vehicle control
0.01	99.21 (38.61) %	0.0072 (0.13) 0.96	Comparable to average blood Hg concentrations in a human cohort
0.05	138.94 (29.64) %	0.40 (0.13) 0.0032 *	Not selected for RNA-seq
0.25	178.52 (79.20) %	0.80 (0.13) 3.84E-08 *	Suggests a significant increase in viability relative to control
1.25	118.40 (20.77) %	0.013 (0.13) 0.92	Not selected for RNA-seq
6.25	38.73 (30.73) %	-0.60 (0.13) 2.20E-05 *	Suggests a significant decrease in viability relative to control
+ Control	3.01 (3.27) %	-0.95 (0.13) 1.83E-10 *	Not selected for RNA-seq

NOTE: Treatments that were significantly different from the negative control are denoted by asterisks (*). Rationale for use in RNA-seq for each treatment group are recorded in the last column if applicable.

Table 4-2: Number of significant differentially expressed genes (DEGs) and enriched Gene Ontology (GO) pathways in treatment groups relative to the untreated control.

Result Type	0.01 μM meHg	0.25 μM meHg	6.25 μM meHg	Significant findings in multiple doses
Differentially Expressed Genes	1	2	5,564	2
Enriched GO Pathways	226	281	1,119	266

Table 4-3: Significantly differentially expressed genes by meHg treatment group (incomplete list).

Gene	Treatment Group	baseMean	log2FoldChange	lfcSE	pvalue	padj
ENSG00000258830	0.01 μ M meHg	43.25	-3.05	0.52	4.06E-09	8.21E-05
ENSG00000258830	6.25 μ M meHg	43.25	1.17	0.36	0.001218	7.03E-03
ENSG00000242479	0.25 μ M meHg	11.36	-6.7	1.3	2.31E-07	4.68E-03
<i>RGPD5</i>	0.25 μ M meHg	549.56	0.94	0.2	1.91E-06	0.019
<i>RGPD5</i>	6.25 μ M meHg	549.56	1.3	0.2	5.27E-11	2.61E-09
<i>CHRNA9</i>	6.25 μ M meHg	286.07	4.74	0.29	5.27E-58	1.05E-53
<i>SELENON</i>	6.25 μ M meHg	6412.03	-1.1	0.07	4.29E-55	4.26E-51
<i>TENT5A</i>	6.25 μ M meHg	1093.17	1.97	0.13	4.55E-53	3.01E-49
<i>MB21D2</i>	6.25 μ M meHg	622.89	2.24	0.15	8.81E-48	4.37E-44
<i>NFASC</i>	6.25 μ M meHg	11260.03	1.28	0.09	6.05E-42	2.4E-38
<i>LUCAT1</i>	6.25 μ M meHg	386.48	6.12	0.45	1.44E-41	4.75E-38
<i>SLCO4A1</i>	6.25 μ M meHg	923.12	1.32	0.1	6.15E-39	1.74E-35
<i>ATF6B</i>	6.25 μ M meHg	2062.01	-1.21	0.09	3.30E-38	8.19E-35
<i>CDKN1A</i>	6.25 μ M meHg	5043.83	2.58	0.2	9.74E-38	2.15E-34
<i>CREM</i>	6.25 μ M meHg	1310.93	2.22	0.18	1.84E-36	3.64E-33
<i>THSD4</i>	6.25 μ M meHg	5096.67	1.48	0.12	2.54E-36	4.58E-33
<i>TXNRD1</i>	6.25 μ M meHg	9798.7	2.18	0.17	3.19E-36	5.28E-33

<i>ABCB6</i>	6.25 µM meHg	2352.68	1.92	0.15	3.63E-36	5.55E-33
<i>CTDSP1</i>	6.25 µM meHg	1121.83	-1.8	0.15	3.12E-35	4.42E-32
<i>HSP90AA1</i>	6.25 µM meHg	65868.19	1.55	0.13	5.11E-34	6.76E-31
<i>TNFRSF10B</i>	6.25 µM meHg	3344.44	1.18	0.1	4.49E-33	5.56E-30
<i>TFPI2</i>	6.25 µM meHg	16932.9	2.502471	0.21	2.25E-32	2.62E-29
<i>RFLNB</i>	6.25 µM meHg	4730.35	1.3	0.11	2.55E-32	2.82E-29
<i>CTSF</i>	6.25 µM meHg	1163.3	-1.72	0.15	1.18E-31	1.18E-28
<i>ENSG00000280385</i>	6.25 µM meHg	740.52	1.58	0.14	1.14E-31	1.18E-28
... 5,544 more rows						

NOTE: Incomplete results. Shows all significant results for the lowest two treatment groups, but only genes with lowest q-values for the highest meHg treatment group. baseMean = average expression count across all samples; lfcSE = log2FoldChange standard error; padj = BH adjusted p-value.

Table 4-4: Top ten enriched GO pathways with the lowest adjusted p-values from GSE analyses for all three treatment groups.

Treatment Group (µM meHg)	Ontology	ID	Description	Set Size	Enrichment Score	NES	pval	qval
0.01	BP	GO:0030855	epithelial cell differentiation	465	0.48	1.93	1.00E-10	6.63E-07
0.01	BP	GO:0001501	skeletal system development	376	0.45	1.81	1.41E-08	3.19E-05
0.01	MF	GO:0004888	transmembrane signaling receptor activity	481	0.43	1.74	1.44E-08	3.19E-05
0.01	BP	GO:0019221	cytokine-mediated signaling pathway	293	0.48	1.88	5.69E-08	9.42E-05
0.01	BP	GO:0018108	peptidyl-tyrosine phosphorylation	186	0.52	1.92	1.21E-07	1.6E-04
0.01	MF	GO:0030527	structural constituent of chromatin	80	-0.66	-2.09	1.64E-07	1.82E-04
0.01	BP	GO:0018212	peptidyl-tyrosine modification	188	0.52	1.91	2.24E-07	1.85E-04
0.01	BP	GO:0043588	skin development	186	0.52	1.9	2.21E-07	1.85E-04
0.01	BP	GO:0009913	epidermal cell differentiation	128	0.58	2.05	3.32E-07	2.45E-04

0.01	BP	GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	470	0.41	1.67	4.18E-07	2.73E-04
0.25	CC	GO:0022626	cytosolic ribosome	110	0.68	2.4	1.00E-10	1.08E-07
0.25	CC	GO:0044391	ribosomal subunit	181	0.62	2.32	1.00E-10	1.08E-07
0.25	CC	GO:0005840	ribosome	225	0.57	2.19	1.00E-10	1.08E-07
0.25	CC	GO:0005743	mitochondrial inner membrane	443	0.48	1.94	1.00E-10	1.08E-07
0.25	CC	GO:0019866	organelle inner membrane	487	0.47	1.91	1.00E-10	1.08E-07
0.25	MF	GO:0003735	structural constituent of ribosome	158	0.63	2.3	1.00E-10	1.08E-07
0.25	BP	GO:0002181	cytoplasmic translation	153	0.59	2.17	2.00E-10	1.85E-07
0.25	BP	GO:0030335	positive regulation of cell migration	397	0.46	1.86	3.03E-10	2.36E-07
0.25	MF	GO:0030527	structural constituent of chromatin	80	0.69	2.3	3.28E-10	2.36E-07
0.25	BP	GO:0040017	positive regulation of locomotion	424	0.45	1.81	4.40E-10	2.70E-07
6.25	BP	GO:0070661	leukocyte proliferation	199	0.58	2.2	1.00E-10	1.90E-08
6.25	BP	GO:0001649	osteoblast differentiation	185	0.58	2.18	1.00E-10	1.90E-08
6.25	BP	GO:0002237	response to molecule of bacterial origin	210	0.55	2.13	1.00E-10	1.90E-08
6.25	BP	GO:0019221	cytokine-mediated signaling pathway	293	0.54	2.12	1.00E-10	1.90E-08
6.25	BP	GO:0001819	positive regulation of cytokine production	304	0.53	2.11	1.00E-10	1.90E-08
6.25	BP	GO:0001503	ossification	320	0.53	2.1	1.00E-10	1.90E-08
6.25	BP	GO:0006954	inflammatory response	481	0.51	2.1	1.00E-10	1.90E-08
6.25	BP	GO:1903131	mononuclear cell differentiation	309	0.53	2.08	1.00E-10	1.90E-08
6.25	BP	GO:0040017	positive regulation of locomotion	424	0.51	2.08	1.00E-10	1.90E-08
6.25	BP	GO:0002521	leukocyte differentiation	405	0.51	2.08	1.00E-10	1.90E-08

NOTE: Complete results for each outcome can be found in Aim 3 Appendix: Tables 4-7 appx, 4-8 appx, and 4-9 appx. BP = Biological Process; MF = Molecular Function; CC = Cellular Component. Set Size = number of genes in the given gene set pathway. Enrichment Score = the degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes (compared to what would be expected by chance). NES = normalized enrichment score; variant of the enrichment score that has been normalized across multiple gene sets and datasets. qval = BH adjusted p-value.

Table 4-5: Summary of total, 5mC, and 5hmC DNA methylation for an average across all CpG sites in an amplicon for data generated through ONT sequencing and GLM results comparing each treatment group to the negative control.

Gene Name	Treatment Group (μ M meHg)	Total DNAm		5mC DNAm		5hmC DNAm	
		Average (S.D.)	GLM β (S.E.) p-value	Average (S.D.)	GLM β (S.E.) p-value	Average (S.D.)	GLM β (S.E.) p-value
GABRG3	0 (- ctrl)	0.55 (1.11) %	NA	0.57 (1.13) %	NA	0.59 (1.05) %	NA
GABRG3	0.01	0.64 (1.19) %	0.10 (0.15) 0.551	0.67 (0.79) %	0.10 (0.34) 0.788	0.47 (1.17) %	-0.14 (0.18) 0.472
GABRG3	0.25	0.64 (0.88) %	0.10 (0.15) 0.551	0.98 (2.23) %	0.40 (0.34) 0.277	0.40 (0.62) %	-0.19 (0.18) 0.332
GABRG3	6.25	0.76 (1.14) %	0.21 (0.15) 0.205	0.93 (2.35) %	0.36 (0.34) 0.332	0.58 (1.09) %	-5.39e-03 (0.18) 0.978
DAAM2	0 (- ctrl)	76.56 (18.82) %	NA	78.56 (18.97) %	NA	2.50 (2.12) %	NA
DAAM2	0.01	78.22 (14.21) %	1.67 (2.89) 0.585	79.33 (21.91) %	0.78 (4.33) 0.863	17.50 (21.92) %	2.90 (12.50) 0.838
DAAM2	0.25	76.78 (18.44) %	0.22 (2.89) 0.941	84.56 (11.26) %	6.0 (4.33) 0.215	8.00 (4.24) %	5.23 (10.63) 0.671
DAAM2	6.25	76.44 (15.84) %	-0.11 (2.89) 0.971	76.89 (22.54) %	-1.67 (4.33) 0.714	13.33 (11.50) %	2.77 (10.63) 0.819
TNFRSF10A	0 (- ctrl)	83.91 (8.79) %	NA	86.80 (9.26) %	NA	3.47 (2.70) %	NA
TNFRSF10A	0.01	84.47 (7.91) %	0.56 (0.87) 0.545	85.84 (8.88) %	-0.96 (1.09) 0.415	4.12 (4.20) %	1.57 (4.40) 0.734
TNFRSF10A	0.25	84.53 (8.84) %	0.62 (0.87) 0.500	85.47 (10.58) %	-1.33 (1.09) 0.268	6.40 (10.84) %	6.50 (4.40) 0.190
TNFRSF10A	6.25	84.27 (8.70) %	0.36 (0.87) 0.696	86.38 (9.04) %	-0.42 (1.09) 0.712	3.27 (3.97) %	0.79 (4.40) 0.863
STAT1	0 (- ctrl)	0.83 (1.34) %	NA	0.80 (1.37) %	NA	0.51 (1.09) %	NA
STAT1	0.01	1.38 (1.88) %	0.55 (0.19) 0.029 *	0.95 (1.43) %	0.14 (0.27) 0.612	0.94 (1.65) %	0.46 (0.25) 0.119
STAT1	0.25	0.78 (1.12) %	-0.05 (0.19) 0.822	0.88 (1.58) %	0.08 (0.27) 0.774	0.46 (0.72) %	-0.02 (0.25) 0.928

<i>STAT1</i>	6.25	0.76 (0.94) %	-0.07 (0.19) 0.720	0.88 (1.37) %	0.08 (0.27) 0.774	0.47 (0.71) %	-0.02 (0.25) 0.945
<i>ENSG00000242479</i>	0 (- ctrl)	94.00 (4.58) %	NA	96.22 (4.76) %	NA	4.67 (6.43) %	NA
<i>ENSG00000242479</i>	0.01	94.33 (2.29) %	0.33 (1.53) 0.835	93.67 (8.14) %	-2.56 (1.25) 0.0864 •	8.50 (6.14) %	8.75 (3.56) 0.0572 •
<i>ENSG00000242479</i>	0.25	96.44 (3.43) %	2.44 (1.53) 0.161	88.67 (9.71) %	-7.56 (1.25) 0.000917 *	10.86 (7.34) %	10.42 (3.56) 0.0327 *
<i>ENSG00000242479</i>	6.25	94.33 (2.87) %	0.33 (1.53) 0.835	94.67 (8.43) %	-1.56 (1.25) 0.259	8.00 (7.48) %	8.08 (3.56) 0.0722 •
<i>ENSG00000258830</i>	0 (- ctrl)	53.56 (12.37) %	NA	44.56 (18.42) %	NA	13.43 (10.37) %	NA
<i>ENSG00000258830</i>	0.01	58.11 (6.21) %	4.56 (3.26) 0.211	49.11 (19.44) %	4.56 (14.77) 0.768	16.17 (10.65) %	2.33 (9.21) 0.810
<i>ENSG00000258830</i>	0.25	59.22 (12.46) %	5.67 (3.26) 0.133	62.67 (27.63) %	18.11 (14.77) 0.266	41.00 (24.04) %	21.86 (10.63) 0.095 •
<i>ENSG00000258830</i>	6.25	47.33 (16.09) %	-6.22 (3.26) 0.105	37.78 (35.31) %	-6.78 (14.77) 0.662	35.50 (20.96) %	14.83 (9.21) 0.168

Table 4-6: Summary of total, 5mC, and 5hmC DNA methylation for specific CpG sites in amplicons for data generated through ONT sequencing and GLM results comparing each treatment group to the negative control.

Gene Name (position in amplicon)	Treatment Group (μM meHg)	Total DNAm		5mC DNAm		5hmC DNAm	
		Average (S.D.)	GLM β (S.E.) p-value	Average (S.D.)	GLM β (S.E.) p-value	Average (S.D.)	GLM β (S.E.) p-value
<i>GABRG3</i> (p44)	0 (- ctrl)	0.67 (0.58) %	NA	0.33 (0.58) %	NA	1.00 (0) %	NA
<i>GABRG3</i> (p44)	0.01	3.33 (2.52) %	2.67 (1.35) 0.096 •	0.67 (0.58) %	0.33 (2.75) 0.907	2.67 (3.06) %	2.65e-18 (1.00e-02) 1.00
<i>GABRG3</i> (p44)	0.25	2.67 (1.15) %	2.00 (1.35) 0.190	6.67 (5.69) %	6.33 (2.75) 0.0605 •	0 (NA) %	-6.00e-02 (1.73e-02) 0.179

GABRG3 (p44)	6.25	1.67 (0.58) %	1.00 (1.35) 0.488	3.00 (3.00) %	2.67 (2.75) 0.369	2.00 (NA) %	5.00e-03 (1.32e-02) 0.770
DAAM2 (p171)	0 (- ctrl)	87.67 (2.08) %	NA	88.67 (3.06) %	NA	NA	NA
DAAM2 (p171)	0.01	85.67 (1.53) %	-2.00 (1.79) 0.307	90.67 (2.08) %	2.00 (5.04) 0.705	NA	NA
DAAM2 (p171)	0.25	83.33 (2.31)%	-4.33 (1.79) 0.052 •	91.33 (1.53) %	2.67 (5.04) 0.616	NA	NA
DAAM2 (p171)	6.25	85.00 (1.73) %	-2.67 (1.79) 0.187	81.67 (10.69) %	-7.00 (5.04) 0.214	NA	NA
TNFRSF10A (p289)	0 (- ctrl)	89.00 (2.65) %	NA	93.67 (1.53) %	NA	0 (NA) %	NA
TNFRSF10A (p289)	0.01	89.33 (0.58) %	0.33 (1.70) 0.851	90.67 (3.06) %	-3.00 (2.05) 0.195	0.5 (0.71) %	3.00 (5.29) 0.672
TNFRSF10A (p289)	0.25	91.33 (2.89) %	2.33 (1.70) 0.219	90.67 (5.86) %	-3.00 (2.05) 0.195	4.5 (6.36) %	7.00 (5.29) 0.412
TNFRSF10A (p289)	6.25	90.33 (2.08) %	1.33 (1.70) 0.463	93.00 (1.00) %	-0.67 (2.05) 0.757	0 (NA) %	-1.41e-15 (5.66) 1.000
STAT1 (p301)	0 (- ctrl)	0 (0) %	NA	0 (0) %	NA	0 (0) %	NA
STAT1 (p301)	0.01	0.67 (1.15) %	0.67 (0.56) 0.280	0.33 (0.58) %	0.33 (0.36) 0.390	1.00 (1.41) %	1.15 (0.61) 0.133
STAT1 (p301)	0.25	0.33 (0.58) %	0.33 (0.56) 0.574	0 (0) %	-1.963e-17 (0.36) 1.000	0.33 (0.58) %	0.33 (0.53) 0.563
STAT1 (p301)	6.25	0 (0) %	1.813e-16 (0.56) 1.000	0.33 (0.58) %	0.33 (0.36) 0.390	0 (0) %	-0.25 (0.61) 0.707

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Appendix

Table 4-7 appx: All enriched GO pathways from GSE analyses for the 0.01 μ M meHg treatment group.

ONTOLOGY	ID	Description	Set Size	Enrichment Score	NES	pvalue	qvalue
BP	GO:0030855	epithelial cell differentiation	465	0.478	1.934	1.00E-10	6.63E-07
BP	GO:0001501	skeletal system development	376	0.453	1.808	1.41E-08	3.19E-05
MF	GO:0004888	transmembrane signaling receptor activity	481	0.432	1.744	1.44E-08	3.19E-05
BP	GO:0019221	cytokine-mediated signaling pathway	293	0.484	1.879	5.69E-08	9.42E-05
BP	GO:0018108	peptidyl-tyrosine phosphorylation	186	0.523	1.922	1.21E-07	0.00016
MF	GO:0030527	structural constituent of chromatin	80	-0.66	-2.09	1.64E-07	0.00018
BP	GO:0018212	peptidyl-tyrosine modification	188	0.517	1.905	2.24E-07	0.00019
BP	GO:0043588	skin development	186	0.517	1.898	2.21E-07	0.00019
BP	GO:0009913	epidermal cell differentiation	128	0.58	2.046	3.32E-07	0.00024
BP	GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	470	0.412	1.668	4.18E-07	0.00027
CC	GO:0000786	nucleosome	110	-0.6	-1.97	4.53E-07	0.00027
BP	GO:0040017	positive regulation of locomotion	424	0.416	1.668	5.07E-07	0.00028
CC	GO:0030312	external encapsulating structure	356	0.442	1.746	6.72E-07	0.00032
CC	GO:0031012	extracellular matrix	356	0.442	1.746	6.72E-07	0.00032
BP	GO:0030335	positive regulation of cell migration	397	0.424	1.7	8.72E-07	0.00039
BP	GO:0048514	blood vessel morphogenesis	444	0.412	1.662	1.61E-06	0.00067
BP	GO:0061448	connective tissue development	201	0.49	1.815	1.81E-06	0.00071
BP	GO:0008544	epidermis development	225	0.474	1.775	2.41E-06	0.00084
MF	GO:0030545	signaling receptor regulator activity	244	0.469	1.766	2.35E-06	0.00084
BP	GO:2000147	positive regulation of cell motility	413	0.415	1.666	2.76E-06	0.00092
BP	GO:0001503	ossification	320	0.438	1.719	3.46E-06	0.00109
BP	GO:0019216	regulation of lipid metabolic process	240	0.471	1.771	3.79E-06	0.00114
BP	GO:0097696	receptor signaling pathway via STAT	103	0.587	2.002	4.90E-06	0.00135
MF	GO:0030546	signaling receptor activator activity	222	0.477	1.784	4.87E-06	0.00135
BP	GO:0007584	response to nutrient	111	0.568	1.953	5.11E-06	0.00135
MF	GO:0048018	receptor ligand activity	217	0.479	1.783	5.94E-06	0.00152
BP	GO:0008202	steroid metabolic process	220	0.469	1.747	6.36E-06	0.00156
BP	GO:0050730	regulation of peptidyl-tyrosine phosphorylation	151	0.524	1.885	6.69E-06	0.00158
BP	GO:0002065	columnar/cuboidal epithelial cell differentiation	83	0.609	1.995	7.66E-06	0.00165
BP	GO:0001525	angiogenesis	383	0.406	1.626	7.59E-06	0.00165

MF	GO:0005216	monoatomic ion channel activity	293	0.437	1.697	7.72E-06	0.00165
BP	GO:0043410	positive regulation of MAPK cascade	312	0.421	1.652	8.23E-06	0.0017
BP	GO:0002521	leukocyte differentiation	405	0.399	1.604	9.02E-06	0.00178
BP	GO:0001817	regulation of cytokine production	495	0.385	1.566	9.15E-06	0.00178
CC	GO:0062023	collagen-containing extracellular matrix	275	0.439	1.692	9.56E-06	0.00181
BP	GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	104	0.574	1.967	1.07E-05	0.00197
BP	GO:0007259	receptor signaling pathway via JAK-STAT	97	0.58	1.947	1.21E-05	0.00218
BP	GO:0070374	positive regulation of ERK1 and ERK2 cascade	122	0.535	1.863	1.54E-05	0.00263
BP	GO:0031589	cell-substrate adhesion	272	0.438	1.682	1.55E-05	0.00263
BP	GO:0045229	external encapsulating structure organization	225	0.452	1.691	1.82E-05	0.00301
BP	GO:0030216	keratinocyte differentiation	82	0.595	1.953	1.99E-05	0.00322
BP	GO:0043062	extracellular structure organization	224	0.451	1.687	2.31E-05	0.00364
BP	GO:0010817	regulation of hormone levels	350	0.401	1.587	2.45E-05	0.00366
BP	GO:0071417	cellular response to organonitrogen compound	495	0.376	1.529	2.43E-05	0.00366
MF	GO:0019838	growth factor binding	105	0.555	1.893	2.49E-05	0.00366
BP	GO:0031667	response to nutrient levels	381	0.391	1.562	3.18E-05	0.00458
BP	GO:0009991	response to extracellular stimulus	401	0.389	1.559	3.86E-05	0.00544
BP	GO:0042509	regulation of tyrosine phosphorylation of STAT protein	41	0.7	2.062	4.67E-05	0.00595
BP	GO:0042531	positive regulation of tyrosine phosphorylation of STAT protein	32	0.728	2.045	4.62E-05	0.00595
BP	GO:0007260	tyrosine phosphorylation of STAT protein	44	0.69	2.032	4.57E-05	0.00595
BP	GO:0045165	cell fate commitment	171	0.475	1.733	4.41E-05	0.00595
BP	GO:0030198	extracellular matrix organization	223	0.447	1.668	4.47E-05	0.00595
BP	GO:0006775	fat-soluble vitamin metabolic process	25	0.792	2.112	5.34E-05	0.00655
BP	GO:0045444	fat cell differentiation	185	0.465	1.707	5.31E-05	0.00655
BP	GO:0071407	cellular response to organic cyclic compound	419	0.378	1.518	5.72E-05	0.0069
BP	GO:0070371	ERK1 and ERK2 cascade	212	0.454	1.689	6.18E-05	0.00691
BP	GO:1901615	organic hydroxy compound metabolic process	407	0.385	1.549	6.04E-05	0.00691
BP	GO:0009611	response to wounding	391	0.386	1.542	6.14E-05	0.00691
MF	GO:0022839	monoatomic ion gated channel activity	222	0.443	1.657	6.17E-05	0.00691
MF	GO:0022836	gated channel activity	223	0.442	1.652	6.26E-05	0.00691
CC	GO:0043235	receptor complex	271	0.427	1.635	6.50E-05	0.00706
BP	GO:0071396	cellular response to lipid	410	0.384	1.542	6.65E-05	0.00711
BP	GO:0001822	kidney development	233	0.431	1.616	7.08E-05	0.00745
BP	GO:1905314	semi-lunar valve development	40	0.699	2.046	7.25E-05	0.0075

BP	GO:1903131	mononuclear cell differentiation	309	0.411	1.607	7.53E-05	0.00768
BP	GO:0003180	aortic valve morphogenesis	32	0.719	2.018	7.83E-05	0.0077
BP	GO:0097530	granulocyte migration	70	0.612	1.94	7.98E-05	0.0077
BP	GO:0016042	lipid catabolic process	236	0.44	1.646	7.87E-05	0.0077
BP	GO:0050673	epithelial cell proliferation	304	0.409	1.593	8.02E-05	0.0077
BP	GO:0006954	inflammatory response	481	0.371	1.497	8.49E-05	0.00793
BP	GO:0007507	heart development	480	0.368	1.488	8.44E-05	0.00793
BP	GO:0001649	osteoblast differentiation	185	0.454	1.666	9.53E-05	0.00877
BP	GO:0061437	renal system vasculature development	22	0.787	2.037	0.0001004	0.00887
BP	GO:0061440	kidney vasculature development	22	0.787	2.037	0.0001004	0.00887
MF	GO:0140375	immune receptor activity	53	0.631	1.913	9.98E-05	0.00887
BP	GO:0006935	chemotaxis	286	0.412	1.6	0.0001035	0.00891
BP	GO:0042330	taxis	286	0.412	1.6	0.0001035	0.00891
BP	GO:0060351	cartilage development involved in endochondral bone morphogenesis	27	0.759	2.058	0.0001086	0.00911
BP	GO:0045216	cell-cell junction organization	153	0.482	1.736	0.0001083	0.00911
BP	GO:0072012	glomerulus vasculature development	20	0.819	2.066	0.0001102	0.00913
BP	GO:0071622	regulation of granulocyte chemotaxis	32	0.709	1.992	0.0001256	0.0098
BP	GO:0016055	Wnt signaling pathway	383	0.378	1.513	0.0001267	0.0098
BP	GO:0032870	cellular response to hormone stimulus	457	0.361	1.464	0.0001256	0.0098
CC	GO:0098552	side of membrane	424	0.369	1.479	0.0001272	0.0098
MF	GO:0015267	channel activity	320	0.401	1.574	0.0001253	0.0098
MF	GO:0022803	passive transmembrane transporter activity	320	0.401	1.574	0.0001253	0.0098
MF	GO:0033293	monocarboxylic acid binding	48	0.645	1.934	0.000139	0.01059
BP	GO:0198738	cell-cell signaling by wnt	385	0.376	1.507	0.0001416	0.01066
BP	GO:0002573	myeloid leukocyte differentiation	164	0.475	1.722	0.0001475	0.01098
CC	GO:0005788	endoplasmic reticulum lumen	225	0.426	1.592	0.0001505	0.01108
CC	GO:0016324	apical plasma membrane	256	0.415	1.575	0.0001573	0.01146
BP	GO:0070372	regulation of ERK1 and ERK2 cascade	193	0.451	1.666	0.0001788	0.01288
BP	GO:0003176	aortic valve development	37	0.701	2.001	0.0001833	0.01302
BP	GO:0045667	regulation of osteoblast differentiation	96	0.545	1.819	0.0001847	0.01302
BP	GO:0071621	granulocyte chemotaxis	56	0.622	1.916	0.0001894	0.01308
BP	GO:0006261	DNA-templated DNA replication	153	-0.48	-1.65	0.0001891	0.01308
BP	GO:0072001	renal system development	238	0.427	1.601	0.0001922	0.01313
BP	GO:0050920	regulation of chemotaxis	158	0.461	1.661	0.0002091	0.01414
BP	GO:0003170	heart valve development	62	0.594	1.868	0.0002113	0.01414
BP	GO:0003179	heart valve morphogenesis	52	0.63	1.908	0.0002311	0.01507
BP	GO:0001667	ameboidal-type cell migration	344	0.393	1.551	0.0002345	0.01507
BP	GO:0042327	positive regulation of	476	0.359	1.449	0.0002388	0.01507

		phosphorylation					
CC	GO:0030133	transport vesicle	366	0.379	1.511	0.0002321	0.01507
MF	GO:0031406	carboxylic acid binding	128	0.497	1.752	0.0002375	0.01507
MF	GO:0005261	monoatomic cation channel activity	228	0.419	1.572	0.0002358	0.01507
BP	GO:0006694	steroid biosynthetic process	127	0.492	1.735	0.0002462	0.01511
BP	GO:0070663	regulation of leukocyte proliferation	152	0.466	1.676	0.0002432	0.01511
BP	GO:0050900	leukocyte migration	227	0.427	1.602	0.0002444	0.01511
BP	GO:0062012	regulation of small molecule metabolic process	250	0.42	1.586	0.0002584	0.01571
BP	GO:0060070	canonical Wnt signaling pathway	258	0.41	1.557	0.0002675	0.01612
BP	GO:0042110	T cell activation	352	0.383	1.516	0.0002785	0.01663
BP	GO:0030111	regulation of Wnt signaling pathway	282	0.399	1.547	0.0002846	0.01684
BP	GO:0002067	glandular epithelial cell differentiation	47	0.648	1.936	0.0003037	0.01769
BP	GO:0070562	regulation of vitamin D receptor signaling pathway	10	0.893	1.872	0.0003043	0.01769
BP	GO:0046890	regulation of lipid biosynthetic process	133	0.479	1.693	0.0003143	0.01811
CC	GO:0045177	apical part of cell	304	0.391	1.525	0.0003297	0.01884
BP	GO:0060348	bone development	158	0.452	1.629	0.0003459	0.01947
BP	GO:0060537	muscle tissue development	316	0.386	1.508	0.0003467	0.01947
BP	GO:0060350	endochondral bone morphogenesis	47	0.645	1.927	0.0003523	0.0195
BP	GO:0030098	lymphocyte differentiation	278	0.4	1.552	0.0003616	0.0195
BP	GO:0010038	response to metal ion	264	0.4	1.53	0.0003619	0.0195
BP	GO:0065004	protein-DNA complex assembly	203	-0.43	-1.53	0.0003578	0.0195
CC	GO:0005774	vacuolar membrane	408	0.363	1.458	0.0003598	0.0195
BP	GO:0098868	bone growth	27	0.73	1.98	0.0003791	0.01989
BP	GO:0071772	response to BMP	127	0.485	1.709	0.0003855	0.01989
BP	GO:0071773	cellular response to BMP stimulus	127	0.485	1.709	0.0003855	0.01989
BP	GO:0050865	regulation of cell activation	405	0.363	1.459	0.0003752	0.01989
MF	GO:0004896	cytokine receptor activity	50	0.617	1.865	0.0003872	0.01989
MF	GO:0043177	organic acid binding	130	0.486	1.72	0.0003838	0.01989
CC	GO:0000793	condensed chromosome	253	-0.42	-1.54	0.0003978	0.02028
BP	GO:0001819	positive regulation of cytokine production	304	0.388	1.514	0.000407	0.02059
BP	GO:0002694	regulation of leukocyte activation	372	0.372	1.49	0.0004178	0.02097
BP	GO:0034103	regulation of tissue remodeling	55	0.602	1.846	0.0004444	0.02214
BP	GO:0007043	cell-cell junction assembly	105	0.504	1.719	0.0004481	0.02216
BP	GO:0050810	regulation of steroid biosynthetic process	54	0.606	1.851	0.0004684	0.02235
BP	GO:0045598	regulation of fat cell differentiation	107	0.5	1.709	0.0004608	0.02235
BP	GO:0051216	cartilage development	140	0.478	1.689	0.0004637	0.02235

BP	GO:1902075	cellular response to salt	154	0.454	1.636	0.0004636	0.02235
BP	GO:0050678	regulation of epithelial cell proliferation	251	0.408	1.542	0.0004688	0.02235
BP	GO:0071459	protein localization to chromosome, centromeric region	40	-0.64	-1.82	0.000475	0.02248
BP	GO:0006721	terpenoid metabolic process	61	0.568	1.777	0.0004813	0.02255
BP	GO:0002064	epithelial cell development	158	0.446	1.607	0.0004831	0.02255
BP	GO:0032835	glomerulus development	51	0.624	1.882	0.0005085	0.02324
BP	GO:0033002	muscle cell proliferation	147	0.46	1.641	0.0005059	0.02324
BP	GO:0043408	regulation of MAPK cascade	460	0.353	1.431	0.000506	0.02324
BP	GO:0007389	pattern specification process	321	0.377	1.478	0.000512	0.02324
BP	GO:0001523	retinoid metabolic process	50	0.607	1.835	0.0005237	0.02339
BP	GO:0007178	transmembrane receptor protein serine/threonine kinase signaling pathway	287	0.386	1.499	0.0005249	0.02339
MF	GO:0005509	calcium ion binding	488	0.352	1.421	0.0005259	0.02339
BP	GO:0032526	response to retinoic acid	75	0.56	1.804	0.0005315	0.02348
MF	GO:0015276	ligand-gated monoatomic ion channel activity	93	0.519	1.722	0.0005395	0.02368
MF	GO:0005501	retinoid binding	22	0.748	1.936	0.0005652	0.02448
MF	GO:0019840	isoprenoid binding	22	0.748	1.936	0.0005652	0.02448
BP	GO:0016101	diterpenoid metabolic process	53	0.592	1.798	0.000579	0.02476
BP	GO:0070661	leukocyte proliferation	199	0.422	1.558	0.0005758	0.02476
BP	GO:0048385	regulation of retinoic acid receptor signaling pathway	15	0.834	1.941	0.0005887	0.02485
BP	GO:0050680	negative regulation of epithelial cell proliferation	94	0.518	1.729	0.0005888	0.02485
BP	GO:0071375	cellular response to peptide hormone stimulus	235	0.4	1.5	0.0005974	0.02506
BP	GO:0044242	cellular lipid catabolic process	177	0.445	1.626	0.0006274	0.02582
BP	GO:0048705	skeletal system morphogenesis	158	0.443	1.598	0.0006262	0.02582
BP	GO:0002683	negative regulation of immune system process	317	0.369	1.445	0.0006269	0.02582
MF	GO:0022834	ligand-gated channel activity	94	0.517	1.725	0.0006312	0.02582
BP	GO:0043297	apical junction assembly	55	0.59	1.808	0.000661	0.02685
BP	GO:0042063	gliogenesis	257	0.396	1.503	0.0006645	0.02685
BP	GO:0048762	mesenchymal cell differentiation	200	0.422	1.56	0.0006764	0.02717
BP	GO:1902074	response to salt	274	0.379	1.458	0.0006805	0.02717
BP	GO:0033273	response to vitamin	60	0.584	1.827	0.0006931	0.0275
BP	GO:0003417	growth plate cartilage development	17	0.803	1.942	0.0007068	0.02788
BP	GO:0003416	endochondral bone growth	25	0.735	1.959	0.000732	0.02871
BP	GO:0060828	regulation of canonical Wnt signaling pathway	217	0.415	1.545	0.0007758	0.03024
BP	GO:0042445	hormone metabolic process	144	0.465	1.657	0.0007871	0.0305
BP	GO:0042060	wound healing	296	0.386	1.502	0.0007967	0.0307
MF	GO:0015318	inorganic molecular entity transmembrane transporter	478	0.343	1.386	0.0008031	0.03076

		activity					
MF	GO:0005201	extracellular matrix structural constituent	104	0.503	1.723	0.000809	0.03081
BP	GO:0048384	retinoic acid receptor signaling pathway	28	0.728	1.994	0.0008163	0.03091
BP	GO:0060349	bone morphogenesis	72	0.551	1.761	0.0008261	0.03111
BP	GO:0072006	nephron development	114	0.48	1.653	0.0008478	0.03174
BP	GO:0007219	Notch signaling pathway	144	0.463	1.649	0.0008905	0.03315
BP	GO:0030336	negative regulation of cell migration	220	0.401	1.495	0.000937	0.03431
BP	GO:0048729	tissue morphogenesis	467	0.347	1.405	0.0009285	0.03431
CC	GO:0005581	collagen trimer	50	0.594	1.796	0.0009348	0.03431
BP	GO:0097529	myeloid leukocyte migration	127	0.468	1.649	0.0009727	0.03526
BP	GO:0090090	negative regulation of canonical Wnt signaling pathway	113	0.474	1.63	0.0009814	0.03526
BP	GO:0060485	mesenchyme development	242	0.393	1.478	0.0009758	0.03526
BP	GO:0007600	sensory perception	332	0.369	1.458	0.0009842	0.03526
BP	GO:0070830	bicellular tight junction assembly	47	0.616	1.841	0.0009914	0.03533
BP	GO:1904892	regulation of receptor signaling pathway via STAT	62	0.551	1.733	0.0010332	0.03642
BP	GO:0010001	glial cell differentiation	192	0.418	1.543	0.001028	0.03642
BP	GO:0045639	positive regulation of myeloid cell differentiation	74	0.544	1.745	0.0010514	0.03654
BP	GO:0051249	regulation of lymphocyte activation	321	0.365	1.431	0.0010531	0.03654
CC	GO:0005911	cell-cell junction	388	0.353	1.409	0.0010456	0.03654
BP	GO:0015698	inorganic anion transport	102	0.496	1.689	0.0010829	0.03719
BP	GO:0030856	regulation of epithelial cell differentiation	105	0.488	1.662	0.0010818	0.03719
BP	GO:0045765	regulation of angiogenesis	198	0.409	1.512	0.0010959	0.03744
BP	GO:0035270	endocrine system development	90	0.51	1.683	0.0011513	0.03903
CC	GO:0009925	basal plasma membrane	203	0.401	1.491	0.0011544	0.03903
BP	GO:0019218	regulation of steroid metabolic process	71	0.55	1.756	0.0011746	0.03952
BP	GO:0045132	meiotic chromosome segregation	66	-0.56	-1.71	0.0011897	0.03982
BP	GO:0051897	positive regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction	114	0.473	1.629	0.0011982	0.03991
BP	GO:0045833	negative regulation of lipid metabolic process	72	0.541	1.729	0.0012217	0.04048
BP	GO:2000146	negative regulation of cell motility	231	0.401	1.501	0.001236	0.04075
BP	GO:0009617	response to bacterium	358	0.365	1.449	0.0012591	0.04131
BP	GO:0043200	response to amino acid	104	0.491	1.683	0.0012693	0.04133
BP	GO:0010810	regulation of cell-substrate adhesion	166	0.43	1.559	0.0012785	0.04133
BP	GO:0045785	positive regulation of cell adhesion	315	0.378	1.479	0.001274	0.04133

BP	GO:1901342	regulation of vasculature development	200	0.411	1.519	0.0012921	0.04157
BP	GO:0071230	cellular response to amino acid stimulus	71	0.546	1.742	0.0012993	0.0416
BP	GO:0040013	negative regulation of locomotion	262	0.377	1.44	0.0013182	0.042
BP	GO:0050801	monoatomic ion homeostasis	439	0.345	1.389	0.0013879	0.04401
BP	GO:0072577	endothelial cell apoptotic process	38	0.644	1.856	0.0014501	0.04445
BP	GO:2000351	regulation of endothelial cell apoptotic process	34	0.644	1.811	0.0014554	0.04445
BP	GO:0002685	regulation of leukocyte migration	141	0.443	1.568	0.0014347	0.04445
BP	GO:0071248	cellular response to metal ion	145	0.434	1.548	0.0014096	0.04445
BP	GO:0030217	T cell differentiation	204	0.409	1.515	0.0014254	0.04445
BP	GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	181	0.413	1.51	0.001442	0.04445
BP	GO:0048732	gland development	326	0.367	1.445	0.0014444	0.04445
CC	GO:0030658	transport vesicle membrane	197	0.406	1.501	0.0014269	0.04445
BP	GO:0002931	response to ischemia	50	0.585	1.768	0.0014657	0.04456
BP	GO:0010663	positive regulation of striated muscle cell apoptotic process	10	0.858	1.797	0.0015344	0.04552
BP	GO:0010666	positive regulation of cardiac muscle cell apoptotic process	10	0.858	1.797	0.0015344	0.04552
BP	GO:2000027	regulation of animal organ morphogenesis	91	0.505	1.674	0.0015455	0.04552
BP	GO:1904019	epithelial cell apoptotic process	96	0.501	1.67	0.0015424	0.04552
BP	GO:0051896	regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction	163	0.436	1.581	0.0015318	0.04552
BP	GO:0002237	response to molecule of bacterial origin	210	0.397	1.479	0.0015091	0.04552
BP	GO:0043434	response to peptide hormone	327	0.354	1.394	0.00152	0.04552
MF	GO:0005125	cytokine activity	86	0.507	1.663	0.0015523	0.04552

Table 4-8 appx: All enriched GO pathways from GSE analyses for the 0.25 μ M meHg treatment group.

ONTOLOGY	ID	Description	setSize	enrichmentScore	NES	pvalue	qvalue
CC	GO:0022626	cytosolic ribosome	110	0.68384	2.4	1.00E-10	1.08E-07
CC	GO:0044391	ribosomal subunit	181	0.61737	2.32	1.00E-10	1.08E-07
CC	GO:0005840	ribosome	225	0.57363	2.19	1.00E-10	1.08E-07
CC	GO:0005743	mitochondrial inner membrane	443	0.47714	1.94	1.00E-10	1.08E-07
CC	GO:0019866	organelle inner membrane	487	0.46748	1.91	1.00E-10	1.08E-07
MF	GO:0003735	structural constituent of ribosome	158	0.62795	2.3	1.00E-10	1.08E-07
BP	GO:0002181	cytoplasmic translation	153	0.59257	2.17	2.00E-10	1.85E-07
BP	GO:0030335	positive regulation of cell migration	397	0.46151	1.86	3.03E-10	2.36E-07
MF	GO:0030527	structural constituent of chromatin	80	0.68803	2.3	3.28E-10	2.36E-07
BP	GO:0040017	positive regulation of locomotion	424	0.44725	1.81	4.40E-10	2.70E-07
CC	GO:0098798	mitochondrial protein-containing complex	275	0.50931	1.98	4.58E-10	2.70E-07
BP	GO:0006935	chemotaxis	286	0.49155	1.92	7.66E-10	3.81E-07
BP	GO:0042330	taxis	286	0.49155	1.92	7.66E-10	3.81E-07
BP	GO:2000147	positive regulation of cell motility	413	0.44438	1.79	1.13E-09	5.21E-07
CC	GO:0015935	small ribosomal subunit	71	0.67973	2.22	2.66E-08	1.15E-05
CC	GO:0000786	nucleosome	110	0.60249	2.11	3.51E-08	1.42E-05
CC	GO:0022627	cytosolic small ribosomal subunit	37	0.77058	2.25	9.13E-08	3.47E-05
CC	GO:0015934	large ribosomal subunit	114	0.58695	2.06	1.22E-07	4.18E-05
CC	GO:0098800	inner mitochondrial membrane protein complex	136	0.56129	2.03	1.23E-07	4.18E-05
MF	GO:0004888	transmembrane signaling receptor activity	481	0.39923	1.63	1.75E-07	5.66E-05
CC	GO:0022625	cytosolic large ribosomal subunit	57	0.69247	2.19	2.48E-07	7.60E-05
MF	GO:0030545	signaling receptor regulator activity	244	0.46194	1.77	2.58E-07	7.60E-05
BP	GO:0060326	cell chemotaxis	179	0.51124	1.92	3.13E-07	8.81E-05
BP	GO:0009060	aerobic respiration	169	0.5123	1.9	3.57E-07	9.62E-05
BP	GO:0050920	regulation of chemotaxis	158	0.5223	1.91	3.96E-07	0.000102
BP	GO:0006119	oxidative phosphorylation	123	0.563	2	4.51E-07	0.000112
MF	GO:0048018	receptor ligand activity	217	0.47226	1.79	6.89E-07	0.000165
MF	GO:0030546	signaling receptor activator activity	222	0.47014	1.8	8.05E-07	0.000186
CC	GO:0009897	external side of plasma membrane	182	0.49607	1.86	8.72E-07	0.000195
BP	GO:0048514	blood vessel morphogenesis	444	0.39383	1.6	1.41E-06	0.000304
CC	GO:0005925	focal adhesion	378	0.41045	1.65	1.62E-06	0.000338
BP	GO:0001525	angiogenesis	383	0.40297	1.62	2.30E-06	0.000465
CC	GO:0030055	cell-substrate junction	385	0.40009	1.61	2.50E-06	0.00049
BP	GO:0050921	positive regulation of chemotaxis	93	0.57714	1.97	2.88E-06	0.000547
BP	GO:0046390	ribose phosphate biosynthetic process	202	0.46889	1.77	6.40E-06	0.001159
BP	GO:0044283	small molecule biosynthetic process	449	0.38458	1.56	6.45E-06	0.001159
BP	GO:0030595	leukocyte chemotaxis	128	0.51881	1.86	7.11E-06	0.001244
BP	GO:0009152	purine ribonucleotide biosynthetic process	183	0.47025	1.76	9.73E-06	0.001657

CC	GO:0098552	side of membrane	424	0.38151	1.54	1.09E-05	0.001807
BP	GO:0019221	cytokine-mediated signaling pathway	293	0.41715	1.64	1.14E-05	0.001845
BP	GO:0045333	cellular respiration	210	0.45362	1.71	1.21E-05	0.001913
BP	GO:0009260	ribonucleotide biosynthetic process	196	0.46241	1.74	1.29E-05	0.001982
BP	GO:0006334	nucleosome assembly	98	0.56303	1.94	1.34E-05	0.002011
BP	GO:0033002	muscle cell proliferation	147	0.49682	1.8	1.40E-05	0.002017
BP	GO:0061448	connective tissue development	201	0.46615	1.76	1.40E-05	0.002017
BP	GO:0030855	epithelial cell differentiation	465	0.37026	1.51	1.45E-05	0.002043
BP	GO:0050900	leukocyte migration	227	0.43839	1.67	1.49E-05	0.002045
BP	GO:0006091	generation of precursor metabolites and energy	427	0.37868	1.53	1.66E-05	0.002239
BP	GO:0060070	canonical Wnt signaling pathway	258	0.42448	1.64	1.76E-05	0.002327
BP	GO:0001503	ossification	320	0.3996	1.58	1.90E-05	0.002376
CC	GO:0030312	external encapsulating structure	356	0.39013	1.55	1.91E-05	0.002376
CC	GO:0031012	extracellular matrix	356	0.39013	1.55	1.91E-05	0.002376
BP	GO:0071621	granulocyte chemotaxis	56	0.62318	1.96	2.12E-05	0.002539
MF	GO:0019955	cytokine binding	90	0.56364	1.92	2.08E-05	0.002539
MF	GO:0046982	protein heterodimerization activity	274	0.41267	1.61	2.19E-05	0.002571
BP	GO:0034728	nucleosome organization	116	0.5143	1.81	2.32E-05	0.002685
BP	GO:0060828	regulation of canonical Wnt signaling pathway	217	0.43837	1.66	2.50E-05	0.002806
BP	GO:0198738	cell-cell signaling by wnt	385	0.37985	1.53	2.52E-05	0.002806
BP	GO:0042773	ATP synthesis coupled electron transport	83	0.5593	1.87	2.97E-05	0.003043
BP	GO:0042775	mitochondrial ATP synthesis coupled electron transport	83	0.5593	1.87	2.97E-05	0.003043
BP	GO:0045598	regulation of fat cell differentiation	107	0.52867	1.85	3.01E-05	0.003043
BP	GO:0070663	regulation of leukocyte proliferation	152	0.48405	1.77	2.98E-05	0.003043
BP	GO:0051216	cartilage development	140	0.4874	1.76	2.93E-05	0.003043
BP	GO:0016055	Wnt signaling pathway	383	0.38273	1.54	2.90E-05	0.003043
BP	GO:0060351	cartilage development involved in endochondral bone morphogenesis	27	0.75631	2.04	3.14E-05	0.003082
CC	GO:0031983	vesicle lumen	229	0.43102	1.65	3.14E-05	0.003082
BP	GO:0070661	leukocyte proliferation	199	0.44451	1.67	3.20E-05	0.003093
BP	GO:0018108	peptidyl-tyrosine phosphorylation	186	0.45262	1.7	3.35E-05	0.003142
BP	GO:0040013	negative regulation of locomotion	262	0.41943	1.63	3.34E-05	0.003142
BP	GO:0019216	regulation of lipid metabolic process	240	0.41914	1.61	3.83E-05	0.003545
BP	GO:0009206	purine ribonucleoside triphosphate biosynthetic process	95	0.54033	1.85	4.33E-05	0.003945
CC	GO:0060205	cytoplasmic vesicle lumen	228	0.43014	1.64	4.65E-05	0.004176
CC	GO:0034774	secretory granule lumen	226	0.4318	1.65	4.90E-05	0.004341
BP	GO:0018212	peptidyl-tyrosine modification	188	0.44775	1.68	5.00E-05	0.004375
BP	GO:0009145	purine nucleoside triphosphate biosynthetic process	96	0.54122	1.86	5.40E-05	0.004657
BP	GO:0009142	nucleoside triphosphate biosynthetic process	109	0.52258	1.83	5.56E-05	0.00473

MF	GO:0050839	cell adhesion molecule binding	472	0.35831	1.46	5.96E-05	0.005007
MF	GO:0005125	cytokine activity	86	0.54419	1.83	6.09E-05	0.005055
BP	GO:0097529	myeloid leukocyte migration	127	0.49433	1.77	6.88E-05	0.005639
BP	GO:0019646	aerobic electron transport chain	75	0.57495	1.89	7.18E-05	0.005732
BP	GO:0002688	regulation of leukocyte chemotaxis	78	0.56253	1.87	7.44E-05	0.005732
BP	GO:0006766	vitamin metabolic process	77	0.55492	1.85	7.41E-05	0.005732
BP	GO:0090090	negative regulation of canonical Wnt signaling pathway	113	0.50685	1.78	7.35E-05	0.005732
CC	GO:0005788	endoplasmic reticulum lumen	225	0.41593	1.59	7.18E-05	0.005732
BP	GO:0006754	ATP biosynthetic process	85	0.5508	1.85	7.62E-05	0.005798
BP	GO:0070665	positive regulation of leukocyte proliferation	95	0.5299	1.82	8.17E-05	0.006075
BP	GO:0009201	ribonucleoside triphosphate biosynthetic process	101	0.52158	1.81	8.11E-05	0.006075
BP	GO:0060485	mesenchyme development	242	0.41311	1.58	8.65E-05	0.006288
BP	GO:0042327	positive regulation of phosphorylation	476	0.35326	1.44	8.62E-05	0.006288
BP	GO:0072522	purine-containing compound biosynthetic process	225	0.41377	1.58	9.03E-05	0.006493
BP	GO:0050670	regulation of lymphocyte proliferation	134	0.47974	1.73	9.57E-05	0.006805
BP	GO:0001667	ameboidal-type cell migration	344	0.38546	1.54	0.000105	0.00738
MF	GO:0140375	immune receptor activity	53	0.60846	1.9	0.000108	0.007481
BP	GO:0002685	regulation of leukocyte migration	141	0.47654	1.72	0.000111	0.007551
BP	GO:0032944	regulation of mononuclear cell proliferation	137	0.47431	1.72	0.000111	0.007551
BP	GO:0043068	positive regulation of programmed cell death	411	0.36372	1.47	0.000123	0.008287
BP	GO:0001501	skeletal system development	376	0.36343	1.46	0.000128	0.008528
CC	GO:0000775	chromosome, centromeric region	250	0.39949	1.53	0.000131	0.008639
BP	GO:0065004	protein-DNA complex assembly	203	0.43113	1.63	0.000139	0.008975
CC	GO:0062023	collagen-containing extracellular matrix	275	0.40385	1.57	0.000139	0.008975
BP	GO:0045653	negative regulation of megakaryocyte differentiation	18	0.79665	1.97	0.000146	0.009034
BP	GO:0071622	regulation of granulocyte chemotaxis	32	0.69738	1.96	0.000147	0.009034
BP	GO:0097530	granulocyte migration	70	0.56374	1.84	0.000147	0.009034
BP	GO:0022904	respiratory electron transport chain	104	0.51286	1.78	0.000146	0.009034
BP	GO:0030111	regulation of Wnt signaling pathway	282	0.39538	1.54	0.000141	0.009034
BP	GO:0003272	endocardial cushion formation	21	0.77075	1.97	0.000151	0.009069
BP	GO:0015980	energy derivation by oxidation of organic compounds	280	0.38695	1.51	0.000151	0.009069
BP	GO:0031667	response to nutrient levels	381	0.35977	1.44	0.000149	0.009069
BP	GO:1901293	nucleoside phosphate biosynthetic process	251	0.39914	1.53	0.000153	0.009109
BP	GO:0002690	positive regulation of leukocyte chemotaxis	57	0.5969	1.89	0.000156	0.009115
BP	GO:0050730	regulation of peptidyl-tyrosine phosphorylation	151	0.46059	1.68	0.000156	0.009115

BP	GO:0071675	regulation of mononuclear cell migration	77	0.54576	1.82	0.00016	0.00922
CC	GO:0043235	receptor complex	271	0.40028	1.56	0.000163	0.009323
BP	GO:1905314	semi-lunar valve development	40	0.64272	1.9	0.000169	0.009514
BP	GO:0006694	steroid biosynthetic process	127	0.48107	1.72	0.000169	0.009514
BP	GO:0048659	smooth muscle cell proliferation	105	0.50604	1.76	0.000173	0.009672
BP	GO:0009991	response to extracellular stimulus	401	0.36589	1.48	0.000177	0.009789
BP	GO:0003170	heart valve development	62	0.57287	1.84	0.000182	0.009998
BP	GO:0048660	regulation of smooth muscle cell proliferation	102	0.50947	1.77	0.000194	0.010394
BP	GO:0046890	regulation of lipid biosynthetic process	133	0.4661	1.68	0.000192	0.010394
BP	GO:0140014	mitotic nuclear division	249	0.39449	1.52	0.000193	0.010394
BP	GO:0098868	bone growth	27	0.72249	1.95	0.000203	0.010607
BP	GO:0070374	positive regulation of ERK1 and ERK2 cascade	122	0.4787	1.7	0.000204	0.010607
BP	GO:0009165	nucleotide biosynthetic process	249	0.39311	1.51	0.000205	0.010607
MF	GO:0004896	cytokine receptor activity	50	0.61565	1.9	0.000205	0.010607
BP	GO:0003179	heart valve morphogenesis	52	0.61082	1.9	0.00021	0.010784
BP	GO:0060973	cell migration involved in heart development	17	0.79424	1.95	0.000216	0.010814
BP	GO:0072132	mesenchyme morphogenesis	43	0.64458	1.93	0.000215	0.010814
BP	GO:0070482	response to oxygen levels	274	0.38811	1.51	0.000213	0.010814
BP	GO:0071674	mononuclear cell migration	107	0.49585	1.73	0.000219	0.010925
BP	GO:0003416	endochondral bone growth	25	0.72922	1.93	0.000223	0.010995
BP	GO:0042110	T cell activation	352	0.36533	1.45	0.00023	0.011172
BP	GO:0010035	response to inorganic substance	395	0.35751	1.44	0.000229	0.011172
BP	GO:0030336	negative regulation of cell migration	220	0.41539	1.58	0.000235	0.011336
CC	GO:0042788	polysomal ribosome	30	0.6849	1.89	0.000237	0.01134
BP	GO:0010977	negative regulation of neuron projection development	124	0.47613	1.7	0.000245	0.01157
BP	GO:0050678	regulation of epithelial cell proliferation	251	0.39271	1.51	0.000246	0.01157
CC	GO:0070469	respirasome	82	0.53344	1.79	0.000247	0.01157
BP	GO:0043065	positive regulation of apoptotic process	399	0.36031	1.46	0.000249	0.011581
BP	GO:0015986	proton motive force-driven ATP synthesis	65	0.57385	1.85	0.000263	0.012088
BP	GO:0010001	glial cell differentiation	192	0.43033	1.62	0.000264	0.012088
BP	GO:0000302	response to reactive oxygen species	160	0.44002	1.61	0.000265	0.012088
BP	GO:0061311	cell surface receptor signaling pathway involved in heart development	19	0.76619	1.92	0.000273	0.012241
BP	GO:0003176	aortic valve development	37	0.64986	1.9	0.000272	0.012241
BP	GO:2000146	negative regulation of cell motility	231	0.40398	1.54	0.000274	0.012241
BP	GO:0072001	renal system development	238	0.40775	1.56	0.000279	0.012265
BP	GO:0001822	kidney development	233	0.40377	1.54	0.000279	0.012265

BP	GO:0042776	proton motive force-driven mitochondrial ATP synthesis	57	0.58488	1.85	0.000293	0.012792
BP	GO:0046165	alcohol biosynthetic process	107	0.4918	1.72	0.000302	0.013133
BP	GO:0072594	establishment of protein localization to organelle	423	0.35146	1.42	0.000306	0.013189
BP	GO:0048729	tissue morphogenesis	467	0.34307	1.39	0.000321	0.013769
BP	GO:0002687	positive regulation of leukocyte migration	93	0.50071	1.71	0.000332	0.014147
CC	GO:0034506	chromosome, centromeric core domain	19	0.76139	1.91	0.000337	0.014262
BP	GO:0022900	electron transport chain	149	0.44419	1.61	0.00034	0.01428
BP	GO:0032943	mononuclear cell proliferation	177	0.43045	1.61	0.000347	0.014401
MF	GO:0005126	cytokine receptor binding	130	0.46723	1.67	0.000347	0.014401
BP	GO:0002062	chondrocyte differentiation	87	0.51781	1.75	0.000375	0.015377
BP	GO:0048762	mesenchymal cell differentiation	200	0.41536	1.56	0.000374	0.015377
BP	GO:0006954	inflammatory response	481	0.3367	1.37	0.000378	0.015393
BP	GO:1903510	mucopolysaccharide metabolic process	72	0.54432	1.78	0.000397	0.016073
BP	GO:0030203	glycosaminoglycan metabolic process	82	0.52526	1.76	0.000406	0.016328
BP	GO:0031214	biomineral tissue development	114	0.47507	1.67	0.000419	0.016716
BP	GO:0001934	positive regulation of protein phosphorylation	434	0.34588	1.4	0.000422	0.016744
BP	GO:0043123	positive regulation of canonical NF-kappaB signal transduction	154	0.43388	1.59	0.000434	0.017135
BP	GO:0048732	gland development	326	0.36282	1.44	0.00044	0.017255
BP	GO:0043410	positive regulation of MAPK cascade	312	0.364	1.44	0.000465	0.018022
CC	GO:0098803	respiratory chain complex	72	0.54118	1.77	0.000463	0.018022
BP	GO:0050768	negative regulation of neurogenesis	120	0.46777	1.66	0.000472	0.018193
BP	GO:0001659	temperature homeostasis	138	0.43762	1.58	0.000485	0.018466
CC	GO:0005746	mitochondrial respirasome	75	0.54084	1.78	0.000483	0.018466
CC	GO:0005844	polysome	64	0.55491	1.79	0.000501	0.018946
BP	GO:0071459	protein localization to chromosome, centromeric region	40	0.61503	1.82	0.000505	0.019005
BP	GO:0006706	steroid catabolic process	13	0.82017	1.87	0.000514	0.019221
BP	GO:0002694	regulation of leukocyte activation	372	0.34967	1.4	0.000523	0.019446
BP	GO:0033108	mitochondrial respiratory chain complex assembly	93	0.49271	1.69	0.000532	0.01968
CC	GO:0098687	chromosomal region	380	0.35794	1.43	0.000539	0.01981
BP	GO:0034695	response to prostaglandin E	17	0.77009	1.89	0.000544	0.019877
BP	GO:0045926	negative regulation of growth	194	0.41505	1.55	0.00057	0.020728
BP	GO:0046651	lymphocyte proliferation	171	0.43117	1.6	0.000583	0.021063
BP	GO:0097305	response to alcohol	181	0.42379	1.59	0.000596	0.021431
BP	GO:0030178	negative regulation of Wnt signaling pathway	140	0.44266	1.6	0.000617	0.022074
BP	GO:0072521	purine-containing compound metabolic process	402	0.34761	1.41	0.000624	0.022202

BP	GO:0015698	inorganic anion transport	102	0.48822	1.69	0.000646	0.022852
BP	GO:0000070	mitotic sister chromatid segregation	181	0.42339	1.59	0.000651	0.022888
BP	GO:0003002	regionalization	286	0.36935	1.44	0.000663	0.02307
BP	GO:0007389	pattern specification process	321	0.35815	1.42	0.00066	0.02307
BP	GO:0032835	glomerulus development	51	0.58935	1.82	0.000689	0.02383
BP	GO:0008202	steroid metabolic process	220	0.40035	1.52	0.000692	0.02383
BP	GO:0061644	protein localization to CENP-A containing chromatin	18	0.76386	1.89	0.000725	0.024308
BP	GO:0051205	protein insertion into membrane	70	0.5305	1.74	0.000722	0.024308
BP	GO:0032496	response to lipopolysaccharide	198	0.40558	1.53	0.000725	0.024308
CC	GO:0043505	CENP-A containing nucleosome	18	0.76386	1.89	0.000725	0.024308
CC	GO:0061638	CENP-A containing chromatin	18	0.76386	1.89	0.000725	0.024308
BP	GO:0001936	regulation of endothelial cell proliferation	93	0.4849	1.66	0.000733	0.024451
BP	GO:0070371	ERK1 and ERK2 cascade	212	0.40284	1.52	0.000749	0.024842
BP	GO:0006821	chloride transport	72	0.5321	1.74	0.000766	0.025025
BP	GO:1901654	response to ketone	148	0.44228	1.6	0.000758	0.025025
BP	GO:0097191	extrinsic apoptotic signaling pathway	183	0.41281	1.55	0.000764	0.025025
BP	GO:0045765	regulation of angiogenesis	198	0.40437	1.52	0.000781	0.025389
BP	GO:0042063	gliogenesis	257	0.37978	1.47	0.000819	0.026386
MF	GO:0015453	oxidoreduction-driven active transmembrane transporter activity	55	0.5748	1.8	0.00082	0.026386
BP	GO:1901342	regulation of vasculature development	200	0.40461	1.52	0.00083	0.026583
BP	GO:0051961	negative regulation of nervous system development	126	0.45427	1.62	0.00084	0.026652
MF	GO:0045296	cadherin binding	306	0.36389	1.44	0.000837	0.026652
BP	GO:0001666	response to hypoxia	237	0.38438	1.47	0.000855	0.026712
BP	GO:0022407	regulation of cell-cell adhesion	305	0.36675	1.45	0.00085	0.026712
BP	GO:0050673	epithelial cell proliferation	304	0.36647	1.44	0.000852	0.026712
CC	GO:1902495	transmembrane transporter complex	283	0.36776	1.43	0.000866	0.026926
BP	GO:0061138	morphogenesis of a branching epithelium	127	0.45148	1.61	0.000875	0.027091
MF	GO:0019843	rRNA binding	63	0.53739	1.73	0.000883	0.027208
BP	GO:0052548	regulation of endopeptidase activity	191	0.40032	1.5	0.000898	0.027273
BP	GO:0010631	epithelial cell migration	240	0.3787	1.45	0.000895	0.027273
BP	GO:0090132	epithelium migration	240	0.3787	1.45	0.000895	0.027273
BP	GO:0003417	growth plate cartilage development	17	0.75787	1.86	0.000909	0.02749
BP	GO:0019751	polyol metabolic process	85	0.49778	1.67	0.000913	0.02749
MF	GO:0015318	inorganic molecular entity transmembrane transporter activity	478	0.32794	1.34	0.000925	0.027716
BP	GO:0051249	regulation of lymphocyte activation	321	0.35356	1.4	0.000935	0.027866
BP	GO:0070372	regulation of ERK1 and ERK2 cascade	193	0.41163	1.54	0.000956	0.028388
BP	GO:0045652	regulation of megakaryocyte differentiation	35	0.6454	1.85	0.000966	0.028549

CC	GO:0031970	organelle envelope lumen	83	0.49746	1.66	0.000975	0.028677
BP	GO:0003180	aortic valve morphogenesis	32	0.65582	1.84	0.000984	0.028803
CC	GO:1990351	transporter complex	307	0.35913	1.42	0.000989	0.028831
BP	GO:0032602	chemokine production	58	0.55436	1.77	0.001011	0.028941
BP	GO:0032642	regulation of chemokine production	58	0.55436	1.77	0.001011	0.028941
BP	GO:0042476	odontogenesis	78	0.51125	1.7	0.001007	0.028941
BP	GO:0032102	negative regulation of response to external stimulus	296	0.36816	1.45	0.001006	0.028941
BP	GO:0003198	epithelial to mesenchymal transition involved in endocardial cushion formation	11	0.8324	1.82	0.001022	0.029129
BP	GO:0014910	regulation of smooth muscle cell migration	53	0.55979	1.75	0.001045	0.029387
BP	GO:0003158	endothelium development	103	0.46406	1.61	0.001043	0.029387
BP	GO:0045637	regulation of myeloid cell differentiation	161	0.42423	1.55	0.001037	0.029387
BP	GO:0035590	purinergic nucleotide receptor signaling pathway	18	0.75401	1.87	0.001052	0.029465
BP	GO:0043062	extracellular structure organization	224	0.38529	1.47	0.001057	0.02947
BP	GO:0019218	regulation of steroid metabolic process	71	0.52849	1.73	0.001083	0.030066
BP	GO:0090130	tissue migration	241	0.38404	1.47	0.001091	0.030162
BP	GO:0006164	purine nucleotide biosynthetic process	216	0.39973	1.51	0.001115	0.030691
BP	GO:0048640	negative regulation of developmental growth	92	0.48596	1.66	0.001149	0.031496
BP	GO:0061383	trabecula morphogenesis	33	0.64986	1.83	0.001173	0.03176
BP	GO:0052547	regulation of peptidase activity	210	0.39568	1.49	0.001172	0.03176
BP	GO:0062012	regulation of small molecule metabolic process	250	0.37156	1.43	0.00117	0.03176
CC	GO:0000313	organellar ribosome	89	0.49647	1.69	0.001191	0.031964
CC	GO:0005761	mitochondrial ribosome	89	0.49647	1.69	0.001191	0.031964
BP	GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	104	0.47595	1.66	0.001204	0.032192
BP	GO:0009259	ribonucleotide metabolic process	321	0.35024	1.39	0.001209	0.032193
BP	GO:0050865	regulation of cell activation	405	0.33792	1.36	0.00122	0.032346
BP	GO:0003203	endocardial cushion morphogenesis	34	0.6304	1.79	0.001232	0.032545
BP	GO:0006820	monoatomic anion transport	108	0.46795	1.64	0.001238	0.032564
BP	GO:0042255	ribosome assembly	59	0.54029	1.73	0.001252	0.0328
BP	GO:0051222	positive regulation of protein transport	246	0.37742	1.44	0.00126	0.032875
BP	GO:0043588	skin development	186	0.40553	1.52	0.001321	0.034172
BP	GO:0001894	tissue homeostasis	203	0.40136	1.51	0.001331	0.034172
BP	GO:0060249	anatomical structure homeostasis	203	0.40136	1.51	0.001331	0.034172
BP	GO:1904951	positive regulation of establishment of protein localization	262	0.37472	1.45	0.001322	0.034172
BP	GO:2000177	regulation of neural precursor cell proliferation	77	0.50286	1.67	0.001339	0.034252
BP	GO:0036293	response to decreased oxygen levels	252	0.37668	1.44	0.001364	0.034751

MF	GO:0015267	channel activity	320	0.35259	1.4	0.001409	0.035625
MF	GO:0022803	passive transmembrane transporter activity	320	0.35259	1.4	0.001409	0.035625
BP	GO:0032722	positive regulation of chemokine production	44	0.57735	1.74	0.001452	0.036413
BP	GO:0046683	response to organophosphorus	89	0.49145	1.67	0.001458	0.036413
BP	GO:0019693	ribose phosphate metabolic process	328	0.35631	1.42	0.001454	0.036413
BP	GO:1901617	organic hydroxy compound biosynthetic process	184	0.40248	1.51	0.001465	0.03645
BP	GO:0006753	nucleoside phosphate metabolic process	447	0.33317	1.36	0.001484	0.036789
BP	GO:2000179	positive regulation of neural precursor cell proliferation	47	0.58333	1.79	0.001521	0.037571
BP	GO:0060914	heart formation	27	0.67224	1.81	0.001528	0.037593
BP	GO:0042098	T cell proliferation	123	0.44442	1.58	0.001535	0.037634
BP	GO:0003418	growth plate cartilage chondrocyte differentiation	10	0.82903	1.78	0.001554	0.037954
BP	GO:0032350	regulation of hormone metabolic process	28	0.65301	1.77	0.001565	0.03807
BP	GO:0050870	positive regulation of T cell activation	150	0.41776	1.51	0.001573	0.038127
BP	GO:0046173	polyol biosynthetic process	49	0.58599	1.81	0.001596	0.038528
MF	GO:0008083	growth factor activity	92	0.47929	1.64	0.001608	0.038685
BP	GO:0030212	hyaluronan metabolic process	21	0.71682	1.83	0.001639	0.039284
BP	GO:0060317	cardiac epithelial to mesenchymal transition	25	0.6852	1.81	0.001727	0.040773
BP	GO:0048645	animal organ formation	43	0.59542	1.78	0.001727	0.040773
BP	GO:0050922	negative regulation of chemotaxis	51	0.56977	1.76	0.001713	0.040773
BP	GO:0030099	myeloid cell differentiation	325	0.35403	1.4	0.001724	0.040773
BP	GO:0009617	response to bacterium	358	0.35081	1.4	0.001752	0.04123
BP	GO:0009117	nucleotide metabolic process	439	0.33406	1.36	0.001789	0.041947
BP	GO:0001704	formation of primary germ layer	92	0.47515	1.62	0.001849	0.043132
BP	GO:0006163	purine nucleotide metabolic process	384	0.33923	1.36	0.001853	0.043132
BP	GO:0009144	purine nucleoside triphosphate metabolic process	142	0.41998	1.52	0.001876	0.043347
BP	GO:0045787	positive regulation of cell cycle	262	0.37024	1.44	0.001869	0.043347
BP	GO:0051055	negative regulation of lipid biosynthetic process	48	0.5623	1.73	0.001912	0.04402

Table 4-9 appx: All enriched GO pathways from GSE analyses for the 6.25 μ M meHg treatment group.

ONTOLOGY	ID	Description	setSize	enrichmentScore	NES (Normalized Enrichment Score)	pvalue	qvalue
BP	GO:0070661	leukocyte proliferation	199	0.575	2.205	1.00E-10	1.90E-08
BP	GO:0001649	osteoblast differentiation	185	0.575	2.181	1.00E-10	1.90E-08
BP	GO:0002237	response to molecule of bacterial origin	210	0.551	2.127	1.00E-10	1.90E-08
BP	GO:0019221	cytokine-mediated signaling pathway	293	0.535	2.117	1.00E-10	1.90E-08
BP	GO:0001819	positive regulation of cytokine production	304	0.532	2.108	1.00E-10	1.90E-08
BP	GO:0001503	ossification	320	0.53	2.105	1.00E-10	1.90E-08
BP	GO:0006954	inflammatory response	481	0.513	2.097	1.00E-10	1.90E-08
BP	GO:1903131	mononuclear cell differentiation	309	0.525	2.084	1.00E-10	1.90E-08
BP	GO:0040017	positive regulation of locomotion	424	0.513	2.081	1.00E-10	1.90E-08
BP	GO:0002521	leukocyte differentiation	405	0.514	2.078	1.00E-10	1.90E-08
BP	GO:0030335	positive regulation of cell migration	397	0.511	2.067	1.00E-10	1.90E-08
BP	GO:2000147	positive regulation of cell motility	413	0.506	2.049	1.00E-10	1.90E-08
BP	GO:0050865	regulation of cell activation	405	0.494	2	1.00E-10	1.90E-08
BP	GO:0002694	regulation of leukocyte activation	372	0.493	1.977	1.00E-10	1.90E-08
BP	GO:0001817	regulation of cytokine production	495	0.483	1.977	1.00E-10	1.90E-08
BP	GO:0060271	cilium assembly	336	-0.41	-1.932	1.00E-10	1.90E-08
BP	GO:0043068	positive regulation of programmed cell death	411	0.475	1.924	1.00E-10	1.90E-08
BP	GO:0043065	positive regulation of apoptotic process	399	0.474	1.917	1.00E-10	1.90E-08
BP	GO:0044782	cilium organization	359	-0.4	-1.916	1.00E-10	1.90E-08
BP	GO:0001525	angiogenesis	383	0.473	1.909	1.00E-10	1.90E-08
BP	GO:0001934	positive regulation of protein phosphorylation	434	0.468	1.903	1.00E-10	1.90E-08
BP	GO:0042327	positive regulation of phosphorylation	476	0.458	1.872	1.00E-10	1.90E-08
BP	GO:0048514	blood vessel morphogenesis	444	0.459	1.867	1.00E-10	1.90E-08
BP	GO:0030855	epithelial cell differentiation	465	0.455	1.855	1.00E-10	1.90E-08
CC	GO:0000786	nucleosome	110	-0.6	-2.464	1.00E-10	1.90E-08
MF	GO:0030527	structural constituent of chromatin	80	-0.64	-2.502	1.00E-10	1.90E-08

MF	GO:0048018	receptor ligand activity	217	0.566	2.185	1.00E-10	1.90E-08
MF	GO:0030546	signaling receptor activator activity	222	0.565	2.183	1.00E-10	1.90E-08
MF	GO:0030545	signaling receptor regulator activity	244	0.538	2.101	1.00E-10	1.90E-08
BP	GO:0002573	myeloid leukocyte differentiation	164	0.579	2.168	1.25E-10	2.22E-08
BP	GO:0032496	response to lipopolysaccharide	198	0.554	2.123	1.24E-10	2.22E-08
BP	GO:0042110	T cell activation	352	0.484	1.938	1.34E-10	2.31E-08
BP	GO:0032103	positive regulation of response to external stimulus	384	0.47	1.897	1.44E-10	2.40E-08
BP	GO:0030099	myeloid cell differentiation	325	0.488	1.945	1.76E-10	2.86E-08
BP	GO:0022407	regulation of cell-cell adhesion	305	0.495	1.959	2.46E-10	3.88E-08
BP	GO:0030098	lymphocyte differentiation	278	0.498	1.957	2.81E-10	4.30E-08
BP	GO:0070663	regulation of leukocyte proliferation	152	0.584	2.176	3.35E-10	5.00E-08
BP	GO:0051249	regulation of lymphocyte activation	321	0.479	1.91	4.26E-10	6.19E-08
BP	GO:0051604	protein maturation	442	0.442	1.8	5.01E-10	7.09E-08
BP	GO:0032943	mononuclear cell proliferation	177	0.559	2.116	5.53E-10	7.63E-08
BP	GO:0009611	response to wounding	391	0.467	1.884	5.72E-10	7.70E-08
BP	GO:0009617	response to bacterium	358	0.473	1.897	6.72E-10	8.83E-08
BP	GO:0006935	chemotaxis	286	0.495	1.95	1.02E-09	1.28E-07
BP	GO:0042330	taxis	286	0.495	1.95	1.02E-09	1.28E-07
BP	GO:0046651	lymphocyte proliferation	171	0.552	2.081	1.15E-09	1.41E-07
CC	GO:0005925	focal adhesion	378	0.461	1.856	1.21E-09	1.45E-07
BP	GO:0002683	negative regulation of immune system process	317	0.479	1.906	1.24E-09	1.45E-07
BP	GO:0097191	extrinsic apoptotic signaling pathway	183	0.543	2.057	1.42E-09	1.60E-07
BP	GO:0002250	adaptive immune response	250	0.506	1.978	1.45E-09	1.60E-07
BP	GO:0050867	positive regulation of cell activation	242	0.506	1.976	1.40E-09	1.60E-07
BP	GO:0043408	regulation of MAPK cascade	460	0.433	1.766	1.58E-09	1.71E-07
BP	GO:0050878	regulation of body fluid levels	247	0.502	1.964	1.78E-09	1.89E-07
BP	GO:0032944	regulation of mononuclear cell proliferation	137	0.581	2.136	1.89E-09	1.94E-07
BP	GO:0002460	adaptive immune response based on somatic recombination of immune receptors	181	0.543	2.059	1.90E-09	1.94E-07

		built from immunoglobulin superfamily domains					
BP	GO:1903706	regulation of hemopoiesis	292	0.487	1.925	2.16E-09	2.16E-07
BP	GO:0031347	regulation of defense response	491	0.429	1.757	2.31E-09	2.27E-07
BP	GO:0009991	response to extracellular stimulus	401	0.453	1.83	2.69E-09	2.60E-07
BP	GO:0050778	positive regulation of immune response	444	0.434	1.765	3.30E-09	3.14E-07
BP	GO:0035082	axoneme assembly	77	-0.6	-2.326	3.93E-09	3.67E-07
BP	GO:0006979	response to oxidative stress	321	0.464	1.851	4.84E-09	4.45E-07
BP	GO:0071496	cellular response to external stimulus	278	0.482	1.895	5.32E-09	4.74E-07
CC	GO:0030055	cell-substrate junction	385	0.451	1.82	5.28E-09	4.74E-07
BP	GO:0007159	leukocyte cell-cell adhesion	248	0.498	1.947	5.91E-09	5.18E-07
BP	GO:1902105	regulation of leukocyte differentiation	208	0.52	2.001	6.15E-09	5.30E-07
BP	GO:0050670	regulation of lymphocyte proliferation	134	0.574	2.102	1.07E-08	8.94E-07
BP	GO:0061448	connective tissue development	201	0.52	1.997	1.07E-08	8.94E-07
BP	GO:0045859	regulation of protein kinase activity	396	0.442	1.786	1.29E-08	1.06E-06
BP	GO:0002696	positive regulation of leukocyte activation	233	0.499	1.938	1.39E-08	1.13E-06
BP	GO:0050920	regulation of chemotaxis	158	0.548	2.045	1.59E-08	1.25E-06
BP	GO:0031667	response to nutrient levels	381	0.448	1.802	1.57E-08	1.25E-06
BP	GO:0043410	positive regulation of MAPK cascade	312	0.466	1.848	1.65E-08	1.28E-06
BP	GO:0009266	response to temperature stimulus	137	0.562	2.066	1.84E-08	1.41E-06
BP	GO:0070482	response to oxygen levels	274	0.477	1.88	2.53E-08	1.91E-06
BP	GO:0044703	multi-organism reproductive process	133	0.565	2.065	2.64E-08	1.97E-06
BP	GO:0048771	tissue remodeling	130	0.568	2.068	2.93E-08	2.15E-06
BP	GO:0030308	negative regulation of cell growth	155	0.545	2.036	3.55E-08	2.58E-06
BP	GO:0006457	protein folding	194	0.514	1.961	3.90E-08	2.76E-06
BP	GO:0001666	response to hypoxia	237	0.485	1.892	3.87E-08	2.76E-06
BP	GO:0045667	regulation of osteoblast differentiation	96	0.617	2.178	4.06E-08	2.81E-06
BP	GO:0071396	cellular response to lipid	410	0.435	1.763	4.07E-08	2.81E-06
BP	GO:0001578	microtubule bundle formation	105	-0.52	-2.125	4.34E-08	2.95E-06

BP	GO:1902107	positive regulation of leukocyte differentiation	123	0.568	2.06	5.09E-08	3.39E-06
BP	GO:1903708	positive regulation of hemopoiesis	123	0.568	2.06	5.09E-08	3.39E-06
BP	GO:0036293	response to decreased oxygen levels	252	0.484	1.892	5.19E-08	3.41E-06
BP	GO:0030162	regulation of proteolysis	473	0.42	1.714	5.29E-08	3.43E-06
BP	GO:0044706	multi-multicellular organism process	140	0.553	2.043	5.95E-08	3.82E-06
BP	GO:0042060	wound healing	296	0.463	1.83	6.61E-08	4.19E-06
BP	GO:0046631	alpha-beta T cell activation	102	0.602	2.145	6.88E-08	4.32E-06
BP	GO:0003341	cilium movement	140	-0.48	-2.038	7.10E-08	4.39E-06
BP	GO:0002252	immune effector process	358	0.444	1.783	7.16E-08	4.39E-06
BP	GO:0018212	peptidyl-tyrosine modification	188	0.512	1.948	8.02E-08	4.81E-06
BP	GO:0030217	T cell differentiation	204	0.503	1.935	7.95E-08	4.81E-06
BP	GO:0050730	regulation of peptidyl-tyrosine phosphorylation	151	0.546	2.031	8.29E-08	4.92E-06
BP	GO:0050727	regulation of inflammatory response	221	0.484	1.87	8.77E-08	5.15E-06
BP	GO:0045639	positive regulation of myeloid cell differentiation	74	0.642	2.197	1.11E-07	6.47E-06
BP	GO:1903037	regulation of leukocyte cell-cell adhesion	224	0.491	1.901	1.28E-07	7.35E-06
BP	GO:0007249	canonical NF-kappaB signal transduction	234	0.481	1.871	1.33E-07	7.55E-06
BP	GO:0018108	peptidyl-tyrosine phosphorylation	186	0.511	1.94	1.49E-07	8.38E-06
BP	GO:0002685	regulation of leukocyte migration	141	0.55	2.028	1.56E-07	8.70E-06
BP	GO:0045785	positive regulation of cell adhesion	315	0.449	1.783	1.64E-07	9.07E-06
BP	GO:0034599	cellular response to oxidative stress	207	0.497	1.913	1.67E-07	9.11E-06
BP	GO:0062197	cellular response to chemical stress	258	0.472	1.849	1.73E-07	9.31E-06
CC	GO:0000502	proteasome complex	57	0.679	2.207	1.74E-07	9.31E-06
BP	GO:0007162	negative regulation of cell adhesion	194	0.502	1.915	1.76E-07	9.32E-06
BP	GO:0016049	cell growth	420	0.417	1.688	1.86E-07	9.76E-06
BP	GO:0048545	response to steroid hormone	239	0.471	1.838	2.10E-07	1.10E-05
BP	GO:0043549	regulation of kinase activity	459	0.413	1.684	2.18E-07	1.13E-05
BP	GO:0007565	female pregnancy	123	0.553	2.004	2.53E-07	1.29E-05
BP	GO:0009612	response to mechanical stimulus	160	0.529	1.976	2.58E-07	1.31E-05

BP	GO:0002286	T cell activation involved in immune response	60	0.662	2.167	2.62E-07	1.31E-05
BP	GO:0022409	positive regulation of cell-cell adhesion	194	0.498	1.898	2.71E-07	1.34E-05
CC	GO:1905369	endopeptidase complex	73	0.632	2.166	2.87E-07	1.41E-05
BP	GO:0033002	muscle cell proliferation	147	0.53	1.967	2.99E-07	1.45E-05
BP	GO:0051251	positive regulation of lymphocyte activation	210	0.489	1.887	3.00E-07	1.45E-05
BP	GO:0048872	homeostasis of number of cells	248	0.469	1.835	3.20E-07	1.54E-05
CC	GO:0031983	vesicle lumen	229	0.469	1.822	3.31E-07	1.57E-05
BP	GO:0040008	regulation of growth	482	0.407	1.664	3.84E-07	1.81E-05
BP	GO:0051385	response to mineralocorticoid	24	0.808	2.236	4.10E-07	1.90E-05
BP	GO:0002688	regulation of leukocyte chemotaxis	78	0.618	2.124	4.16E-07	1.90E-05
BP	GO:0050866	negative regulation of cell activation	124	0.545	1.977	4.12E-07	1.90E-05
MF	GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	334	0.432	1.726	4.17E-07	1.90E-05
BP	GO:0071260	cellular response to mechanical stimulus	52	0.678	2.169	4.32E-07	1.95E-05
BP	GO:0061077	chaperone-mediated protein folding	69	0.629	2.118	4.57E-07	2.05E-05
MF	GO:0004888	transmembrane signaling receptor activity	481	0.402	1.642	4.98E-07	2.22E-05
BP	GO:0050673	epithelial cell proliferation	304	0.442	1.752	5.53E-07	2.44E-05
BP	GO:0034605	cellular response to heat	56	0.676	2.191	5.67E-07	2.48E-05
BP	GO:0070664	negative regulation of leukocyte proliferation	49	0.683	2.153	6.00E-07	2.60E-05
BP	GO:0048732	gland development	326	0.437	1.742	6.04E-07	2.60E-05
BP	GO:0007169	transmembrane receptor protein tyrosine kinase signaling pathway	470	0.408	1.665	6.19E-07	2.65E-05
BP	GO:0043122	regulation of canonical NF-kappaB signal transduction	208	0.484	1.863	6.53E-07	2.77E-05
BP	GO:0050863	regulation of T cell activation	234	0.467	1.818	6.67E-07	2.77E-05
CC	GO:0005930	axoneme	125	-0.47	-1.951	6.69E-07	2.77E-05
MF	GO:0005126	cytokine receptor binding	130	0.538	1.959	6.68E-07	2.77E-05
BP	GO:0050679	positive regulation of epithelial cell proliferation	140	0.527	1.944	7.18E-07	2.96E-05

BP	GO:0050729	positive regulation of inflammatory response	75	0.621	2.125	8.13E-07	3.32E-05
BP	GO:0001890	placenta development	114	0.552	1.983	8.21E-07	3.33E-05
BP	GO:0002695	negative regulation of leukocyte activation	110	0.558	2.001	8.42E-07	3.37E-05
BP	GO:0031214	biomineral tissue development	114	0.551	1.981	8.43E-07	3.37E-05
BP	GO:0002687	positive regulation of leukocyte migration	93	0.582	2.044	8.58E-07	3.38E-05
BP	GO:0048659	smooth muscle cell proliferation	105	0.563	2.011	8.58E-07	3.38E-05
BP	GO:0031349	positive regulation of defense response	296	0.446	1.761	8.71E-07	3.41E-05
BP	GO:0051092	positive regulation of NF-kappaB transcription factor activity	107	0.57	2.039	8.80E-07	3.42E-05
CC	GO:0060205	cytoplasmic vesicle lumen	228	0.471	1.824	8.86E-07	3.42E-05
BP	GO:0002274	myeloid leukocyte activation	128	0.541	1.977	9.04E-07	3.44E-05
BP	GO:0032102	negative regulation of response to external stimulus	296	0.445	1.759	9.01E-07	3.44E-05
BP	GO:0035966	response to topologically incorrect protein	148	0.516	1.92	9.50E-07	3.57E-05
BP	GO:0045926	negative regulation of growth	194	0.488	1.861	9.48E-07	3.57E-05
BP	GO:1903039	positive regulation of leukocyte cell-cell adhesion	162	0.511	1.909	1.01E-06	3.75E-05
BP	GO:0045765	regulation of angiogenesis	198	0.484	1.855	1.01E-06	3.75E-05
BP	GO:1901342	regulation of vasculature development	200	0.481	1.847	1.04E-06	3.81E-05
CC	GO:0009897	external side of plasma membrane	182	0.488	1.849	1.10E-06	4.02E-05
BP	GO:1901654	response to ketone	148	0.514	1.912	1.11E-06	4.03E-05
BP	GO:0002690	positive regulation of leukocyte chemotaxis	57	0.659	2.14	1.14E-06	4.10E-05
BP	GO:0002761	regulation of myeloid leukocyte differentiation	84	0.593	2.06	1.18E-06	4.24E-05
CC	GO:0034774	secretory granule lumen	226	0.472	1.825	1.22E-06	4.34E-05
BP	GO:0045637	regulation of myeloid cell differentiation	161	0.51	1.903	1.27E-06	4.50E-05
CC	GO:0035869	ciliary transition zone	68	-0.55	-2.089	1.29E-06	4.53E-05
BP	GO:0032870	cellular response to hormone stimulus	457	0.397	1.616	1.32E-06	4.60E-05
CC	GO:0097014	ciliary plasm	126	-0.46	-1.954	1.33E-06	4.61E-05

BP	GO:0045862	positive regulation of proteolysis	280	0.44	1.732	1.53E-06	5.23E-05
BP	GO:0001667	ameboidal-type cell migration	344	0.421	1.681	1.53E-06	5.23E-05
MF	GO:0001216	DNA-binding transcription activator activity	338	0.428	1.709	1.52E-06	5.23E-05
BP	GO:0072539	T-helper 17 cell differentiation	19	0.823	2.156	1.58E-06	5.34E-05
BP	GO:0072538	T-helper 17 type immune response	30	0.755	2.16	1.63E-06	5.48E-05
BP	GO:0051090	regulation of DNA-binding transcription factor activity	285	0.442	1.74	1.75E-06	5.85E-05
BP	GO:0035265	organ growth	121	0.547	1.981	1.93E-06	6.37E-05
BP	GO:0050817	coagulation	153	0.505	1.884	1.93E-06	6.37E-05
BP	GO:0034103	regulation of tissue remodeling	55	0.648	2.097	2.05E-06	6.72E-05
BP	GO:0032945	negative regulation of mononuclear cell proliferation	45	0.682	2.125	2.15E-06	6.89E-05
BP	GO:0050672	negative regulation of lymphocyte proliferation	45	0.682	2.125	2.15E-06	6.89E-05
BP	GO:0007599	hemostasis	156	0.503	1.876	2.15E-06	6.89E-05
BP	GO:0050900	leukocyte migration	227	0.464	1.797	2.16E-06	6.89E-05
BP	GO:0042692	muscle cell differentiation	304	0.433	1.715	2.16E-06	6.89E-05
BP	GO:0009408	response to heat	84	0.586	2.037	2.31E-06	7.25E-05
BP	GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	104	0.559	1.994	2.32E-06	7.25E-05
BP	GO:0008544	epidermis development	225	0.468	1.81	2.33E-06	7.25E-05
BP	GO:0060537	muscle tissue development	316	0.43	1.711	2.33E-06	7.25E-05
BP	GO:0071216	cellular response to biotic stimulus	147	0.511	1.899	2.34E-06	7.25E-05
BP	GO:0070371	ERK1 and ERK2 cascade	212	0.467	1.803	2.43E-06	7.48E-05
BP	GO:0014812	muscle cell migration	69	0.608	2.048	2.48E-06	7.60E-05
BP	GO:0045860	positive regulation of protein kinase activity	209	0.474	1.824	2.73E-06	8.32E-05
BP	GO:0045732	positive regulation of protein catabolic process	183	0.483	1.83	2.90E-06	8.78E-05
BP	GO:0061512	protein localization to cilium	66	-0.56	-2.094	3.09E-06	9.31E-05
BP	GO:0043086	negative regulation of catalytic activity	354	0.422	1.691	3.22E-06	9.65E-05
BP	GO:0010720	positive regulation of cell development	335	0.42	1.677	3.33E-06	9.94E-05
CC	GO:1904813	ficolin-1-rich granule lumen	109	0.552	1.978	3.41E-06	0.000101

MF	GO:0005125	cytokine activity	86	0.577	2.008	3.56E-06	0.000105
BP	GO:0071356	cellular response to tumor necrosis factor	148	0.502	1.867	3.71E-06	0.000109
BP	GO:0052547	regulation of peptidase activity	210	0.463	1.786	3.78E-06	0.00011
BP	GO:0042176	regulation of protein catabolic process	322	0.422	1.684	3.88E-06	0.000113
BP	GO:0060326	cell chemotaxis	179	0.479	1.816	3.92E-06	0.000113
BP	GO:0007596	blood coagulation	151	0.507	1.887	4.16E-06	0.000119
BP	GO:0032602	chemokine production	58	0.644	2.094	4.20E-06	0.00012
BP	GO:0032642	regulation of chemokine production	58	0.644	2.094	4.20E-06	0.00012
BP	GO:0048729	tissue morphogenesis	467	0.388	1.581	4.26E-06	0.000121
BP	GO:0050921	positive regulation of chemotaxis	93	0.564	1.982	4.30E-06	0.000121
BP	GO:0002287	alpha-beta T cell activation involved in immune response	44	0.669	2.076	4.75E-06	0.00013
BP	GO:0002293	alpha-beta T cell differentiation involved in immune response	44	0.669	2.076	4.75E-06	0.00013
BP	GO:0002294	CD4-positive, alpha-beta T cell differentiation involved in immune response	44	0.669	2.076	4.75E-06	0.00013
BP	GO:0031960	response to corticosteroid	96	0.567	2.002	4.72E-06	0.00013
BP	GO:0062012	regulation of small molecule metabolic process	250	0.447	1.749	4.66E-06	0.00013
CC	GO:0101002	ficolin-1-rich granule	141	0.513	1.893	4.85E-06	0.000133
BP	GO:0002263	cell activation involved in immune response	171	0.473	1.783	4.92E-06	0.000134
BP	GO:0032722	positive regulation of chemokine production	44	0.668	2.074	4.96E-06	0.000134
BP	GO:0006986	response to unfolded protein	126	0.529	1.925	5.12E-06	0.000138
BP	GO:0071674	mononuclear cell migration	107	0.551	1.969	5.21E-06	0.000139
BP	GO:0045444	fat cell differentiation	185	0.474	1.799	5.22E-06	0.000139
BP	GO:0009896	positive regulation of catabolic process	465	0.389	1.587	5.24E-06	0.000139
MF	GO:0051082	unfolded protein binding	104	0.551	1.964	5.32E-06	0.00014
BP	GO:0032620	interleukin-17 production	25	0.781	2.181	5.46E-06	0.000143
BP	GO:0032660	regulation of interleukin-17 production	25	0.781	2.181	5.46E-06	0.000143
BP	GO:0001558	regulation of cell growth	351	0.413	1.655	5.58E-06	0.000145
BP	GO:0097696	receptor signaling pathway via STAT	103	0.545	1.939	5.60E-06	0.000145

BP	GO:0048660	regulation of smooth muscle cell proliferation	102	0.554	1.975	5.64E-06	0.000145
BP	GO:0048762	mesenchymal cell differentiation	200	0.464	1.78	5.77E-06	0.000148
BP	GO:0046632	alpha-beta T cell differentiation	75	0.597	2.041	5.91E-06	0.000151
CC	GO:0015629	actin cytoskeleton	408	0.393	1.592	6.18E-06	0.000157
BP	GO:1901652	response to peptide	385	0.404	1.632	6.29E-06	0.000159
BP	GO:0071219	cellular response to molecule of bacterial origin	123	0.518	1.879	7.02E-06	0.000176
BP	GO:0034612	response to tumor necrosis factor	165	0.491	1.844	7.02E-06	0.000176
BP	GO:0051250	negative regulation of lymphocyte activation	94	0.565	1.99	7.10E-06	0.000177
BP	GO:0048661	positive regulation of smooth muscle cell proliferation	58	0.637	2.073	7.64E-06	0.00019
BP	GO:0001912	positive regulation of leukocyte mediated cytotoxicity	28	0.746	2.105	8.14E-06	0.0002
BP	GO:0045621	positive regulation of lymphocyte differentiation	84	0.568	1.972	8.09E-06	0.0002
BP	GO:0070374	positive regulation of ERK1 and ERK2 cascade	122	0.528	1.914	8.19E-06	0.0002
BP	GO:0001763	morphogenesis of a branching structure	136	0.507	1.859	8.23E-06	0.0002
BP	GO:0071417	cellular response to organonitrogen compound	495	0.384	1.571	8.24E-06	0.0002
MF	GO:0140416	transcription regulator inhibitor activity	17	0.832	2.139	8.43E-06	0.000204
MF	GO:0019955	cytokine binding	90	0.556	1.941	8.73E-06	0.00021
BP	GO:0001909	leukocyte mediated cytotoxicity	60	0.62	2.031	8.79E-06	0.000211
BP	GO:0002449	lymphocyte mediated immunity	162	0.49	1.83	8.91E-06	0.000213
BP	GO:0140467	integrated stress response signaling	36	0.697	2.08	9.05E-06	0.000215
BP	GO:0006775	fat-soluble vitamin metabolic process	25	0.772	2.157	9.64E-06	0.000227
BP	GO:0002819	regulation of adaptive immune response	119	0.52	1.88	9.61E-06	0.000227
BP	GO:0002253	activation of immune response	337	0.413	1.649	9.69E-06	0.000227
BP	GO:0002443	leukocyte mediated immunity	220	0.449	1.732	9.80E-06	0.000229
BP	GO:0044409	entry into host	103	0.539	1.918	9.96E-06	0.000232
BP	GO:0043368	positive T cell selection	20	0.81	2.168	1.07E-05	0.000249
BP	GO:0030316	osteoclast	75	0.587	2.008	1.17E-05	0.000269

		differentiation					
BP	GO:0060485	mesenchyme development	242	0.442	1.724	1.17E-05	0.000269
BP	GO:0002009	morphogenesis of an epithelium	392	0.396	1.6	1.17E-05	0.000269
MF	GO:0061135	endopeptidase regulator activity	80	0.565	1.947	1.19E-05	0.000272
BP	GO:0061138	morphogenesis of a branching epithelium	127	0.511	1.865	1.20E-05	0.000273
BP	GO:0072001	renal system development	238	0.437	1.704	1.23E-05	0.000277
BP	GO:0032612	interleukin-1 production	63	0.613	2.02	1.25E-05	0.000279
BP	GO:0032652	regulation of interleukin-1 production	63	0.613	2.02	1.25E-05	0.000279
BP	GO:2001233	regulation of apoptotic signaling pathway	316	0.415	1.65	1.24E-05	0.000279
BP	GO:0043588	skin development	186	0.471	1.786	1.25E-05	0.000279
BP	GO:0002262	myeloid cell homeostasis	141	0.504	1.86	1.33E-05	0.000295
BP	GO:0043534	blood vessel endothelial cell migration	94	0.556	1.957	1.37E-05	0.000301
BP	GO:0097305	response to alcohol	181	0.468	1.775	1.37E-05	0.000301
BP	GO:1904019	epithelial cell apoptotic process	96	0.553	1.952	1.38E-05	0.000302
BP	GO:0014909	smooth muscle cell migration	59	0.623	2.028	1.40E-05	0.000306
MF	GO:0008083	growth factor activity	92	0.554	1.938	1.42E-05	0.000308
BP	GO:0003179	heart valve morphogenesis	52	0.63	2.016	1.46E-05	0.000315
BP	GO:0033674	positive regulation of kinase activity	249	0.439	1.718	1.46E-05	0.000315
BP	GO:0030216	keratinocyte differentiation	82	0.566	1.953	1.51E-05	0.000325
BP	GO:0010721	negative regulation of cell development	209	0.455	1.752	1.65E-05	0.000353
BP	GO:0046718	viral entry into host cell	97	0.541	1.911	1.68E-05	0.000357
BP	GO:0001910	regulation of leukocyte mediated cytotoxicity	38	0.679	2.054	1.72E-05	0.000365
BP	GO:0007259	receptor signaling pathway via JAK-STAT	97	0.54	1.908	1.77E-05	0.000374
BP	GO:0022408	negative regulation of cell-cell adhesion	117	0.519	1.865	1.78E-05	0.000376
BP	GO:0030225	macrophage differentiation	44	0.65	2.016	1.79E-05	0.000376
BP	GO:0030856	regulation of epithelial cell differentiation	105	0.524	1.871	1.81E-05	0.000376
BP	GO:0061458	reproductive system development	212	0.449	1.732	1.81E-05	0.000376

BP	GO:0032350	regulation of hormone metabolic process	28	0.731	2.065	1.84E-05	0.00038
BP	GO:0090130	tissue migration	241	0.436	1.7	1.84E-05	0.00038
MF	GO:0050839	cell adhesion molecule binding	472	0.379	1.548	1.90E-05	0.000391
BP	GO:0003300	cardiac muscle hypertrophy	66	0.6	1.999	1.95E-05	0.000398
BP	GO:0002699	positive regulation of immune effector process	149	0.496	1.844	1.95E-05	0.000398
MF	GO:0002020	protease binding	109	0.532	1.908	1.95E-05	0.000398
BP	GO:0019216	regulation of lipid metabolic process	240	0.444	1.734	1.97E-05	0.000399
BP	GO:0031668	cellular response to extracellular stimulus	234	0.438	1.704	1.99E-05	0.000402
BP	GO:0070372	regulation of ERK1 and ERK2 cascade	193	0.459	1.745	2.00E-05	0.000403
BP	GO:0050678	regulation of epithelial cell proliferation	251	0.432	1.689	2.08E-05	0.000417
BP	GO:0071887	leukocyte apoptotic process	84	0.556	1.93	2.12E-05	0.000424
BP	GO:0051085	chaperone cofactor-dependent protein refolding	30	0.72	2.06	2.13E-05	0.000425
BP	GO:0051146	striated muscle cell differentiation	220	0.441	1.702	2.20E-05	0.000437
BP	GO:0042093	T-helper cell differentiation	43	0.665	2.049	2.28E-05	0.000448
BP	GO:0002366	leukocyte activation involved in immune response	168	0.469	1.76	2.28E-05	0.000448
BP	GO:0007178	transmembrane receptor protein serine/threonine kinase signaling pathway	287	0.423	1.668	2.27E-05	0.000448
BP	GO:0045619	regulation of lymphocyte differentiation	135	0.503	1.842	2.34E-05	0.000458
MF	GO:0140678	molecular function inhibitor activity	335	0.4	1.595	2.36E-05	0.00046
BP	GO:0031638	zymogen activation	39	0.676	2.044	2.37E-05	0.00046
BP	GO:0008625	extrinsic apoptotic signaling pathway via death domain receptors	71	0.583	1.987	2.53E-05	0.000489
BP	GO:0001837	epithelial to mesenchymal transition	132	0.498	1.817	2.55E-05	0.000492
BP	GO:0042098	T cell proliferation	123	0.502	1.82	2.58E-05	0.000496
BP	GO:0071453	cellular response to oxygen levels	138	0.493	1.815	2.61E-05	0.000499
BP	GO:0002697	regulation of immune effector process	209	0.451	1.736	2.62E-05	0.000499
BP	GO:0032611	interleukin-1 beta production	55	0.619	2.002	2.64E-05	0.000501

BP	GO:0032651	regulation of interleukin-1 beta production	55	0.619	2.002	2.64E-05	0.000501
MF	GO:0045296	cadherin binding	306	0.417	1.653	2.67E-05	0.000505
BP	GO:1905314	semi-lunar valve development	40	0.668	2.027	2.70E-05	0.000506
BP	GO:0042129	regulation of T cell proliferation	98	0.526	1.859	2.70E-05	0.000506
BP	GO:0050870	positive regulation of T cell activation	150	0.482	1.794	2.82E-05	0.000528
BP	GO:0045670	regulation of osteoclast differentiation	45	0.648	2.019	2.92E-05	0.000544
BP	GO:0009913	epidermal cell differentiation	128	0.504	1.841	2.93E-05	0.000544
BP	GO:0001916	positive regulation of T cell mediated cytotoxicity	19	0.782	2.049	3.00E-05	0.000555
BP	GO:2001236	regulation of extrinsic apoptotic signaling pathway	125	0.504	1.833	3.08E-05	0.000568
BP	GO:0030101	natural killer cell activation	44	0.642	1.992	3.14E-05	0.000578
BP	GO:0051091	positive regulation of DNA-binding transcription factor activity	175	0.469	1.773	3.31E-05	0.000606
BP	GO:0035710	CD4-positive, alpha-beta T cell activation	72	0.579	1.973	3.39E-05	0.000613
BP	GO:0030595	leukocyte chemotaxis	128	0.502	1.835	3.37E-05	0.000613
BP	GO:0048608	reproductive structure development	209	0.447	1.722	3.38E-05	0.000613
BP	GO:0052548	regulation of endopeptidase activity	191	0.451	1.718	3.39E-05	0.000613
BP	GO:0036294	cellular response to decreased oxygen levels	126	0.505	1.837	3.46E-05	0.000623
BP	GO:0042445	hormone metabolic process	144	0.489	1.814	3.47E-05	0.000624
MF	GO:0061134	peptidase regulator activity	113	0.512	1.846	3.67E-05	0.000658
BP	GO:0043367	CD4-positive, alpha-beta T cell differentiation	57	0.615	1.997	3.73E-05	0.000666
BP	GO:0003170	heart valve development	62	0.602	1.979	3.78E-05	0.000673
BP	GO:0002292	T cell differentiation involved in immune response	47	0.642	2.01	3.88E-05	0.000683
BP	GO:0046634	regulation of alpha-beta T cell activation	63	0.595	1.959	3.86E-05	0.000683
MF	GO:0004896	cytokine receptor activity	50	0.626	1.979	3.88E-05	0.000683
BP	GO:0042130	negative regulation of T cell proliferation	37	0.668	2.006	4.03E-05	0.000704

BP	GO:0014897	striated muscle hypertrophy	67	0.579	1.941	4.05E-05	0.000704
BP	GO:0070167	regulation of biomineral tissue development	69	0.573	1.931	4.05E-05	0.000704
BP	GO:0071456	cellular response to hypoxia	116	0.511	1.836	4.07E-05	0.000704
BP	GO:0001822	kidney development	233	0.43	1.673	4.06E-05	0.000704
BP	GO:0002831	regulation of response to biotic stimulus	335	0.394	1.573	4.04E-05	0.000704
BP	GO:0045646	regulation of erythrocyte differentiation	38	0.667	2.017	4.22E-05	0.000728
CC	GO:0098552	side of membrane	424	0.382	1.549	4.31E-05	0.000742
BP	GO:0071675	regulation of mononuclear cell migration	77	0.566	1.939	4.38E-05	0.00075
BP	GO:0000302	response to reactive oxygen species	160	0.48	1.793	4.43E-05	0.000755
BP	GO:0043123	positive regulation of canonical NF-kappaB signal transduction	154	0.474	1.765	4.43E-05	0.000755
BP	GO:0002703	regulation of leukocyte mediated immunity	131	0.485	1.767	4.55E-05	0.000773
BP	GO:0002822	regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	107	0.522	1.865	4.60E-05	0.000779
BP	GO:0014706	striated muscle tissue development	189	0.453	1.724	4.75E-05	0.000801
BP	GO:0007507	heart development	480	0.373	1.526	4.80E-05	0.000808
BP	GO:0006458	'de novo' protein folding	39	0.665	2.011	4.85E-05	0.000813
MF	GO:0044183	protein folding chaperone	57	0.61	1.982	4.87E-05	0.000815
BP	GO:0051345	positive regulation of hydrolase activity	358	0.392	1.575	4.89E-05	0.000815
BP	GO:0090494	dopamine uptake	10	-0.86	-2.081	4.91E-05	0.000816
BP	GO:0048638	regulation of developmental growth	248	0.419	1.64	4.97E-05	0.000823
BP	GO:0010565	regulation of cellular ketone metabolic process	99	0.524	1.856	5.08E-05	0.000839
BP	GO:0006816	calcium ion transport	312	0.404	1.602	5.30E-05	0.000872
BP	GO:0019882	antigen processing and presentation	74	0.568	1.941	5.41E-05	0.000889
BP	GO:0043434	response to peptide hormone	327	0.398	1.588	5.56E-05	0.00091
CC	GO:1905368	peptidase complex	106	0.516	1.841	5.59E-05	0.000912
BP	GO:0006334	nucleosome assembly	98	-0.45	-1.818	5.62E-05	0.000915

BP	GO:0002763	positive regulation of myeloid leukocyte differentiation	39	0.66	1.996	5.96E-05	0.000967
BP	GO:0002757	immune response-activating signaling pathway	300	0.409	1.618	6.05E-05	0.000976
MF	GO:0140375	immune receptor activity	53	0.623	1.991	6.04E-05	0.000976
BP	GO:0030279	negative regulation of ossification	24	0.733	2.027	6.08E-05	0.000977
BP	GO:0042100	B cell proliferation	50	0.618	1.954	6.17E-05	0.00098
BP	GO:0044458	motile cilium assembly	57	-0.52	-1.929	6.15E-05	0.00098
BP	GO:0031589	cell-substrate adhesion	272	0.416	1.633	6.14E-05	0.00098
MF	GO:0005179	hormone activity	48	0.632	1.98	6.18E-05	0.00098
MF	GO:0031625	ubiquitin protein ligase binding	287	0.413	1.627	6.17E-05	0.00098
BP	GO:0003176	aortic valve development	37	0.661	1.985	6.21E-05	0.000982
BP	GO:0070665	positive regulation of leukocyte proliferation	95	0.539	1.9	6.31E-05	0.000991
BP	GO:0048738	cardiac muscle tissue development	175	0.463	1.751	6.31E-05	0.000991
BP	GO:0019883	antigen processing and presentation of endogenous antigen	17	0.799	2.054	6.37E-05	0.000999
BP	GO:0031100	animal organ regeneration	45	0.632	1.97	6.45E-05	0.001006
BP	GO:0071222	cellular response to lipopolysaccharide	118	0.509	1.831	6.45E-05	0.001006
BP	GO:0007566	embryo implantation	41	0.646	1.969	6.50E-05	0.001009
BP	GO:0007129	homologous chromosome pairing at meiosis	33	-0.63	-2.057	6.56E-05	0.001017
BP	GO:0045668	negative regulation of osteoblast differentiation	39	0.657	1.987	6.65E-05	0.001017
BP	GO:0032609	type II interferon production	58	0.604	1.967	6.61E-05	0.001017
BP	GO:0032649	regulation of type II interferon production	58	0.604	1.967	6.61E-05	0.001017
BP	GO:0032635	interleukin-6 production	99	0.52	1.843	6.66E-05	0.001017
BP	GO:0032675	regulation of interleukin-6 production	99	0.52	1.843	6.66E-05	0.001017
BP	GO:0045598	regulation of fat cell differentiation	107	0.514	1.839	6.70E-05	0.001018
BP	GO:0051403	stress-activated MAPK cascade	182	0.443	1.679	6.70E-05	0.001018
MF	GO:0140662	ATP-dependent protein folding chaperone	33	0.69	2.036	6.96E-05	0.001054
BP	GO:0042026	protein refolding	22	0.756	2.046	7.00E-05	0.001058
BP	GO:0001959	regulation of cytokine-	113	0.503	1.811	7.09E-05	0.001068

		mediated signaling pathway					
BP	GO:0060560	developmental growth involved in morphogenesis	213	0.433	1.674	7.12E-05	0.001071
BP	GO:0045648	positive regulation of erythrocyte differentiation	27	0.717	2.025	7.27E-05	0.001085
BP	GO:1904035	regulation of epithelial cell apoptotic process	71	0.565	1.926	7.28E-05	0.001085
BP	GO:0048598	embryonic morphogenesis	449	0.375	1.527	7.24E-05	0.001085
BP	GO:0031669	cellular response to nutrient levels	214	0.436	1.684	7.40E-05	0.0011
BP	GO:0043030	regulation of macrophage activation	31	0.692	2.006	7.45E-05	0.001104
BP	GO:0001818	negative regulation of cytokine production	175	0.461	1.745	7.61E-05	0.001125
MF	GO:0042605	peptide antigen binding	14	0.821	1.993	7.66E-05	0.001129
BP	GO:0022612	gland morphogenesis	93	0.522	1.833	7.86E-05	0.001156
BP	GO:0030278	regulation of ossification	88	0.529	1.844	7.93E-05	0.001164
BP	GO:0071407	cellular response to organic cyclic compound	419	0.382	1.546	7.99E-05	0.001169
BP	GO:0045058	T cell selection	29	0.719	2.042	8.11E-05	0.001184
BP	GO:0032946	positive regulation of mononuclear cell proliferation	85	0.549	1.907	8.28E-05	0.001201
MF	GO:0004857	enzyme inhibitor activity	253	0.412	1.614	8.25E-05	0.001201
BP	GO:0042073	intraciliary transport	44	-0.57	-1.985	8.37E-05	0.001212
BP	GO:0046683	response to organophosphorus	89	0.534	1.865	8.50E-05	0.001225
BP	GO:0044000	movement in host	126	0.494	1.795	8.50E-05	0.001225
BP	GO:0031098	stress-activated protein kinase signaling cascade	189	0.448	1.707	8.59E-05	0.001233
BP	GO:0002764	immune response-regulating signaling pathway	311	0.401	1.591	8.84E-05	0.001267
BP	GO:0050671	positive regulation of lymphocyte proliferation	82	0.538	1.857	8.99E-05	0.001282
MF	GO:0044389	ubiquitin-like protein ligase binding	305	0.409	1.619	8.99E-05	0.001282
CC	GO:0022626	cytosolic ribosome	110	0.502	1.803	9.21E-05	0.00131
BP	GO:0030282	bone mineralization	86	0.532	1.851	9.35E-05	0.001322
BP	GO:1901653	cellular response to peptide	278	0.402	1.58	9.34E-05	0.001322
BP	GO:0001936	regulation of endothelial cell proliferation	93	0.519	1.821	9.38E-05	0.001324

BP	GO:2001234	negative regulation of apoptotic signaling pathway	197	0.437	1.674	9.58E-05	0.001348
BP	GO:0001776	leukocyte homeostasis	82	0.536	1.85	9.77E-05	0.001372
BP	GO:0048863	stem cell differentiation	192	0.445	1.693	9.85E-05	0.001379
BP	GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	25	0.736	2.056	9.91E-05	0.001384
BP	GO:0010631	epithelial cell migration	240	0.429	1.674	0.0001	0.001396
BP	GO:0090132	epithelium migration	240	0.429	1.674	0.0001	0.001396
BP	GO:0062014	negative regulation of small molecule metabolic process	80	0.538	1.853	0.000102	0.001408
BP	GO:0032872	regulation of stress-activated MAPK cascade	143	0.469	1.736	0.000103	0.001429
BP	GO:0032436	positive regulation of proteasomal ubiquitin-dependent protein catabolic process	84	0.531	1.844	0.000104	0.001438
BP	GO:0001659	temperature homeostasis	138	0.476	1.752	0.000105	0.00145
BP	GO:0002285	lymphocyte activation involved in immune response	120	0.497	1.8	0.000106	0.001458
BP	GO:0010611	regulation of cardiac muscle hypertrophy	43	0.641	1.976	0.000108	0.001463
BP	GO:0032640	tumor necrosis factor production	104	0.51	1.817	0.000108	0.001463
BP	GO:0032680	regulation of tumor necrosis factor production	104	0.51	1.817	0.000108	0.001463
BP	GO:0046890	regulation of lipid biosynthetic process	133	0.477	1.741	0.000107	0.001463
BP	GO:0048568	embryonic organ development	326	0.391	1.561	0.000107	0.001463
BP	GO:0051347	positive regulation of transferase activity	317	0.393	1.564	0.000108	0.001466
BP	GO:0002011	morphogenesis of an epithelial sheet	50	0.612	1.933	0.00011	0.001485
BP	GO:0071559	response to transforming growth factor beta	212	0.428	1.652	0.00011	0.001485
BP	GO:0051084	'de novo' post-translational protein folding	35	0.676	2.005	0.000113	0.001519
BP	GO:0071560	cellular response to transforming growth factor beta stimulus	207	0.433	1.668	0.000113	0.001519
CC	GO:0005838	proteasome regulatory particle	19	0.759	1.987	0.000115	0.001542

BP	GO:0002062	chondrocyte differentiation	87	0.529	1.841	0.000124	0.001645
BP	GO:0071706	tumor necrosis factor superfamily cytokine production	107	0.504	1.801	0.000124	0.001645
BP	GO:1903555	regulation of tumor necrosis factor superfamily cytokine production	107	0.504	1.801	0.000124	0.001645
BP	GO:0002429	immune response-activating cell surface receptor signaling pathway	184	0.443	1.675	0.000124	0.001647
BP	GO:0035270	endocrine system development	90	0.525	1.833	0.000126	0.001657
BP	GO:0048754	branching morphogenesis of an epithelial tube	106	0.504	1.798	0.000127	0.001677
BP	GO:2001237	negative regulation of extrinsic apoptotic signaling pathway	77	0.55	1.885	0.000129	0.001698
BP	GO:0042509	regulation of tyrosine phosphorylation of STAT protein	41	0.634	1.932	0.000132	0.001734
BP	GO:0002428	antigen processing and presentation of peptide antigen via MHC class Ib	10	0.864	1.941	0.000133	0.001734
BP	GO:0002064	epithelial cell development	158	0.455	1.697	0.000133	0.001734
BP	GO:0001933	negative regulation of protein phosphorylation	248	0.409	1.6	0.000133	0.001734
BP	GO:0031343	positive regulation of cell killing	30	0.687	1.966	0.000136	0.001763
BP	GO:0002548	monocyte chemotaxis	20	0.761	2.039	0.00014	0.001802
CC	GO:0016234	inclusion body	63	0.572	1.885	0.000139	0.001802
BP	GO:0002711	positive regulation of T cell mediated immunity	36	0.651	1.942	0.000142	0.001832
CC	GO:0036038	MKS complex	13	-0.79	-2.085	0.000142	0.001832
BP	GO:0045669	positive regulation of osteoblast differentiation	47	0.622	1.949	0.000145	0.001855
BP	GO:0014896	muscle hypertrophy	68	0.561	1.887	0.000146	0.001866
BP	GO:0032757	positive regulation of interleukin-8 production	39	0.638	1.93	0.000151	0.001926
CC	GO:0043235	receptor complex	271	0.407	1.6	0.000151	0.001927
MF	GO:0004930	G protein-coupled receptor activity	236	0.41	1.597	0.000156	0.001981
BP	GO:0030879	mammary gland development	102	0.509	1.816	0.000158	0.002001
BP	GO:0045766	positive regulation of angiogenesis	115	0.496	1.784	0.00016	0.002025

BP	GO:1904018	positive regulation of vasculature development	115	0.496	1.784	0.00016	0.002025
BP	GO:0046635	positive regulation of alpha-beta T cell activation	40	0.638	1.935	0.000163	0.002052
BP	GO:0045582	positive regulation of T cell differentiation	74	0.552	1.886	0.000164	0.002066
BP	GO:0030323	respiratory tube development	141	0.472	1.741	0.000168	0.002106
BP	GO:1903038	negative regulation of leukocyte cell-cell adhesion	79	0.536	1.847	0.000169	0.002118
BP	GO:0070192	chromosome organization involved in meiotic cell cycle	45	-0.56	-1.943	0.000173	0.00216
BP	GO:0042113	B cell activation	185	0.437	1.656	0.000174	0.002167
BP	GO:0070059	intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress	58	0.589	1.917	0.000178	0.002206
BP	GO:0060759	regulation of response to cytokine stimulus	121	0.483	1.75	0.000178	0.002206
CC	GO:0036064	ciliary basal body	156	-0.37	-1.617	0.000179	0.002219
BP	GO:0014074	response to purine-containing compound	102	0.508	1.809	0.000182	0.00224
BP	GO:0070302	regulation of stress-activated protein kinase signaling cascade	146	0.466	1.733	0.000182	0.00224
BP	GO:0002227	innate immune response in mucosa	12	-0.8	-2.048	0.000182	0.00224
BP	GO:0043370	regulation of CD4-positive, alpha-beta T cell differentiation	31	0.677	1.963	0.000185	0.002245
BP	GO:0032637	interleukin-8 production	49	0.603	1.901	0.000184	0.002245
MF	GO:0003823	antigen binding	25	0.723	2.02	0.000185	0.002245
MF	GO:0001618	virus receptor activity	50	0.603	1.905	0.000184	0.002245
MF	GO:0140272	exogenous protein binding	50	0.603	1.905	0.000184	0.002245
BP	GO:1903034	regulation of response to wounding	114	0.483	1.736	0.000185	0.002247
BP	GO:0007260	tyrosine phosphorylation of STAT protein	44	0.613	1.902	0.00019	0.002299
BP	GO:0043369	CD4-positive or CD8-positive, alpha-beta T cell lineage commitment	14	0.804	1.951	0.000191	0.002307
BP	GO:0002483	antigen processing and presentation of endogenous peptide antigen	15	0.804	1.989	0.000196	0.002352

BP	GO:0019885	antigen processing and presentation of endogenous peptide antigen via MHC class I	15	0.804	1.989	0.000196	0.002352
BP	GO:2000514	regulation of CD4-positive, alpha-beta T cell activation	44	0.613	1.901	0.0002	0.002393
BP	GO:0016485	protein processing	179	0.436	1.651	0.0002	0.002393
CC	GO:0031514	motile cilium	182	-0.35	-1.554	0.0002	0.002393
BP	GO:0042542	response to hydrogen peroxide	80	0.526	1.812	0.000201	0.002395
BP	GO:0031341	regulation of cell killing	42	0.623	1.91	0.000202	0.002399
BP	GO:0014743	regulation of muscle hypertrophy	44	0.612	1.897	0.000207	0.002452
MF	GO:0070851	growth factor receptor binding	81	0.54	1.86	0.000207	0.002452
BP	GO:0007160	cell-matrix adhesion	179	0.435	1.649	0.000209	0.00246
BP	GO:0031330	negative regulation of cellular catabolic process	172	0.437	1.647	0.000209	0.00246
BP	GO:0051384	response to glucocorticoid	81	0.539	1.856	0.00021	0.002473
BP	GO:0045132	meiotic chromosome segregation	66	-0.49	-1.841	0.000211	0.002474
BP	GO:0030888	regulation of B cell proliferation	39	0.63	1.906	0.000213	0.002496
BP	GO:0006469	negative regulation of protein kinase activity	147	0.452	1.677	0.000215	0.002511
BP	GO:0060541	respiratory system development	158	0.451	1.681	0.000217	0.002528
BP	GO:0045936	negative regulation of phosphate metabolic process	310	0.39	1.548	0.000218	0.002542
BP	GO:0030324	lung development	138	0.466	1.716	0.000221	0.002561
BP	GO:0002363	alpha-beta T cell lineage commitment	14	0.801	1.945	0.000223	0.002579
BP	GO:1901800	positive regulation of proteasomal protein catabolic process	104	0.498	1.776	0.000223	0.002579
BP	GO:0001935	endothelial cell proliferation	110	0.492	1.766	0.000224	0.002588
BP	GO:0002475	antigen processing and presentation via MHC class Ib	12	0.827	1.932	0.000228	0.002623
BP	GO:0033209	tumor necrosis factor-mediated signaling pathway	83	0.519	1.798	0.000229	0.00263
BP	GO:0032677	regulation of interleukin-8 production	48	0.609	1.908	0.000236	0.002703
BP	GO:0050922	negative regulation of chemotaxis	51	0.595	1.889	0.00024	0.002744

BP	GO:0043535	regulation of blood vessel endothelial cell migration	71	0.542	1.848	0.00024	0.002744
BP	GO:0001938	positive regulation of endothelial cell proliferation	68	0.554	1.861	0.000241	0.002749
BP	GO:0002042	cell migration involved in sprouting angiogenesis	43	0.626	1.931	0.000244	0.002774
BP	GO:0060294	cilium movement involved in cell motility	104	-0.41	-1.698	0.000247	0.002801
BP	GO:0090329	regulation of DNA-templated DNA replication	42	-0.57	-1.946	0.000248	0.002807
BP	GO:0051701	biological process involved in interaction with host	140	0.466	1.719	0.00025	0.002823
BP	GO:0072012	glomerulus vasculature development	20	0.751	2.01	0.00025	0.002824
BP	GO:0033032	regulation of myeloid cell apoptotic process	22	0.731	1.979	0.000252	0.002842
BP	GO:0042326	negative regulation of phosphorylation	269	0.4	1.571	0.000262	0.002943
BP	GO:0002931	response to ischemia	50	0.597	1.887	0.000263	0.00295
BP	GO:1903306	negative regulation of regulated secretory pathway	16	0.771	1.949	0.000265	0.002964
BP	GO:0002065	columnar/cuboidal epithelial cell differentiation	83	0.517	1.792	0.000266	0.002973
BP	GO:0010817	regulation of hormone levels	350	0.383	1.531	0.00027	0.003009
BP	GO:0001913	T cell mediated cytotoxicity	28	0.68	1.921	0.000274	0.003049
BP	GO:0032740	positive regulation of interleukin-17 production	16	0.769	1.945	0.000276	0.003062
BP	GO:0071375	cellular response to peptide hormone stimulus	235	0.406	1.581	0.000276	0.003062
BP	GO:0031400	negative regulation of protein modification process	370	0.375	1.503	0.000279	0.003075
MF	GO:0051015	actin filament binding	178	0.429	1.626	0.000279	0.003075
BP	GO:0003351	epithelial cilium movement involved in extracellular fluid movement	37	-0.57	-1.925	0.000282	0.003102
BP	GO:0045580	regulation of T cell differentiation	116	0.484	1.738	0.000282	0.003102
BP	GO:0046425	regulation of receptor signaling pathway via JAK-STAT	57	0.579	1.879	0.000283	0.003102

BP	GO:0014910	regulation of smooth muscle cell migration	53	0.596	1.905	0.000285	0.003119
BP	GO:0032732	positive regulation of interleukin-1 production	41	0.619	1.886	0.000287	0.003126
BP	GO:0034341	response to type II interferon	80	0.52	1.79	0.000286	0.003126
BP	GO:0043372	positive regulation of CD4-positive, alpha-beta T cell differentiation	18	0.754	1.965	0.00029	0.003155
BP	GO:0042180	cellular ketone metabolic process	157	0.447	1.665	0.000294	0.003197
BP	GO:0002755	MyD88-dependent toll-like receptor signaling pathway	15	0.792	1.959	0.000295	0.0032
BP	GO:2000516	positive regulation of CD4-positive, alpha-beta T cell activation	23	0.707	1.936	0.000296	0.0032
BP	GO:0045165	cell fate commitment	171	0.425	1.604	0.000297	0.003205
BP	GO:0050868	negative regulation of T cell activation	73	0.536	1.836	0.000309	0.003325
BP	GO:0046637	regulation of alpha-beta T cell differentiation	41	0.617	1.88	0.000311	0.003326
BP	GO:0001539	cilium or flagellum-dependent cell motility	109	-0.41	-1.712	0.000311	0.003326
BP	GO:0060285	cilium-dependent cell motility	109	-0.41	-1.712	0.000311	0.003326
CC	GO:0022624	proteasome accessory complex	23	0.704	1.93	0.000311	0.003326
BP	GO:0002833	positive regulation of response to biotic stimulus	237	0.402	1.568	0.000312	0.003327
BP	GO:0002063	chondrocyte development	27	0.688	1.944	0.000319	0.0034
BP	GO:0061437	renal system vasculature development	22	0.727	1.969	0.000324	0.003443
BP	GO:0061440	kidney vasculature development	22	0.727	1.969	0.000324	0.003443
BP	GO:0048146	positive regulation of fibroblast proliferation	42	0.614	1.882	0.000326	0.003445
BP	GO:0010563	negative regulation of phosphorus metabolic process	311	0.39	1.546	0.000326	0.003445
BP	GO:0070841	inclusion body assembly	21	0.735	1.987	0.000327	0.003446
BP	GO:0034109	homotypic cell-cell adhesion	69	0.541	1.821	0.000329	0.00346
BP	GO:0030903	notochord development	14	0.795	1.93	0.000333	0.003499
BP	GO:0003180	aortic valve morphogenesis	32	0.663	1.936	0.000337	0.003523
BP	GO:0072006	nephron development	114	0.474	1.702	0.000336	0.003523

BP	GO:2000628	regulation of miRNA metabolic process	70	0.542	1.832	0.000341	0.003564
CC	GO:0098858	actin-based cell projection	181	0.435	1.647	0.000343	0.003572
BP	GO:0010035	response to inorganic substance	395	0.368	1.488	0.000344	0.003582
BP	GO:0001780	neutrophil homeostasis	15	0.788	1.949	0.000354	0.003679
BP	GO:0045137	development of primary sexual characteristics	166	0.434	1.627	0.000356	0.003695
BP	GO:0002385	mucosal immune response	15	-0.74	-2.04	0.000358	0.003702
CC	GO:0043292	contractile fiber	178	0.426	1.617	0.000367	0.003789
BP	GO:0034728	nucleosome organization	116	-0.4	-1.648	0.000368	0.003796
BP	GO:0002228	natural killer cell mediated immunity	35	0.654	1.941	0.000372	0.003826
BP	GO:0042267	natural killer cell mediated cytotoxicity	35	0.654	1.941	0.000372	0.003826
BP	GO:0045103	intermediate filament-based process	44	0.599	1.859	0.000382	0.003916
CC	GO:0072562	blood microparticle	51	0.584	1.854	0.000394	0.004031
BP	GO:0010586	miRNA metabolic process	89	0.508	1.774	0.000405	0.004142
BP	GO:0006909	phagocytosis	160	0.446	1.669	0.000411	0.004189
BP	GO:0035051	cardiocyte differentiation	119	0.47	1.698	0.000417	0.00424
BP	GO:0045088	regulation of innate immune response	282	0.386	1.521	0.000417	0.00424
BP	GO:0030502	negative regulation of bone mineralization	10	0.84	1.885	0.000431	0.00436
BP	GO:0035904	aorta development	49	0.586	1.847	0.000431	0.00436
BP	GO:1903510	mucopolysaccharide metabolic process	72	0.533	1.819	0.000431	0.00436
BP	GO:2000319	regulation of T-helper 17 cell differentiation	12	0.814	1.901	0.000433	0.004368
BP	GO:0051897	positive regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction	114	0.47	1.69	0.000439	0.004413
MF	GO:0060590	ATPase regulator activity	47	0.603	1.889	0.000439	0.004413
BP	GO:0098868	bone growth	27	0.681	1.925	0.000444	0.00445
BP	GO:0007548	sex differentiation	192	0.429	1.634	0.000448	0.004484
MF	GO:0031072	heat shock protein binding	111	0.483	1.735	0.000451	0.004504
BP	GO:0070168	negative regulation of biomineral tissue development	17	0.758	1.95	0.000456	0.004551
BP	GO:0002768	immune response-regulating cell surface receptor signaling pathway	194	0.42	1.603	0.000457	0.004553

CC	GO:0005879	axonemal microtubule	28	-0.62	-1.956	0.000461	0.004578
BP	GO:1904892	regulation of receptor signaling pathway via STAT	62	0.561	1.845	0.000463	0.004599
BP	GO:0033028	myeloid cell apoptotic process	28	0.672	1.898	0.000478	0.004735
BP	GO:0050909	sensory perception of taste	22	-0.66	-2.007	0.00048	0.004749
BP	GO:0061684	chaperone-mediated autophagy	15	0.782	1.934	0.000484	0.004773
MF	GO:0004866	endopeptidase inhibitor activity	67	0.535	1.793	0.000485	0.004783
BP	GO:0051260	protein homooligomerization	153	0.438	1.634	0.00049	0.004818
BP	GO:0072109	glomerular mesangium development	12	0.811	1.895	0.000499	0.00489
BP	GO:0046633	alpha-beta T cell proliferation	30	0.66	1.889	0.000498	0.00489
BP	GO:0040014	regulation of multicellular organism growth	50	0.584	1.847	0.000501	0.004901
CC	GO:0045177	apical part of cell	304	0.384	1.521	0.000506	0.00494
BP	GO:2000106	regulation of leukocyte apoptotic process	61	0.561	1.846	0.000509	0.004958
BP	GO:0007179	transforming growth factor beta receptor signaling pathway	167	0.43	1.615	0.000511	0.004971
BP	GO:0001894	tissue homeostasis	203	0.411	1.582	0.00052	0.00504
BP	GO:0060249	anatomical structure homeostasis	203	0.411	1.582	0.00052	0.00504
BP	GO:0046578	regulation of Ras protein signal transduction	157	0.44	1.64	0.000529	0.005118
BP	GO:0032755	positive regulation of interleukin-6 production	69	0.531	1.79	0.00053	0.005124
BP	GO:0040013	negative regulation of locomotion	262	0.399	1.564	0.000548	0.005283
BP	GO:1905515	non-motile cilium assembly	61	-0.48	-1.776	0.000549	0.00529
BP	GO:0001655	urogenital system development	45	0.594	1.852	0.000551	0.005299
BP	GO:0030203	glycosaminoglycan metabolic process	82	0.509	1.756	0.000553	0.005304
BP	GO:0001656	metanephros development	59	0.559	1.819	0.000554	0.005308
BP	GO:0038034	signal transduction in absence of ligand	53	0.582	1.862	0.000559	0.005336
BP	GO:0097192	extrinsic apoptotic signaling pathway in absence of ligand	53	0.582	1.862	0.000559	0.005336
BP	GO:0071346	cellular response to type II interferon	67	0.532	1.783	0.000564	0.005362
BP	GO:0043542	endothelial cell	168	0.425	1.594	0.000563	0.005362

		migration					
BP	GO:0034620	cellular response to unfolded protein	83	0.505	1.748	0.000566	0.005377
BP	GO:2000379	positive regulation of reactive oxygen species metabolic process	47	0.597	1.871	0.00057	0.005386
CC	GO:0042470	melanosome	106	0.479	1.709	0.000569	0.005386
CC	GO:0048770	pigment granule	106	0.479	1.709	0.000569	0.005386
BP	GO:0002709	regulation of T cell mediated immunity	48	0.594	1.861	0.000579	0.00546
BP	GO:0042531	positive regulation of tyrosine phosphorylation of STAT protein	32	0.646	1.888	0.000584	0.005501
BP	GO:0060575	intestinal epithelial cell differentiation	19	0.716	1.876	0.000589	0.005531
BP	GO:1903707	negative regulation of hemopoiesis	75	0.526	1.801	0.00059	0.005531
MF	GO:0005035	death receptor activity	11	0.828	1.912	0.000597	0.005591
BP	GO:1990868	response to chemokine	39	0.607	1.837	0.000602	0.005614
BP	GO:1990869	cellular response to chemokine	39	0.607	1.837	0.000602	0.005614
CC	GO:0005912	adherens junction	155	0.44	1.643	0.000601	0.005614
BP	GO:0090083	regulation of inclusion body assembly	15	0.775	1.917	0.00061	0.00567
BP	GO:0006022	aminoglycan metabolic process	87	0.503	1.753	0.000613	0.005693
BP	GO:0048588	developmental cell growth	206	0.415	1.598	0.000615	0.005704
BP	GO:0006858	extracellular transport	40	-0.55	-1.87	0.000619	0.005727
BP	GO:0035967	cellular response to topologically incorrect protein	103	0.478	1.703	0.00062	0.005728
BP	GO:0001914	regulation of T cell mediated cytotoxicity	22	0.706	1.91	0.000622	0.005735
BP	GO:0046885	regulation of hormone biosynthetic process	18	0.737	1.919	0.000629	0.005793
BP	GO:0046173	polyol biosynthetic process	49	0.58	1.827	0.000631	0.005806
BP	GO:0010657	muscle cell apoptotic process	52	0.572	1.829	0.000641	0.005879
BP	GO:0045616	regulation of keratinocyte differentiation	26	0.693	1.941	0.000645	0.005908
BP	GO:0046850	regulation of bone remodeling	38	0.614	1.858	0.000654	0.005945
BP	GO:0032526	response to retinoic acid	75	0.524	1.792	0.000654	0.005945
BP	GO:0030317	flagellated sperm motility	87	-0.43	-1.696	0.000654	0.005945
BP	GO:0097722	sperm motility	87	-0.43	-1.696	0.000654	0.005945
BP	GO:0045089	positive regulation of innate immune	226	0.403	1.56	0.000653	0.005945

		response					
CC	GO:0032432	actin filament bundle	64	0.547	1.809	0.000661	0.005995
BP	GO:0009615	response to virus	297	0.378	1.494	0.000662	0.006001
BP	GO:0060425	lung morphogenesis	37	0.615	1.846	0.000664	0.006006
BP	GO:0090504	epiboly	31	0.645	1.87	0.000668	0.006034
BP	GO:0031348	negative regulation of defense response	185	0.419	1.589	0.000669	0.006034
BP	GO:0046697	decidualization	18	0.734	1.913	0.000683	0.006147
BP	GO:0046638	positive regulation of alpha-beta T cell differentiation	30	0.654	1.87	0.000692	0.006213
BP	GO:0007265	Ras protein signal transduction	299	0.379	1.501	0.000693	0.006213
BP	GO:0045143	homologous chromosome segregation	42	-0.54	-1.866	0.000709	0.006354
BP	GO:0071677	positive regulation of mononuclear cell migration	42	0.603	1.849	0.000718	0.006385
BP	GO:0051591	response to cAMP	58	0.565	1.837	0.000717	0.006385
BP	GO:0042440	pigment metabolic process	63	0.539	1.776	0.000716	0.006385
BP	GO:0051259	protein complex oligomerization	203	0.407	1.566	0.000717	0.006385
BP	GO:0002456	T cell mediated immunity	66	0.539	1.796	0.00072	0.006396
BP	GO:0032434	regulation of proteasomal ubiquitin-dependent protein catabolic process	128	0.459	1.675	0.000722	0.006406
BP	GO:1901673	regulation of mitotic spindle assembly	20	0.723	1.936	0.00073	0.006464
BP	GO:0002706	regulation of lymphocyte mediated immunity	94	0.495	1.742	0.000731	0.006465
BP	GO:0044403	biological process involved in symbiotic interaction	212	0.406	1.566	0.000733	0.006472
BP	GO:0008406	gonad development	162	0.438	1.635	0.000735	0.006482
BP	GO:0070304	positive regulation of stress-activated protein kinase signaling cascade	93	0.484	1.7	0.000737	0.006486
BP	GO:2000060	positive regulation of ubiquitin-dependent protein catabolic process	101	0.483	1.723	0.000742	0.006517
BP	GO:0003416	endochondral bone growth	25	0.695	1.941	0.00076	0.006658
BP	GO:0002437	inflammatory response to antigenic stimulus	42	0.602	1.846	0.000759	0.006658
BP	GO:0043373	CD4-positive, alpha-beta T cell lineage	12	0.805	1.88	0.000763	0.006671

		commitment					
BP	GO:0043491	phosphatidylinositol 3-kinase/protein kinase B signal transduction	198	0.406	1.556	0.000767	0.006693
BP	GO:0051216	cartilage development	140	0.45	1.66	0.000773	0.006736
CC	GO:0030016	myofibril	172	0.419	1.579	0.000776	0.006753
BP	GO:0060749	mammary gland alveolus development	13	0.775	1.842	0.00078	0.006768
BP	GO:0061377	mammary gland lobule development	13	0.775	1.842	0.00078	0.006768
BP	GO:0051348	negative regulation of transferase activity	190	0.415	1.581	0.000785	0.006802
BP	GO:0006925	inflammatory cell apoptotic process	16	0.746	1.886	0.000789	0.00682
BP	GO:0045176	apical protein localization	13	0.774	1.839	0.000796	0.006875
BP	GO:0021515	cell differentiation in spinal cord	31	0.639	1.852	0.000803	0.006914
BP	GO:0070527	platelet aggregation	54	0.559	1.802	0.000803	0.006914
BP	GO:0061614	miRNA transcription	56	0.556	1.8	0.000824	0.007084
BP	GO:0060135	maternal process involved in female pregnancy	40	0.603	1.829	0.000828	0.007101
BP	GO:0030500	regulation of bone mineralization	57	0.557	1.809	0.000835	0.007155
BP	GO:1902893	regulation of miRNA transcription	55	0.556	1.8	0.00084	0.007183
MF	GO:0030414	peptidase inhibitor activity	71	0.521	1.775	0.000841	0.007183
BP	GO:0032147	activation of protein kinase activity	64	0.542	1.795	0.000847	0.007212
BP	GO:0045861	negative regulation of proteolysis	160	0.437	1.635	0.000848	0.007212
BP	GO:0051896	regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction	163	0.432	1.616	0.000847	0.007212
BP	GO:0032874	positive regulation of stress-activated MAPK cascade	91	0.489	1.71	0.000857	0.007272
BP	GO:0097396	response to interleukin-17	11	0.823	1.898	0.000869	0.007325
BP	GO:0097398	cellular response to interleukin-17	11	0.823	1.898	0.000869	0.007325
BP	GO:0002708	positive regulation of lymphocyte mediated immunity	68	0.529	1.777	0.00087	0.007325
BP	GO:0001892	embryonic placenta development	67	0.522	1.751	0.000871	0.007325
BP	GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	428	0.35	1.423	0.000869	0.007325

CC	GO:0031674	I band	111	0.468	1.683	0.000869	0.007325
BP	GO:0009409	response to cold	37	0.608	1.825	0.000873	0.007329
BP	GO:2000630	positive regulation of miRNA metabolic process	51	0.57	1.807	0.000874	0.007329
BP	GO:0045124	regulation of bone resorption	32	0.636	1.858	0.000876	0.007329
CC	GO:0030027	lamellipodium	182	0.408	1.547	0.00088	0.007354
BP	GO:0072132	mesenchyme morphogenesis	43	0.6	1.851	0.000887	0.007407
CC	GO:0005839	proteasome core complex	17	0.739	1.9	0.000901	0.007512
MF	GO:0061133	endopeptidase activator activity	12	0.8	1.869	0.000904	0.007518
BP	GO:0061005	cell differentiation involved in kidney development	41	0.593	1.807	0.00092	0.007632
BP	GO:0016032	viral process	326	0.369	1.47	0.00092	0.007632
BP	GO:0048008	platelet-derived growth factor receptor signaling pathway	50	0.574	1.813	0.000924	0.007652
BP	GO:0006766	vitamin metabolic process	77	0.51	1.749	0.000934	0.007721
BP	GO:0044319	wound healing, spreading of cells	30	0.646	1.849	0.000944	0.007786
BP	GO:0090505	epiboly involved in wound healing	30	0.646	1.849	0.000944	0.007786
BP	GO:0060840	artery development	76	0.508	1.742	0.000962	0.007921
BP	GO:0060351	cartilage development involved in endochondral bone morphogenesis	27	0.663	1.874	0.000965	0.007938
BP	GO:0002360	T cell lineage commitment	19	0.701	1.837	0.000968	0.007946
BP	GO:0035914	skeletal muscle cell differentiation	51	0.567	1.799	0.000984	0.008056
BP	GO:0032868	response to insulin	218	0.404	1.558	0.000984	0.008056
BP	GO:0032570	response to progesterone	30	0.645	1.844	0.001002	0.008179
BP	GO:0071248	cellular response to metal ion	145	0.441	1.636	0.001004	0.008179
BP	GO:0030522	intracellular receptor signaling pathway	278	0.371	1.459	0.001003	0.008179
BP	GO:0018210	peptidyl-threonine modification	98	0.47	1.659	0.001008	0.008205
BP	GO:0090399	replicative senescence	15	0.763	1.888	0.00102	0.008284
BP	GO:0010506	regulation of autophagy	321	0.363	1.447	0.001023	0.008297
BP	GO:0050913	sensory perception of bitter taste	16	-0.71	-1.97	0.001039	0.008415
BP	GO:0010952	positive regulation of peptidase activity	126	0.453	1.647	0.00105	0.008494
BP	GO:0007606	sensory perception of	41	-0.53	-1.813	0.001052	0.008499

		chemical stimulus					
BP	GO:0097028	dendritic cell differentiation	21	0.707	1.912	0.00106	0.008551
BP	GO:0007584	response to nutrient	111	0.464	1.668	0.001064	0.008569
BP	GO:0045920	negative regulation of exocytosis	24	0.672	1.859	0.001066	0.008572
BP	GO:0010614	negative regulation of cardiac muscle hypertrophy	22	0.694	1.877	0.001081	0.008667
BP	GO:0002824	positive regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	73	0.516	1.769	0.00108	0.008667
BP	GO:0003417	growth plate cartilage development	17	0.733	1.886	0.001086	0.008698
BP	GO:0034614	cellular response to reactive oxygen species	123	0.446	1.616	0.001098	0.008779
BP	GO:0060444	branching involved in mammary gland duct morphogenesis	16	0.74	1.87	0.001101	0.008791
BP	GO:1902532	negative regulation of intracellular signal transduction	447	0.348	1.417	0.001103	0.008795
MF	GO:0000774	adenyl-nucleotide exchange factor activity	15	0.761	1.881	0.001114	0.008871
BP	GO:0046849	bone remodeling	71	0.517	1.762	0.001124	0.008931
BP	GO:0044057	regulation of system process	370	0.361	1.446	0.001126	0.008937
BP	GO:2000316	regulation of T-helper 17 type immune response	19	0.696	1.825	0.001138	0.009022
BP	GO:0045216	cell-cell junction organization	153	0.425	1.584	0.001146	0.009071
BP	GO:0061041	regulation of wound healing	84	0.492	1.71	0.00116	0.009169
BP	GO:0006271	DNA strand elongation involved in DNA replication	15	-0.71	-1.944	0.001162	0.009173
BP	GO:0045104	intermediate filament cytoskeleton organization	43	0.593	1.827	0.001181	0.009289
BP	GO:0030168	platelet activation	91	0.481	1.681	0.001182	0.009289
BP	GO:0019058	viral life cycle	230	0.395	1.533	0.001179	0.009289
BP	GO:0071622	regulation of granulocyte chemotaxis	32	0.625	1.828	0.001231	0.00965
BP	GO:0002507	tolerance induction	13	0.765	1.818	0.00123	0.00965
BP	GO:0002526	acute inflammatory response	48	0.575	1.803	0.001248	0.009768

BP	GO:0050907	detection of chemical stimulus involved in sensory perception	20	-0.66	-1.943	0.001252	0.009786
BP	GO:0007492	endoderm development	59	0.543	1.766	0.001276	0.009957
BP	GO:0001501	skeletal system development	376	0.352	1.414	0.001296	0.010097
BP	GO:0048873	homeostasis of number of cells within a tissue	28	0.648	1.83	0.001313	0.010216
BP	GO:1900046	regulation of hemostasis	44	0.574	1.781	0.001321	0.010261
BP	GO:0045109	intermediate filament organization	24	0.666	1.842	0.001334	0.010352
CC	GO:0030018	Z disc	104	0.467	1.665	0.001343	0.010403
MF	GO:0045499	chemorepellent activity	23	0.664	1.819	0.001375	0.010641
BP	GO:0032835	glomerulus development	51	0.561	1.779	0.001383	0.01069
BP	GO:0071241	cellular response to inorganic substance	168	0.412	1.549	0.001392	0.010742
BP	GO:1990845	adaptive thermogenesis	130	0.437	1.594	0.001396	0.010756
BP	GO:0070286	axonemal dynein complex assembly	31	-0.57	-1.832	0.0014	0.010769
BP	GO:0106106	cold-induced thermogenesis	117	0.459	1.647	0.001409	0.010811
BP	GO:0120161	regulation of cold-induced thermogenesis	117	0.459	1.647	0.001409	0.010811
BP	GO:0140747	regulation of ncRNA transcription	76	0.498	1.707	0.001414	0.010838
BP	GO:0010955	negative regulation of protein processing	23	0.663	1.816	0.00142	0.010853
BP	GO:1903318	negative regulation of protein maturation	23	0.663	1.816	0.00142	0.010853
BP	GO:0010632	regulation of epithelial cell migration	180	0.407	1.541	0.001427	0.010888
BP	GO:0007264	small GTPase mediated signal transduction	434	0.351	1.429	0.001446	0.011017
MF	GO:1901981	phosphatidylinositol phosphate binding	150	0.422	1.57	0.001468	0.011172
BP	GO:0097529	myeloid leukocyte migration	127	0.437	1.595	0.001472	0.011184
BP	GO:0031954	positive regulation of protein autophosphorylation	23	0.662	1.814	0.00148	0.011234
BP	GO:0002821	positive regulation of adaptive immune response	78	0.499	1.713	0.001501	0.011377
CC	GO:0098636	protein complex involved in cell adhesion	39	0.584	1.767	0.001505	0.01139
BP	GO:0045651	positive regulation of macrophage differentiation	13	0.762	1.809	0.00152	0.011487
BP	GO:0001704	formation of primary germ layer	92	0.481	1.685	0.001525	0.01151

BP	GO:0042116	macrophage activation	57	0.542	1.762	0.001545	0.011625
BP	GO:0016064	immunoglobulin mediated immune response	82	0.486	1.678	0.001544	0.011625
BP	GO:0070588	calcium ion transmembrane transport	252	0.385	1.504	0.001548	0.011636
BP	GO:0032729	positive regulation of type II interferon production	39	0.583	1.763	0.001561	0.011719
BP	GO:0048565	digestive tract development	93	0.472	1.656	0.001581	0.011852
BP	GO:1905048	regulation of metallopeptidase activity	13	0.761	1.808	0.001584	0.011859
BP	GO:0045600	positive regulation of fat cell differentiation	54	0.547	1.762	0.001588	0.011868
BP	GO:0010466	negative regulation of peptidase activity	77	0.499	1.708	0.001618	0.012075
BP	GO:0055006	cardiac cell development	65	0.521	1.733	0.001667	0.012409
BP	GO:0009410	response to xenobiotic stimulus	303	0.367	1.454	0.001666	0.012409
CC	GO:0030670	phagocytic vesicle membrane	67	0.51	1.708	0.001672	0.012428
BP	GO:0048640	negative regulation of developmental growth	92	0.479	1.678	0.001702	0.012617
BP	GO:0003007	heart morphogenesis	202	0.399	1.533	0.0017	0.012617
BP	GO:0045599	negative regulation of fat cell differentiation	43	0.585	1.803	0.001728	0.012788
CC	GO:0035577	azurophil granule membrane	45	0.571	1.78	0.001729	0.012788
BP	GO:0038166	angiotensin-activated signaling pathway	11	0.803	1.852	0.001746	0.012879
BP	GO:0007018	microtubule-based movement	343	-0.28	-1.33	0.001745	0.012879
CC	GO:0005667	transcription regulator complex	412	0.355	1.435	0.00178	0.013113
BP	GO:0090022	regulation of neutrophil chemotaxis	21	0.694	1.877	0.001794	0.013193
BP	GO:1902807	negative regulation of cell cycle G1/S phase transition	69	0.506	1.704	0.001801	0.013227
BP	GO:0090066	regulation of anatomical structure size	402	0.351	1.418	0.00181	0.013281
BP	GO:0061136	regulation of proteasomal protein catabolic process	176	0.407	1.54	0.00182	0.013337
BP	GO:1902106	negative regulation of leukocyte differentiation	70	0.511	1.727	0.001824	0.013348
BP	GO:0035924	cellular response to vascular endothelial	50	0.559	1.767	0.001829	0.013362

		growth factor stimulus					
BP	GO:0090649	response to oxygen-glucose deprivation	13	0.756	1.796	0.001859	0.013565
BP	GO:0050864	regulation of B cell activation	89	0.48	1.676	0.001863	0.013575
BP	GO:0060538	skeletal muscle organ development	131	0.429	1.563	0.001882	0.013694
CC	GO:0098576	luminal side of membrane	23	0.656	1.798	0.001888	0.013723
BP	GO:0048675	axon extension	110	0.457	1.639	0.001894	0.013748
BP	GO:0061180	mammary gland epithelium development	52	0.541	1.731	0.001903	0.013795
CC	GO:0001725	stress fiber	57	0.536	1.741	0.001947	0.014081
CC	GO:0097517	contractile actin filament bundle	57	0.536	1.741	0.001947	0.014081
BP	GO:0003352	regulation of cilium movement	22	-0.62	-1.887	0.001963	0.014174
BP	GO:0046636	negative regulation of alpha-beta T cell activation	28	0.638	1.802	0.00199	0.014333
BP	GO:0031952	regulation of protein autophosphorylation	32	0.614	1.794	0.001989	0.014333
BP	GO:0019724	B cell mediated immunity	83	0.478	1.656	0.002009	0.014454
BP	GO:0007409	axonogenesis	375	0.351	1.409	0.002017	0.014487
BP	GO:0001580	detection of chemical stimulus involved in sensory perception of bitter taste	14	-0.7	-1.884	0.002034	0.014587
BP	GO:0044786	cell cycle DNA replication	41	-0.51	-1.753	0.002036	0.014587
BP	GO:0051402	neuron apoptotic process	230	0.387	1.502	0.002044	0.014624
BP	GO:0032731	positive regulation of interleukin-1 beta production	35	0.614	1.821	0.002049	0.014642
MF	GO:0140776	protein-containing complex destabilizing activity	13	0.754	1.791	0.002053	0.014651
CC	GO:0032838	plasma membrane bounded cell projection cytoplasm	212	-0.31	-1.398	0.002064	0.014713
BP	GO:0010660	regulation of muscle cell apoptotic process	46	0.559	1.746	0.002077	0.014784
BP	GO:1903587	regulation of blood vessel endothelial cell proliferation involved in sprouting angiogenesis	12	0.78	1.821	0.002083	0.014806
BP	GO:0007369	gastrulation	143	0.426	1.575	0.002088	0.014825
CC	GO:0045335	phagocytic vesicle	117	0.45	1.617	0.002095	0.014856
CC	GO:0045178	basal part of cell	215	0.389	1.503	0.002104	0.014903

MF	GO:0042826	histone deacetylase binding	105	0.449	1.601	0.002125	0.015031
BP	GO:0002715	regulation of natural killer cell mediated immunity	19	0.681	1.785	0.002132	0.015042
BP	GO:0042269	regulation of natural killer cell mediated cytotoxicity	19	0.681	1.785	0.002132	0.015042
CC	GO:0032809	neuronal cell body membrane	19	-0.64	-1.905	0.002135	0.015046
BP	GO:0045494	photoreceptor cell maintenance	35	-0.55	-1.836	0.002158	0.015185
BP	GO:2001185	regulation of CD8-positive, alpha-beta T cell activation	13	0.752	1.786	0.002182	0.015296
BP	GO:1903052	positive regulation of proteolysis involved in protein catabolic process	122	0.449	1.628	0.00218	0.015296
CC	GO:0005902	microvillus	71	0.506	1.724	0.002181	0.015296
BP	GO:0003413	chondrocyte differentiation involved in endochondral bone morphogenesis	14	0.748	1.815	0.002191	0.015322
BP	GO:2000737	negative regulation of stem cell differentiation	23	0.652	1.785	0.00219	0.015322
BP	GO:0072678	T cell migration	42	0.571	1.749	0.002198	0.015352
BP	GO:0060317	cardiac epithelial to mesenchymal transition	25	0.662	1.848	0.002211	0.01536
BP	GO:0010950	positive regulation of endopeptidase activity	113	0.448	1.616	0.002203	0.01536
BP	GO:0033673	negative regulation of kinase activity	160	0.422	1.578	0.002209	0.01536
MF	GO:0008514	organic anion transmembrane transporter activity	165	0.417	1.567	0.00221	0.01536
BP	GO:0045834	positive regulation of lipid metabolic process	98	0.456	1.611	0.002226	0.015444
CC	GO:0016323	basolateral plasma membrane	178	0.398	1.508	0.002239	0.01552
BP	GO:0022616	DNA strand elongation	37	-0.51	-1.714	0.002266	0.015682
BP	GO:0090493	catecholamine uptake	11	-0.75	-1.853	0.002283	0.015782
BP	GO:0032689	negative regulation of type II interferon production	18	0.707	1.842	0.002292	0.015806
CC	GO:0097729	9+2 motile cilium	120	-0.37	-1.529	0.002292	0.015806
BP	GO:0002221	pattern recognition receptor signaling pathway	168	0.405	1.52	0.002298	0.015821
CC	GO:0042641	actomyosin	63	0.518	1.705	0.0023	0.015821
BP	GO:0030212	hyaluronan metabolic process	21	0.683	1.846	0.002315	0.015904

BP	GO:0014902	myotube differentiation	88	0.468	1.632	0.002325	0.015956
BP	GO:0045807	positive regulation of endocytosis	105	0.447	1.595	0.002346	0.01608
CC	GO:0090575	RNA polymerase II transcription regulator complex	216	0.389	1.5	0.002356	0.016126
BP	GO:0055001	muscle cell development	140	0.436	1.611	0.00236	0.016131
MF	GO:0033038	bitter taste receptor activity	10	-0.77	-1.871	0.00237	0.016184
MF	GO:0004970	ionotropic glutamate receptor activity	15	-0.69	-1.897	0.00238	0.016228
BP	GO:0034976	response to endoplasmic reticulum stress	240	0.383	1.494	0.00239	0.016279
MF	GO:0019838	growth factor binding	105	0.446	1.593	0.00242	0.016462
BP	GO:0043281	regulation of cysteine-type endopeptidase activity involved in apoptotic process	128	0.436	1.593	0.002435	0.016525
BP	GO:0065004	protein-DNA complex assembly	203	-0.32	-1.42	0.002434	0.016525
BP	GO:0048710	regulation of astrocyte differentiation	21	0.681	1.84	0.002468	0.016728
BP	GO:0002705	positive regulation of leukocyte mediated immunity	81	0.495	1.705	0.002477	0.016764
BP	GO:0045622	regulation of T-helper cell differentiation	22	0.672	1.819	0.002481	0.01677
BP	GO:2000377	regulation of reactive oxygen species metabolic process	107	0.456	1.629	0.00249	0.016817
BP	GO:0060350	endochondral bone morphogenesis	47	0.564	1.769	0.002499	0.016852
BP	GO:0003171	atrioventricular valve development	23	0.648	1.775	0.002522	0.016991
BP	GO:0042742	defense response to bacterium	120	0.444	1.607	0.002558	0.017212
BP	GO:0002040	sprouting angiogenesis	95	0.476	1.679	0.002564	0.017227
BP	GO:0090084	negative regulation of inclusion body assembly	10	0.795	1.785	0.002573	0.017269
BP	GO:0060395	SMAD protein signal transduction	62	0.529	1.739	0.0026	0.017426
BP	GO:0003014	renal system process	78	0.483	1.66	0.002605	0.01744
BP	GO:0001961	positive regulation of cytokine-mediated signaling pathway	40	0.573	1.739	0.00263	0.017589
BP	GO:0003206	cardiac chamber morphogenesis	91	0.465	1.625	0.002653	0.017717
BP	GO:0034101	erythrocyte homeostasis	114	0.444	1.596	0.002681	0.017882
BP	GO:0035929	steroid hormone secretion	16	0.712	1.801	0.002684	0.017883

BP	GO:0051346	negative regulation of hydrolase activity	140	0.434	1.603	0.002693	0.017922
CC	GO:0030990	intraciliary transport particle	20	-0.63	-1.87	0.002702	0.017957
BP	GO:0071214	cellular response to abiotic stimulus	263	0.378	1.481	0.002719	0.018028
BP	GO:0104004	cellular response to environmental stimulus	263	0.378	1.481	0.002719	0.018028
CC	GO:0022625	cytosolic large ribosomal subunit	57	0.527	1.713	0.002726	0.018056
CC	GO:0016324	apical plasma membrane	256	0.371	1.452	0.002747	0.018174
BP	GO:0048145	regulation of fibroblast proliferation	73	0.49	1.678	0.002763	0.018258
BP	GO:0003197	endocardial cushion development	43	0.573	1.768	0.002795	0.018444
BP	GO:2000179	positive regulation of neural precursor cell proliferation	47	0.56	1.756	0.002834	0.018611
BP	GO:0003018	vascular process in circulatory system	188	0.398	1.512	0.00283	0.018611
BP	GO:0001701	in utero embryonic development	324	0.356	1.419	0.002828	0.018611
MF	GO:0042056	chemoattractant activity	17	0.701	1.801	0.002834	0.018611
BP	GO:0070098	chemokine-mediated signaling pathway	32	0.604	1.764	0.002876	0.018841
BP	GO:0010977	negative regulation of neuron projection development	124	0.428	1.553	0.002873	0.018841
BP	GO:0090257	regulation of muscle system process	161	0.416	1.553	0.00289	0.018912
BP	GO:0033189	response to vitamin A	11	0.787	1.817	0.00291	0.018967
BP	GO:0003198	epithelial to mesenchymal transition involved in endocardial cushion formation	11	0.787	1.817	0.00291	0.018967
BP	GO:0061311	cell surface receptor signaling pathway involved in heart development	19	0.673	1.762	0.00291	0.018967
BP	GO:0072348	sulfur compound transport	39	0.566	1.713	0.002912	0.018967
BP	GO:0043217	myelin maintenance	16	0.71	1.794	0.002935	0.019095
BP	GO:0060413	atrial septum morphogenesis	12	0.771	1.801	0.002949	0.01914
BP	GO:2000116	regulation of cysteine-type endopeptidase activity	144	0.419	1.553	0.002949	0.01914
CC	GO:0031091	platelet alpha granule	60	0.518	1.697	0.002964	0.019214
CC	GO:0016235	aggresome	29	0.635	1.803	0.002983	0.019315
BP	GO:0070431	nucleotide-binding oligomerization domain containing 2	15	0.735	1.818	0.003002	0.019345

		signaling pathway					
BP	GO:0050818	regulation of coagulation	46	0.55	1.717	0.002998	0.019345
BP	GO:0033077	T cell differentiation in thymus	62	0.521	1.715	0.003005	0.019345
BP	GO:0031099	regeneration	137	0.423	1.553	0.003002	0.019345
MF	GO:0038187	pattern recognition receptor activity	15	0.735	1.817	0.003002	0.019345
BP	GO:1901655	cellular response to ketone	79	0.483	1.665	0.003022	0.019429
BP	GO:1902895	positive regulation of miRNA transcription	40	0.567	1.721	0.003058	0.019615
BP	GO:0048144	fibroblast proliferation	90	0.472	1.648	0.003057	0.019615
CC	GO:0098553	luminal side of endoplasmic reticulum membrane	15	0.734	1.814	0.003066	0.019644
BP	GO:0030850	prostate gland development	33	0.615	1.814	0.003086	0.019752
BP	GO:0009895	negative regulation of catabolic process	292	0.362	1.43	0.003093	0.019775
MF	GO:0140297	DNA-binding transcription factor binding	404	0.34	1.376	0.0031	0.019793
CC	GO:0009925	basal plasma membrane	203	0.387	1.487	0.003116	0.019872
BP	GO:0014741	negative regulation of muscle hypertrophy	23	0.643	1.762	0.003127	0.019919
BP	GO:0003281	ventricular septum development	57	0.523	1.7	0.003158	0.020096
BP	GO:0034694	response to prostaglandin	22	0.663	1.795	0.00319	0.02024
CC	GO:0042581	specific granule	101	0.454	1.622	0.003192	0.02024
MF	GO:0001968	fibronectin binding	23	0.642	1.76	0.003187	0.02024
MF	GO:0051087	protein-folding chaperone binding	118	0.444	1.598	0.003222	0.020405
BP	GO:0070613	regulation of protein processing	50	0.545	1.722	0.003227	0.020413
BP	GO:0018107	peptidyl-threonine phosphorylation	90	0.471	1.646	0.003259	0.020594
BP	GO:0046660	female sex differentiation	86	0.466	1.62	0.003268	0.020598
BP	GO:0051056	regulation of small GTPase mediated signal transduction	256	0.368	1.44	0.003267	0.020598
MF	GO:0005178	integrin binding	114	0.441	1.585	0.003271	0.020598
BP	GO:0060841	venous blood vessel development	13	0.738	1.754	0.003281	0.020641
BP	GO:0010810	regulation of cell-substrate adhesion	166	0.398	1.494	0.003303	0.020752
BP	GO:0035850	epithelial cell differentiation involved in kidney development	34	0.607	1.801	0.003312	0.020774
BP	GO:0002532	production of	53	0.54	1.728	0.003314	0.020774

		molecular mediator involved in inflammatory response					
BP	GO:0002224	toll-like receptor signaling pathway	52	0.526	1.684	0.003329	0.020848
BP	GO:0071243	cellular response to arsenic-containing substance	18	0.698	1.818	0.003353	0.02094
BP	GO:0043537	negative regulation of blood vessel endothelial cell migration	28	0.623	1.758	0.003348	0.02094
BP	GO:0048846	axon extension involved in axon guidance	37	0.573	1.719	0.003359	0.02094
BP	GO:1902284	neuron projection extension involved in neuron projection guidance	37	0.573	1.719	0.003359	0.02094
BP	GO:0001885	endothelial cell development	52	0.526	1.682	0.003384	0.021073
MF	GO:0005164	tumor necrosis factor receptor binding	20	0.676	1.809	0.003409	0.021201
BP	GO:0042088	T-helper 1 type immune response	20	0.675	1.808	0.00347	0.02151
BP	GO:0042063	gliogenesis	257	0.366	1.436	0.003467	0.02151
CC	GO:0098878	neurotransmitter receptor complex	35	-0.54	-1.795	0.003465	0.02151
BP	GO:0045604	regulation of epidermal cell differentiation	41	0.562	1.712	0.003488	0.021597
CC	GO:0045121	membrane raft	204	0.381	1.463	0.003513	0.02173
BP	GO:0055123	digestive system development	101	0.452	1.613	0.003533	0.021826
MF	GO:0008066	glutamate receptor activity	23	-0.59	-1.783	0.003549	0.021901
BP	GO:0045682	regulation of epidermis development	46	0.547	1.709	0.003558	0.021931
BP	GO:0061564	axon development	422	0.342	1.387	0.003574	0.021983
CC	GO:0031093	platelet alpha granule lumen	42	0.559	1.714	0.003574	0.021983
BP	GO:0046642	negative regulation of alpha-beta T cell proliferation	12	0.767	1.79	0.003583	0.022012
BP	GO:0043618	regulation of transcription from RNA polymerase II promoter in response to stress	31	0.602	1.745	0.003589	0.022023
BP	GO:0033280	response to vitamin D	27	0.623	1.762	0.003602	0.022033
BP	GO:0042446	hormone biosynthetic process	41	0.559	1.702	0.003601	0.022033
MF	GO:0097718	disordered domain specific binding	27	0.624	1.763	0.003602	0.022033
BP	GO:0031639	plasminogen activation	16	0.702	1.774	0.003628	0.022164

BP	GO:0060287	epithelial cilium movement involved in determination of left/right asymmetry	11	-0.73	-1.809	0.00364	0.022214
BP	GO:0030330	DNA damage response, signal transduction by p53 class mediator	65	0.503	1.674	0.003671	0.022381
BP	GO:0030193	regulation of blood coagulation	42	0.556	1.705	0.003687	0.022448
BP	GO:0018209	peptidyl-serine modification	258	0.365	1.43	0.003691	0.022448
CC	GO:0062023	collagen-containing extracellular matrix	275	0.355	1.398	0.003695	0.02245
BP	GO:1900047	negative regulation of hemostasis	28	0.62	1.752	0.003704	0.022453
BP	GO:0050851	antigen receptor-mediated signaling pathway	122	0.439	1.593	0.003704	0.022453
BP	GO:0001893	maternal placenta development	27	0.62	1.753	0.003781	0.022709
BP	GO:0016525	negative regulation of angiogenesis	67	0.496	1.662	0.003783	0.022709
BP	GO:0014032	neural crest cell development	69	0.485	1.633	0.003777	0.022709
BP	GO:0035150	regulation of tube size	90	0.467	1.633	0.003764	0.022709
BP	GO:0035296	regulation of tube diameter	90	0.467	1.633	0.003764	0.022709
BP	GO:0097746	blood vessel diameter maintenance	90	0.467	1.633	0.003764	0.022709
BP	GO:0043500	muscle adaptation	76	0.476	1.631	0.003782	0.022709
CC	GO:0098857	membrane microdomain	205	0.383	1.474	0.003779	0.022709
MF	GO:0005161	platelet-derived growth factor receptor binding	11	0.778	1.795	0.003777	0.022709
BP	GO:0055078	sodium ion homeostasis	23	0.638	1.747	0.003795	0.022755
BP	GO:0003013	circulatory system process	413	0.342	1.383	0.003861	0.023129
BP	GO:0071900	regulation of protein serine/threonine kinase activity	224	0.374	1.448	0.003904	0.023361
BP	GO:2001222	regulation of neuron migration	42	0.555	1.702	0.003912	0.023381
BP	GO:0030214	hyaluronan catabolic process	12	0.764	1.785	0.003918	0.023392
BP	GO:0030336	negative regulation of cell migration	220	0.378	1.459	0.003927	0.023423
BP	GO:0010755	regulation of plasminogen activation	14	0.725	1.758	0.003954	0.023559
BP	GO:0032958	inositol phosphate biosynthetic process	20	0.668	1.788	0.003961	0.023576
BP	GO:0090594	inflammatory response to wounding	15	0.726	1.796	0.003991	0.023719
BP	GO:0007250	activation of NF-	13	0.734	1.745	0.003996	0.023719

		kappaB-inducing kinase activity					
BP	GO:0001706	endoderm formation	41	0.556	1.693	0.003998	0.023719
BP	GO:0045649	regulation of macrophage differentiation	18	0.692	1.802	0.004042	0.023874
BP	GO:0003272	endocardial cushion formation	21	0.665	1.798	0.004036	0.023874
BP	GO:0010812	negative regulation of cell-substrate adhesion	45	0.554	1.729	0.004032	0.023874
MF	GO:0004867	serine-type endopeptidase inhibitor activity	36	0.576	1.718	0.004042	0.023874
BP	GO:0019932	second-messenger-mediated signaling	210	0.377	1.455	0.004069	0.024006
BP	GO:0120255	olefinic compound biosynthetic process	17	0.687	1.766	0.004077	0.02403
CC	GO:0005766	primary lysosome	118	0.439	1.58	0.004107	0.024154
CC	GO:0042582	azurophil granule	118	0.439	1.58	0.004107	0.024154
BP	GO:0062013	positive regulation of small molecule metabolic process	101	0.448	1.6	0.004118	0.024193
BP	GO:0033260	nuclear DNA replication	37	-0.49	-1.656	0.004127	0.024223
BP	GO:0070935	3'-UTR-mediated mRNA stabilization	17	0.685	1.761	0.004139	0.024267
BP	GO:0048708	astrocyte differentiation	59	0.512	1.666	0.004173	0.024437
BP	GO:1904044	response to aldosterone	13	0.732	1.74	0.004191	0.024518
BP	GO:0002758	innate immune response-activating signaling pathway	176	0.391	1.481	0.004197	0.02453
BP	GO:0001906	cell killing	79	0.476	1.64	0.00421	0.024576
BP	GO:0032814	regulation of natural killer cell activation	19	0.662	1.733	0.004223	0.024626
BP	GO:1903317	regulation of protein maturation	54	0.525	1.693	0.004227	0.024626
BP	GO:2000134	negative regulation of G1/S transition of mitotic cell cycle	64	0.507	1.679	0.004232	0.024627
BP	GO:0050777	negative regulation of immune response	120	0.433	1.567	0.004264	0.024788
BP	GO:0010038	response to metal ion	264	0.365	1.43	0.00427	0.024797
BP	GO:0055080	monoatomic cation homeostasis	431	0.334	1.36	0.004301	0.024922
CC	GO:0044298	cell body membrane	21	-0.62	-1.863	0.004296	0.024922
BP	GO:0032733	positive regulation of interleukin-10 production	24	0.633	1.751	0.004306	0.024925
BP	GO:0098742	cell-cell adhesion via plasma-membrane adhesion molecules	200	0.38	1.457	0.00433	0.025038
BP	GO:0001783	B cell apoptotic	22	0.651	1.763	0.004366	0.025223

		process					
BP	GO:0045685	regulation of glial cell differentiation	55	0.52	1.682	0.004371	0.025227
BP	GO:0010460	positive regulation of heart rate	17	0.682	1.753	0.004388	0.025297
BP	GO:0061383	trabecula morphogenesis	33	0.605	1.784	0.004413	0.025387
BP	GO:0060993	kidney morphogenesis	69	0.481	1.619	0.004411	0.025387
BP	GO:0045672	positive regulation of osteoclast differentiation	15	0.723	1.788	0.004438	0.025503
BP	GO:0097306	cellular response to alcohol	73	0.48	1.646	0.004468	0.025623
BP	GO:0048864	stem cell development	73	0.48	1.645	0.004468	0.025623
BP	GO:0046330	positive regulation of JNK cascade	65	0.498	1.658	0.004475	0.025638
BP	GO:0072079	nephron tubule formation	10	0.776	1.742	0.004504	0.025769
MF	GO:0061629	RNA polymerase II-specific DNA-binding transcription factor binding	289	0.359	1.416	0.004507	0.025769
CC	GO:0005911	cell-cell junction	388	0.345	1.393	0.00454	0.025927
MF	GO:0098641	cadherin binding involved in cell-cell adhesion	15	0.722	1.787	0.004566	0.026047
BP	GO:2000736	regulation of stem cell differentiation	67	0.49	1.641	0.004576	0.026055
BP	GO:0010498	proteasomal protein catabolic process	491	0.33	1.351	0.004573	0.026055
BP	GO:2000146	negative regulation of cell motility	231	0.372	1.443	0.004592	0.026118
BP	GO:0019217	regulation of fatty acid metabolic process	65	0.497	1.655	0.004636	0.02634
BP	GO:0045453	bone resorption	51	0.526	1.668	0.004643	0.026354
BP	GO:0048534	hematopoietic or lymphoid organ development	68	0.487	1.636	0.004676	0.026456
BP	GO:1901343	negative regulation of vasculature development	68	0.487	1.636	0.004676	0.026456
BP	GO:2000181	negative regulation of blood vessel morphogenesis	68	0.487	1.636	0.004676	0.026456
CC	GO:0030017	sarcomere	155	0.41	1.531	0.004693	0.0265
MF	GO:0036002	pre-mRNA binding	38	0.563	1.703	0.004693	0.0265
BP	GO:0007176	regulation of epidermal growth factor-activated receptor activity	16	0.693	1.752	0.0047	0.026514
BP	GO:0048844	artery morphogenesis	52	0.516	1.652	0.004705	0.026514
BP	GO:1902042	negative regulation of extrinsic apoptotic signaling pathway via	23	0.631	1.728	0.004766	0.02683

		death domain receptors					
CC	GO:0060170	ciliary membrane	61	-0.43	-1.575	0.004811	0.027054
BP	GO:1903036	positive regulation of response to wounding	54	0.52	1.678	0.004827	0.027119
BP	GO:0007519	skeletal muscle tissue development	123	0.418	1.516	0.004872	0.027342
BP	GO:0030890	positive regulation of B cell proliferation	31	0.593	1.719	0.004909	0.027524
BP	GO:0036037	CD8-positive, alpha-beta T cell activation	16	0.691	1.746	0.004953	0.027629
BP	GO:0008637	apoptotic mitochondrial changes	95	0.46	1.624	0.004953	0.027629
BP	GO:0098781	ncRNA transcription	119	0.42	1.52	0.004946	0.027629
BP	GO:0010508	positive regulation of autophagy	131	0.412	1.499	0.004953	0.027629
BP	GO:0002218	activation of innate immune response	192	0.388	1.476	0.004947	0.027629
BP	GO:0072567	chemokine (C-X-C motif) ligand 2 production	13	0.726	1.725	0.004971	0.027671
BP	GO:2000341	regulation of chemokine (C-X-C motif) ligand 2 production	13	0.726	1.725	0.004971	0.027671
MF	GO:0046943	carboxylic acid transmembrane transporter activity	116	0.431	1.549	0.004999	0.0278
BP	GO:0055007	cardiac muscle cell differentiation	95	0.459	1.62	0.005053	0.028042
CC	GO:0030139	endocytic vesicle	266	0.369	1.448	0.005052	0.028042
BP	GO:0071621	granulocyte chemotaxis	56	0.514	1.664	0.005063	0.02807
BP	GO:0060191	regulation of lipase activity	45	0.545	1.699	0.005088	0.028183
BP	GO:0000101	sulfur amino acid transport	10	0.772	1.733	0.005105	0.028191
BP	GO:0043011	myeloid dendritic cell differentiation	10	0.771	1.731	0.005105	0.028191
BP	GO:0006109	regulation of carbohydrate metabolic process	145	0.416	1.54	0.005099	0.028191
BP	GO:0007288	sperm axoneme assembly	26	-0.56	-1.75	0.005175	0.028524
BP	GO:0070293	renal absorption	22	0.646	1.749	0.005171	0.028524
BP	GO:0072210	metanephric nephron development	27	0.613	1.732	0.00521	0.028666
BP	GO:0050819	negative regulation of coagulation	30	0.603	1.724	0.005212	0.028666
BP	GO:0097193	intrinsic apoptotic signaling pathway	262	0.367	1.438	0.005224	0.028707
BP	GO:0090190	positive regulation of branching involved in ureteric bud morphogenesis	10	0.77	1.729	0.005238	0.028738

MF	GO:0048029	monosaccharide binding	55	0.512	1.657	0.00524	0.028738
BP	GO:1903053	regulation of extracellular matrix organization	47	0.544	1.704	0.005247	0.028744
MF	GO:0042887	amide transmembrane transporter activity	31	0.589	1.707	0.005254	0.028754
BP	GO:0018105	peptidyl-serine phosphorylation	246	0.36	1.407	0.005273	0.028804
CC	GO:0032591	dendritic spine membrane	13	-0.7	-1.837	0.005272	0.028804
BP	GO:0035720	intraciliary anterograde transport	16	-0.66	-1.826	0.00531	0.028975
BP	GO:0007517	muscle organ development	259	0.364	1.425	0.005317	0.028985
BP	GO:0010968	regulation of microtubule nucleation	19	0.655	1.717	0.005349	0.029072
BP	GO:0002043	blood vessel endothelial cell proliferation involved in sprouting angiogenesis	19	0.655	1.715	0.005349	0.029072
BP	GO:0060348	bone development	158	0.4	1.491	0.005343	0.029072
CC	GO:0032590	dendrite membrane	28	-0.55	-1.734	0.005354	0.029072
BP	GO:2001056	positive regulation of cysteine-type endopeptidase activity	96	0.45	1.587	0.005382	0.029193
BP	GO:0008045	motor neuron axon guidance	18	0.684	1.783	0.005421	0.029357
BP	GO:0006534	cysteine metabolic process	10	0.769	1.727	0.005438	0.029357
BP	GO:0002407	dendritic cell chemotaxis	10	0.768	1.726	0.005438	0.029357
BP	GO:2000058	regulation of ubiquitin-dependent protein catabolic process	159	0.402	1.502	0.005436	0.029357
CC	GO:0005774	vacuolar membrane	408	0.331	1.337	0.005428	0.029357
BP	GO:0008207	C21-steroid hormone metabolic process	21	0.658	1.779	0.005454	0.029381
BP	GO:0003222	ventricular trabecula myocardium morphogenesis	11	0.768	1.772	0.005448	0.029381
BP	GO:0033627	cell adhesion mediated by integrin	60	0.505	1.654	0.005466	0.029417
BP	GO:0003205	cardiac chamber development	125	0.421	1.532	0.005483	0.029484
BP	GO:0043620	regulation of DNA-templated transcription in response to stress	36	0.565	1.686	0.005532	0.029715
MF	GO:0043394	proteoglycan binding	26	0.637	1.785	0.005537	0.029715
BP	GO:0035590	purinergic nucleotide receptor signaling pathway	18	0.683	1.78	0.005546	0.029733

MF	GO:0008527	taste receptor activity	11	-0.72	-1.775	0.00558	0.029888
BP	GO:0008630	intrinsic apoptotic signaling pathway in response to DNA damage	89	0.455	1.588	0.005605	0.02999
BP	GO:0042594	response to starvation	187	0.381	1.448	0.00561	0.02999
BP	GO:0072234	metanephric nephron tubule development	14	0.712	1.728	0.005626	0.030007
BP	GO:0050918	positive chemotaxis	40	0.553	1.678	0.00563	0.030007
BP	GO:1900076	regulation of cellular response to insulin stimulus	55	0.51	1.65	0.005621	0.030007
BP	GO:0009158	ribonucleoside monophosphate catabolic process	11	0.767	1.77	0.005649	0.03005
BP	GO:0030540	female genitalia development	11	0.767	1.77	0.005649	0.03005
BP	GO:0001889	liver development	113	0.427	1.538	0.005654	0.03005
BP	GO:0021983	pituitary gland development	23	0.624	1.71	0.005677	0.030089
BP	GO:0050770	regulation of axonogenesis	143	0.405	1.497	0.005671	0.030089
MF	GO:0071889	14-3-3 protein binding	30	0.601	1.718	0.005678	0.030089
BP	GO:0050767	regulation of neurogenesis	317	0.35	1.392	0.00571	0.030229
BP	GO:0002021	response to dietary excess	22	0.643	1.739	0.005729	0.030301
BP	GO:0050912	detection of chemical stimulus involved in sensory perception of taste	15	-0.66	-1.812	0.005748	0.030376
BP	GO:0043255	regulation of carbohydrate biosynthetic process	77	0.469	1.606	0.005802	0.030632
BP	GO:0001773	myeloid dendritic cell activation	14	0.71	1.723	0.005819	0.03069
BP	GO:0048002	antigen processing and presentation of peptide antigen	42	0.545	1.669	0.005826	0.030698
BP	GO:0043502	regulation of muscle adaptation	61	0.495	1.627	0.005914	0.031131
BP	GO:0003203	endocardial cushion morphogenesis	34	0.589	1.747	0.005962	0.031354
BP	GO:0072073	kidney epithelium development	109	0.43	1.541	0.005981	0.031423
BP	GO:0120162	positive regulation of cold-induced thermogenesis	76	0.461	1.581	0.006028	0.031643
BP	GO:0070741	response to interleukin-6	23	0.622	1.705	0.006041	0.03168
BP	GO:0003188	heart valve formation	14	0.708	1.718	0.006076	0.031803
BP	GO:0060143	positive regulation of syncytium formation	13	0.717	1.703	0.006075	0.031803

		by plasma membrane fusion					
MF	GO:0005342	organic acid transmembrane transporter activity	117	0.429	1.541	0.006088	0.031833
BP	GO:0071364	cellular response to epidermal growth factor stimulus	40	0.549	1.667	0.006146	0.032106
BP	GO:0046427	positive regulation of receptor signaling pathway via JAK-STAT	27	0.609	1.72	0.006163	0.032107
BP	GO:0030195	negative regulation of blood coagulation	27	0.608	1.718	0.006163	0.032107
MF	GO:0051539	4 iron, 4 sulfur cluster binding	43	-0.47	-1.635	0.006155	0.032107
BP	GO:2000177	regulation of neural precursor cell proliferation	77	0.466	1.598	0.00622	0.032375
BP	GO:0048384	retinoic acid receptor signaling pathway	28	0.603	1.703	0.006253	0.032451
BP	GO:0050829	defense response to Gram-negative bacterium	28	0.603	1.701	0.006253	0.032451
MF	GO:0008047	enzyme activator activity	483	0.324	1.327	0.006249	0.032451
BP	GO:0090100	positive regulation of transmembrane receptor protein serine/threonine kinase signaling pathway	83	0.454	1.574	0.00628	0.03256
BP	GO:0006026	aminoglycan catabolic process	20	0.652	1.745	0.006357	0.032932
BP	GO:0003279	cardiac septum development	86	0.452	1.571	0.006364	0.032937
BP	GO:0006261	DNA-templated DNA replication	153	-0.32	-1.362	0.006396	0.033068
BP	GO:0003418	growth plate cartilage chondrocyte differentiation	10	0.761	1.708	0.006439	0.033263
BP	GO:0042448	progesterone metabolic process	11	0.764	1.762	0.006451	0.033292
BP	GO:0006873	intracellular monoatomic ion homeostasis	384	0.336	1.356	0.006517	0.033603
CC	GO:0005882	intermediate filament	61	0.491	1.614	0.006565	0.033816
BP	GO:0042730	fibrinolysis	13	0.715	1.698	0.006595	0.033876
BP	GO:0090025	regulation of monocyte chemotaxis	13	0.714	1.698	0.006595	0.033876
BP	GO:0002181	cytoplasmic translation	153	0.391	1.459	0.006593	0.033876
BP	GO:0035264	multicellular organism growth	122	0.429	1.556	0.006665	0.034206
BP	GO:2000209	regulation of anoikis	19	0.651	1.705	0.006727	0.034426
BP	GO:0010829	negative regulation of	13	0.713	1.693	0.006725	0.034426

		glucose transmembrane transport					
MF	GO:0015172	acidic amino acid transmembrane transporter activity	13	0.712	1.693	0.006725	0.034426
BP	GO:0061900	glial cell activation	31	0.583	1.689	0.006751	0.034518
BP	GO:0003181	atrioventricular valve morphogenesis	22	0.637	1.725	0.006784	0.034623
BP	GO:0072207	metanephric epithelium development	16	0.681	1.72	0.006784	0.034623
BP	GO:0008202	steroid metabolic process	220	0.368	1.421	0.006835	0.03485
BP	GO:0050810	regulation of steroid biosynthetic process	54	0.513	1.654	0.006852	0.034886
BP	GO:0007204	positive regulation of cytosolic calcium ion concentration	90	0.451	1.575	0.006855	0.034886
BP	GO:0071379	cellular response to prostaglandin stimulus	18	0.674	1.757	0.006864	0.034903
BP	GO:0032760	positive regulation of tumor necrosis factor production	68	0.475	1.596	0.006955	0.035332
BP	GO:0051131	chaperone-mediated protein complex assembly	20	0.65	1.741	0.006975	0.035398
BP	GO:0060070	canonical Wnt signaling pathway	258	0.356	1.393	0.006984	0.035412
BP	GO:0019748	secondary metabolic process	41	0.533	1.624	0.00701	0.035482
BP	GO:0072593	reactive oxygen species metabolic process	165	0.4	1.501	0.007004	0.035482
BP	GO:0042472	inner ear morphogenesis	71	0.475	1.619	0.007037	0.035584
BP	GO:0043433	negative regulation of DNA-binding transcription factor activity	112	0.433	1.561	0.007049	0.035613
BP	GO:0008209	androgen metabolic process	17	0.666	1.712	0.007068	0.035644
BP	GO:0045912	negative regulation of carbohydrate metabolic process	40	0.542	1.646	0.007065	0.035644
CC	GO:0098797	plasma membrane protein complex	401	0.332	1.343	0.007076	0.035648
BP	GO:0060760	positive regulation of response to cytokine stimulus	45	0.537	1.674	0.007097	0.035724
BP	GO:0051145	smooth muscle cell differentiation	46	0.529	1.652	0.007151	0.035962
MF	GO:0005184	neuropeptide hormone activity	14	0.699	1.695	0.007172	0.036004
MF	GO:0160041	neuropeptide activity	14	0.699	1.695	0.007172	0.036004
BP	GO:0071229	cellular response to acid chemical	76	0.458	1.571	0.007182	0.036018

BP	GO:0009267	cellular response to starvation	157	0.393	1.466	0.007229	0.036223
BP	GO:0070562	regulation of vitamin D receptor signaling pathway	10	0.757	1.7	0.00731	0.036593
BP	GO:0003209	cardiac atrium morphogenesis	22	0.634	1.716	0.007406	0.036991
BP	GO:0060603	mammary gland duct morphogenesis	22	0.634	1.715	0.007406	0.036991
BP	GO:1902041	regulation of extrinsic apoptotic signaling pathway via death domain receptors	40	0.54	1.637	0.007409	0.036991
CC	GO:0030312	external encapsulating structure	356	0.339	1.36	0.007427	0.037013
CC	GO:0031012	extracellular matrix	356	0.339	1.36	0.007427	0.037013
BP	GO:0120316	sperm flagellum assembly	36	-0.49	-1.669	0.007438	0.037029
MF	GO:0000979	RNA polymerase II core promoter sequence-specific DNA binding	17	0.661	1.701	0.007444	0.037029
BP	GO:0009069	serine family amino acid metabolic process	32	0.577	1.685	0.007455	0.037053
BP	GO:1903556	negative regulation of tumor necrosis factor superfamily cytokine production	34	0.582	1.725	0.007583	0.037603
BP	GO:1901741	positive regulation of myoblast fusion	10	0.755	1.695	0.007578	0.037603
BP	GO:0038061	non-canonical NF-kappaB signal transduction	92	0.445	1.557	0.007586	0.037603
BP	GO:0001767	establishment of lymphocyte polarity	12	0.743	1.735	0.007605	0.037638
BP	GO:0010718	positive regulation of epithelial to mesenchymal transition	47	0.531	1.664	0.007613	0.037638
BP	GO:0071774	response to fibroblast growth factor	78	0.459	1.579	0.00762	0.037638
BP	GO:0002753	cytosolic pattern recognition receptor signaling pathway	113	0.42	1.514	0.007621	0.037638
BP	GO:0036336	dendritic cell migration	13	0.706	1.678	0.00764	0.037664
BP	GO:0072676	lymphocyte migration	64	0.495	1.64	0.007639	0.037664

Table 4-10 appx: Complete list of GO pathways significantly enriched in GSE analyses of all three treatment groups.

ONTOLOGY	ID	Description	setSize
BP	GO:0003417	growth plate cartilage development	17
BP	GO:0003416	endochondral bone growth	25
BP	GO:0060351	cartilage development involved in endochondral bone morphogenesis	27
BP	GO:0098868	bone growth	27
BP	GO:0003180	aortic valve morphogenesis	32
BP	GO:0071622	regulation of granulocyte chemotaxis	32
BP	GO:0003176	aortic valve development	37
BP	GO:1905314	semi-lunar valve development	40
MF	GO:0004896	cytokine receptor activity	50
BP	GO:0032835	glomerulus development	51
BP	GO:0003179	heart valve morphogenesis	52
MF	GO:0140375	immune receptor activity	53
BP	GO:0071621	granulocyte chemotaxis	56
BP	GO:0003170	heart valve development	62
MF	GO:0030527	structural constituent of chromatin	80
MF	GO:0005125	cytokine activity	86
BP	GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	104
BP	GO:0045598	regulation of fat cell differentiation	107
CC	GO:0000786	nucleosome	110
BP	GO:0070374	positive regulation of ERK1 and ERK2 cascade	122
BP	GO:0097529	myeloid leukocyte migration	127
BP	GO:0046890	regulation of lipid biosynthetic process	133
BP	GO:0051216	cartilage development	140
BP	GO:0002685	regulation of leukocyte migration	141
BP	GO:0033002	muscle cell proliferation	147
BP	GO:0050730	regulation of peptidyl-tyrosine phosphorylation	151
BP	GO:0070663	regulation of leukocyte proliferation	152
BP	GO:0050920	regulation of chemotaxis	158
BP	GO:0018108	peptidyl-tyrosine phosphorylation	186
BP	GO:0043588	skin development	186
BP	GO:0018212	peptidyl-tyrosine modification	188
BP	GO:0070372	regulation of ERK1 and ERK2 cascade	193
BP	GO:0045765	regulation of angiogenesis	198
BP	GO:0070661	leukocyte proliferation	199
BP	GO:0048762	mesenchymal cell differentiation	200
BP	GO:1901342	regulation of vasculature development	200
BP	GO:0061448	connective tissue development	201
BP	GO:0065004	protein-DNA complex assembly	203
BP	GO:0070371	ERK1 and ERK2 cascade	212

MF	GO:0048018	receptor ligand activity	217
BP	GO:0008202	steroid metabolic process	220
BP	GO:0030336	negative regulation of cell migration	220
MF	GO:0030546	signaling receptor activator activity	222
BP	GO:0050900	leukocyte migration	227
BP	GO:2000146	negative regulation of cell motility	231
BP	GO:0001822	kidney development	233
BP	GO:0072001	renal system development	238
BP	GO:0019216	regulation of lipid metabolic process	240
BP	GO:0060485	mesenchyme development	242
MF	GO:0030545	signaling receptor regulator activity	244
BP	GO:0062012	regulation of small molecule metabolic process	250
BP	GO:0050678	regulation of epithelial cell proliferation	251
BP	GO:0042063	gliogenesis	257
BP	GO:0060070	canonical Wnt signaling pathway	258
BP	GO:0040013	negative regulation of locomotion	262
CC	GO:0043235	receptor complex	271
CC	GO:0062023	collagen-containing extracellular matrix	275
BP	GO:0006935	chemotaxis	286
BP	GO:0042330	taxis	286
BP	GO:0019221	cytokine-mediated signaling pathway	293
BP	GO:0050673	epithelial cell proliferation	304
BP	GO:0043410	positive regulation of MAPK cascade	312
BP	GO:0001503	ossification	320
BP	GO:0051249	regulation of lymphocyte activation	321
BP	GO:0048732	gland development	326
BP	GO:0001667	ameboidal-type cell migration	344
BP	GO:0042110	T cell activation	352
CC	GO:0030312	external encapsulating structure	356
CC	GO:0031012	extracellular matrix	356
BP	GO:0009617	response to bacterium	358
BP	GO:0002694	regulation of leukocyte activation	372
BP	GO:0001501	skeletal system development	376
BP	GO:0031667	response to nutrient levels	381
BP	GO:0001525	angiogenesis	383
BP	GO:0030335	positive regulation of cell migration	397
BP	GO:0009991	response to extracellular stimulus	401
BP	GO:0050865	regulation of cell activation	405
BP	GO:2000147	positive regulation of cell motility	413
BP	GO:0040017	positive regulation of locomotion	424
CC	GO:0098552	side of membrane	424
BP	GO:0048514	blood vessel morphogenesis	444
BP	GO:0030855	epithelial cell differentiation	465

BP	GO:0048729	tissue morphogenesis	467
BP	GO:0042327	positive regulation of phosphorylation	476
MF	GO:0004888	transmembrane signaling receptor activity	481
BP	GO:0006954	inflammatory response	481

Table 4-11 appx: Forward and reverse primer sequences and general information for genes used for targeted DNA methylation analysis.

Gene	Primer	Primer Sequence	Melting Temperature (deg. C)	Amplicon Length	Number of CpG sites in amplicon
GABRG3	Forward	GTTGGAGGAAATTTGTATTG	56.6	229	14 (+1 in primer's region)
	Reverse	CRAAAAAAAAAATTTCCCAATTAC	59.9		
POLD4	Forward	TTGGAGAAGTTTATGGGTTTT	59.5	392	6 (+0 in primer's region)
	Reverse	CATTTAACCTATACTTCCTACTTTC	58.1		
DAAM2	Forward	TATTAGGTTTTAAGGGATAGAG	57.7	348	3 (+0 in primer's region)
	Reverse	TCCCTTCACCTTAAAATAAACTT	60.6		
TNFRSF10A	Forward	GTTYGGTTTATTTTGTTTTGGTT	61.2	356	15 (+1 in primer's region)
	Reverse	TTTCTCTTACCCACTCCTCA	59.6		
Stat1	Forward	TTTTTGTTYGGTTTGGGGTTTGT	63.7	383	37 (+1 in primer's region)
	Reverse	ACCTCCAAATTTAAACTTCT	57.5		
EIF2AK1	Forward	GGATTTTGTATTTTGGGATTGT	59.1	319	3 (+0 in primer's region)
	Reverse	CTTCTATTAATTTCTTTTAAAAATCC	60.4		
ENSG00000258830	Forward	GAAGTAGAGGAAGAAATATAAAG	57.3	392	3 (+0 in primer's region)
	Reverse	CCAAAAATATACAACCAAATCAC	58.5		

Chapter 5

Discussion

General Overview

The long-term goal of this research is to characterize the behavior of Hg and DNA methylation (DNAm) in the human body and discover potential mechanisms by which they can impart neurological changes. Within the scope of this dissertation, we utilized an innovative epigenetic approach that explored this topic via a two-tiered study design comprising three aims. The central hypotheses of these aims respectively predict: (1) DNAm marks at certain sites in adolescence would be associated with CPT3 (Conners Continuous Performance Test) scores reflective of one or more aspects of attention in a Mexican cohort, (2) childhood mercury exposure and DNAm at certain sites would interact to affect CPT3 scores in the same cohort, and (3) genes involved in cognition & neurodevelopment would be (3A) expressed and (3B) methylated differentially in neurons treated with different concentrations of mercury in a dose-dependent manner. The results generated by this dissertation present interesting findings to support these hypotheses. In this discussion, we will first describe the implications of these main findings as they relate to Hg exposure, then as they relate to epigenetics. After outlining the neurological effects of these two predictors individually, we will deliberate about the combined effects and what they might mean for human health.

Overview of Results: the Associations of Mercury Exposure with Neurodevelopment and Function

Mercury (Hg) is a potent environmental neurotoxicant and, despite recent efforts to reduce its emission, it is still a very pressing public health concern [1]. Hg exposure is associated with reduced cognition & hearing, behavioral changes, and impaired coordination in children of various ages in both cross-sectional and longitudinal studies [2]. The results of this dissertation, taken together with prior publications by other research groups, suggest that childhood Hg exposure may be an important risk factor for ADHD, which in and of itself presents a critical public health crisis. Because the etiology of ADHD and the potential effects of low-levels of Hg on the developing human brain are not completely understood, we explored this research question by conducting a transcriptomic analysis on human dopaminergic-like neurons treated with biologically relevant and cytotoxic doses of meHg and compared their RNA expression to that of an untreated control (Aim 3A). Significant findings for gene expression and functional pathway enrichment were identified in all three treatment groups in this RNA sequencing experiment, but the lower two doses (0.01 and 0.25 μ M meHg) are of particular interest; they are similar to the average blood mercury levels in cohorts of human children around the world. In fact, the lowest of these two treatment groups is of particular relevance to this dissertation, as it was calculated based on the average blood Hg levels recorded for child participants in the ELEMENT (Early Life Exposure in Mexico to ENvironmental Toxicants) cohort who were used in the epidemiological tier of this dissertation (Aims 1 and 2). Only one significant differentially expressed gene (DEG, $p_{\text{adj}} < 0.05$) was identified in this treatment group. It was a novel gene (ENSG00000258830) annotated to a gene ontology (GO) term related to nucleic acid binding and was associated with lower expression levels ($\log_2$ Fold change = -3.05 (SE=0.52), $p_{\text{adj}} =$

8.21E-05) compared to the untreated control. Although ENSG00000258830 expression was not significant in the 0.25 μ M meHg treatment group (0.25 (0.37), 1.00), it was associated with higher expression levels in the 6.25 μ M treatment (1.17 (0.36), 7.03E-03). Having an opposite direction of effect in the high and low dose groups suggests that the brain may respond differently to very low levels of meHg than it would to higher dose exposures. Regardless, nucleic acid binding activity may be dysregulated in response to meHg. Another gene that responded notably differently to lower doses of meHg than to higher levels was ENSG00000242479. The expression of this pseudogene was significantly reduced in the 0.25 μ M meHg treatment group (-6.7 (1.3), 4.68E-03), but the expression in the lowest and highest treatment groups did not appear to be significantly different to that of untreated cells, which presents a non-monotonic dose-response for this gene. Interestingly, a similar pattern was observed in the PrestoBlue viability assay, which demonstrated a significant increase in metabolic activity for low and medium doses and a significant decrease for higher doses of meHg. It is important to note that these results reflect only a 48-hour exposure window, and these outcomes do not translate directly to neurobehavioral or functional outcomes, so it is difficult to discern whether the results presented in this set of experiments are related to toxic or protective mechanisms. We also cannot posit that these effects would be maintained if the presence of meHg were to persist into a more chronic and realistic exposure or that they would translate to an adverse neurobehavioral effect. Regardless, the gene set enrichment analysis from the RNA-seq did provide some functional implications across all treatment groups regarding the effect of meHg exposure on dopaminergic-like neurons, exhibiting enrichment for pathways related to cell signaling and migration, inflammation, and immune response, as well as protein-DNA complex assembly.

While the *in vitro* analysis provided a more direct assessment of the effects of meHg on the brain, the epidemiological analysis in aim 2 of this dissertation was able to model more realistic and complex associations and provide valuable insight regarding how exposure to meHg may relate to ADHD-like behaviors. Our results in ELEMENT participants are comparable to findings from previous studies, as they exhibit gender-specific and both positive and negative associations, with some results even suggesting a beneficial effect of this potent neurotoxicant on attention-related behaviors. While we hypothesized that consideration of variables for neuro-beneficial seafood nutrients that are widely co-exposed with meHg would help elucidate these counterintuitive findings, generally, the inclusion of PUFA (polyunsaturated fatty acids) nutrients did little to explain the complex relationships outlined in the results of aim 2. One exception to this, was the association between urine Hg levels and vigilance (CPT3 ISI change score) in males, which increased from an effect size of 1.31 (S.E.= 0.65, $p = 0.045^*$) in the main models to 2.03 (0.80, 0.013*) with the inclusion of PUFAs in the sensitivity models (Aim 2 Tables 3-2 and 3-3). This suggests that exposure to higher levels of mercury in male children is associated with poorer vigilance scores and that co-exposure to PUFA nutrients can help to mitigate this adverse relationship. In addition to PUFAs (ie. Omega(ω)-3 and ω 6-PUFAs), seafood is rich in several other highly beneficial nutrients including selenium (Se), vitamins D, and B2, which may also be promoting healthy brain development and/or protecting against toxic insult by meHg [3, 4]. Notably, in the selection of covariates for the analyses in aim 2, we considered the use of Se levels, which was calculated from the same food frequency questionnaire (FFQ) from which the PUFA variable was derived. Inclusion of variables for PUFA, Se, or both, resulted in very similar model outputs in the linear regression analyses outlining the relationship between Hg exposure and CPT3 performance. We decided to only

control for the PUFA variable, which was correlated with Se, as it tended to result in a slightly better model fit, and by including both we stood the risk of overfitting our models. Regardless, there are other potentially protective nutrients, exposures, and variables that may be contributing to these seemingly beneficial effects. One factor that may contribute to inter-individual variability of response to Hg exposure and susceptibility to ADHD is epigenetics.

Overview of Results: the Associations of DNAm with Neurodevelopment and Function

Through aim 1 of this dissertation, we first assessed the associations between the epigenetic mechanism DNAm and CPT3 performance in ELEMENT children without the consideration of Hg exposure. In this epigenome-wide association study (EWAS), we identified a number of significantly differentially methylated CpG sites and differentially enriched pathways. Hypermethylation in sites annotated to genes *ZNF814*, *ELF4*, and *OR6K6* were significantly related to reduced vigilance in full population models (ISI change main model). Higher methylation levels in *ZNF814* were also related to increased impulsivity (perseverations sensitivity model) and decreased sustained attention (HRT block change main and sensitivity models). Significant gender-specific associations were also identified. One of such examples was hypomethylation at the *GLI3* gene (encoding GLI Family Zinc Finger Protein 3), which was related to decreased sustained attention in females, but not in males. Both *ZNF814* and *GLI3* encode zinc finger proteins belonging to the C2H2-type family, which are believed to regulate transcription activation via sequence specific DNA binding activity. These results suggest that aberrant methylation levels at genes encoding zinc finger proteins may contribute to ADHD etiology by dysregulation of DNA binding.

The results of this analysis are particularly interesting when taken in context with the *in vitro* RNA sequencing results from Aim 3A, which suggests nucleic acid binding, and thus regulation of expression of many downstream genes, may be dysregulated in response to biologically relevant doses of meHg. While this hypothesis was not directly tested one may conjecture that aberrant nucleic acid binding activity related to meHg exposure, DNAm, or both may translate to altered expression of key downstream genes and ultimately adverse neurodevelopment in children and adolescents. The functional pathways enrichment analysis from the EWAS in aim 1 also produced interesting findings that are comparable to the meHg-induced changes in gene expression identified in the *in vitro* tier. In analyses for all CPT3 outcomes tested in aim 1, enrichment for gene pathways pertaining to inflammation, immune response, and neurotransmission were identified. Another pathway of particular note was ferroptosis, as it was enriched in both perseverations and HRT ISI change EWASs. Unlike the previously mentioned pathways, ferroptosis was not directly enriched by meHg in the transcriptomic analysis, but numerous pathways related to ferroptosis (ie. inflammation, oxidative stress, MAPK and JAK-STAT signaling pathways) were [5-8]. Clearly, DNAm and biologically relevant levels of meHg are related to very similar biological mechanism, pathways, and outcomes and are likely important predictors of ADHD etiology.

Synthesis of Results: the Combined Effects of DNAm and Hg Exposure on the Developing Human Brain

While DNAm and Hg exposure have both been studied as risk factors in ADHD etiology, very few studies consider the effects of both variables in concert with one another on neurobehavior and development. This dissertation addresses this gap in the literature and goes

one step further by modeling the combined effects of these two main predictors together under one model through interaction. Notably, most combined analyses previously described in the literature explore the potential mediating role of DNAm in the relationship between Hg exposure and neurodevelopmental outcomes [9, 10]. The present study, however, provides an innovative approach which probes the question of whether epigenetic patterns may influence an individual's susceptibility to the neurotoxic effects of Hg. This novel concept describing one's epigenetic susceptibility or predisposition to toxicant-related diseases is under explored and, to our knowledge, has never been modeled in this way. In full population models from Aim 2 of this dissertation, significant interactions ($q_{\text{int}} < 0.10$) were identified between childhood mercury exposure and DNAm at three CpG sites in models of their relationship with CPT3 performance in ELEMENT adolescents. Methylation at one of these CpG sites, which is annotated to the *ABCA3* gene (encoding the protein ATP-binding cassette subfamily A, member 3), interacted with hair mercury levels in models of associations with HRT ISI change scores ($\beta_{\text{int}} = 5.01$, $\text{SE} = 1.24$, $q_{\text{int}} = 0.067$). *ABCA3* is an important lipid transporter involved in phosphatidylcholine and phosphatidylglycerol trafficking. While it is more highly expressed in lung tissue, low expression levels have been reported in various CNS cells including in neurons, oligodendrocytes, astrocytes, microglia, and endothelial cells comprising cerebral vessels. While the functional role of *ABCA3* in the brain specifically remains poorly understood, ABC transporters as a general class of proteins characteristically utilize ATP to actively efflux small lipophilic molecules that may otherwise passively diffuse across biological membranes, many of which have demonstrated a functional role in blood brain barrier (BBB) permeability [11-13]. With this knowledge it is reasonable to hypothesize that *ABCA3* may be involved in the efflux of lipophilic toxicants like meHg and/or trafficking of important lipids like PUFAs in the brain. The

other two significant interactions identified in the full population models of Aim 2 were between urine Hg exposure and DNAm in their association with inattentive behaviors in adolescence (CPT3 omissions errors score). One significant CpG site (probe ID cg10396484) was annotated to the *LBX1* gene ($\beta_{\text{int}}=0.048$, $\text{SE}=0.012$, $q=0.057$) and the second site (cg21442182) was not annotated to any gene at all ($\beta_{\text{int}}=-0.013$, $\text{SE}=0.0033$, $q=0.075$). The *LBX1* gene encodes a Ladybird Homeobox Protein Homolog 1, which is a highly conserved homeodomain-containing protein related to GO terms for DNA-binding transcription factor activity and sequence-specific DNA binding. This transcription factor plays a very important role in regulating neuron-specific gene expression patterns that influence cell fate during neurodevelopment. It is also involved in regulating cell migration and is essential for GABAergic interneuron development in the dorsal horn of the spinal cord [14]. The second site (cg21442182), though not annotated to a gene in the reference genome, may also be related to transcriptional regulation of gene expression. H3K27Ac mark enrichment was observed at the position of this CpG site when viewed on the UCSC Genome Browser (Figure 5-1). H3K27Ac represents regions of the genome where histone 3 is acetylated at lysine 27, which is usually indicative of active or accessible chromatin associated with transcriptional regulation. While this information suggests cg21442182 may play a role in regulating transcription, to our knowledge it is not considered to be located within a known enhancer or promoter.

Gender modified the independent effects of DNAm and mercury exposure in ELEMENT children and adolescents and the majority of significant interactions between the Hg and DNAm were identified in the gender stratified models from Aim 2. In models of perseveration among the males, significant interactions were identified between urine Hg levels and three specific CpG sites, all of which are suspected to effect nucleic acid binding activity and play a role in

regulation of gene expression. Conversely, in the female only omissions errors models, 157 significant interactions between DNAm and hair mercury levels were identified. Because urine is a better biomarker for inorganic mercury and hair better reflects organic mercury, our results may suggest that females are more sensitive to the effects of meHg and males may be more sensitive to organic mercury when relationships are modified by DNAm levels. Another notable difference in males compared to females is the specific model outcome for which significant results were identified. The significant interactions in males were identified in the perseveration models only. This outcome is reflective of hyperactivity and impulsivity (ADHD-HI), which is consistent with clinical trends of males being more frequently diagnosed with the ADHD-HI subtype than females. Conversely, the significant interactions in females were only identified in the omissions error outcome (related to inattentiveness). Again, current data suggest that females are more often afflicted with the predominantly inattentive ADHD subtype (ADHD-PI)[15, 16]. While this analysis of epigenetic susceptibility was important to help us conceptualize the potential neurobehavioral effects of the two main predictors in children, it is important to remember that in the epidemiological tier of this dissertation neither DNAm nor Hg levels were quantified in the tissue of interest—the brain. If DNAm levels for specific sites in blood and brain are correlated with one another the results from blood may provide some mechanistic insight regarding how Hg is affecting the brain, but such inferences would need to be assessed in experimental models. Regardless of potential functional implications in brain, these results could also serve as biomarkers of ADHD in blood, a biologically available tissue. Prior to use in a clinical setting, proposed biomarkers would need to be validated, replicating results in various cohorts throughout the world.

Both Hg exposure and DNAm were considered in the *in vitro* tier of this dissertation, as well. In contrast to the previous analysis, however, DNAm was modeled as a dependent variable due to the nature of the experiment. As previously described, differential expression was identified in cultured human dopaminergic-like neurons treated with biologically relevant doses of meHg. DNA extracted from the same cells used in the RNA-sequencing analysis was subjected to polymerase chain reactions and subsequent sequencing of amplicons in analyses of differential total, 5mC (5-methylcytosine), and 5hmC (5-hydroxy-methylcytosine) methylation using Oxford NanoPore Technologies. Unlike analyses from tier one of this dissertation, distinguishing between 5mC and 5hmC adds an additional layer of rigor to this experiment, as the marks frequently serve distinct roles in gene regulation and have the potential to counter the effects of the other, masking significant associations when modeled together. Conversely, there are some examples when the reverse is also true, wherein both marks contribute to a common outcome and associations can only be identified when 5mC and 5hmC are modeled jointly in an analysis of total methylation. That said, there is value in all three types of analysis, 5mC, 5hmC, and total methylation, and future analyses in the ELEMENT or other cohort studies should consider modeling 5mC and 5hmC separately, which may elucidate some of the findings from tier 1 of this dissertation.

In Aim 3A, two novel genes in the 0.01 μ M (ENSG00000258830) and 0.25 μ M (ENSG00000242479) meHg treatment groups were significantly differentially expressed via a stringent significance threshold of $p_{\text{adj}} < 0.05$ & $\text{abs}(\log_2\text{FoldChange}) > 1$ and thus were selected for the targeted methylation analysis in aim 3B. No significant findings were identified in the total methylation analysis for either amplicon, but when distinctions were made between 5mC and 5hmC associations began to reveal themselves. One marginally significant finding was

identified for ENSG00000258830 in the 0.25 μ M treatment group of the 5hmC analysis (21.86 (10.63), 0.095). While this did not align with the expression patterns observed for this gene, it could still be influencing gene expression via transcriptional regulation of other neighboring genes or could poise the gene for altered regulation following a future challenge. While higher 5mC levels (especially in promoter regions) is usually related to reduced expression of that gene, 5hmC has been reported to influence gene expression in a number of different ways [17].

Notably, unlike the ENSG00000258830 (whose amplicon was located in the gene body), the ENSG00000242479 primers were designed to target a sequence near the transcription start site that's likely in the gene promoter. The findings from the methylation analysis for ENSG00000242479, on the other hand, were very interesting when taken in context with the expression data. While exposure to 0.25 μ M meHg was associated with decreased expression, it was also associated with decreased 5mC levels (-7.56 (1.25), 0.000917), which is the opposite of what is expected based on traditional trends. Conversely, there was a significant increase in 5hmC (10.42 (3.56), 0.0327) levels in this treatment group. Across all meHg treatment groups for this amplicon, 5mC and 5hmC exhibited opposite directions of effect, being consistently negative and positive for each mark respectively. Notably, the associations were marginally significant for both marks in the 0.01 μ M meHg treatment group and for 5hmC in the 6.25 μ M group. Though not always significant, the magnitude of effects were comparable within each treatment group for expression, 5mC, and 5hmC data alike, with the lowest and highest groups exhibiting a small effect and the middle dose exhibiting a large effect. This data presents a non-monotonic effect (Table 5-1). Other genes were selected for target methylation analysis based on functional relevance of the transcript as previously identified in the literature and suggested through significant results identified in other parts of this dissertation. Through these analyses,

we identified differential methylation of *STAT1*, *GABRG3*, and *DAAM2* in response to biologically relevant doses of meHg, but not to high-dose exposures (6.25 μ M). Future research is needed to discern whether these changes promote toxic or protective mechanisms.

Innovation and Implications for Human Health

While the associations of either DNAm or meHg exposure with adverse neurological outcomes is well-documented, only a handful of studies have examined these risk factors in concert with one another. This was addressed in the present dissertation by considering both variables simultaneously via *in vitro* in tandem with epidemiological methodologies. For both meHg and DNAm, a greater emphasis has been placed on the prenatal timepoint in previous research, leaving their effects during childhood and adolescence underexplored, despite it being an essential period in the development of the frontal lobe of the brain and executive functioning skills. This window of susceptibility was analyzed in a Hispanic cohort by leveraging mercury data (measured in hair and urine during childhood), CPT3 scores (administered during adolescence), and DNAm levels (quantified in adolescent blood). We also endeavored to explore this timepoint *in vitro* by exposing dopaminergic-like neurons to meHg after rather than during differentiation, the latter being a common approach to model *in utero* exposures. Having identified significant findings in both tiers of this dissertation, we have greater confidence in our results, which suggest that DNAm and Hg exposure may influence neurodevelopment during childhood and adolescence. To verify this evidence, we recommend exploring these timepoints in other human populations as well as through *in vivo* systems.

Our *in vitro* RNA sequencing and differential methylation results suggest that dopaminergic-like neurons respond differently to meHg exposure at biologically relevant and

cytotoxic doses and expression and epigenetics may not respond in a traditional monotonic manner. This idea was also supported in a test of metabolic activity, which demonstrated a significant increase in metabolism in the low to medium meHg doses and a significant decrease in the higher dose. This does not mean that meHg is beneficial at low doses, but rather suggests excitotoxicity. Notably, this hypothesis was not directly tested. It is important to remember that meHg is a known neurotoxicant, but also that there are a lot of beneficial nutrients in seafood (the main source of meHg exposure in humans) that promote healthy neurodevelopment. While exposure to low levels of meHg do not consistently associate with adverse neurodevelopmental outcomes, which was corroborated by our analyses in ELEMENT children from aim 2, we do not believe meHg at any level supports healthy brain development. Because seafood is so rich in nutrients, we do not recommend ELEMENT participants remove it from their diet in efforts to reduce meHg intake. A better approach would be to modify the types of fish consumed based on the ratio of meHg levels relative to seafood nutrients. Sardines, for example, are believed to have the ideal balance of low meHg and high PUFA levels in Mexico City [18].

Through the epidemiological tier of this dissertation, we identified specific CpG sites where DNAm significantly associated with CPT3 scores and other positions where it significantly interacted with Hg exposure in their associations with the same outcomes. In both cases, these results may serve as future biomarkers of ADHD. It also implicated several significant pathways that may be involved in ADHD etiology, including inflammation, immune response, and ferroptosis. These findings may inform future research of potential clinical relevance. While DNAm was shown to be an important variable in these epidemiological models, analyses could have been improved by distinguishing between 5mC and 5hmC. By measuring them together as we did in these epidemiological models, some associations may have

been masked. This was the case in another study within the ELEMENT cohort, where prenatal lead exposure was associated with differential blood leukocyte DNAm at adolescence, but many associations were only identified after 5mC and 5hmC marks were assessed separately [19]. This was further evidenced by our *in vitro* analysis of differential DNAm in neurons, which employed a novel approach that used bisulfite/ oxidative bisulfite conversion reactions paired with ONT sequencing techniques to distinguish between these two similar marks. The majority of significant findings were identified in either 5hmC or 5mC models but were masked in total DNAm models, demonstrating that despite their similarities these are indeed distinct marks and should be modeled separately. Past research has shown that hydroxymethylation (5hmC) is particularly abundant in neurons, especially dopaminergic neurons, and plays specific biological roles depending upon the position of the 5hmC mark in the genome and the developmental timepoint that it is present [17]. While 5hmC used to be regarded solely as an intermediate in demethylation with no true functional relevance, our research contributes to a growing body of literature identifying it as an independent and functionally important mark. Future research, especially in neurobiology, needs to normalize the independent assessment of these two epigenetic marks.

Lastly, the interaction analysis of aim 2 of this dissertation is particularly innovative, as it probes a novel concept which we refer to as epigenetic susceptibility. Examples of genetic susceptibility to Hg related ADHD symptoms have been documented, but to our knowledge the epigenetic influence has never been modeled in this way. Neither the exposome nor the epigenome is static, so it would stand to reason that more dynamic interactions and associations are possible. As a scientific community we need to dare to reach beyond conventional gene x environment interaction (G x E) studies and engage the idea of epigenetic x environment

interaction ($E \times E$). Results from $G \times E$ studies are often easier to interpret since they utilize a binary variable that can be stratified to assess each genetic variant separately. Results generated by $E \times E$ studies are difficult to interpret, however, they are also relevant, because DNAm is not static, making it a great potential target for intervention. Better understanding how our epigenomes are interacting with the environment to influence health and well-being could refine our approach to risk assessment and inform how we develop interventions.

Future Directions

While we have already provided some recommendations for future studies, this dissertation lays the foundation for several other potential avenues of research as well. One weakness of the *in vitro* tier is that it only assessed one cell type and was not associated with a clinical outcome. The brain is incredibly complex and various different regions are known to be affected by ADHD. Replication of these experiments in other cell lines representing different cell types (ie. glutamatergic or GABAergic neurons) derived from both males and females is recommended. A cell line of particular interest is an induced pluripotent stem cell (iPSC) line derived from a teenaged boy diagnosed with ADHD [20]. These cells are unique because they are derived from blood cells of an adolescent patient but are able to be differentiated to different types of cells, including cortical neurons [20-22]. In addition to this, another similar set of iPSCs were derived from his twin brother who does not have ADHD [20, 23]. Baseline comparisons could be made between ADHD and non-ADHD methylation/expression profiles and could help draw inferences about susceptibility to neurotoxicity. For instance, expression differences related to genes involved in detoxification of exposures like meHg may be augmented in the ADHD line relative to the non-ADHD line. This would suggest that something in the ADHD cell-line (ie. methylation profiles) predisposes them to a greater adverse effect to meHg. Conversely, if there

is not an apparent toxic effect in the ADHD-line but the mercury-treated non-ADHD cells have similar methylation/expression as the untreated ADHD line, this may indicate that mercury is inducing changes similar to an ADHD phenotype. Importantly, exploring gene ontology could provide insights as to whether ADHD predisposes up/downregulation of certain pathways in cortical neurons in response to Hg. By then relating it back to DNAm in a subsequent analysis, it will allow us to discern whether DNAm is facilitating the changes in gene expression seen in the RNAseq. In order to truly demonstrate that changes in DNAm are causing the expression changes for a gene or related molecular pathway, one would need to impede a cell's ability to methylate or demethylate DNA. This can be accomplished by knocking down DNA methyltransferases (DNMT) and ten-eleven translocation methyl cytosine dioxygenases (TET) that facilitate these two processes respectively. We recommend conducting similar experiments *in vivo*, as well. This would be able to examine meHg's effects on multiple cell types, the systems and tissues with which they interact, and whether these effects result in behavioral changes and/or structural alterations in the brain similar to that of an ADHD patient.

In regard to the epidemiological tier, one recommendation would be to compare DNAm levels at adolescence and at birth. Through this, one could discern whether the significant marks identified in this dissertation are specific to adolescence or were maintained from birth. The same could be done for Hg exposure. While our results indicate that Hg exposure and DNAm at childhood are important predictors of adolescent ADHD symptoms, we cannot assume these effects are necessarily specific to childhood without also examining their effects at birth. Additionally, future research in the ELEMENT cohort quantifying 5mC and 5hmC separately is warranted. Evidence from Aim 3B of this dissertation suggests that the two marks may serve differential roles in their relationships with meHg *in vitro* and there is value in modeling them

separately; the same is likely true in the epidemiological analyses, as well. Notably, modification is not the only way Hg exposure and DNAm could jointly affect ADHD etiology. Mediation analysis is another potential approach to examine their combined effects, and this is the typical paradigm used in the environmental epigenetics field whereby epigenetic effects are thought to be the mechanism linking an exposure to an outcome. Another consideration is that no exposure exists in isolation, and it is likely Hg is being co-exposed with several other toxicants. This could potentially be addressed through exposure mixtures analyses.

While there are numerous ways that we could have examined our primary research question, this dissertation uniquely balanced the strengths and weakness of various study designs and employed innovative modeling and laboratory techniques. We identified significant associations in all three aims which suggest epigenetic mechanisms, inflammation/immune response, nucleic acid binding, and cell migration are all dysregulated by Hg exposure and DNAm and likely play a role in how they influence ADHD etiology. Future research is needed to better characterize these mechanistic implications. Regardless, this project is deeply significant, as it aims to understand how/why Hg impacts neurocognition, and this is considered in an understudied Hispanic population. It takes an innovative approach, assessing often-overlooked epigenetic profiles (via total, 5mC, and 5hmC DNAm) as potential mechanisms modulating the effects of mercury on the brain, and can inform future interventions by identifying relevant risk factors for ADHD. Lastly, this dissertation presents a novel concept which we have coined “epigenetic susceptibility” that is similar in nature to G x E interaction studies. This research could serve as a model for future E x E studies using other toxicants and health outcomes and help us as a scientific community better understand the specific ways in which our epigenomes interact with the environment.

Figures and Tables



Figure 5-1: Location of Significant Probe cg21442182 Where DNA Methylation Significantly Interacted with Urine Hg Levels in Their Effect on Omissions Error Scores in ELEMENT Children.

Red dashed line indicates the location of the CpG site of interest (chromosome 13 position 31,446,420). Image modified from UCSC Genome Browser track: https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr13%3A31445920%2D31446920&hgsid=2255629648_E8FtbVqXGD76GOWN5LtyJDizmaay

Table 5-1: Differential expression and DNA methylation (DNAm) in response to biologically relevant doses of meHg for two novel genes identified in RNA-seq.

Gene	Analysis	baseMean	0.01 uM	0.25 uM	6.25 uM
ENSG00000242479	Gene Expression log2FoldChange (lfcSE) p-value	11.36	-0.938 (0.60) 0.117	-6.699 (1.29) 2.31E-07 **	-0.186 (0.58) 0.749
	5mC DNAm GLM β (S.E.) p-value	93.31 %	-2.56 (1.25) 0.0864 •	-7.56 (1.25) 0.000917 *	-1.56 (1.25) 0.259
	5hmC DNAm GLM β (S.E.) p-value	8.01 %	8.75 (3.56) 0.0572 •	10.42 (3.56) 0.0327 *	8.08 (3.56) 0.0722 •
	Total DNAm GLM β (S.E.) p-value	94.78 %	0.33 (1.53) 0.835	2.44 (1.53) 0.161	0.33 (1.53) 0.835
ENSG00000258830	Gene Expression log2FoldChange (lfcSE) p-value	43.25	-3.05 (0.52) 4.06E-09 **	0.25 (0.37) 0.494	1.17 (0.36) 0.00122 **
	5mC DNAm GLM β (S.E.) p-value	48.53 %	4.56 (14.77) 0.768	18.11 (14.77) 0.266	-6.78 (14.77) 0.662
	5hmC DNAm GLM β (S.E.) p-value	26.53 %	2.33 (9.21) 0.810	21.86 (10.63) 0.095 •	14.83 (9.21) 0.168
	Total DNAm GLM β (S.E.) p-value	54.56%	4.56 (3.26) 0.211	5.67 (3.26) 0.133	-6.22 (3.26) 0.105

Note: * significant based on a raw p-value less than 0.05; ** significant based on q-value less than 0.05 (expression data only); • significant based on raw less than p-value 0.10

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