ABILITY OF DIET TO INDUCE HERITABLE EPIGENETIC MODIFICATIONS ASSOCIATED WITH ALZHEIMER'S DISEASE

by

BRIDEY ELIZABETH BROWN

Bachelor of Science, Biology, 2022
Texas Christian University
Fort Worth, TX

Submitted to the Graduate Faculty of the
College of Science and Engineering
Texas Christian University
In partial fulfillment of the requirements
For the degree of

Master of Science

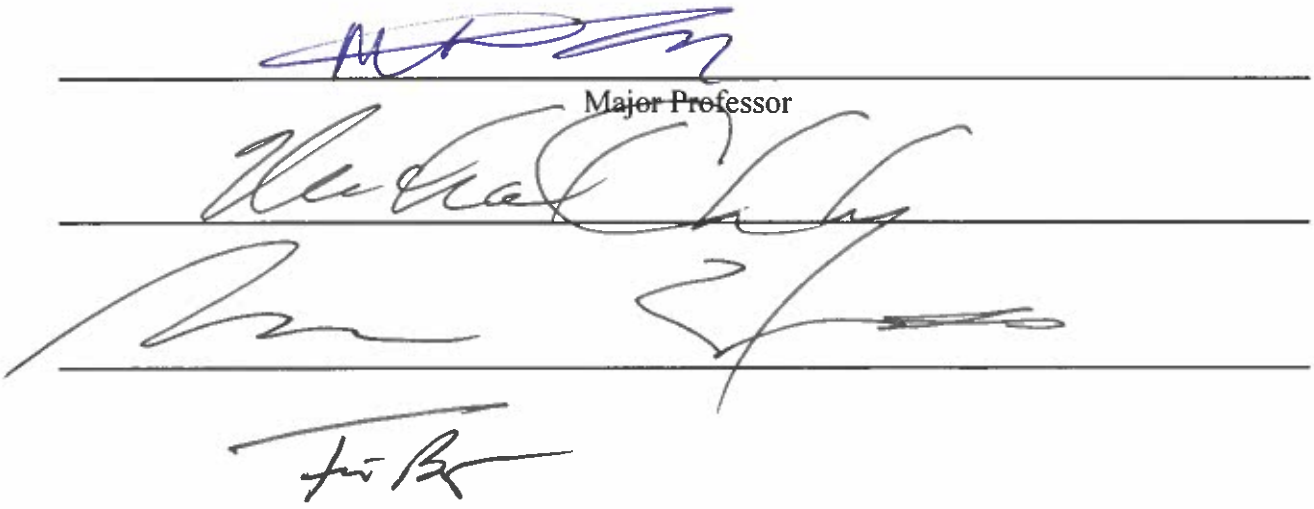
May 2024

ABILITY OF DIET TO INDUCE HERITABLE EPIGENETIC MODIFICATIONS ASSOCIATED WITH ALZHEIMER'S DISEASE

by

Bridey Elizabeth Brown

Dissertation approved:



Major Professor

For The College of Science and Engineering

Acknowledgements

I would first and foremost like to thank Dr. Matt Hale, who has truly been the best advisor I could have asked for during my time as a graduate and undergraduate student at TCU. He taught me the skills necessary to become a confident scientist, teacher, and researcher, and I will forever treasure my time in his lab. I would also like to thank my committee members: Dr. Michael Chumley for welcoming me with open arms into his lab and allowing me to collaborate on a project, and Dr. Mikaela Stewart for always encouraging me to think critically and creatively.

I would also like to extend my immense gratitude to undergraduate researcher Asher Smith, who has played a pivotal role in the collection of data and analysis for this thesis. This project would not have been possible without his support, and I am truly grateful to have had such a wonderful and intelligent lab partner and friend by my side. Furthermore, I would like to thank Dr. Paige Braden-Kuhle for being an incredible mentor, always having a positive attitude, and allowing me to collaborate with her on her doctoral research. Ms. Kira Gangbin and Mr. Alex Caron also deserve my thanks, as they have not only helped me craft this thesis, but also been two of my dearest friends. I would also like to thank Ms. Morgan Bertrand and Ms. Vivienne Lacy, as well as the other members of the Chumley lab, for their contributions and allowing me to find a second home in their lab.

Lastly, I would not be here if not for the support of my wonderful parents, Marnie and Terry Brown, who have always encouraged me to dream big. They have been instrumental in instilling within me the confidence to follow my passions, and I am forever grateful for their unwavering love and support. And of course I would like to thank my little brother and forever partner-in-crime, Brighton Brown, for always making me laugh.

Table of Contents

List of Figures

List of Tables

Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease that is characterized by the accumulation of beta-amyloid (Aβ) plaques and neurofibrillary tangles in the brain (Hanseeuw et al., 2019; Villemagne et al., 2013). AD is the most common cause of dementia, and it was the seventh leading cause of death in Americans in 2020 (Murphy et al., 2021). As of 2022, an estimated 6.5 million Americans aged 65 and older suffer from AD ("2022 Alzheimer's disease facts and figures," 2022). Barring any developments in disease prevention or treatment, that number is expected to reach 13.8 million by 2060 (Rajan et al., 2021). There are two types of AD: early-onset Alzheimer's disease (EOAD) and late-onset Alzheimer's disease (LOAD). EOAD occurs in individuals younger than 65, and is the result of a mutation in one of three genes (either *app*, *psen1*, or *psen2*) in 14-82% of cases (Campion et al., 1999; Janssen et al., 2003; Lanoiselée et al., 2017; Raux et al., 2005; Theuns et al., 2000). These three genes are involved in the Amyloid Precursor Protein processing pathway, and mutations in any of them result in increased Aβ plaque formation, and an increased likelihood of AD development (Gordon et al., 2018). EOAD only accounts for approximately 1% of all AD cases (Bekris et al., 2010). In contrast, LOAD cases make up the majority of AD cases and are characteristically sporadic, likely due to the complex interplay of a variety of genetic and environmental influences (Rabinovici, 2019). Because the etiology of LOAD is complex and not fully understood, developing treatment plans is difficult. There are currently seven FDA-approved drugs for AD treatment: two of which are able to target the underlying physiology of AD by removing Aβ, but they must be delivered via intravenous infusion. The remaining five drugs mitigate disease symptoms temporarily, but do not target the underlying physiology (*Medications for memory, cognition and dementia-*

related behaviors, 2023). In the absence of effective treatment options, the best alternative is developing preventative strategies, however, our inadequate understanding of the mechanisms that mediate LOAD make this difficult.

One factor that has been proven to influence the propensity for developing LOAD, and therefore a potential method that individuals can use to reduce their risk, is dietary practices. One potential dietary strategy for the prevention of LOAD is a traditional Mediterranean diet (MD), as studies have shown that adherence to a MD is associated with reduced risk of developing AD (Scarmeas et al., 2006; Singh et al., 2014; van den Brink et al., 2019), and reduced risk of mortality due to AD (Scarmeas et al., 2007; Trichopoulou et al., 2003). Initial evidence suggesting the MD may confer neuroprotective effects throughout aging comes from the world's five Blue Zones, which are regions where people live the longest and healthiest lives (Buettner, 2017). Two of these Blue Zones are found in the Mediterranean region – one in Sardinia, Italy and the other in Ikaria, Greece – and the increased longevity of their residents is often attributed to their diet, which is high in fiber, unsaturated fats, and complex carbohydrates (Trichopoulou et al., 2005). Frequent consumption of individual components of the MD, such as fruits, vegetables, and fish, is also associated with reduced risk of AD (Barberger-Gateau et al., 2007). Increasing MD adherence has also been associated with a decreased rate of cerebral Aβ accumulation in adults who already had a high Aβ burden (and were therefore on track to develop AD; Gordon et al., 2018). Of the individual MD components analyzed in Gordon et al., high fruit intake was the strongest contributor to decreased Aβ accumulation (Gordon et al., 2018). Agarwal et al. (2023) found an inverse correlation between MD adherence and Aβ load in the

postmortem brain tissue of older adults (Agarwal et al., 2023). Therefore, not only is the MD associated with decreased risk of AD development and morbidity, but it has also been shown to ameliorate key pathologies of AD.

Further support for utilizing diet as a potential AD prevention strategy comes from the effects of consuming a Western diet (WD), as components of the WD such as high intake of saturated fats and cholesterol has been found to be associated with increased AD risk and prevlance over the age of 55 (Kalmijn et al. 1997). In countries like Japan where dietary patterns have recently trended toward a WD (indicated primarily by an increased consumption of animal fat), there has been a correlated increase in AD prevalence (Grant, 2014). Furthermore, dietary studies in mice have demonstrated that the WD and its components can lead to increased and/or accelerated accumulation of brain Aβ and neurofibrillary tangles (Więckowska-Gacek et al., 2021). This same pattern can also be seen in humans when diets contain increased WD components, such as high glycemic diets (Taylor et al., 2021; Taylor et al., 2017), clearly indicating an increased propensity to developing LOAD with components of the WD.

One limitation with previous studies that have investigated dietary influences on AD is the nature of the diet, which was supplemented with a single component of the MD or WD rather than targeting all components of both diets, or relied on a WD with fat levels that far exceed a typical American diet. To address this, the Neurobiology of Aging Lab at TCU designed holistic versions of a MD and a typical American diet (TAD, i.e., WD) that are more representative of human dietary consumption on a daily basis (Braden-Kuhle, 2023).

From there, they sought to directly compare the MD and TAD in regards to the development of various AD pathologies in C57BL/6J mice. One of their findings was that mice on the TAD had significantly more soluble Aβ in the prefrontal cortex compared to the MD, and that male mice on the TAD had significantly more soluble Aβ in the hippocampus than males on the MD (Braden-Kuhle, 2023). Furthermore, the Aβ levels were directly correlated to the level of cognitive dysfunction demonstrated in the mice. In addition, it was also found that two genes encoding for pro-inflammatory cytokines (TNF-α and IL-1β) were differentially expressed between diets in the hippocampus of male mice, wherein males on the MD had significantly lower expression compared to males on the TAD upon LPS injection (Braden-Kuhle, 2023). As AD has also been linked to neuroinflammation, the decreased expression of pro-inflammatory cytokines as a result of the MD indicates that the MD may be neuroprotective against inflammation and AD.

Although Braden-Kuhle (2023) suggests that both the MD and TAD can influence AD development and pathology, the underlying mechanisms behind their contribution to AD development/prevention remain unclear. One potential mechanism could be via diet-induced epigenetic modification to genes associated with AD. The most studied epigenetic modification is DNA methylation of cytosines, and 75% of cytosine methylation in brain tissue occurs in the CpG context, wherein both cytosines on either complimentary strand of a CpG dinucleotide are methylated (Lister et al., 2013). DNA methylation has been known to alter gene expression levels (Suzuki & Bird, 2008) and other studies have found a link between differential methylation and the development of AD (Zhang et al., 2020). Previously collected data from multiple genes shows that MD can induce methylation changes in genes

involved in inflammation, immunocompetence, neuronal function, and synaptic plasticity (Arpon et al., 2016; Luceri et al., 2017). Therefore, studies indicate that not only can dietary factors induce differential methylation of genes involved in AD, but they can also alter expression and propensity for developing AD. However, none of these studies attempted to draw a direct link between holistic MD-induced methylation differences and resulting gene expression relating to AD, and certainly not compared to a TAD.

These alterations in methylation patterns, and subsequent changes in gene expression, as a result of consumption of a MD or TAD could also result in transgenerational effects, as methylation patterns are known to be heritable (Villicaña & Bell, 2021). In other diseases such as obesity, high blood pressure, and metabolic syndrome, ancestral diet has been linked to epigenetic modifications associated with the disease that are then passed on to offspring and influence their disease risk (King & Skinner, 2020; Masuyama & Hiramatsu, 2012). Studies investigating the WD and MD separately have found that maternal exposure to the diets induces DNA methylation changes in offspring (Dudley et al., 2011; Gali Ramamoorthy et al., 2018; Gonzalez-Nahm et al., 2017; House et al., 2018). Transgenic mouse models capable of producing measurable Aβ in their brains have also demonstrated that maternal supplementation with choline (a common MD component) is able to reduce hippocampal Aβ load by 44% in F_1 offspring who were only exposed to the diet during gestation and lactation (Velazquez et al., 2020). F_1 individuals were bred to produce an F_2 generation that was never exposed to the diet, and they found a 29% reduction in hippocampal Aβ load in F_2 offspring whose grandparents had choline supplementation (Velazquez et al., 2020). In the same study, 27 genes involved in brain immune response and regulation of neuronal death were found to

be differentially expressed in F_1 offspring based on whether their parents had choline supplementation, and this differential expression carried over to the F_2 offspring (Velazquez et al., 2020). Therefore, research in this field has shown that 1) epigenetic modifications can be associated with AD, 2) dietary factors can induce epigenetic modifications that can be transmitted to subsequent generations, and 3) certain diets can influence risk of AD development. However, to the best of my knowledge, no previous study has investigated whether these factors intersect. Therefore, here I will investigate if the holistic MD, when compared to the TAD, is associated with epigenetic modifications that then induce changes in gene expression and subsequently influence the development of AD related pathologies in a mouse model. Moreover, this study explores whether any such epigenetic modifications are heritable in subsequent generations, so this research could be of fundamental importance to determine predisposition of offspring to AD development or protection.

Methods

Sample Collection

The parental (P) generation of mice were weaned on postnatal day 21 and randomly assigned to either the MD or the TAD (Table 1). Mice assigned to the MD group consumed their diet for four months, at which point they were paired for breeding while continuing diet consumption. Mice assigned to the TAD group consumed their diet for three months before being paired for breeding because there were no viable pups produced from breeders paired after four months of diet consumption. Each breeding pair shared the same dietary condition (i.e., females on the MD were paired with males on the MD, etc.) to produce the F_1 generation. All breeders continued their experimental diets throughout breeding, gestation,

and lactation. When they reached nine months of age (8 months of diet consumption), their hippocampal tissue was collected and used for DNA extraction, while their prefrontal cortex was collected and stored in RNALater at -80ºC for RNA extraction. Meanwhile, all members of the F_1 generation were weaned on low-fat diet (LFD) as a control (because it is a standard rodent chow used in most research labs), but remained separated in groups based on the dietary condition of their parents in order to examine the transgenerational effects of each diet. When the F_1s reached four months of age, hippocampal tissue was collected for DNA extraction and prefrontal cortex was collected for RNA extraction.

Table 1. Composition of C57BL/6J mice in the parental generation paired to breed and produce the F_1 generation.

Parental Generation (P)	MD	TAD
Female, C57BL/6J	n = 5	n = 5
Male, C57BL/6J	n = 3	n = 3

DNA Extraction

DNA was extracted from hippocampal tissues of three mice of each sex on each of the experimental diets from each generation (24 mice in total) using Qiagen's DNeasy Blood and Tissue kit according to the manufacturer's protocol. Nanodrop was used to quantify DNA, and samples were stored at -20ºC for later analysis.

Epigenetic Array and Analysis

DNA samples for each diet group were sent to the University of Texas Southwestern Medical Center Microarray Core Facility (Dallas, TX), where all 24 DNA samples were run on an Illumina Infinium Mouse Methylation BeadChip (also known as the MM285 chip). This chip queried more than 285,000 methylation sites across the methylome per sample and

produced an output of intensity data (IDAT) files that can be used to determine metylation patterns. Analysis of the IDATs was carried out in R version 4.3.1 with the R-package Sensible Step-wise Analysis of DNA Methylation BeadChips (*SeSAMe*) version 1.18.4 (Zhou et al., 2018). Default data preprocessing codes were used to perform quality masking (*qualityMask*), non-linear dye-bias correction (*dyeBiasNL*), detection p-value masking using oob (*pOOBAH*), and background subtraction using oob (*noob*). Methylation levels at each methylation site, which are reported as β-values (the ratio of the intensity of the methylated bead type to the combined locus intensity), were retrieved using the *openSesame* command. All CpG probes that reported "NA" values in any samples were eliminated from further analysis. Parental females on the TAD (n = 5) were set as the reference group, wherein methylation levels for all other samples would be compared back to them. The *DML* command was used to compare β-values and determine differentially methylated loci (DML), indicating one CpG site that was differentially methylated, according to diet between parental samples. DMLs were then filtered such that only DMLs with a p-value of less than 0.0001 or an effect size of greater than 10% remained. This process was repeated for F_1 samples, and the results were compared to determine whether the differential methylation patterns in the parents were passed on to the offspring. DMLs with either a p-val<0.0001 or effect size of 10% were cross-referenced with the MM285 Manifest to determine which DMLs were found within or near protein-coding genes. A literature review was then conducted to identify protein coding genes potentially implicated in AD development or pathology. Six genes with a p-val<0.0001 and five genes with an effect size > 10% were selected for further gene expression analysis.

RNA Extraction and Conversion of RNA to cDNA

RNALater was removed from prefrontal cortex tissues of the same 24 mice and resuspended in homogenization solution. Tissue was homogenized using a tissue sonicator set to pulse three times at an amplitude of 65%. RNA extraction was subsequently performed on the Maxwell RSC Instrument using Promega's Maxwell RSC simplyRNA Tissue Kit according to the manufacturer's protocol. Nanodrop was used to quantify RNA, and 3µL of each sample was immediately converted into cDNA using the Invitrogen SuperScript III First-Strand kit according to manufacturer's protocol. Remaining RNA samples were stored in -80ºC for later analysis, and cDNA samples were stored in -20ºC for later analysis.

qPCR

One-in-ten dilutions of cDNA were made for qPCR use. Pre-established qPCR primers for DMLs and a reference gene (*β-actin*) were found via the *PrimerBank* database (https://pga.mgh.harvard.edu/primerbank/) using NCBI GeneID's for each protein-coding gene (Wang et al., 2011). Primers used in this study and specific assay conditions can be found in Table S1. qPCR of samples was performed on an Applied Biosystems StepOnePlus Real-Time PCR System using PerfeCTa SYBR Green FastMix ROX. qPCR assay mixtures were made with 1µL cDNA, 3.28µL NF Water, 5µL SYBR, and 0.36µL of each primer. Assays were performed in triplicate, and the fluorescence threshold (Cq) was averaged between the triplicates. In the cases where the standard deviation was greater than 0.5 and there existed an obvious outlier in the triplicates, it was removed from analysis. Gene expression differences within each generation were calculated using $\Delta\Delta C_t$ between dietary groups for each gene of interest, collapsing by sex, with all TAD animals as the reference

group. Differences in expression were tested using an independent t-test assuming equal variance and with alpha set to 0.05.

Results

Differential Methlyation

After quality control and eliminating probes that reported "NA" values in any samples, 263,451 probes remained for *DML* analysis. Differential methylation was determined by p-value and effect size. However, no DMLs had both a p-val < 0.0001 and an effect size greater than 10% (see Figure 1) and no DMLs were stastically significant after a Benjamini and Hochberg false-discovery rate correction. Instead, I focused on loci with either a significant p-value or an effect size greater than 10% for further analyses. Fourty-nine DMLs showed a statistically significant difference between diets in the parental generation. Of which, 37 were hypermethylated and 12 were hypomethylated in MD relative to TAD (Figure 1). Of those, 28 were located within the body of a protein-coding gene, while 15 were located near a protein-coding gene. Six of the DMLs associated with protein-coding genes were selected for gene expression analysis based off of their heatmaps (Figure 2) and potential contribution to AD pathology based on Gene Ontology (GO) Terms and AD implication in other studies (Table 2).

There were also 11 DMLs with an effect size of at least 10%, eight of which were hypermethylated and three were hypomethylated in MD relative to TAD (Figure 1). Of the DMLs with a large effect size, five were located within the body of a protein-coding gene, while three were located near a protein-coding gene. Five of the DMLs associated with

protein-coding genes were selected for gene expression analysis based off of their heatmaps (Figure 2), average beta-values (Figure 3), and potential contribution to AD pathology based on GO Terms and AD implication in other studies (Table 2).

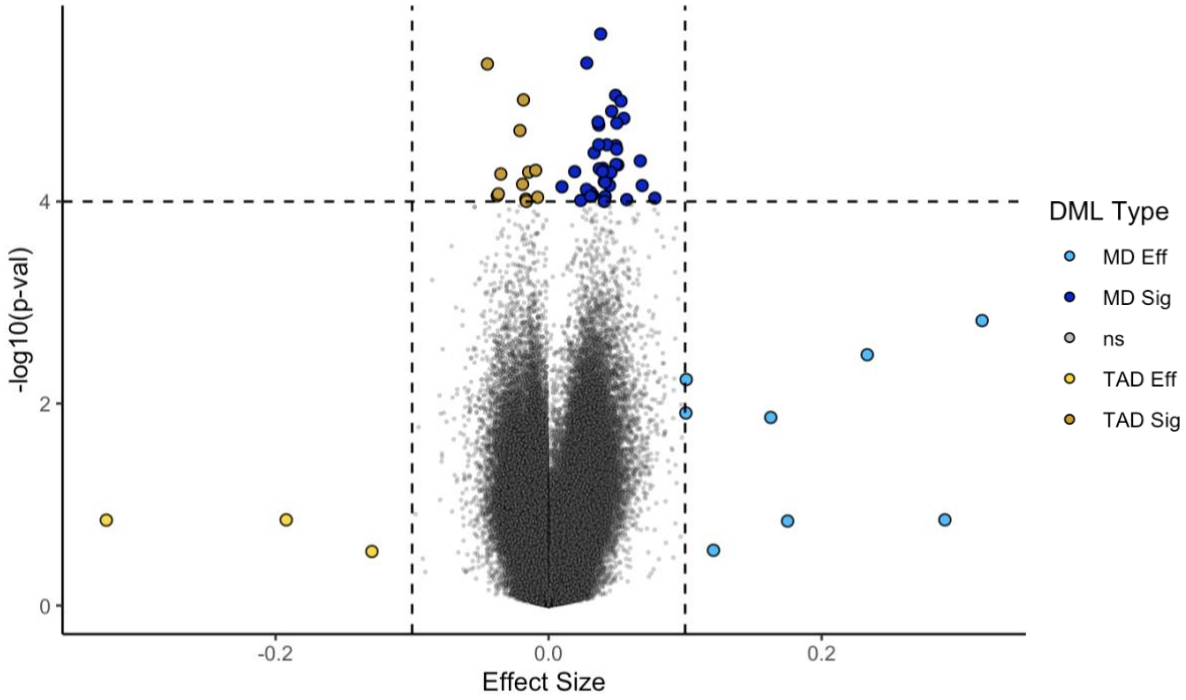


Figure 1. Volcano plot of DMLs based off of diet according to effect size (≥10%) and statistical significance (p-val≤0.0001).

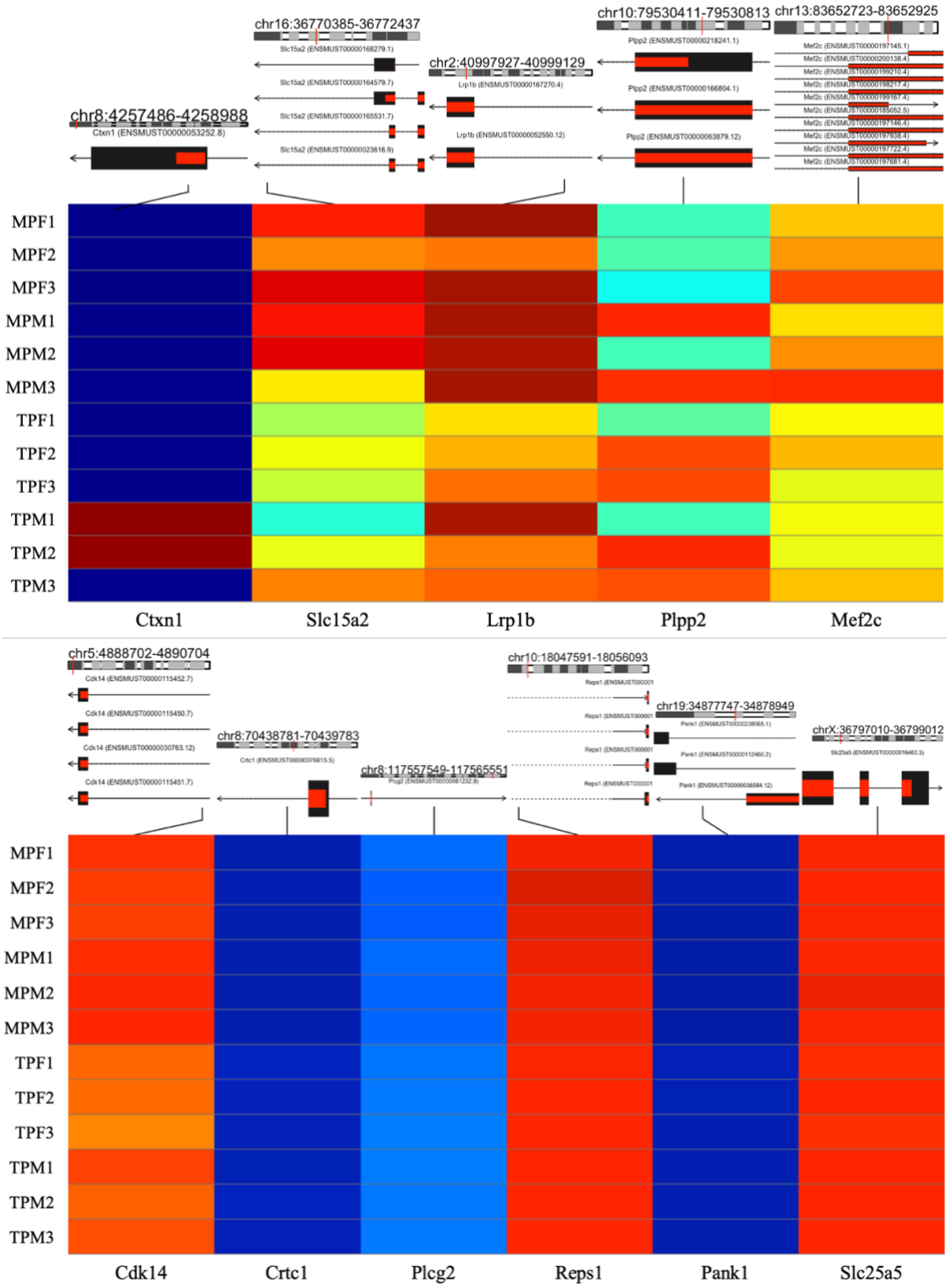


Figure 2. Methylation heatmaps of DMLs selected for qPCR, split based off of statistical significance (p-val <0.0001) or effect size >10%. Color represents DNA methylation β-

values, where blue = zero methylation and red = full methylation. Sample names are assigned such that the first letter indicates diet (M=MD and T=TAD), the second letter indicates generation (P=parental generation), and the third letter indicates sex (F=female and M=male). Numbers represent biological replicates.

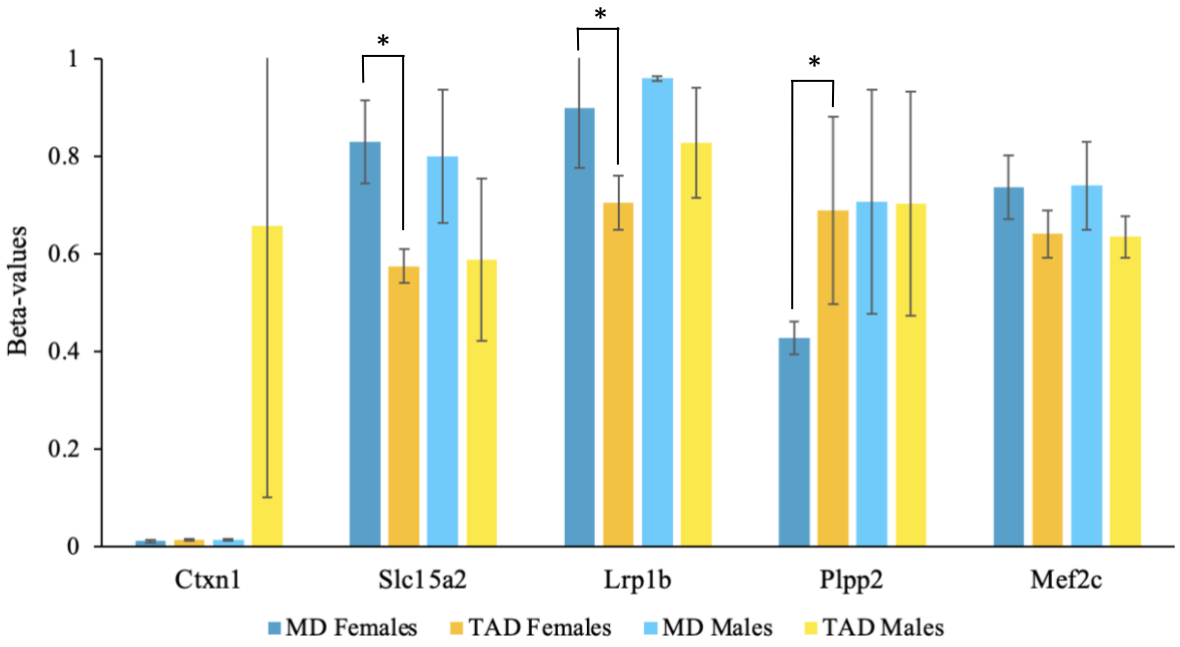


Figure 3. Average parental beta-values for effect size genes according to diet and sex. Error bars depict standard deviation. Differences in beta-values were tested using a one-tailed independent t-test assuming equal variance and with alpha set to 0.05. *Slc15a2*, *Lrp1b*, and *Plpp2* were found to have significantly different beta-values ($p<0.04$) between MD females and TAD females. No genes were found to have significantly different beta-values between MD males and TAD males.

Table 2. Loci chosen for qPCR, which were either statistically significant (p-val<0.0001) or had an effect size >10%.

Significant DMLs

Locus	p-value	Effect Size	Hypo- vs. Hyper-Methylated Relative to TAD	Site of Methylation Relative to TSS	EnsReg Build	GO Terms
Cdk14	7.292E-05	0.056	Hyper	375,563 bp's downstream	N/A	• G2/M transition of mitotic cell cycle • Regulation of canonical Wnt signaling pathway

Crtc1	5.381E-05	0.009	Hypo	298 bp's downstream	Promoter	• Memory • positive regulation of CREB transcription factor activity
Plcg2	3.396E-05	0.021	Hypo	63,259 bp's downstream	Promoter Flanking Region	• Stimulatory C-type lectin receptor signaling pathway • toll-like receptor signaling pathway
Reps1	2.007E-05	0.040	Hyper	7,848 bp's upstream	N/A	• Endocytosis • Endosomal Transport
Pank1	6.768E-05	0.010	Hypo	298 bp's upstream	Promoter	• Coenzyme A biosynthetic process • Phosphorylation
Slc25a5	5.806E-05	0.030	Hyper	2,360 bp's downstream	N/A	• B cell differentiation • Erythrocyte differentiation
Effect Size DMLs						
Ctxn1	0.115	0.324	Hypo	788 bp's downstream	CTCF Binding Site	• N/A
Slc15a2	0.005	0.233	Hyper	14,385 bp's downstream	CTCF Binding Site	• Immune system process • Oligopeptide transport
Lrp1b	0.009	0.163	Hyper	790,151 bp's downstream	N/A	• Endocytosis • Protein transport
Plpp2	0.279	0.129	Hypo	3,149 bp's downstream	N/A	• Phospholipid metabolic process • Sphingosine metabolic process • Ceramide metabolic process
Mef2c	0.018	0.101	Hyper	27,221 bp's downstream	Open Chromatin	• MAPK Cascade • Neuron migration • Negative regulation of transcription by RNA polymerase II

Differential Expression

Calculation of $\Delta\Delta C_t$ revealed that none of the selected genes were differentially expressed in either the parents or the offspring (Figure 4). Expression of the housekeeping gene, β-actin, was consistent in both dietary conditions and both generations ($p > 0.05$).

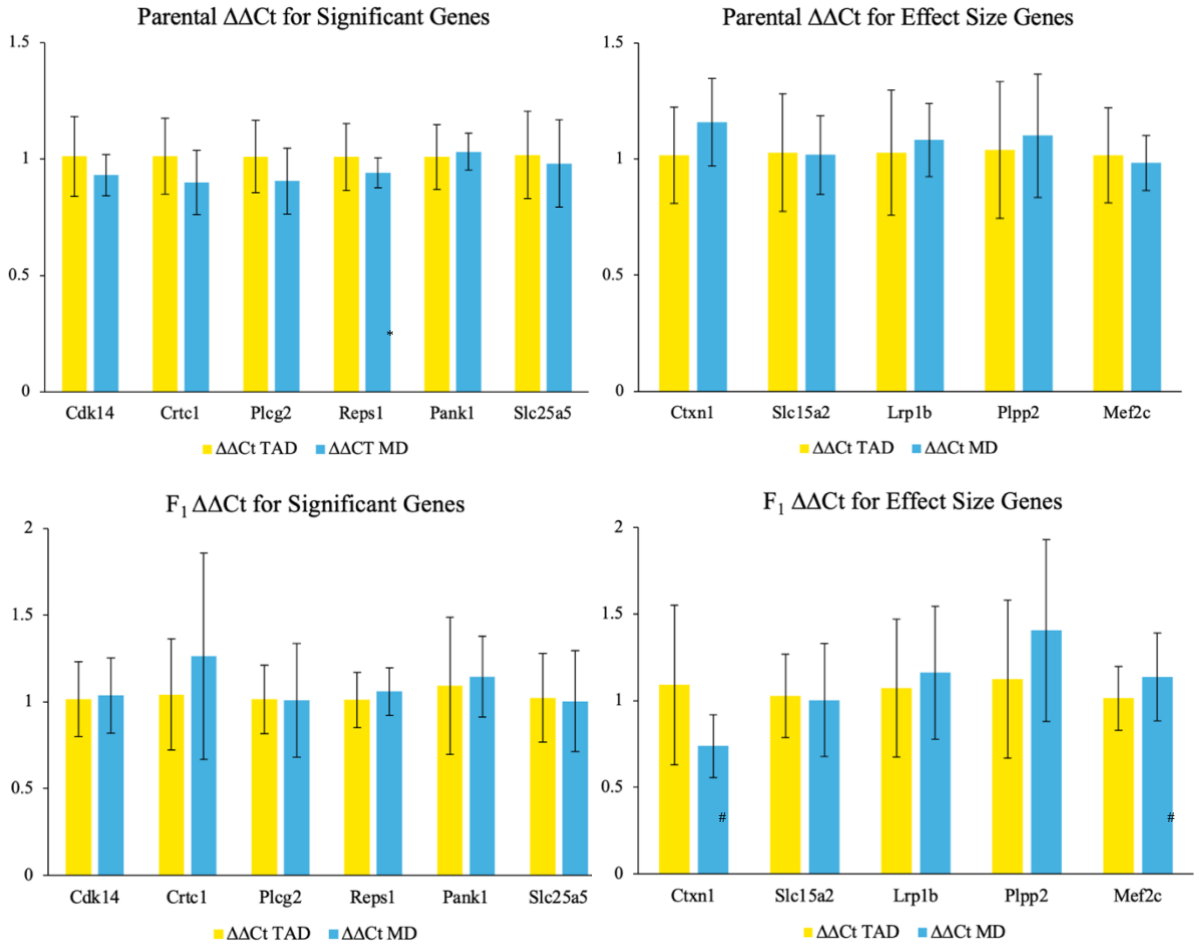


Figure 4. $\Delta\Delta C_t$ scores for selected loci for both generations (collapsing by sex) with TAD set as the reference group. Error bars depict standard deviation, which was >0.064 for all genes in the parental group and >0.137 for all genes in the F_1 group. No loci were found to be significantly differentially expressed when sexes were pooled. Genes found to be significantly differentially expressed in females only are indicated by #, whereas genes found to be significantly differentially expressed in males only are indicated by *.

Discussion

This study sought to determine if patterns of DNA methylation associated with diet were an underlying cause of the physiological differences found between MD and TAD animals in Braden-Kuhle (2023). However, no locus was found to be significantly differentially methylated with an effect size of at least 10% suggesting any underlying differences in methylation pattern are either highly variable, or of small effect. In order to locate loci that might be affected by dietary variation, I decided to investigate statistical significance and effect size of any candidate DML in isolation, producing two different categories of DMLs to analyze with gene expression. Although we failed to find any DML both statistically associated with diet and with a large effect size, the study design did allows us to answer a series of interesting questions: is statistical significance or large effect size more biologically relevant when it comes to alterations in methylation pattern and their effects on gene expression? Or, are both required in order to see a difference in expression levels? I hypothesized that if either were able to influence gene expression in isolation, the genes with larger effect size would be more likely to cause changes in gene expression and subsequent effects on the physiology of the organism. I also hypothesized that the lack of statistical significance in conjunction with large effect size is likely due to a small number of biological replicates (i.e., small sample size) that reduces power and increases the amount of variance within a sample group as shown above in Figure 3.

DMLs were determined to be statistically significant if they had a p-val < 0.0001 (as no loci were significant after a Benjamini-Hochberg correction for multiple testing). Of the 49 significant DML's, 43 were associated with a protein coding gene (either within the gene

or nearby). Following a literature review, six genes were selected because of their previous implication in AD or other neurodegenerative diseases, or association with pathways related to AD development. Of those six genes, three were hypermethylated (*Cdk14, Reps1,* and *Slc25a5*), indicating increased methylation levels in MD compared to TAD. *Cdk14* encodes for a pro-inflammatory regulator, and even though it was not found to be differentially expressed in this study, another study found that *Cdk14* was downregulated in the temporal cortex of female non-human primates on a Mediterranean diet in comparison to a Western diet (Shively et al., 2023). This expression change is consistent with the hypermethylation in MD mice reported herein, which is typically associated with decreased gene expression. *Reps1* encodes for a signaling adaptor protein that is involved in clathrin-mediated endocytosis, and has been implicated in bioinformatic analyses as a possible biomarker for AD (Luo et al., 2022). Therefore, the slight hypermethylation I observed in the MD could contribute to decreased *Reps1* expression and decreased AD development, but note that my gene expression assay did not find *Reps1* to be differentially expressed (when pooling sexes). *Slc25a5* encodes for a solute carrier protein that mediates ADP import and ATP export from the mitochondrial matrix. Other Slc25A proteins have been linked to expression of apolipoprotein E (APOE), which is a known risk factor for AD development, wherein mutation of other Slc25A proteins has been shown to significantly increase APOE expression (Wynne et al., 2023). This seems counterintuitive to the hypermethylation I observed, because I would anticipate that it would cause decreased expression of *Slc25a5*, and therefore increase amounts of APOE if it functions in a manner similar to the other Slc25A proteins. However, it is possible that the location of the hypermethlation could play a role in influencing gene expression, which is discussed further below.

Three of the statistically significant genes were hypomethylated (*Pank1*, *Crtc1*, and *Plcg2*), indicating decreased methylation in MD compared to TAD. *Pank1* encodes for an enzyme responsible for catalyzing coenzyme A biosynthesis, and *Pank1* knockout mice exhibited gene expression patterns similar to those seen in neurodegenerative diseases (Subramanian et al., 2020). Therefore, hypomethylation of the *Pank1* promoter, and supposed increased expression of *Pank1* in response to MD, could be neuroprotective. *Plcg2* is involved in production of anti-inflammatory cytokines, and has been found to be upregulated in the brains of AD patients (Tsai et al., 2020). So while hypomethylation would initially seem to be neurodegenerative, it is possible that the location of methylation could play a role in the direction gene expression is altered, as discussed below. *Crtc1* plays a role in synaptic plasticity and long-term memory formation in the hippocampus, and its promoters have been found to be hypomethylated in the hippocampus of AD patients, but that hypomethylation was associated with decreased expression of *Crtc1* rather than increased expression (Mendioroz et al., 2016). In control patients, however, Mendioroz et al. (2016) observed that hypomethylation would result in increased expression of *Crtc1*, which could be neuroprotective. This indicates that epigenetic regulation of *Crtc1* functions as expected until AD pathology develops, at which point it becomes dysregulated, so more research is necessary to determine the effects of methylation on *Crtc1* expression.

I also identified 11 DMLs with an effect size >10%, eight of which were associated with a protein coding gene (either within the gene or nearby). Following a literature review, five genes were selected because of their previous implication in AD or other

neurodegenerative diseases, or association with pathways related to AD development. Three were hypermethylated (*Slc15a2*, *Mef2c*, and *Lrp1b*), while two were hypomethylated (*Ctxn1* and *Plpp2*). *Slc15a2* encodes for a proton-coupled oligopeptide transporter that facilitates transport of peptides from cerebrospinal fluid into the blood (Smith et al., 2004). *Slc15a2* has been implicated as a possible biomarker for AD in a microarray study where its expression decreased with AD progression (Arisi et al., 2011). *Mef2c* encodes for a transcription enhancer factor that plays a role in hippocampal-dependent learning and memory. Sao et al. (2018) found that expression *Mef2c* in peripheral leukocytes was significantly lower in AD patients compared to controls, but found no differences in methylation between the groups. Another study found that decreased expression of *Mef2c* via a knockdown exacerbates AD pathology (Ren et al., 2022). *Lrp1b* encodes for a low density lipoprotein receptor, and it has been shown to be part of a neuroprotective response and is upregulated in neurons in response to Aβ-induced damage (Benoit et al., 2013). The remaining two genes in the effect size group, *Ctxn1* and *Plpp2*, were hypomethylated. *Ctxn1* may be involved in signaling of cortical neurons during forebrain development, and machine learning analyses have identified *Ctxn1* expression as a predictor for AD (Cheng et al., 2021). *Plpp2* is involved in glycerophospholipid synthesis and was found to be upregulated in the cerebral cortex of AD patients (Zeng et al., 2023). Based on their functions, the methylation patterns of all five genes initially seem counterproductive to the neuroprotective goal of the MD, but further analysis of the location of these methylation changes may elucidate neuroprotective capabilities.

While it is generally thought that hypermethylation of a locus is associated with decreased expression of a gene, that assumption is based on data obtained from the effects increased methylation at the promoter. However, CpG island methylation is not restricted to gene control regions, and can also occur within the body of the gene itself (Liang et al., 1998). Furthermore, hypermethylation within the body of a gene not only allows for the gene to be expressed (Larsen et al., 1993), but can even cause expression of the gene to increase (Gonzalez-Zulueta et al., 1995; Salem et al., 2000). As such, there seems to be a paradox wherein hypermethylation of transcriptional promoter regions suppresses expression, whereas hypermethylation within the transcribed portion of the gene is associated with increased expression (Jones, 1999). This would indicate that the location of methylation plays an important role in influencing gene expression. In relation to the data generated herein, all five of the DMLs with effect size >10% and three of the DMLs with p-val<0.0001 occurred within the body of the gene rather than the promoter region. As such, the hypermethylation within the bodies of *Slc15a2*, *Mef2c*, *Lrp1b,* and *Slc25a5* would likely *increase* their expression, allowing for them to contribute to neuroprotection in the MD. This trend was observed to a small extent in *Lrp1b*, wherein the MD had increased methylation and somewhat increased expression, though neither were statistically significant. Conversely, hypomethylation in the bodies of *Ctxn1, Plpp2*, and *Plcg2* would likely decrease their expression, subsequently hindering their neurodegenerative effects in the MD. The only DML that appears to be neurodegenerative under this paradox is *Cdk14* because hypermethylation in the body of the gene would likely increase its expression, contrary to other studies that have found decreased expression of *Cdk14* in MD compared to WD (Negrey et al., 2023). That said, Negrey et al. (2023) only looked at differences in

expression, not differences in methylation, so it is possible that a mechanism other than DNA methylation regulates expression of *Cdk14*. DNA methylation is a mechanism of cis-regulation of gene expression, but there could also be trans-regulation of gene expression from transcription factors. *Mef2c* and *Crtc1* both encode for transcription factors (transcriptional enhancer and transcriptional co-activator, respectively), suggesting that they are likely involved in regulating transcription of other genes.

The same seems to hold true in this study, as none of the genes selected for qPCR were significantly differentially expressed between MD and TAD when sexes were pooled. Some expression patterns seemed to have a trend, but it is possible that the limited sample size in this study made it nearly impossible to detect slight gene expression differences because of high amounts of variance within treatment groups (e.g., Figures 3 and 4 above). Furthermore, the DNA methylation data in this study was gathered from hippocampal tissue, whereas the tissue for qPCR was obtained from the prefrontal cortex. Therefore, it is possible that the methylation differences seen in the hippocampus were not shared with the prefrontal cortex, and if so, it is perhaps unsurprising that these methylation patterns were not coupled with differential expression. A major assumption of this project is that the hippocampus is the brain region that would even respond to dietary influences and induce differential methylation. However, it is entirely possible that other regions of the brain implicated in AD development, such as the prefrontal cortex and temporal cortex, are responsible for inducing the methylation changes associated with physiological effects, and I would not observe them because only the hippocampal methylome was analyzed (De Jager et al., 2014; Mastroeni et al., 2009; Zhang et al., 2020). Finally, in order to increase sample size, $\Delta\Delta C_t$ scores between

diets were calculated by pooling sexes. However, Braden-Kuhle (2023) did find sex differences in response to these diets, including different amounts of Aβ: males on the MD had less Aβ than TAD in the hippocampus *and* the prefrontal cortex, while females on the MD only had less Aβ than TAD in the prefrontal cortex, not the hippocampus. Braden-Kuhle (2023) also observed sex differences in gene expression in the hippocampus: males on the MD had decreased expression of pro-inflammatory cytokines (TNF-α and IL-1β) compared to TAD when treated with LPS to induce an inflammatory response, but females had no such difference. Females on the MD also had lower baseline expression of beta-secretase, which is involved in the production of Aβ peptides, than TAD, while males had no such difference between diets. In this study, when β-values were split out based off of sex, females on the MD had significantly higher β-values for *Slc15a2* (p=0.004) and *Lrp1b* (p=0.033) and a significantly lower β-values for *Plpp2* (p=0.040) (Figure 3). Meanwhile, none of the β-values were significantly different between males on either diet, but that is likely due to the large standard deviation bars around the mean because of the small sample size (n = 3: Figure 3).

These data illustrate the nuanced nature of dietary effects on methylation, gene expression, and physiological differences according to sex. This is supported by the fact that when $\Delta\Delta C_t$ scores were calculated between parents on diets within each sex, expression of one gene, *Reps1*, was significantly decreased in parental males on the MD (p=0.047), but not in females on the MD (p=0.460) (Figure 4). *Reps1,* as previously discussed, is a possible biomarker for AD, so decreased expression may indicate slight neuroprotection in males on the MD compared to TAD, but not females. In F_1 individuals whose parents were on the MD, *Ctxn1* expression was significantly decreased in females (p=0.003), but not in males

(p=0.228), whereas *Mef2c* expression was significantly increased in females (p=0.045), but not in males (p=0.199) (Figure 4). As previously discussed, increased *Ctxn1* and/or decreased *Mef2c* may be predictors for AD, so decreased *Ctxn1* expression and increased *Mef2c* expression in female F_1 offspring of mice on the MD compared to TAD may indicate transgenerational neuroprotection due to the MD, but only in females. Therefore, based on prior observed sex differences in response to diet, it appears that pooling sexes in order to increase sample size could be another factor diminishing the effects of the diet on methylation and gene expression in this study.

Additionally, the same was true of the offspring of the parents on either the MD or the TAD: there were no DMLs that were significantly differentially methylated with an effect size >10%, nor were the selected genes differentially expressed. While other studies have shown that some methylation changes can be heritable, the lack of methylation differences found in the parents meant it was unlikely any were passed on to the offspring. Although epigenetic modifications are often thought to be heritable, previous studies suggest this is not always the case, e.g., McRae et al (2014) found average narrow-sense heritability of methylation changes of 0.187. Furthermore, the heritability of methylation patterns within the brain is often smaller, as only 3-4% of CpG loci with genetic variation in the brain are heritable (Quon et al., 2013). This decreased heritability of methylation patterns in the brain may be attributed to the large-scale epigenomic reconfiguration that mammalian brains undergo during development (Lister et al., 2013). That said, there is still a definite link between parental exposure to a particular diet and subsequent changes in offspring predisposition, as discussed previously (Dudley et al., 2011; Gali Ramamoorthy et al., 2018;

Gonzalez-Nahm et al., 2017; House et al., 2018; Velazquez et al., 2020). It is possible that parental dietary exposure to a particular diet only confers predisposition in offspring if the parental gametes underwent methylation changes, and it is unknown whether the methylation changes in the gametes would match those in the brain of parents. Future studies should investigate methylomic differences between brain tissues and gametes of parents and compare them to the methylome of offspring brain tissue. This would provide a better understanding of whether dietary influences induce different methylation patterns in various tissues, and provide an explanation as to why methylation patterns of the parents in a given tissue do not necessarily match the methylation patterns of the offspring in that same tissue. Additionally, it is also possible that even if the offspring did inherit the slight methylation differences exhibited by their parents and those differences persisted throughout development, their consumption of a control diet may have effectively offset the inherited methylation changes. A similar study could be conducted using an alternative control diet for the F_1 generation, or by collecting brain tissue samples in some animals prior to weaning, to determine if the control diet diminishes the transgenerational effects of the parental diet.

The results of this study support the idea that statistical significance and large effect size are both required for differential methylation patterns to cause changes in gene expression. However, I do not believe that the potential neuroprotective effects of the MD, nor neurodegenerative effects of the TAD, should be discounted. Compared to the MD, the TAD clearly had a negative impact on offspring the longer the parents consumed the diet, as none of the pups from breeders who had consumed the TAD for four months were viable. And, based on the range of the raw β-values, there seems to be a difference between

methylation levels of a given locus on either diet (Figure 3). However, the low sample size, subsequently high amount of variance, and possible sex effects may have limited the power of this study and prevented the detection of significant methylation differences. Future studies should implement these holistic diets with a much larger sample group, as this should assist in increasing the power of detecting differential methylation and expression, as well as teasing out possible sex effects in response to diet. Furthermore, methylation of cytosines is merely one of several known epigenetic modifications that could be heritable and/or influence gene expression, so subsequent studies investigating the influence of these diets on other epigenetic marks (such as histone modification or regulation of microRNAs) could also be fruitful.

References

2022 Alzheimer's disease facts and figures. (2022). *Alzheimers Dement*, *18*(4), 700-789. https://doi.org/10.1002/alz.12638

Agarwal, P., Leurgans, S. E., Agrawal, S., Aggarwal, N., Cherian, l. J., James, B. D., Dhana, K., Barnes, L. L., Bennett, D. A., & Schneider, J. A. (2023). Association of Mediterranean-DASH Intervention for Neurodegenerative Delay and Mediterranean Diets With Alzheimer Disease Pathology. *Neurology*, 10.1212/WNL.0000000000207176. https://doi.org/10.1212/wnl.0000000000207176

Arisi, I., D'Onofrio, M., Brandi, R., Felsani, A., Capsoni, S., Drovandi, G., Felici, G., Weitschek, E., Bertolazzi, P., & Cattaneo, A. (2011). Gene expression biomarkers in the brain of a mouse model for Alzheimer's disease: mining of microarray data by logic classification and feature selection. *J Alzheimers Dis*, *24*(4), 721-738. https://doi.org/10.3233/jad-2011-101881

Arpon, A., Riezu-Boj, J. I., Milagro, F. I., Marti, A., Razquin, C., Martinez-Gonzalez, M. A., Corella, D., Estruch, R., Casas, R., Fito, M., Ros, E., Salas-Salvado, J., & Martinez, J. A. (2016). Adherence to Mediterranean diet is associated with methylation changes in inflammation-related genes in peripheral blood cells. *Journal of Physiology and Biochemistry*, *73*(3), 445-455. https://doi.org/10.1007/s13105-017-0552-6

Barberger-Gateau, P., Raffaitin, C., Letenneur, L., Berr, C., Tzourio, C., Dartigues, J. F., & Alpérovitch, A. (2007). Dietary patterns and risk of dementia: the Three-City cohort

study. *Neurology*, *69*(20), 1921-1930. https://doi.org/10.1212/01.wnl.0000278116.37320.52

Bekris, L. M., Yu, C. E., Bird, T. D., & Tsuang, D. W. (2010). Genetics of Alzheimer disease. *J Geriatr Psychiatry Neurol*, *23*(4), 213-227. https://doi.org/10.1177/0891988710383571

Benoit, M. E., Hernandez, M. X., Dinh, M. L., Benavente, F., Vasquez, O., & Tenner, A. J. (2013). C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid-β neurotoxicity. *J Biol Chem*, *288*(1), 654-665. https://doi.org/10.1074/jbc.M112.400168

Braden-Kuhle, P. N. (2023). *Food for thought: exploring multigenerational effects of a comprehensive Mediterranean diet on cognition and biomarkers of Alzheimer's disease* [Doctoral dissertation, Texas Christian University]. TCU Digital Repository.

Buettner, D. (2017). *The Blue Zones Solution*. Penguin USA.

Campion, D., Dumanchin, C., Hannequin, D., Dubois, B., Belliard, S., Puel, M., Thomas-Anterion, C., Michon, A., Martin, C., Charbonnier, F., Raux, G., Camuzat, A., Penet, C., Mesnage, V., Martinez, M., Clerget-Darpoux, F., Brice, A., & Frebourg, T. (1999). Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. *Am J Hum Genet*, *65*(3), 664-670. https://doi.org/10.1086/302553

Cheng, J., Liu, H.-P., Lin, W.-Y., & Tsai, F.-J. (2021). Machine learning compensates fold-change method and highlights oxidative phosphorylation in the brain transcriptome of

Alzheimer's disease. *Scientific Reports*, *11*(1), 13704. https://doi.org/10.1038/s41598-021-93085-z

De Jager, P. L., Srivastava, G., Lunnon, K., Burgess, J., Schalkwyk, L. C., Yu, L., Eaton, M. L., Keenan, B. T., Ernst, J., McCabe, C., Tang, A., Raj, T., Replogle, J., Brodeur, W., Gabriel, S., Chai, H. S., Younkin, C., Younkin, S. G., Zou, F., . . . Bennett, D. A. (2014). Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. *Nature Neuroscience*, *17*(9), 1156-1163. https://doi.org/10.1038/nn.3786

Dudley, K. J., Sloboda, D. M., Connor, K. L., Beltrand, J., & Vickers, M. H. (2011). Offspring of mothers fed a high fat diet display hepatic cell cycle inhibition and associated changes in gene expression and DNA methylation. *PloS one*, *6*(7), e21662. https://doi.org/10.1371/journal.pone.0021662

Gali Ramamoorthy, T., Allen, T.-J., Davies, A., Harno, E., Sefton, C., Murgatroyd, C., & White, A. (2018). Maternal overnutrition programs epigenetic changes in the regulatory regions of hypothalamic Pomc in the offspring of rats. *International Journal of Obesity*, *42*(8), 1431-1444. https://doi.org/10.1038/s41366-018-0094-1

Gonzalez-Nahm, S., Mendez, M. A., Robinson, W. R., Murphy, S. K., Hoyo, C., Hogan, V. K., & Rowley, D. L. (2017). Low maternal adherence to a Mediterranean diet is associated with increase in methylation at the MEG3-IG differentially methylated region in female infants. *Environmental Epigenetics*, *3*(2), Article dvx007. https://doi.org/10.1093/eep/dvx007

Gonzalez-Zulueta, M., Bender, C. M., Yang, A. S., Nguyen, T., Beart, R. W., Van Tornout, J. M., & Jones, P. A. (1995). Methylation of the 5′ CpG Island of the p16/CDKN2

Tumor Suppressor Gene in Normal and Transformed Human Tissues Correlates with Gene Silencing1. *Cancer Research*, *55*(20), 4531-4535.

Gordon, B. A., Blazey, T. M., Su, Y., Hari-Raj, A., Dincer, A., Flores, S., Christensen, J., McDade, E., Wang, G., Xiong, C., Cairns, N. J., Hassenstab, J., Marcus, D. S., Fagan, A. M., Jack, C. R., Jr., Hornbeck, R. C., Paumier, K. L., Ances, B. M., Berman, S. B., . . . Benzinger, T. L. S. (2018). Spatial patterns of neuroimaging biomarker change in individuals from families with autosomal dominant Alzheimer's disease: a longitudinal study. *Lancet Neurol*, *17*(3), 241-250. https://doi.org/10.1016/S1474-4422(18)30028-0

Grant, W. B. (2014). Trends in Diet and Alzheimer's Disease During the Nutrition Transition in Japan and Developing Countries. *Journal of Alzheimer's Disease*, *38*, 611-620. https://doi.org/10.3233/JAD-130719

Hanseeuw, B. J., Betensky, R. A., Jacobs, H. I. L., Schultz, A. P., Sepulcre, J., Becker, J. A., Cosio, D. M. O., Farrell, M., Quiroz, Y. T., Mormino, E. C., Buckley, R. F., Papp, K. V., Amariglio, R. A., Dewachter, I., Ivanoiu, A., Huijbers, W., Hedden, T., Marshall, G. A., Chhatwal, J. P., . . . Johnson, K. (2019). Association of Amyloid and Tau With Cognition in Preclinical Alzheimer Disease: A Longitudinal Study. *JAMA Neurol*, *76*(8), 915-924. https://doi.org/10.1001/jamaneurol.2019.1424

House, J. S., Mendez, M., Maguire, R. L., Gonzalez-Nahm, S., Huang, Z., Daniels, J., Murphy, S. K., Fuemmeler, B. F., Wright, F. A., & Hoyo, C. (2018). Periconceptional Maternal Mediterranean Diet Is Associated With Favorable Offspring Behaviors and Altered CpG Methylation of Imprinted Genes [Original Research]. *Frontiers in Cell and Developmental Biology*, *6*. https://doi.org/10.3389/fcell.2018.00107

Janssen, J. C., Beck, J. A., Campbell, T. A., Dickinson, A., Fox, N. C., Harvey, R. J., Houlden, H., Rossor, M. N., & Collinge, J. (2003). Early onset familial Alzheimer's disease: Mutation frequency in 31 families. *Neurology*, *60*(2), 235-239. https://doi.org/10.1212/01.wnl.0000042088.22694.e3

Jones, P. A. (1999). The DNA methylation paradox. *Trends in Genetics*, *15*(1), 34-37. https://doi.org/https://doi.org/10.1016/S0168-9525(98)01636-9

King, S. E., & Skinner, M. K. (2020). Epigenetic Transgenerational Inheritance of Obesity Susceptibility. *Trends in Endocrinology & Metabolism*, *31*(7), 478-494. https://doi.org/https://doi.org/10.1016/j.tem.2020.02.009

Lanoiselée, H. M., Nicolas, G., Wallon, D., Rovelet-Lecrux, A., Lacour, M., Rousseau, S., Richard, A. C., Pasquier, F., Rollin-Sillaire, A., Martinaud, O., Quillard-Muraine, M., de la Sayette, V., Boutoleau-Bretonniere, C., Etcharry-Bouyx, F., Chauviré, V., Sarazin, M., le Ber, I., Epelbaum, S., Jonveaux, T., . . . Campion, D. (2017). APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Med*, *14*(3), e1002270. https://doi.org/10.1371/journal.pmed.1002270

Larsen, F., Solheim, J., & Prydz, H. (1993). A methylated CpG island 3' in the apolipoprotein-E gene does not repress its transcription. *Human Molecular Genetics*, *2*(6), 775-780. https://doi.org/10.1093/hmg/2.6.775

Liang, G., Salem, C. E., Yu, M. C., Nguyen, H. D., Gonzales, F. A., Nguyen, T. T., Nichols, P. W., & Jones, P. A. (1998). DNA Methylation Differences Associated with Tumor Tissues Identified by Genome Scanning Analysis. *Genomics*, *53*(3), 260-268. https://doi.org/https://doi.org/10.1006/geno.1998.5502

Lister, R., Mukamel, E. A., Nery, J. R., Urich, M., Puddifoot, C. A., Johnson, N. D., Lucero, J., Huang, Y., Dwork, A. J., Schultz, M. D., Yu, M., Tonti-Filippini, J., Heyn, H., Hu, S., Wu, J. C., Rao, A., Esteller, M., He, C., Haghighi, F. G., . . . Ecker, J. R. (2013). Global epigenomic reconfiguration during mammalian brain development. *Science*, *341*(6146), 1237905. https://doi.org/10.1126/science.1237905

Luceri, C., Bigagli, E., Pitozzi, V., & Giovannelli, L. (2017). A nutrigenomics approach for the study of anti-aging interventions: olive oil phenols and the modulation of gene and microRNA expression profiles in mouse brain. *Eur J Nutr*, *56*(2), 865-877. https://doi.org/10.1007/s00394-015-1134-4

Luo, J., Chen, L., Huang, X., Xie, J., Zou, C., Pan, M., Mo, J., & Zou, D. (2022). REPS1 as a Potential Biomarker in Alzheimer's Disease and Vascular Dementia [Original Research]. *Frontiers in Aging Neuroscience*, *14*. https://doi.org/10.3389/fnagi.2022.894824

Mastroeni, D., McKee, A., Grover, A., Rogers, J., & Coleman, P. D. (2009). Epigenetic differences in cortical neurons from a pair of monozygotic twins discordant for Alzheimer's disease. *PloS one*, *4*(8), e6617. https://doi.org/10.1371/journal.pone.0006617

Masuyama, H., & Hiramatsu, Y. (2012). Effects of a high-fat diet exposure in utero on the metabolic syndrome-like phenomenon in mouse offspring through epigenetic changes in adipocytokine gene expression. *Endocrinology*, *153*(6), 2823-2830. https://doi.org/10.1210/en.2011-2161

Medications for memory, cognition and dementia-related behaviors. (2023). 2023 Alzheimer's Association. https://www.alz.org/alzheimers-dementia/treatments/medications-for-memory#Types%20of%20drugs

Mendioroz, M., Celarain, N., Altuna, M., Sánchez-Ruiz de Gordoa, J., Zelaya, M. V., Roldán, M., Rubio, I., Larumbe, R., Erro, M. E., Méndez, I., & Echávarri, C. (2016). CRTC1 gene is differentially methylated in the human hippocampus in Alzheimer's disease. *Alzheimer's Research & Therapy*, *8*(1), 15. https://doi.org/10.1186/s13195-016-0183-0

Murphy, S. L., Kochanek, K. D., Xu, J., & Arias, E. (2021). Mortality in the United States, 2020. *NCHS Data Brief*(427), 1-8.

Negrey, J. D., Frye, B. M., Johnson, C. S. C., Kim, J., Barcus, R. A., Lockhart, S. N., Whitlow, C. T., Sutphen, C., Chiou, K. L., Snyder-Mackler, N., Montine, T. J., Craft, S., Shively, C. A., & Register, T. C. (2023). Mediterranean Diet Protects Against a Neuroinflammatory Cortical Transcriptome: Associations with Brain Volumetrics, Peripheral Inflammation, Social Isolation and Anxiety. *bioRxiv*, 2023.2011.2001.565068. https://doi.org/10.1101/2023.11.01.565068

Quon, G., Lippert, C., Heckerman, D., & Listgarten, J. (2013). Patterns of methylation heritability in a genome-wide analysis of four brain regions. *Nucleic Acids Research*, *41*(4), 2095-2104. https://doi.org/10.1093/nar/gks1449

Rabinovici, G. D. (2019). Late-onset Alzheimer Disease. *Continuum (Minneap Minn)*, *25*(1), 14-33. https://doi.org/10.1212/con.0000000000000700

Rajan, K. B., Weuve, J., Barnes, L. L., McAninch, E. A., Wilson, R. S., & Evans, D. A. (2021). Population estimate of people with clinical Alzheimer's disease and mild

cognitive impairment in the United States (2020-2060). *Alzheimers Dement*, *17*(12), 1966-1975. https://doi.org/10.1002/alz.12362

Raux, G., Guyant-Maréchal, L., Martin, C., Bou, J., Penet, C., Brice, A., Hannequin, D., Frebourg, T., & Campion, D. (2005). Molecular diagnosis of autosomal dominant early onset Alzheimer's disease: an update. *J Med Genet*, *42*(10), 793-795. https://doi.org/10.1136/jmg.2005.033456

Ren, J., Zhang, S., Wang, X., Deng, Y., Zhao, Y., Xiao, Y., Liu, J., Chu, L., & Qi, X. (2022). MEF2C ameliorates learning, memory, and molecular pathological changes in Alzheimer's disease in vivo and in vitro. *Acta Biochim Biophys Sin (Shanghai)*, *54*(1), 77-90. https://doi.org/10.3724/abbs.2021012

Salem, C. E., Markl, I. D. C., Bender, C. M., Gonzales, F. A., Jones, P. A., & Liang, G. (2000). PAX6 methylation and ectopic expression in human tumor cells. *International Journal of Cancer*, *87*(2), 179-185. https://doi.org/https://doi.org/10.1002/1097-0215(20000715)87:2<179::AID-IJC4>3.0.CO;2-X

Sao, T., Yoshino, Y., Yamazaki, K., Ozaki, Y., Mori, Y., Ochi, S., Yoshida, T., Mori, T., Iga, J.-i., & Ueno, S.-i. (2018). MEF2C mRNA expression and cognitive function in Japanese patients with Alzheimer's disease. *Psychiatry and Clinical Neurosciences*, *72*(3), 160-167. https://doi.org/https://doi.org/10.1111/pcn.12618

Scarmeas, N., Luchsinger, J. A., Mayeux, R., & Stern, Y. (2007). Mediterranean diet and Alzheimer disease mortality. *Neurology*, *69*(11), 1084-1093. https://doi.org/10.1212/01.wnl.0000277320.50685.7c

Scarmeas, N., Stern, Y., Tang, M.-X., Mayeux, R., & Luchsinger, J. A. (2006). Mediterranean diet and risk for Alzheimer's disease. *Annals of Neurology*, *59*(6), 912-921. https://doi.org/https://doi.org/10.1002/ana.20854

Shively, C. A., Negrey, J. D., Frye, B. M., Johnson, C. S. C., Kim, J., Barcus, R. A., Lockhart, S. N., Whitlow, C. T., Chiou, K. L., Snyder-Mackler, N., Montine, T. J., Craft, S., & Register, T. C. (2023). Mediterranean diet protects against neuroinflammation and degeneration in female nonhuman primates. *Alzheimer's & Dementia*, *19*(S10), e081770. https://doi.org/https://doi.org/10.1002/alz.081770

Singh, B., Parsaik, A. K., Mielke, M. M., Erwin, P. J., Knopman, D. S., Petersen, R. C., & Roberts, R. O. (2014). Association of mediterranean diet with mild cognitive impairment and Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis*, *39*(2), 271-282. https://doi.org/10.3233/jad-130830

Smith, D. E., Johanson, C. E., & Keep, R. F. (2004). Peptide and peptide analog transport systems at the blood-CSF barrier. *Adv Drug Deliv Rev*, *56*(12), 1765-1791. https://doi.org/10.1016/j.addr.2004.07.008

Subramanian, C., Yao, J., Frank, M. W., Rock, C. O., & Jackowski, S. (2020). A pantothenate kinase-deficient mouse model reveals a gene expression program associated with brain coenzyme a reduction. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1866*(5), 165663. https://doi.org/https://doi.org/10.1016/j.bbadis.2020.165663

Suzuki, M. M., & Bird, A. (2008). DNA methylation landscapes: provocative insights from epigenomics. *Nature Reviews Genetics*, *9*(6), 465-476. https://doi.org/10.1038/nrg2341

Taylor, M. K., Sullivan, D. K., Morris, J. K., Vidoni, E. D., Honea, R. A., Mahnken, J. D., & Burns, J. M. (2021). High Glycemic Diet Is Related to Brain Amyloid Accumulation Over One Year in Preclinical Alzheimer's Disease [Original Research]. *Frontiers in Nutrition*, *8*. https://doi.org/10.3389/fnut.2021.741534

Taylor, M. K., Sullivan, D. K., Swerdlow, R. H., Vidoni, E. D., Morris, J. K., Mahnken, J. D., & Burns, J. M. (2017). A high-glycemic diet is associated with cerebral amyloid burden in cognitively normal older adults. *Am J Clin Nutr*, *106*(6), 1463-1470. https://doi.org/10.3945/ajcn.117.162263

Theuns, J., Del-Favero, J., Dermaut, B., Duijn, C. M. v., Backhovens, H., Broeck, M. V. d., Serneels, S., Corsmit, E., Broeckhoven, C. V., & Cruts, M. (2000). Genetic variability in the regulatory region of presenilin 1 associated with risk for Alzheimer's disease and variable expression. *Human Molecular Genetics*, *9*(3), 325-331. https://doi.org/10.1093/hmg/9.3.325

Trichopoulou, A., Costacou, T., Bamia, C., & Trichopoulos, D. (2003). Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med*, *348*(26), 2599-2608. https://doi.org/10.1056/NEJMoa025039

Trichopoulou, A., Orfanos, P., Norat, T., Bueno-de-Mesquita, B., Ocké, M. C., Peeters, P. H., Schouw, Y. T. v. d., Boeing, H., Hoffmann, K., Boffetta, P., Nagel, G., Masala, G., Krogh, V., Panico, S., Tumino, R., Vineis, P., Bamia, C., Naska, A., Benetou, V., . . . Trichopoulos, D. (2005). Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. *BMJ*, *330*(7498), 991. https://doi.org/10.1136/bmj.38415.644155.8F

Tsai, A. P., Dong, C., Preuss, C., Moutinho, M., Lin, P. B.-C., Hajicek, N., Sondek, J., Bissel, S. J., Oblak, A. L., Carter, G. W., Liu, Y., Landreth, G. E., Lamb, B. T., & Nho, K. (2020). PLCG2 as a Risk Factor for Alzheimer's Disease. *bioRxiv*, 2020.2005.2019.104216. https://doi.org/10.1101/2020.05.19.104216

van den Brink, A. C., Brouwer-Brolsma, E. M., Berendsen, A. A. M., & van de Rest, O. (2019). The Mediterranean, Dietary Approaches to Stop Hypertension (DASH), and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) Diets Are Associated with Less Cognitive Decline and a Lower Risk of Alzheimer's Disease-A Review. *Adv Nutr*, *10*(6), 1040-1065. https://doi.org/10.1093/advances/nmz054

Velazquez, R., Ferreira, E., Winslow, W., Dave, N., Piras, I. S., Naymik, M., Huentelman, M. J., Tran, A., Caccamo, A., & Oddo, S. (2020). Maternal choline supplementation ameliorates Alzheimer's disease pathology by reducing brain homocysteine levels across multiple generations. *Molecular Psychiatry*, *25*(10), 2620-2629. https://doi.org/10.1038/s41380-018-0322-z

Villemagne, V. L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K. A., Salvado, O., Szoeke, C., Macaulay, S. L., Martins, R., Maruff, P., Ames, D., Rowe, C. C., & Masters, C. L. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurol*, *12*(4), 357-367. https://doi.org/10.1016/s1474-4422(13)70044-9

Villicaña, S., & Bell, J. T. (2021). Genetic impacts on DNA methylation: research findings and future perspectives. *Genome Biology*, *22*(1), 127. https://doi.org/10.1186/s13059-021-02347-6

Wang, X., Spandidos, A., Wang, H., & Seed, B. (2011). PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update. *Nucleic Acids Research*, *40*(D1), D1144-D1149. https://doi.org/10.1093/nar/gkr1013

Więckowska-Gacek, A., Mietelska-Porowska, A., Wydrych, M., & Wojda, U. (2021). Western diet as a trigger of Alzheimer's disease: From metabolic syndrome and systemic inflammation to neuroinflammation and neurodegeneration. *Ageing Research Reviews*, *70*, 101397. https://doi.org/https://doi.org/10.1016/j.arr.2021.101397

Wynne, M. E., Ogunbona, O., Lane, A. R., Gokhale, A., Zlatic, S. A., Xu, C., Wen, Z., Duong, D. M., Rayaprolu, S., Ivanova, A., Ortlund, E. A., Dammer, E. B., Seyfried, N. T., Roberts, B. R., Crocker, A., Shanbhag, V., Petris, M., Senoo, N., Kandasamy, S., . . . Faundez, V. (2023). APOE expression and secretion are modulated by mitochondrial dysfunction. *eLife*, *12*, e85779. https://doi.org/10.7554/eLife.85779

Zeng, Y., Cao, S., Li, N., Tang, J., & Lin, G. (2023). Identification of key lipid metabolism-related genes in Alzheimer's disease. *Lipids in Health and Disease*, *22*(1), 155. https://doi.org/10.1186/s12944-023-01918-9

Zhang, L., Silva, T. C., Young, J. I., Gomez, L., Schmidt, M. A., Hamilton-Nelson, K. L., Kunkle, B. W., Chen, X., Martin, E. R., & Wang, L. (2020). Epigenome-wide meta-analysis of DNA methylation differences in prefrontal cortex implicates the immune processes in Alzheimer's disease. *Nature Communications*, *11*(1), 6114. https://doi.org/10.1038/s41467-020-19791-w

Zhou, W., Triche, T. J., Jr, Laird, P. W., & Shen, H. (2018). SeSAMe: reducing artifactual detection of DNA methylation by Infinium BeadChips in genomic deletions. *Nucleic Acids Research*, *46*(20), e123-e123. https://doi.org/10.1093/nar/gky691

Supplemental Material

Supplemental Table 1: Primer sequences and amplification conditions for qPCR

Locus	Forward Primer (5' -> 3")	Reverse Primer (5' -> 3")	Annealing
Ctxn1	AGTTCGGCGTGGACACTATC	GCTGTAGGGGTCTAGCAGTATG	57ºC
Slc15a2	AAAGCGACAACATTGGCTAGA	AAATCCCAAATCGCCATCCAT	55ºC
Lrp1b	CGACCATGTGACTTGTGTCTC	CTCGGGACAGGTATCTAAAGACT	55ºC
Plpp2	CCAAGTACATGATTGGCCGTC	GGCCTCCGTGACATTAGCA	57ºC
Mef2c	ATCCCGATGCAGACGATTCAG	AACAGCACACAATCTTTGCCT	57ºC
Cdk14	AAGAGCAAAATCCGTCCCTAGC	TCATCTCAACGAAGATACAGCCA	57ºC
Crtc1	TGCCCAACGTGAACCAGATT	CCCATGATGTCGTGTGGTCC	57ºC
Plcg2	GTGGACACCCTTCCAGAATATG	ACCTGCCGAGTCTCCATGAT	57ºC
Reps1	CTCCCACGATTTGTTGCTTCA	CACCCGAGTAAGAGCCCTG	57ºC
Pank1	GTTCGCCCAGCATGATTCTC	CTTAACCAGGGTTCCACCGAT	57ºC
Slc25a5	CAAGACAGCGGTAGCACCC	CGCAGTCTATGATGCCCTTGTA	57ºC
Beta-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT	57ºC

Vita

Bridey Elizabeth Brown was born on April 19th, 2000, in Salt Lake City, Utah. She is the daughter of Marnie Brown and Terry Brown. A 2018 graduate of Flower Mound High School, Flower Mound, Texas, she received her Bachelor of Science degree with a major in Biology and minor in Journalism from Texas Christian University, Fort Worth, in 2022. During her time as an undergraduate, she was a Teaching Assistant for Introductory Biology Labs I & II (2019), as well as Genetics and Fundamentals of Biochemistry (2021-2022).

Upon completing her undergraduate degree, Bridey stayed at Texas Christian University to continue her graduate education, receiving her Master of Science in Biology in 2024. While earning her Master in Biology, she held a Teaching Assistantship and continued serving as the Teaching Assistant for both Genetics and Fundamentals of Biochemistry. She also earned the Adkins Fellowship and a Science & Engineering Research Center (SERC) Grant to fund her research project.

After earning her master, Bridey will be pursuing a future in medicine and applying to medical school.

Abstract

ABILITY OF DIET TO INDUCE HERITABLE EPIGENETIC MODIFICATIONS ASSOCIATED WITH ALZHEIMER'S DISEASE

By Bridey Elizabeth Brown, M.S., 2024
Department of Biology
Texas Christian University

Thesis Advisor: Matt Hale Ph.D., Associate Professor & Graduate Program Director

Alzheimer's disease (AD) is a neurodegenerative disease that is the most common cause of dementia, for which there is currently no cure. The Mediterranean diet (MD) is a candidate prevention strategy because it has been shown to reduce risk of AD development/mortality. In comparison, a typical American diet (TAD) has been shown to increase risk of AD development. However, the underlying mechanism is unknown. One largely unexplored mechanism of dietary-induced AD prevalence is epigenetic modification to genes associated with AD. Therefore, this study aims to determine whether dietary influences can induce epigenetic modifications, and subsequently modify expression of genes associated with AD. To do this, DNA was extracted from hippocampal tissue of mice on either a MD or TAD, as well as their offspring who were consuming a control diet. The offspring were included to determine if differential methylation patterns are heritable, thereby implicating transgenerational effects and predisposition to AD development or protection. An epigenetic array was used to identify loci that were differentially methylated between diets, and qPCR was used to determine if differential methylation resulted in significant differences in gene expression. No loci were found to be significantly differentially methylated ($p\text{-val}<0.0001$) with an effect size of 10% or more, nor differentially expressed upon qPCR analysis.