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Role of diet and genetics on aging brain and cognition

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Role of diet and genetics on aging brain and cognition

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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NOMENCLATURE

AD	Alzheimer's disease
FH	Family History
FI	Fluid Intelligence
SEM	Structural Equation Modeling
rsfMRI	Resting State Functional Magnetic Resonance Imaging
SNP	Single Nucleotide Polymorphism

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ABSTRACT

Cognitive decline in old age is normative, but decline can be exacerbated in individuals with Alzheimer's disease (AD) risk factors. Recent studies suggest that certain dietary regimens may slow or exacerbate this decline. However, it is uncertain how changes in whole food consumption affects fluid intelligence (FI) performance, an index of reasoning ability, in adults with or without AD genetic risk over time. Genetic risk for Alzheimer's disease (AD) is mostly found on Chromosome 19 in the *APOE-TOMM40-APOC1* region. Specifically, the Apolipoprotein E ε4 (*APOE4*) haplotype confers the greatest genetic risk for late-onset Alzheimer's disease (AD).

Food Frequency Questionnaire and Fluid Intelligence Test scores, assessed three times across 6 years, were obtained from UK Biobank to cross-sectionally and longitudinally examine which whole foods were most related to FI. Results indicated that both non-carriers (*APOE4-*) and especially carriers (*APOE4+*) showed increased FI with daily cheese and alcohol consumption at baseline. Conversely, decreased FI was seen among *APOE4-* with daily vegetable consumption over time. Among *APOE4+*, regular salt use showed worse FI scores over time. Our findings broadly suggest that reducing FI-related cognitive decline may be related to limiting meat, salt, and vegetable consumption, while increasing intake of wheat products, cheese, as well as the consumption of alcohol in moderation. Food recommendations, with AD genetic and family history factors in consideration, may minimize cognitive decline.

Translocase of Outer Mitochondrial Membrane-40 (*TOMM40*) is the only nuclear-encoded gene that controls mitochondrial protein transport, which is critical for maintaining cellular bioenergetics and is progressively disrupted in AD. *TOMM40* rs2075650 ('650) is one of the most consistent loci to show associations with several neural and cognitive outcomes relevant

to AD. For example, *TOMM40* ‘650 genotypes might affect neural network strength, an early brain marker that is disrupted along the AD continuum. Therefore, 21 orthogonally derived neural networks were examined among 8,222 participants in the UK Biobank cohort. Results indicated that *TOMM40* ‘650 G allele may be related to functional connectivity in auditory and language comprehension areas. This relationship may be modified by sex interactions. Differences were observed between G carriers and non-carriers among males, but not females. Not surprisingly, *APOE4* was associated with several neural networks that share brain topology generally affected by AD pathology.

CHAPTER 1. GENERAL INTRODUCTION

The 2018 US Census Bureau predicts that the population size of older adults will exceed that of young children. Aging raises vulnerability to cognitive decline and dementia. Typically, older adulthood is accompanied by diminishing quality of life. Genetic and lifestyle factors can contribute to brain health and dementia. The common type of dementia is Alzheimer's disease, a neurodegenerative disorder related to multiple components of known and unknown etiology. This has been a costly public health challenge to find the cure. Dietary interventions with genetic risk factors in consideration could improve brain health and slow or prevent cognitive decline.

CHAPTER 2. MORE CHEESE, LESS SALT: CONSUMPTION OF WHOLE FOODS, CHANGES IN FLUID INTELLIGENCE, AND THE INFLUENCE OF ALZHEIMER'S DISEASE GENETIC FACTORS

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Introduction

Normal aging is characterized by relatively modest loss in verbal memory and crystallized intelligence, which encompasses facts, experiences, and use of prior experience to inform actions. By contrast, progressive decline occurs in raw processing speed and higher-order cognitive domains, such as executive function (Glisky, 2007). Executive function consists of distinctive, multi-component processes such as response inhibition, attention, judgment, and regulation of other cognitive domains (Salthouse et al., 2003), which typically peaks in the late teens and shows decline in mid- and late life (Lewis and Todd, 2007; Verhaeghen and Cerella, 2002). The most adversely affected executive function by age is arguably fluid intelligence (FI),

which is the capacity for abstract reasoning and problem-solving without background information (Cattell, 1971).

Alzheimer's disease (AD) is a global public health concern (Brookmeyer et al., 2018), in part because executive function deficits often occur in concert with progressive learning and memory deficits (Karantzoulis and Galvin, 2011). Additionally, otherwise asymptomatic carriers of AD genetic risk factors like apolipoprotein E ε4 allele (*APOE*4), or a parental family history (FH) of AD, tend to show cognitive decline (Bendlin et al., 2010; Bretsky et al., 2003; Small et al., 2004; Wisdom et al., 2011). Observing changes in executive function over time in normal aging vs. genetically at-risk adults may offer valuable insight into subtle cognitive impairments that may serve as early cognitive markers of AD (Clark et al., 2012). Likewise, examining modifiable lifestyle factors that impact FI would be useful for future hypothesis testing.

Recently, lifestyle modification therapies, such as the Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) diet, has been associated with slower cognitive decline and reduced incidence of AD (Morris et al., 2015b, 2015a). More cortical thickness in temporal and frontal areas relevant to AD is also seen in non-demented adults who maintain a higher adherence to a Mediterranean diet (Staubo et al., 2017), which is rich in plant-based food sources and healthy fats (monounsaturated and polyunsaturated fats) and lower in red meats that are not lean. Thus, improving one's dietary practices in mid- or late-life may be beneficial for preserving cognition (Lehtisalo et al., 2019). Importantly, *APOE*4 status or parental FH of AD may influence how dietary patterns are related to cognition over time. For example, semi-weekly consumption of fatty fish may be a protective factor against dementia but only for non-*APOE*4 carriers (Huang et al., 2005). Since current therapeutic

strategies to modulate AD have not been effective so far (Cummings et al., 2014), targeting specific aspects of one's diet may be a potential preventative strategy to consider.

Our main objectives in this study were to test: 1) associations between total FI score and self-reported whole food intake, in contrast to predefined dietary patterns or nutrients, at baseline and 2) associations between total FI score and self-reported whole-food intake gathered over a six-year period at 3 visits for the following subgroups (APOE4+ vs APOE4-; FH+ vs FH-). We considered FI over other cognitive domains because a factor analytic study using UK Biobank demonstrated that FI had the strongest association with other cognitive measures, especially numeric memory scores (Lyall et al., 2016).

Materials and Methods

Cohort

The United Kingdom (UK) Biobank cohort includes a half million participants, aged 40 to 80 years, from 22 assessment centers located in the United Kingdom. The study design has been described elsewhere (Sudlow et al., 2015). Participants completed informed consent prior to baseline examination. Participants (n=1,929) had complete cognitive, demographic, dietary, and physical activity data (see **Supplementary Figure 2.1**).

Longitudinal Measurement of Fluid Intelligence

Participants completed the Fluid Intelligence Test (FIT) as part of a touchscreen questionnaire at baseline and two follow-up assessments (2008, 2012, and 2014). The FIT score is quantified by how many numeric, logic, and syntactic questions (out of 13 total questions) that participants were able to answer correctly within two minutes (Lyall et al., 2016).

Assessment of Long-term Dietary Patterns

Participants answered questions about their food and alcohol consumption as part of a touchscreen questionnaire at baseline and two follow-up assessments (2008, 2012, and 2014). The Food Frequency Questionnaire inquired about their intake of fresh fruit, dried fruit, raw vegetables and salad, cooked vegetables, oily fish, lean fish, processed meat, poultry, beef, lamb, pork, cheese, bread, cereal, beer and cider, red wine, white wine and champagne, and liquor. Bread, cereal, fruit, and vegetable responses were recorded in integer units (slices per week, bowls per week, pieces per day, and tablespoons per day, respectively). Intakes of meat, fish, and cheese responses were recorded as dichotomous variables ("*less than once a week*", "*once a week*", "*two to four times a week*", "*five or six times a week*", "*once or more daily*") that were contrasted to those who recorded they never consumed the respective food. Alcohol consumption responses were recorded by type as an average weekly intake (in pints for beer and cider, glasses for red and white wine and champagne, and measures for liquor). Frequency of alcohol intake was also recorded as dichotomous variables ("daily or almost daily", "three or four times a week", "once or twice a week", "special occasions only") that were contrasted to those who recorded they never consumed alcohol. Bread and cereal intake categories were combined to estimate total grain intake. Fresh and dried fruit intake categories were combined to estimate total fruit intake. Raw and cooked vegetable intake categories were combined to estimate total vegetable intake.

Considerations of Medical Exclusion Criteria

Many conditions, particularly in midlife to late-life adults with obesity, are related to chronic systemic inflammation, which may influence cognitive function. Consequently, to explore if these conditions influenced initial model fitting, we excluded participants with

International Classification of Diseases, Tenth Revision (ICD-10) codes reflecting a systemic inflammatory condition in their medical record since 2007. Examples included most diseases of the digestive system (XI), such as ulcerative colitis (K51) or diverticulitis (K57); chronic lower respiratory diseases (J40-J47); and malignant neoplasms of the digestive organs (C15-C26). However, because our results did not significantly differ based on these exclusion criteria, data from these participants were included in our final models. We also initially excluded participants who had any cerebrovascular disease (I60-I69) or disorder of the nervous system (G00-G99). However, our pattern of results did not significantly differ based on these excluded participants. Consequently, data from these participants were included in the final models as well.

Genetic Factors - APOE and AD Family History

All UK Biobank genome-wide association study (GWAS) data have been processed as previously described (Sudlow et al., 2015). Briefly, APOE haplotype was determined using allele variation on rs429358 and rs7412 (G. Davies et al., 2014). APOE was further stratified as whether participant has at least one ε4 allele (ε2/ε4, ε3/ε4, and ε4/ε4) or not at all (ε2/ε2, ε2/ε3, and ε3/ε3). AD family history was classified according to the participants' self-report responses of the presence or absence of AD in their family history on the touchscreen questionnaire. Specifically, participants were queried about family history via the question, "Has/did your father/mother ever suffer from:", followed by a list of chronic diseases, including 'Alzheimer's disease/dementia.'

Statistical Analyses

Longitudinal Modeling

For FIT, we used difference equations to compute each individual's linear change over time (velocity) to enhance model fit (Shaw et al., 2006). Initial values observed and velocity were modeled together as cognitive outcomes in a system of equations in order to test how dietary parameters influence the trajectory of cognitive decline throughout the range of age observed here. For dietary predictors, we used difference equations to compute each individual's across-time averages and linear change over time. We then integrated these values to derive average levels and the sum of changes in order to estimate the total amount consumed for each product (Pruessner et al., 2003). This method has demonstrated superior goodness-of-fit, increasing testing power, and has elucidating relationships between variables more robust by capturing both within- and between-subject variation over time (Duncan and Duncan, 2004; Hu and Bentler, 1999; Klinedinst, 2017; Preacher, 2008).

Outlier Analysis

In order to ensure that our models were generalizable to at least 99% of the sample population, 1% quantiles were computed, and participants beyond 99% of the sample distribution of the mean among any variable were removed from further analysis. This resulted in a final sample size of 1,787 and 1,326 for FH and *APOE* models, respectively.

Group-stratified Structural Equation Models

Structural equation modeling (SEM) was done using lavaan package from R 3.4.1 (R Foundation for Statistical Programming, Vienna, Austria) (Rosseel, 2012). Graphs were prepared in ggplot2 3.1.1 (Wickham, 2019). Four separate SEMs were built to identify differences

between: 1) Family History Negative; 2) Family History Positive, 3) ApoE4 Negative, and 4) ApoE4 Positive participants. To account for confounding demographics and other lifestyle variables, models controlled for education, sex, social class as defined by Townsend Deprivation Index, body mass index (BMI), and tobacco use. Education was separated into three variables and coded as whether they had the following or not: college or higher qualifications; post-secondary or vocational; and secondary. Townsend Deprivation Index is a standardized score that reflects poverty as measured by material deprivation in a population per zip code (Phillimore et al., 1994).

Variable Selection

An empirical model-building approach was employed to select the most salient variables that predicted FI for each subgroup. In this backward elimination approach a full, "all variables in" model was built, and the least significant variable was removed one at a time until all variables remaining reached $p<0.05$.

Parameter Estimation, ANOVA, Uncertainty Analysis

Standardized parameter estimates (β), which were interpreted as the change in outcome variable per standard deviation change in the predictor, were estimated using a maximum likelihood approach. ANOVA was reported as the overall portion of variation in FI scores that is explained (R^2). Uncertainty analysis relied upon standard errors and *p*-values estimates, where results were considered statistically significant at $p<0.001$ (***), $p<0.01$ (**), and $p<0.05$ (*). Only participants with all available data were considered for analysis (i.e., no missingness imputation was done).

Post Hoc Analysis

To assess whether any observations were driving the results, the Cook's Distance was calculated for each observation in the base and moderation models. The largest value in the base model and moderation model was 0.012 and 0.015, respectively. Any values less than 1.0 are of no concern, and we therefore made no further adjustments to the models.

Results

Demographics and Data Summaries

The demographics for APOE and FH subgroups were presented in **Table 2.1.** There was slight male majority across all four subgroups. The majority of each subgroup were highly educated. We did not observe major differences between groups in BMI, FI score at baseline nor Townsend Deprivation Index.

Main Effects on Fluid Intelligence Scores

We examined differences of dietary effect on FI based on *APOE* haplotype (ε4 carriage versus non-carriage) or family history of AD. Aside from food, those who attended college or post-college had strong positive significant association with FI at baseline regardless of *APOE*4 status, as well as family history ($p<0.001$).

Associations with Diet by Genetic Factors Subgroups

The standardized beta estimates are presented in **Table 2.2.** For *APOE4* status, both non-carriers (*APOE4-*) and especially carriers (*APOE4+*) showed increased FI with daily cheese (*APOE4-*: **β**=.073, *p*=.008; *APOE4+*: **β**=.162, *p*=.001) at baseline, but no relation was observed over time (see **Figure 2.1**). Though among *APOE4+* only, there was a positive association

between FI and weekly cheese over time (**β**=.109, *p*=.025). Daily alcohol consumption was related to greater FI at baseline among only *APOE4+* (**β**=.101, *p*=.023) (see **Figure 2.2**). Greater red wine consumption was related to increased FI at baseline in *APOE4-* instead, (**β**=.059, *p*=.039). Conversely, decreased FI was seen in *APOE4-* with daily vegetable consumption (**β**=-.070, *p*=.023) over time, while for *APOE4+* usually salting showed worse FI scores (**β**=-.121, *p*=.009) over time (see **Figure 2.3**).

Associations with Diet by Family History Subgroups

The standardized beta estimates are presented in **Table 2.3.** For adults without family history, increased FI was related to daily cheese intake (**β**=.207, *p*<.001) at baseline, while daily poultry (**β**=-.096, *p*=.003) or vegetable (**β**=-.072, *p*=.005) consumption was associated with more rapid decline at baseline. Significant associations of these variables and changes in FI over time were not observed. For adults with AD family history, daily whole grain (**β**=.080, *p*=.035) at baseline and red wine (**β**=.100, *p*=.014) consumption predicted higher FI levels over time. FI had a negative association with tea intake (**β**=-.090, *p*=.015) and processed meat intake (**β**=-.098, *p*=.006) at baseline. More sporadic intake of poultry and salt were also associated with worse FI scores. **Figure 2.3** illustrated the association of added salt frequency on change in fluid intellect.

Discussion

The objective of this study was to better understand the relation between whole-foods diet composition and changes in FI, among participants stratified by *APOE4* carriage status and family history status. Our results suggested that whole-foods could affect FI both cross-sectionally and longitudinally. However, differences are present between subgroups for *APOE*

and FH. Additionally, there are variables that appeared influential at baseline but not over time and vice-versa.

Greater dairy intake may be associated with declining memory in older adults who have heart failure, possibly due to the high fat contained in dairy products. Dairy products may then, in turn, contribute to an alteration in blood pressure, consequently affecting memory (Garcia et al., 2015). This association may suggest cheese intake to be unhealthy. On the other hand, dairy consumption may prevent cognitive decline in men without cardiovascular disease sequelae due to calcium and vitamin B12, independent of genetic and family environmental factors (Ogata et al., 2016). Differences in the fat content of dairy products may be related to improvements in spatial working memory (Crichton et al., 2012). However, it is largely unknown how whole fat vs. low fat or nonfat dairy options would affect such outcomes (Rubin, 2018). Thus, dairy consumption may be beneficial in other ways. For example, lactopeptides may improve cognition in older adults (Ano et al., 2018), and probiotics may attenuate depression (Steenbergen et al., 2015).

We also found that, when distinguishing different sources of alcohol, greater red wine consumption over time was related to better FIT scores. Among cognitively normal aged adults, low, but not moderate or heavy consumption of wine, has been associated with improved cognitive performance, white matter integrity, and cerebral blood flow (Haller et al., 2018). Another observational study found that, in contrast to abstainers, light to moderate beer consumption was associated with a higher risk of dementia, while light to moderate wine consumption was associated with a significantly lower risk (Deng et al., 2006). This is consistent with the findings of one large observational, cross-sectional cohort study consisting of 1,624 older participants (mean age±SD = 73.2±9.3 years), which found a link between the amount and

frequency of alcohol intake and cognitive function (Reas et al., 2016). Interestingly, the study found that moderate, regular alcohol consumption related to improved cognitive function in comparison to abstainers of alcohol, supporting that utilizing alcohol in moderation is unlikely to result in adverse effects in the geriatric population. Furthermore, when assessed by APOE status, the study found no significant difference between APOE genotype and the alcohol-cognition relationship, which contradicts our findings. The mechanisms underlying how moderate alcohol consumption may be neuroprotective have yet to be fully elucidated. However, research has shown that alcohol used in moderation may have beneficial cardiovascular-associated effects (including higher high-density lipoprotein, decreased blood pressure, and increased anti-inflammatory properties), which, in turn, may result in better cognitive function (Hines and Rimm, 2001; Ronksley et al., 2011). Alternatively, the alcohol-cognitive link may also be explained by prior evidence suggesting that alcohol could be directly mediated by certain neuroprotective factors, including the reduction of neuroinflammation, oxidative stress, and apoptosis (Bate and Williams, 2011; Liao et al., 2003). However, more research is needed to elucidate the biological underpinnings of alcohol's effects on cognition.

It is important to note that the Reas et al. study failed to differentiate between the types of alcohol consumed (wine, beer, and liquor) in the results. This is noteworthy, as prior literature has shown that this benefit is more pronounced with wine consumption than with beer or liquor consumption (Arntzen et al., 2010; Truelsen et al., 2002). Although alcohol induces oxidative stress, red wine may counteract this because of its polyphenolic content (Berman et al., 2017; Hernández et al., 2016). Furthermore, we did observe a significant association between red wine and higher FI in our data, but only in those with a *APOE4*- genotype or a family history of

Alzheimer's disease. Those who were *APOE4+* or had no family history of Alzheimer's disease, on the other hand, showed no such association.

Across participants without the aforementioned AD risk factors, vegetable consumption was negatively correlated with FI over time for *APOE4-* adults and at baseline for FH- adults. Cruciferous vegetables (i.e. broccoli and kale) are beneficial because they are rich in micronutrients, flavonoids, and antioxidants (Morris et al., 2018). Contrarily, starch-rich vegetables have a higher glycemic load and may elevate the risk for hypertension by inducing oxidative stress (Huang et al., 2019; Lea et al., 2016), which in turn may drive cognitive impairment and AD progression (Sultana and Butterfield, 2010). Thus, vegetable associations may be driven by starch-rich or fried vegetables. However, our study could not discern the type.

Our data supports that excess dietary salt intake appears to harm cognition, especially for individuals with the presence of FH or *APOE4*. Prior research has shown a link between salt-rich diets and cognitive decline. For example, an experimental study using mice who were fed either eight-fold or sixteen-fold over the recommended amount of dietary salt in a mouse's daily diet that after approximately eight to 12 weeks of following this diet, the mice began manifesting cognitive decline and memory difficulties (Faraco et al., 2018). For instance, they have increasing difficulty with differentiating new vs. familiar objects, completing mazes, and building nests. Although the mechanisms behind how salt negatively affects brain health are not fully understood, the authors purported that physiological disturbances such as hinderance of resting cerebral blood flow and endothelial function caused by excess salt intake is likely to be applicable to humans as well.

However, the studies examining associations between dietary salt consumption and cognition appear to be inconclusive in humans (Kendig and Morris, 2019). Stratification of

dataset into AD risk factors, such as done in our study, potentially elucidated this relationship. For instance, we found that FI scores were consistent regardless of the amount of salt consumed for participants lacking either FH AD or *APOE4*, but always salting appeared to be detrimental for participants who were found to have either aforementioned AD risk factor. Individuals with FH of AD, specifically maternal, who were cognitively unimpaired were shown to have reduced brain glucose metabolism (Mosconi et al., 2007). Thus, this predisposition may not handle high salt intake and therefore impair cognition. However, more research is needed to elucidate the mechanisms that may be involved in the correlation between dietary salt intake and cognition in humans.

FH is a unique risk factor that may reflect developmental and or environmental influences, such as dietary habits, which could modulate neurodegeneration (Donix et al., 2012). Although Donix and colleagues found that subjects with FH had lower baseline scores in general cognitive performance, no significant decline were detected longitudinally. However, the difference in findings may be due to the length of time with their study having one follow-up assessments over a period of approximately two years versus ours having two follow-up assessments over a period of six years. Gut microbiota and the brain conjunctively form an axis and have interlinked health outcomes via vagal afferents (Ambrosini et al., 2019; Mayer et al., 2015). Greater diversity in the microbiome has been associated with reduced risk of AD (Vogt et al., 2017) and better ability to digest nutrients (Petersen et al., 2019). Not only does the intestinal microbiome affect absorption potential, but diet can also impact the diversity and metabolic capacity of gut flora and alter body composition (Turnbaugh et al., 2009). A FH of AD may modify how the brain utilizes β-carotene and may be related to other beneficial effects, such as higher regional glucose metabolism in AD-sensitive brain areas (Mosconi et al., 2014). In other

words, FH of AD may moderate how diet and cognition are related because it reflects inter-individual differences in the ability to harbor a healthy diversity of gut bacteria, which alters how food is metabolized and which nutrients are extracted.

There are several limitations that should be considered in our study. Although we utilized longitudinal data for predictors and outcomes, this is an observational study and the direction of causality cannot be inferred. Additionally, associations may also reflect effects from an unmodeled third variable. Our study had nevertheless several strengths worth noting. First, unlike cross-sectional self-reporting of food intake studies, which may underestimate or overestimate consumption, we accounted for individual variation and reduced error variance by considering dietary recall at three time-points over six years. In addition, we also used agnostic statistical analyses to parse which food groups were related to FIT scores, rather than derive scores for adherence to a specific diet type. By identifying effects from individual food categories, Mediterranean and MIND diet recommendations could be further improved.

In summary, our findings suggest that weekly cheese intake over six years is associated with better fluid intelligence performance for *APOE4+* adults. Though daily cheese intake appeared beneficial regardless of *APOE4* carrier status at baseline. The association between FI and certain food may differ between people stratified by AD family history and between people stratified by *APOE4* carrier status. Thus, meal plans may need to be modified to meet individual needs in order to potentially minimize cognitive decline. Future work may examine how other cognitive domains (i.e. spatial memory), brain volume and white matter microstructure are affected by whole food dietary components.

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Figures and Tables

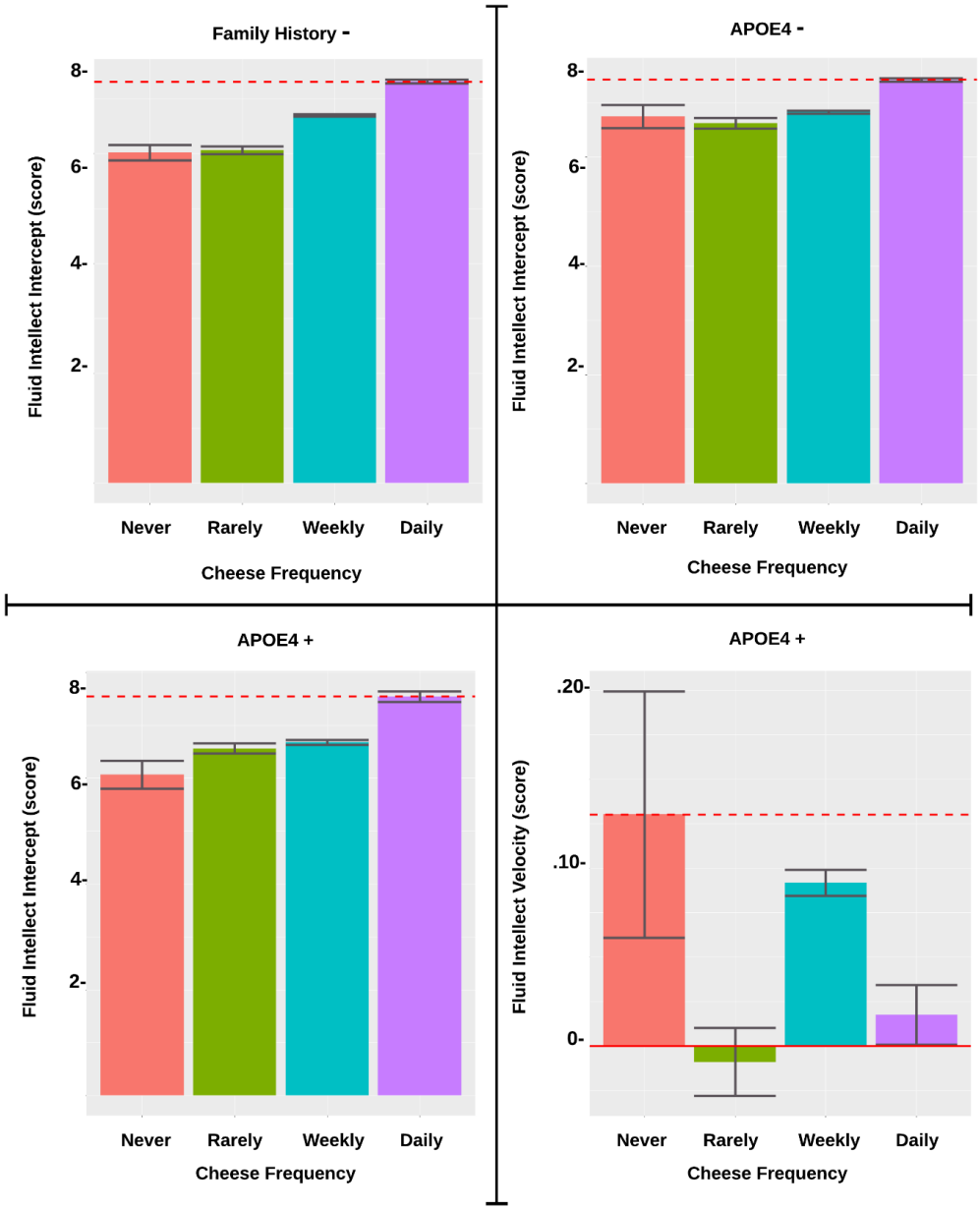


Figure 2.1. Association of Cheese Frequency on Fluid Intellect by FH and APOE4 Subgroup.

Velocity is change over time. The red dotted lines represent the performing group with highest fluid intellect level at baseline.

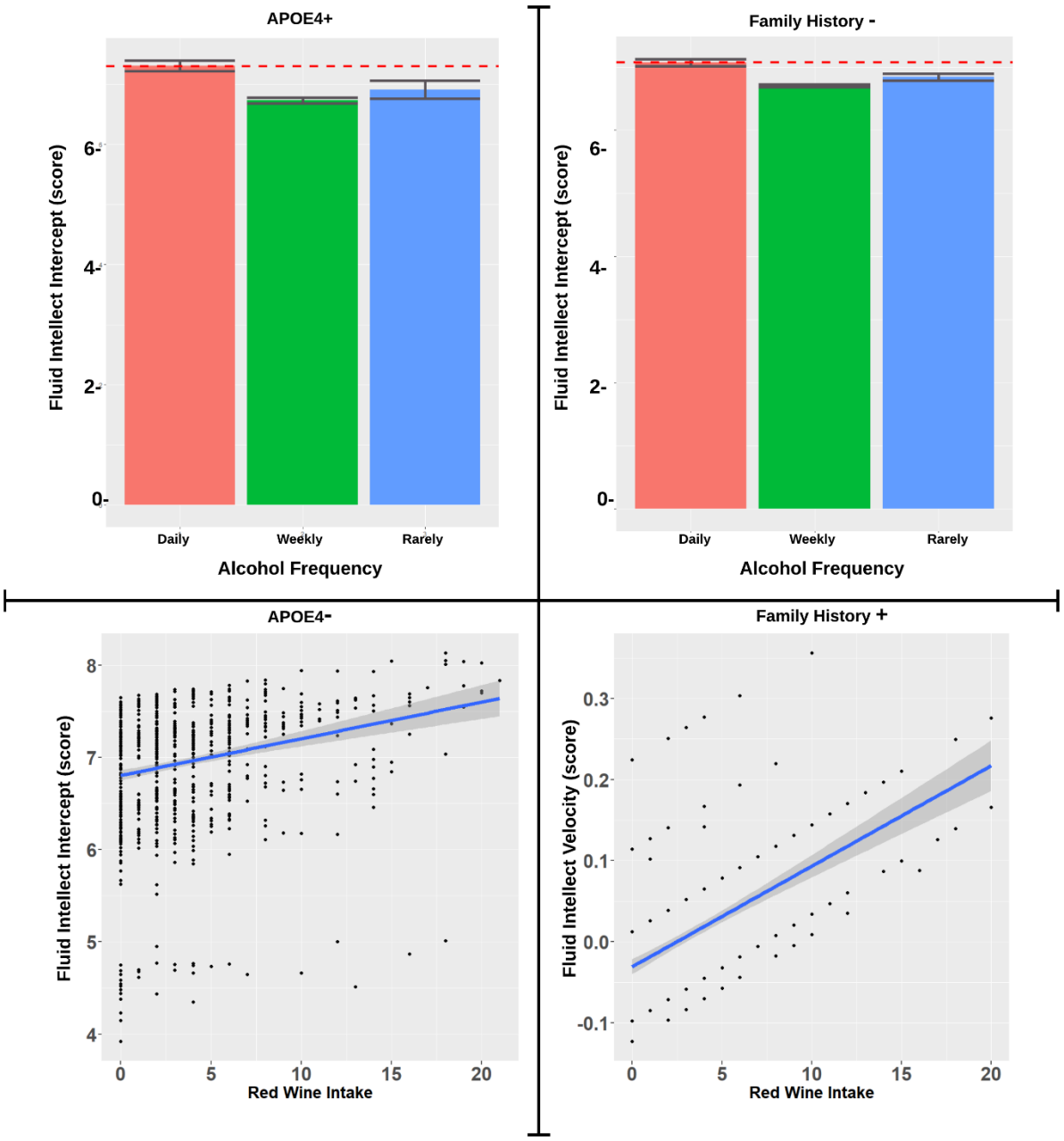


Figure 2.2. Association of Alcohol Frequency and Red Wine Intake on Fluid Intellect by FH and APOE4 Subgroup.

Velocity is change over time. The red dotted lines represent the performing group with highest fluid intellect level at baseline.

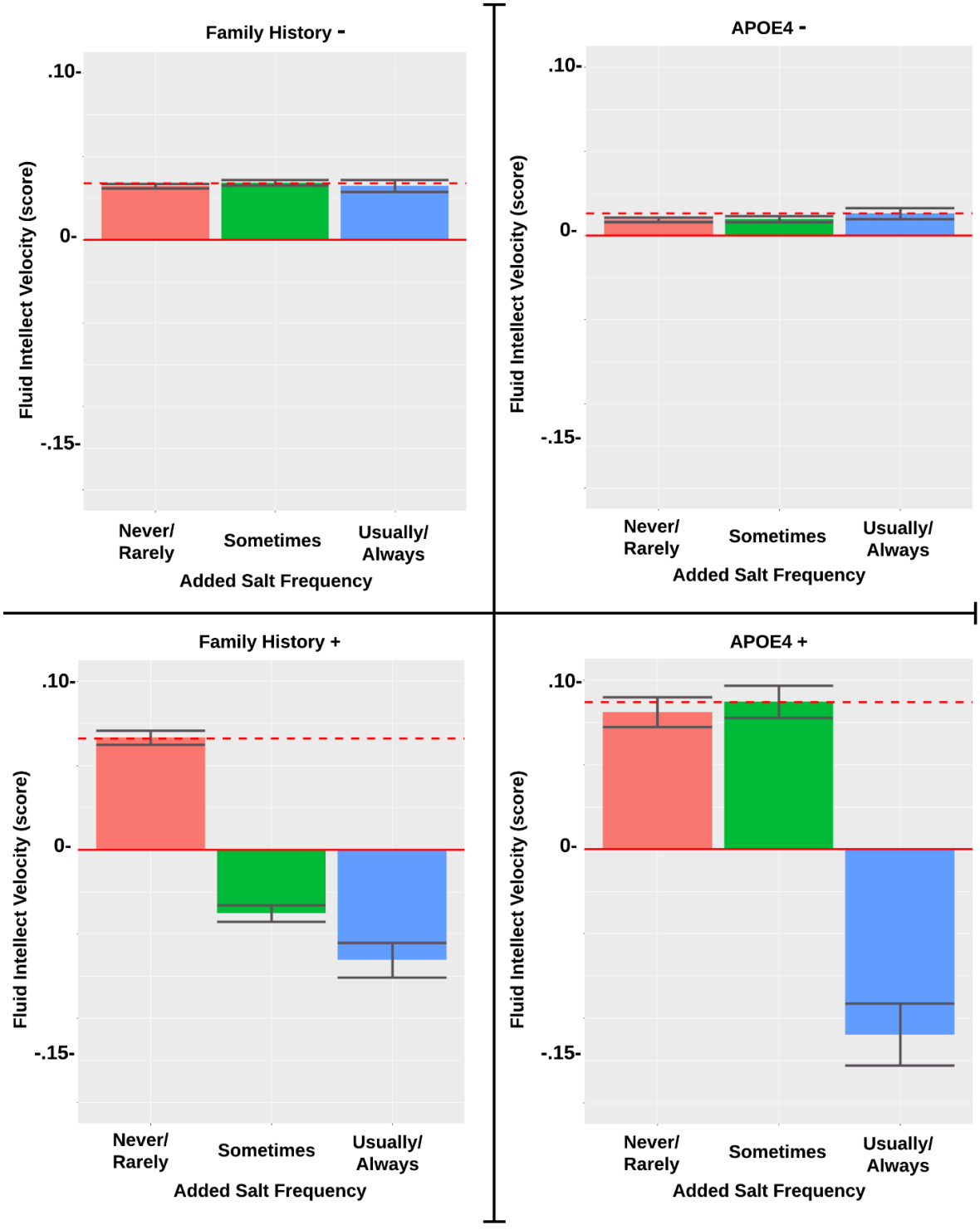


Figure 2.3. Association of Added Salt Frequency on Fluid Intellect by FH and APOE4 Subgroup.

Velocity is change over time. The red dotted lines represent the performing group with highest fluid intellect level at baseline.

Table 2.1. Data summary stratified by APOE subgroups and Family History Factors

Data, measurement unit	**APOE4-**	**APOE4+**	**FH-**	**FH+**
Sample Size (n)	967	359	1305	482
Age (y)	63 ± 7.4	62 ± 7.2	63 ± 7.6	64 ± 6.5
Sex (Female:Male)	470:497	164:195	619:686	226:256
College Plus (n, %)	651 (67.3%)	253 (70.5%)	861 (66.0%)	345 (71.6%)
BMI	26 ± 4.2	26 ± 3.8	26 ± 4.2	26 ± 4.0
Fluid Intellect Baseline	6.9 ± 1.9	6.8 ± 2.1	6.8 ± 2.0	7.0 ± 1.9
Townsend Deprivation Index	-2.4 ± 2.4	-2.4 ± 2.3	-2.3 ± 2.4	-2.3 ± 2.5

The values are mean ± SD unless stated otherwise. Years is abbreviated y. Body mass index is abbreviated BMI.

Table 2.2. Standardized Estimates of predictors on fluid intelligence by APOE4 status

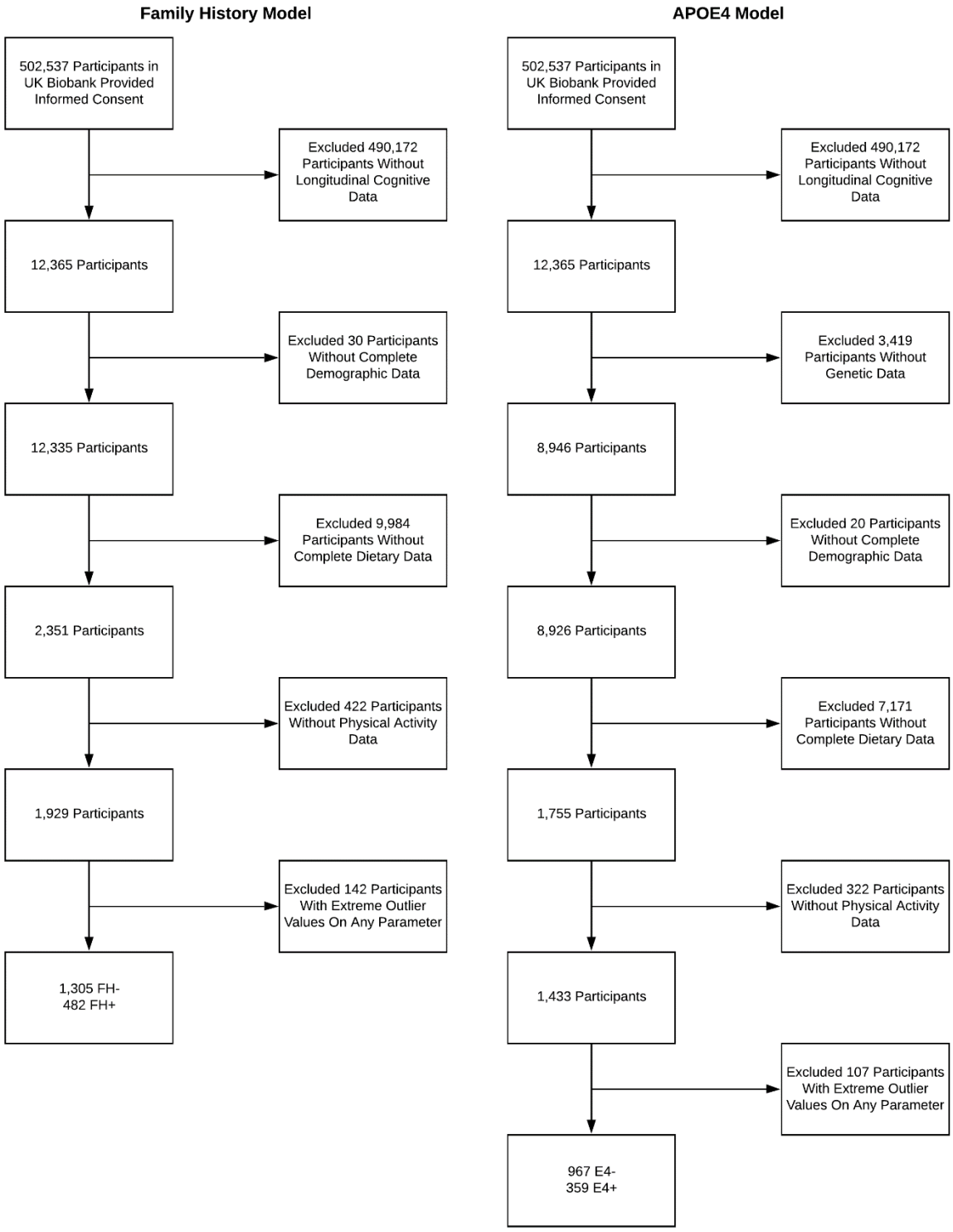
Predictor	Models			
	APOE4- (n=967)		APOE4+ (n=359)	
	Intercept	Velocity	Intercept	Velocity
Cheese Daily	0.073**		0.162**	
Red Wine	0.059*			
Vegetables		-0.070*		
Moderate Activity	-0.082**		-0.149***	
Poultry Rarely			-0.134**	0.134**
Lamb Rarely			0.097*	
Processed Meat Rarely			-0.276**	0.287***
Processed Meat Weekly			-0.370***	0.286***
Processed Meat Daily			-0.179**	0.126*
Daily Alcohol			0.101*	
Usually Salts				-0.121**
Cheese Weekly				0.109*
Lean Fish Rarely				-0.326**
Lean Fish Weekly				-0.245**

*p<.05, **p<.01, ***p<.001.

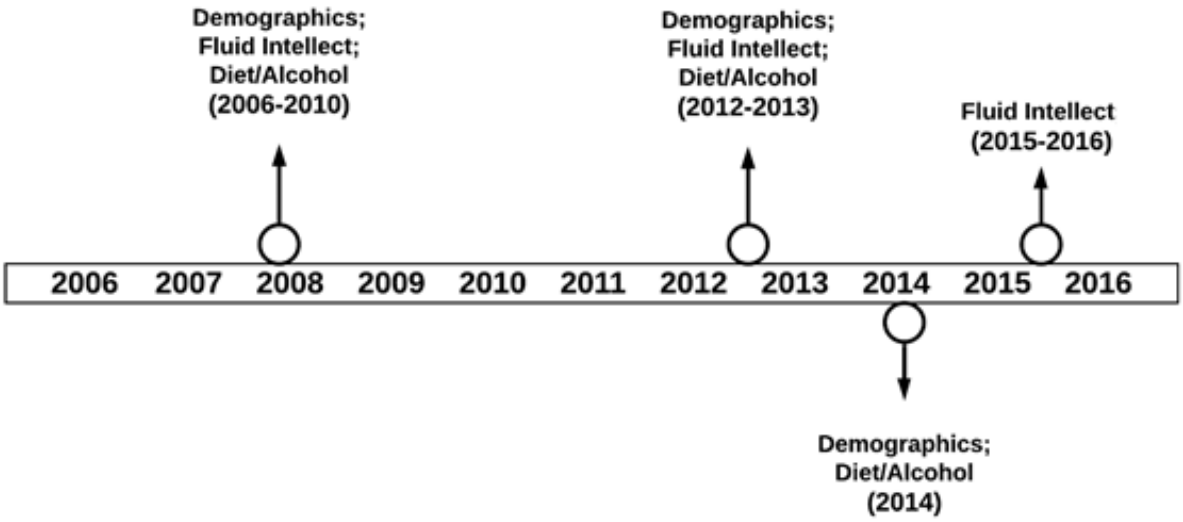
Table 2.3. Standardized Estimates of predictors on fluid intelligence by Family History status

Predictor	Models			
	FH- (n=1305)		FH+ (n=482)	
	Intercept	Velocity	Intercept	Velocity
Cheese Weekly	0.124**			
Lamb Rarely	0.066**			
White Wine	0.064**	-0.054#		
Processed Meat Rarely	0.051*		-0.098**	
Weekly Alcohol	-0.051*			
Vegetables		-0.072**		
Poultry Daily	-0.096**			
Moderate Activity	-0.097***			
Poultry Rarely	-0.103**		-0.107*	
Poultry Weekly	-0.152***		-0.127**	
Grain Substitute				0.100*
Red Wine				0.100*
Whole Grains			0.080*	
Usually Salts				-0.079*
Tea			-0.090*	
Sometimes Salts				-0.114**

Values are standardized betas. *p<.05, **p<.01, ***p<.001.



Supplementary Figure 2.1. Flowchart Diagram of participant selection and exclusion



Supplementary Figure 2.2. Timeline of Data Collection

CHAPTER 3. MAIN EFFECT OF *APOE4 AND TOMM40* '650 POLYMORPHISM WITH NEURAL NETWORK FUNCTIONAL CONNECTIVITY

Modified from a manuscript to be submitted to Neurobiology of Aging

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Introduction

The Apolipoprotein E ε4 (*APOE* ε4*)* haplotype is the strongest genetic risk factor for late-onset Alzheimer's disease (AD), a neurodegenerative disorder characterized by progressive cognitive impairment and reduced brain activity both at rest and during task performance (Agosta et al., 2012; Corder et al., 1993; Farrer et al., 1997; McKenna et al., 2016). In the absence of a task, resting state functional magnetic resonance imaging (rsfMRI) is used to examine spontaneous changes over time in the amount of deoxygenated vs. oxygenated blood used to meet metabolic demand, a proxy for neural activity at baseline with no task (Ma et al., 2016). These spontaneous fluctuations are highly correlated within and across certain brain regions, resulting in consistent neural networks (Damoiseaux et al., 2006). Neural network strength (i.e., functional connectivity or coupling) can discern AD patients from healthy controls, as well as individuals with mild cognitive impairment (MCI) who are stable from those who later

convert to AD (Binnewijzend et al., 2012; Sorg et al., 2007). Further, these links to less neural network strength in older unimpaired adults with *APOE4* can manifest without amyloid deposition (Sheline et al., 2010), suggesting such changes occur years before AD neuropathology begins in earnest and that network strength can act as an early AD neural biomarker.

The common approach is to focus on one specific network, the Default Mode Network (DMN). DMN is an important network to consider because of its vulnerability to AD (Buckner et al., 2009). Specifically, this network robustly shows progressively less activity in adults who are at-risk, MCI, and AD. DMN consists of bilateral parietal cortex, precuneus and posterior cingulate cortex, anterior cingulate cortex, medial prefrontal cortex, hippocampus, and thalamus (Binnewijzend et al., 2012), all of which show less glucose metabolism across the AD continuum. Hence, the current study examined neural network strength in the DMN, but also explore a comprehensive set of other neural networks in a robust sample. Such networks underlie neural activity that regulates executive function, emotion regulation, memory recognition, association, and other behaviors or internal processes relevant to AD.

Likewise in genomics, the APOE ε4 allele receives the overwhelming vast majority of attention, despite the other two APOE haplogroups. Specifically, it is unclear how the protective APOE ε2 allele (Corder et al., 1994) and "neutral risk" ε3 allele differ with regard to activity in these non-DMN networks, particularly in the context of other AD risk factors such as age and sex. Most especially, it is worthwhile to consider that adjacent genes on Chromosome 19 may also be relevant to detecting early hypoactivation in these networks. While several loci on these genes are in moderate linkage disequilibrium with *APOE*, at-risk genotypes may nonetheless have effects on neural network function. This would be critically important for early detection of participants who will eventually go on to develop AD.

In particular, Translocase of Outer Mitochondrial Membrane 40kD (*TOMM40*) is the only nuclear-encoded gene that facilitates mitochondrial protein trafficking and translation, a process critical for cellular bioenergetics (Humphries et al., 2005). Many studies have shown that a G vs. A single nucleotide polymorphism (SNP) on rs2075650 (‘650) increases the risk for normal cognitive decline but also AD (Bagnoli et al., 2013; Harold et al., 2009; Omoumi et al., 2014; Valant et al., 2012). It is still open to debate whether the aforementioned *TOMM40* associations merely reflect LD with *APOE* (Davies et al., 2014; Yu et al., 2007). These studies only covaried the number of ε4 alleles, however, without a more careful consideration of the 3 haplogroups that make up the *APOE* factor. It may be that *APOE* ε4 shows independent relationships itself, in conjunction with *TOMM40* ‘650, or possibly both scenarios depending on the phenotype. It may be easier to disentangle such effects using neural networks sensitive to AD vs. normal aging, rather than AD risk or cognition which have much more error variance.

Accordingly, using genetic and rsfMRI data from the UK Biobank cohort (Sudlow et al., 2015), our objectives were to test the following effects on neural network strength at rest: 1) the main effects of the APOE haplotypes and interactions with sex and age; and 2) the main effects of *TOMM40* ‘650 polymorphism and interactions with sex and age, among all participants. This study will clarify how *APOE* and *TOMM40* risk groups influence early neural markers of AD, which in turn could improve precision medicine for disease treatment.

Materials and Methods

Cohort and Participants

The UK Biobank cohort includes a half million participants, aged 40 to 80 years, from 22 assessment centers located in the United Kingdom (Sudlow et al., 2015). The current study examined a sub-cohort of 8,222 participants with genomics, MRI, and demographics data.

Participants completed informed consent prior to baseline examination. Participants with central nervous system disorders, cerebrovascular diseases, and any of the dementias were excluded from the sample (see **Supplementary Figure 3.1).**

Genotyping

For *TOMM40*, genotype data for the SNP '650 were extracted for analyses using PLINK version 1.90 (https://www.cog-genomics.org/plink/1.9/). *TOMM40* '650 status was coded as those who were non-G carriers (AA homozygotes) versus G-carriers (GA and GG). G has been credited as being an AD risk allele (Elias-Sonnenschein et al., 2013). The Hardy-Weinberg equilibrium (HWE) for *TOMM40* '650 was p = 0.6976, indicating that frequency counts for these genotypes were in line with the general population. For *APOE* haplotype, a similar process was used for SNPs rs429358 and rs7412. Participants were categorized as having ε2, ε3, or ε4 isoforms. Participants classified as *APOE* ε2/ε4 were excluded from analyses.

Resting State fMRI – Acquisition and Processing

As described in previous publications (Miller et al., 2016), participants were scanned on one of three Siemens Skyra 3T units with a 32-channel RF receiver head coil (Siemens Medical Solutions, Erlangen, Germany). Participants were briefly instructed to simply focus on a crosshair and not think about anything specifically, and therefore had eyes open. Scan duration was 6 minutes and 10 seconds, to acquire 490 images with the following acquisition parameters: TR = 735*ms*; TE = 39*ms*; 2.4 x 2.4 x 2.4mm voxel resolution; 88 x 88 x 64 matrix, multiband factor = 8, in-plane acceleration factor = 1, flip angle 52°. Preprocessing and quality control measures are described in UK Biobank white papers (https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/brain_mri.pdf). Briefly, using FSL tools, the 4D dataset was motion-corrected, grand-mean intensity normalized, high-pass temporal filtered

(with sigma = 50.0s), EPI and GDC unwarped and denoised (using ICA+FIX processing). Group Principal Component Analysis and Independent Component Analysis through FMRIB's MELODIC were then used to derive 21 spatially orthogonal, non-noise, distinctive Independent Components (ICs) that represent resting neural networks. Each participant has a Z-score for a given IC, representing the degree of activation relative to the group mean. An expert (AAW) then viewed the activation maps and described the neural networks (see **Supplementary Table 3.1**).

Covariates

For main effect analyses of *APOE* or *TOMM40* '650 genotype, covariates included sex, age at recruitment, social class, education level, and family history of AD. Social class was categorically derived from gross annual household income between 2008 and 2014 as "lower class" (<£18,000), "middle class" (£18,000-£51,999), and "upper class" (£52 000 to > £100,000). Education level was categorized as: college or higher qualifications (3); post-secondary or vocational (2); secondary (1); and, none of the above (0).

Statistical analysis

Linear multiple regression equations were modeled using R, version 3.6.1 (R Foundation for Statistical Programming, Vienna, Austria). Initial analyses tested the main effect of APOE haplotype type (ε2, ε3, ε4) on all 21 neural networks, followed by interactions with age in years and sex. The ε3 allele group was set as the reference group. ANCOVA was used in follow-up tests to examine differences among APOE groups. Next, we examined the main effect of *TOMM40* '650 G carriage and interactions with age in years and sex. *APOE* was added to the *TOMM40* models as a covariate and recoded as a dichotomous variable based on whether the participant carried ε4 or not (*APOE4+* vs *APOE4-*). These analyses were conducted across all

participants. ANCOVA was used in follow-up tests to examine differences among ‘650 G carriage. To address power, sensitivity analyses was conducted for each dataset using GPower 3.1 (Faul et al., 2009). Significant effects were corrected for multiple comparison using the Scheffe method. Family-wise Alpha was set at p=0.05. Estimated marginal means were calculated using emmeans (Lenth et al., 2020). The figures were produced using ggplot2 (Wickham, 2019).

Results

Descriptive Statistics

Our sample included 8,222 participants. Demographic and clinical data are presented in **Table 3.1**. The average age of participants was 55 years (SD = 7.47) and approximately 52% were female. Most participants were highly educated. Slightly more than half participants reported to be in middle class.

APOE Main Effects and Interactions

In contrast with ε3 carriers, ε4 carriers had less functional connectivity in executive function, memory retrieval, memory storage, motor planning, and language processing networks (see **Figure 3.1** and **Table 3.2). Figure 3.2** illustrates functional connectivity in posterior default mode network across APOE groups. Conversely, ε2 carriers had more functional connectivity in memory storage and retrieval (see **Table 3.2)**. No age or sex interactions were observed with *APOE ε*4 carriers for any resting state network. Exploratory three-way interactions also yielded non-significant results for any network.

TOMM40 ‘650 Main Effects and Interactions

We found an effect of the G-allele in neural networks involved with auditory and language comprehension (see **Table 3.3**). **Figure 3.3** illustrated the main effect of G carriage and *APOE*

ε4 carriage separately on auditory/language comprehension areas and posterior default mode network. The estimated difference between carrier vs. non-carrier men was significant ($M_{diff=}$.072, SE=0.0232, p=0.02) in the language comprehension neural network. However, this did not apply among women (M_{diff}=0.007, SE=0.0223, p>0.10). Additionally, there were no significant interactions between *TOMM40* '650 and age in years for any resting state network. Three-way interactions were also examined but yielded no statistically significant effect for any resting state network.

Discussion

Our objectives for the study was to examine the main effect of *APOE* and *TOMM40* on neural network strength. *APOE4* was related to less functional connectivity in several resting state networks: motor planning, left auditory processing, memory consolidation, primary visual, central executive function, cerebellum, posterior default mode network, and top-down cognitive processing. Cortical auditory processing has been deemed a relevant biomarker for preclinical AD, but not with *APOE4* (Tuwaig et al., 2017). Nonetheless, this risk allele has been associated with motor decline and worse episodic memory (Buchman et al., 2009; Lim et al., 2017).

To our knowledge, this was the first study to examine the association between *TOMM40* '650 and resting state neural networks. *TOMM40* '650 G carriage appears to specifically affect neural networks involved in auditory and language comprehension. Language comprehension is tied with Wernicke's area and has been shown to have predominant long-range connectivity (Tomasi and Volkow, 2012). Overall, this may support any effect *TOMM40* does have on AD vulnerable areas is due to LD with *APOE4*.

The effect of *TOMM40* '650 G carrier status that was observed may be due to sex differences seen between G carriers and non-carriers in men but not women. We expected weakened

functional connectivity for G carriers, but the effect appeared to be greater for males. Another UK Biobank Study observed sex differences in resting state connectivity such as men having stronger connectivity between unimodal sensory and motor cortices (Ritchie et al., 2018). While we did observe significant sex effects across all neural networks, sex alone is not enough to distinguish individual patterns (Weis et al., 2020). Unsurprisingly, age was significantly associated with less functional connectivity which has been supported elsewhere (Varangis et al., 2019). However, the lack of three-way interactions observed could support that multiple mechanisms affect functional connectivity separately.

Despite our study's strength of a large cohort, there are notable limitations to address. Since our sample comprises of British Europeans, our results may not generalize to the larger population. Although *TOMM40* '650 polymorphisms contributing similarly to AD was observed in Caucasian and Asian populations (Huang et al., 2016), this study does not encompass other race or ethnicities. The correlations between neural networks may either underestimate or overestimate the effect of '650 G carriage on one neural network. The current study was cross-sectional so we could not capture potential changes in functional connectivity. In addition, causality cannot be implied because the study was observational.

The current study may reflect that more neural networks are vulnerable to *APOE4* than *TOMM40* polymorphisms while the latter may have an effect in areas related to language comprehension in males. Though, this association may be beneficial or compensatory. A thorough examination of the structure among the '650 polymorphisms may be able to delineate the sensitivity of the Wernicke's area. Another research suggestion is to genotype older male adults without hearing impairment for *TOMM40* '650 and compare how G carriers understand language in contrast to non-G carriers. Future work should examine the associations observed in

our study among other ethnic groups to assess generalizability. Assessment of functional connectivity at multiple time points may uncover changes with brain atrophy and cognitive decline. Novel AD genetic risk factors without LD to APOE should also be examined with resting state functional connectivity as an outcome, as aforementioned studies besides the current study have demonstrated its utility (Binnewijzend et al., 2012; Sorg et al., 2007).

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Figures and Tables

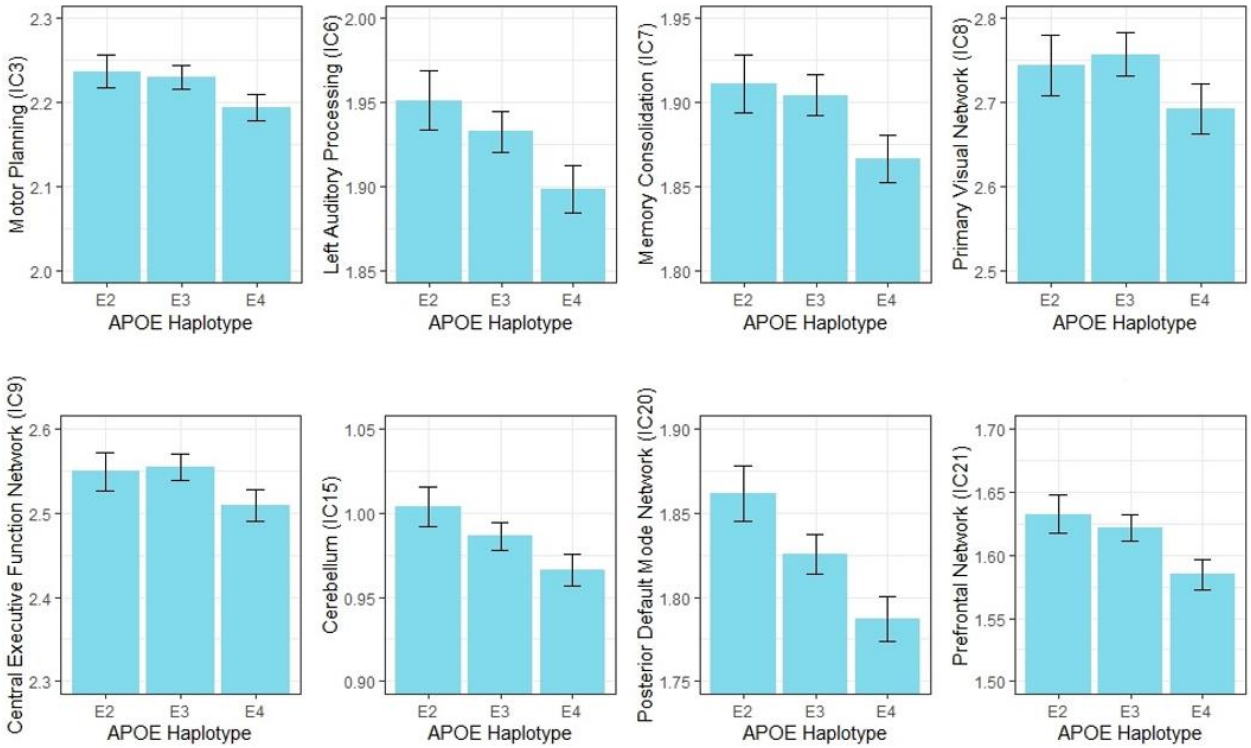


Figure 3.1. APOE Allele Carriage and Neural Network Strength In Several Components.

Refer to Supplementary Table 2.1. Independent Component is abbreviated as IC. Only components where significant ε4 were shown. The ε3 haplotype is the reference group.

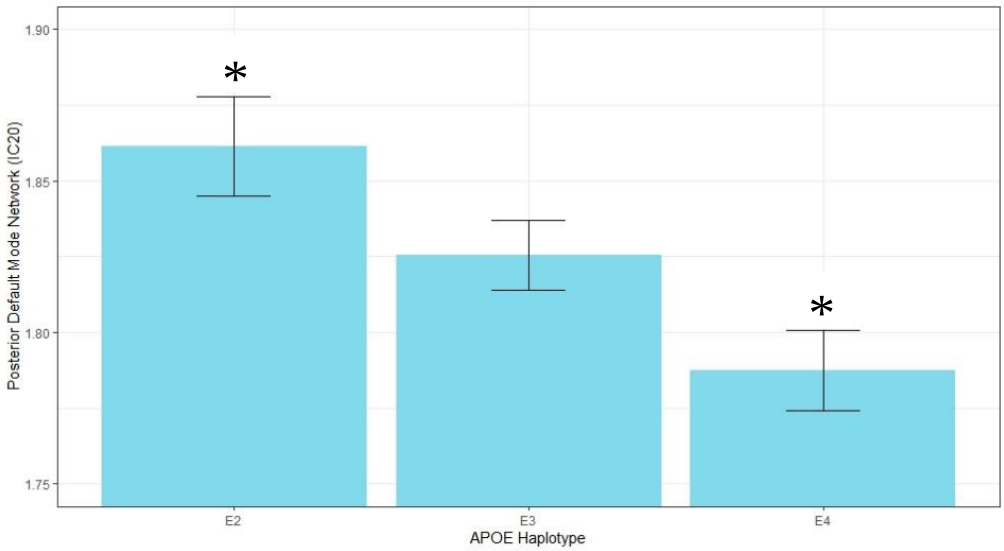


Figure 3.2. APOE Allele Carriage and Neural Network Strength in Posterior Default Mode Network

The ε3 haplotype is the reference group. Independent Component is abbreviated as IC.

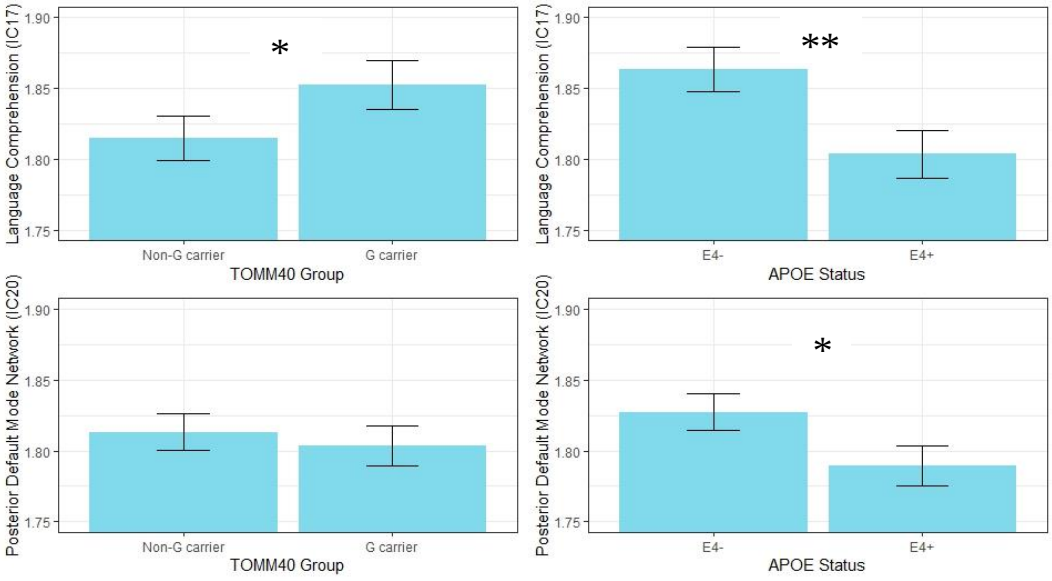


Figure 3.3. TOMM40 '650 or APOE non-risk vs. risk genotypes and neural network strength in Auditory/Language Comprehension Areas and Posterior Default Mode Network

Independent Component is abbreviated as IC.

Table 3.1. Participant composition and data characteristics

Age at recruitment, years	55.12 ± 7.47
Sex, no (%)	
Female	4269 (51.9)
Male	3953 (48.1)
Education, no (%)	
College or higher qualifications	5338 (64.9)
Post-secondary or vocational	1526 (18.6)
Secondary	943 (11.5)
None of the above	415 (5.1)
Social Class, no (%)	
Lower	3644 (44.3)
Middle	4264 (51.9)
Upper	314 (3.8)
Positive Family History of AD, no. (%)	2043 (24.8)
TOMM40 G carrier, no. (%)	2356 (25.4)
APOE ε4 carrier, no. (%)	2135 (26.0)

Table 3.2. Main Effects and Interactions of *APOE* Haplotype Groups on Neural Network Strength

	Model I				Model II	
	APOE ε2 Group		*APOE* ε4 Group		*APOE* ε4 Group * Sex	
Neural Network	β	SE	β	SE	β	SE
1	0.012	0.0189	-0.026	0.0147*	-0.025	0.0293
2	-0.007	0.0219	-0.015	0.0171	-0.009	0.0341
3	0.004	0.0169	**-0.030**	**0.0132***	-0.027	0.0263
4	-0.010	0.0207	-0.020	0.0161	-0.004	0.0322
5	0.007	0.0165	-0.024	0.0129*	-0.031	0.0256
6	0.014	0.0152	**-0.032**	**0.0118****	-0.017	0.0236
7	0.005	0.0149	**-0.036**	**0.0117****	-0.025	0.0232
8	-0.005	0.0318	**-0.029**	**0.0248****	-0.006	0.0495
9	-0.003	0.0198	**-0.032**	**0.0154****	-0.013	0.0308
10	0.005	0.0197	-0.015	0.0153	-0.019	0.0306
11	0.012	0.0144	-0.013	0.0112	-0.019	0.0224
12	0.016	0.0158	-0.022	0.0123	-0.023	0.0246
13	-0.004	0.0124	-0.024	0.0097*	-0.020	0.0193
14	0.022	0.0100*	-0.004	0.0078	-0.012	0.0156
15	0.019	0.0101	**-0.028**	**0.0079***	-0.018	0.0157
16	0.020	0.0144	-0.011	0.0112	-0.025	0.0224
17	0.015	0.0170	-0.024	0.0133*	-0.015	0.0265
18	0.009	0.0094	-0.016	0.0073	-0.016	0.0146
19	-0.006	0.0253	-0.027	0.0197*	0.001	0.0393
20	**0.028**	**0.0142***	**-0.038**	**0.0111*****	-0.009	0.0221
21	0.010	0.0129	**-0.040**	**0.0101*****	-0.033	0.0201

See Supplementary Table 2.1 for descriptions of all 21 independent neural networks. For model 1, APOE ε3 is the reference group for comparisons with ε2 and ε4. Beta weights are significant predictors in the model denoted as: *p<.05, **p<.01, ***p<.001. Estimates that survive correction are indicated by bold text.

Model I: *APOE* Groups + Age + Sex + Education + Social Class + Family History AD

Model II: *APOE* Groups + Sex + *APOE**Sex + Age + Education + Social Class + Family History AD

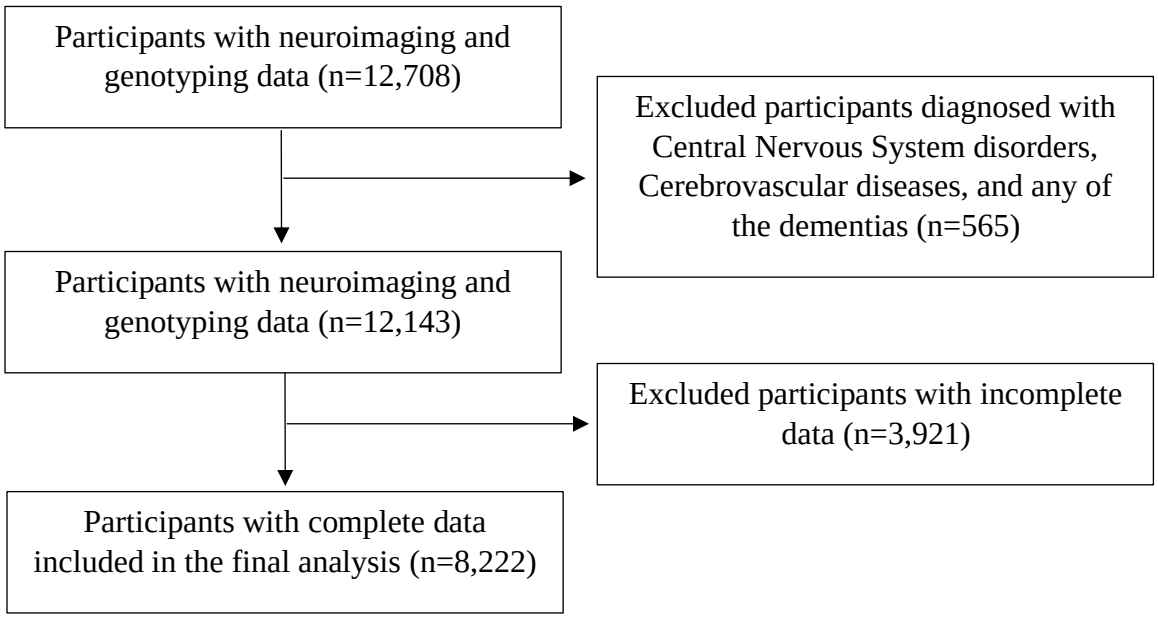
Table 3.3. Main Effects and Interactions of *TOMM40* ‘650 G Carriage on Neural Network Strength

	Model I				Model II	
	‘650 G Carriage		*APOE* ε4 Carriage		G Carriage * Sex	
Neural Networks	β	SE	β	SE	β	SE
1	0.005	0.0208	**-0.033**	**0.0207***	-0.015	0.0287
2	-0.011	0.0242	-0.005	0.0240	-0.041	0.0334*
3	0.028	0.0187	**-0.051**	**0.0186****	-0.020	0.0258
4	-0.016	0.0228	-0.006	0.0227	-0.016	0.0315
5	0.021	0.0182	**-0.041**	**0.0181****	-0.033	0.0251
6	0.000	0.0167	**-0.035**	**0.0166***	-0.002	0.0231
7	0.015	0.0165	**-0.048**	**0.0164****	-0.021	0.0227
8	-0.008	0.0350	-0.023	0.0349	-0.020	0.0484
9	-0.012	0.0218	-0.023	0.0217	-0.044	0.0301*
10	-0.018	0.0217	-0.003	0.0216	-0.024	0.0299
11	-0.007	0.0158	-0.011	0.0157	-0.036	0.0219*
12	-0.021	0.0174	-0.010	0.0173	-0.035	0.0240*
13	0.010	0.0136	-0.031	0.0136	-0.017	0.0189
14	0.002	0.0110	-0.011	0.0110	-0.026	0.0153
15	-0.011	0.0112	-0.025	0.0111	-0.026	0.0154
16	-0.014	0.0158	-0.005	0.0158	-0.012	0.0219
17	**0.031**	**0.0187***	**-0.050**	**0.0186****	**-0.042**	**0.0259***
18	-0.019	0.0103	-0.004	0.0103	-0.039	0.0142*
19	-0.015	0.0278	-0.015	0.0277	-0.023	0.0385
20	-0.010	0.0156	**-0.038**	**0.0155***	-0.023	0.0216
21	0.003	0.0142	**-0.044**	**0.0142****	-0.027	0.0197

See Supplementary Table 2.1 for descriptions of all 21 independent neural networks. Beta weights are significant predictors in the model denoted as: *p<.05, **p<.01, ***p<.001. Estimates that survive correction are indicated by bold text.

Model I: ‘650 G Carrier Status + Age + Sex + Education + Social Class + Family History AD + *APOE e4 status*.

Model II: ‘650 G Carrier Status + Sex + ‘650 G Carrier Status*Sex + Age Group + Education + Social Class + Family History AD + *APOE* ε4 status



Supplementary Figure 3.1. Flowchart Diagram of participant selection and exclusion

Supplementary Table 3.1. Interpretation of 21 Independent Components that Constitute Neural Networks in UK Biobank

Independent Component	Neural Network Description
1	Anterior and Posterior Default Mode Network
2	'Where' and 'What' Pathways
3	Motor Planning Network
4	Extrastriate Visual Network
5	Left Executive Function Network
6	Left Auditory Processing and Speech Production Network
7	Memory Consolidation Network
8	Primary Visual Network
9	Central Executive Function Network
10	Affect Processing Network
11	Motor Execution Network
12	Sensorimotor Network
13	Right Executive Function Network
14	Fronto-Cingular Network
15	Cerebellum Network
16	Frontopolar Network
17	Auditory/Language Comprehension
18	Substantia Nigra Cortico-Striatal Loop
19	Primary Visual Network
20	Posterior Default Mode Network
21	Prefrontal and 'What' Pathway Network

Papaya viewer was used to examine the 21 non-noise, orthogonalized neural network maps from the 25 Independent Component (IC) solution set originally estimated in 4,181 UK Biobank participants (see https://www.fmrib.ox.ac.uk/ukbiobank/group_means/rfMRI_ICA_d25_good_nodes.html for link to the viewer and maps). An imaging and neuroanatomy expert (AAW) cross-referenced these maps with established networks in the literature, as UK Biobank neural network have to our knowledge not been extensively described.

IC1 was "classic" Default Mode Network, with activation in medial prefrontal cortex and the posterior cingulate/precuneus region. IC2 showed lateral extrastriate activation in temporo-occipital junctions, moving down to inferior temporal gyrus and up to superior temporal gyrus, areas which are consistent with the 'where' and 'what' pathways respectively grading for spatial and object elements of visual stimuli. IC3 was predominantly composed of bilateral primary motor, pre-motor, and Supplementary Motor Area (SMA) cortices, cerebellum, medial cingulate cortex, and posterior insula, which may collectively grade for input and output relevant to motor function. IC4 was composed of extrastriate regions with some impingement on primary visual areas, suggesting a network focused on multi-modal visual processing. IC5 was "classic" executive network restricted to the left hemisphere, composed of inferior frontal and parietal gyri. IC6 had activation in bilateral inferior frontal gyri, pre-SMA, and cerebellum, suggesting a network focused on speech production and interpretation. IC7 was a memory consolidation network composed of activation in precuneus, posterior cingulate, and lateral parietal

cortices that streamed down to medial temporal lobe including parahippocampal gyrus and entorhinal cortex, with little activity in hippocampus proper. IC8 showed activation exclusively in posteromedial occipital gyrus, suggesting primary visual processing in V1 rather than extrastriate areas seen in IC4. IC9 was "classic" central executive network and composed of bilateral inferior frontal and parietal gyri. IC10 had a contiguous region in either hemisphere comprising operculum, primary sensory and motor cortices, mid-frontal gyrus, and anterior insula, which may represent synthesis of internal states to give rise to emotional processing and interpretation. IC11 was a motor execution network comprised almost exclusively of pre-central gyrus activity, thalamus, and both anterior and posterior medial cerebellum. IC12 was a sensorimotor network that almost exclusively included pre- and post-central gyri. IC13 was a right executive function network comprised of inferior and parietal gyri. IC14 was a fronto-cingular network composed of all segments of cingulate gyrus except for the rostrum, as well as medial frontal gyrus. IC15 was a cerebellar network that exclusively encompassed cerebellum. IC16 was comprised of dorsolateral prefrontal and anterior cingulate cortices, suggesting that this may be a frontopolar network involving in top-down control of cognitive and/or affect processes. IC17 comprised activation exclusively in superior temporal sulcus, suggesting a network revolving around Wernicke's area and therefore comprehension and production of coherent speech. IC18 touched on basal ganglia, motor, and pre-motor regions, suggesting a motor coordination loop. IC19 had activation almost exclusively in primary visual cortex with no impingement on extrastriate regions, which was highly posterior compared to IC8 which did not have the most posterior part of occipital cortex. IC20 comprised the posterior portion of Default Mode Network. IC21 was a complex neural network to interpret, with activation hot-spots predominantly in bilateral inferior frontal gyrus, as well as left superior mediofrontal and orbitofrontal gyri, precuneus, and left medial temporal cortex. This network may involve top-down cognitive processing of object stimuli in the 'what' pathway.

CHAPTER 4. GENERAL CONCLUSION

Cognitive dysfunction accompanies older adults with AD. Overall, it seems that some genotypes are at higher risk for AD, which is further exacerbated by diet and lifestyle. Potentially detrimental *TOMM40* '650 genotypes may reflect issues with mitochondrial function, glucose regulation, and other issues that are similarly dysregulated by less healthy or poor dietary choices. Thorough examination of neural networks with genetic variation could uncover the mechanism behind dysregulated neural processing, and by extension establish or validate factors that influence cognition.

First, Study 1 indicated that daily cheese intake was related to increased FI at baseline regardless of *APOE4* carriage status. Though daily cheese intake was related to increased FI for adults without family history, but this association was not observed in adults with family history. Additionally, weekly cheese intake had a positive association with FI over time for *APOE4+* only. Since whole-foods may be related to FI cross-sectionally and over time, dietary interventions should be implemented with AD risk factors in consideration.

Second, Study 2 suggested that *APOE* haplotype was related with functional connectivity. Specifically, ε4 in contrast with ε3, was related to reduced functional connectivity in several resting neural networks such as memory consolidation, posterior default mode network and top-down cognitive processing. On the other hand, *TOMM40* '650 G allele was associated with increased functional connectivity in men. Functional connectivity appears to be a useful biomarker to discern impact of genetic risk factors.