Full Title: Causal associations between potentially modifiable risk factors and the Alzheimer's phenome: A Mendelian randomization study

Short Title: Causal effect of modifiable risk factors on the Alzheimer's phenome

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Abstract

Background: Potentially modifiable risk factors have been associated with Alzheimer's disease (AD). However, the causality of these risk factors on AD is unclear. Using Mendelian randomization we evaluated the causal effect of potentially modifiable risk factors on AD and its associated endophenotypes to inform the development of lifestyle interventions that could reduce risk of developing AD.

Methods and Findings: Genetic instruments for the exposures were selected from genome wide association studies (GWAS) for traits previously linked to AD in observational studies including alcohol intake, physical activity, lipid traits, blood pressure traits, type 2 diabetes, body-mass index (BMI), depression, sleep, social isolation, smoking, oily fish intake, and educational attainment. The outcomes included AD status, AD age of onset survival (AAOS), hippocampal volume, CSF levels of $A\beta_{42}$, tau, and $ptau_{181}$, and neuropathological burden of neuritic plaques, neurofibrillary tangles, and vascular brain injury (VBI). MR estimates were calculated using an inverse variance weighted approach. Genetically predicted educational attainment (OR [CI]: 0.7 [0.63, 0.78]), diastolic blood pressure (DBP) (OR [CI]: 0.99 [0.98, 0.99]), systolic blood pressure (SBP) (OR [CI]: 0.99 [0.99, 1]) and physical activity (OR [CI]: 2.5 [1.47, 4.23]) were causally associated with AD risk. Genetically predicted BMI (HR [CI]: 1.13 [1.07, 1.2]) and educational attainment (HR [CI]: 0.74 [0.68, 0.82]) were causally associated with AAOS. Genetically predicted alcohol consumption (β [CI]: -0.15 [-0.25, -0.05]), broad depressive symptoms (β [CI]: 0.5 [0.2, 0.8]), major depressive disorder (β [CI]: 0.12 [0.05, 0.18]) and physical activity were causally associated with CSF $A\beta_{42}$ (β [CI]: 0.25 [0.1, 0.39]). Genetically predicted DBP was causally associated with CSF total Tau (β [CI]: -0.005 [-0.007, -0.002]). Increased risk of VBI were observed for genetically predicted DBP (OR [CI]: 1.05 [1.02, 1.08]), SBP (OR [CI]: 1.06 [1.03, 1.1]), and pulse pressure (OR [CI]: 1.03 [1.01, 1.05]). Increased risk of neuritic plaque burden were observed for genetically predicted LDL-cholesterol (OR [CI]: 1.87

[1.3, 2.69]) and total cholesterol (OR [CI]: 2.03 [1.44, 2.85]). Genetically predicted insomnia symptoms (β [CI]: -0.2 [-0.34, -0.06]) and total cholesterol were associated (β [CI]: -0.06 [-0.1, -0.03]) with hippocampal volume. Potential limitations include weak instrument bias, non-homogenous samples and other implicit limitations of MR analysis.

Conclusions: Demonstration of a causal relationship between blood pressure, cholesterol levels, BMI, depression, insomnia symptoms, physical activity and educational attainment on the AD phenome strongly support public health programs to educate the public about these preventable causes of AD.

Keywords: Alzheimer's disease; endophenotypes; Mendelian randomization; risk factors

Introduction

Late-onset Alzheimer's disease (AD) is a debilitating neurological condition characterized by progressive deterioration in cognitive function and concomitant functional decline [1]. The primary neuropathological hallmarks of AD are the aggregation of extracellular amyloid-β (Aβ) peptides into amyloid plaques and of intracellular hyperphosphorylated tau into neurofibrillary tau tangles (NFTs), accompanied by gliosis and neurodegeneration [1,2].

In the absence of any pharmacotherapeutic intervention, the number of people living with dementia – of which AD accounts for ~70% of cases – is expected to exceed 130 million by 2050 [3]. Observational studies have identified potentially modifiable risk factors that could be targeted in intervention studies to reduce the risk of dementia [4]. From these studies it has been estimated that 35% of AD cases may be attributable to preventable causes such as low educational attainment, hearing loss, hypertension, obesity, smoking depression physical inactivity, social isolation and diabetes [5]. This suggests that interventions that target modifiable risk factors could significantly reduce the population prevalence of AD and related dementias.

Lifestyle interventions that target modifiable risk factors are entirely dependent on accurate causal relationships being established between modifiable risk factors and AD. In observational studies, a correlation between a risk factor and AD cannot be reliably interpreted as evidence of a causal relationship. First, this can be due to confounding, where some or all of the correlation can be due to a third confounding variable that is correlated to both the putative risk factor and AD. Second, the observed correlation between a risk factor and dementia may be due to reverse causation, where the neurodegenerative and cerebrovascular changes that underlie dementia begin decades before the onset of clinical symptoms. As such, the lifestyle risk factors that are associated with the development of dementia in late-life may themselves be a

consequence of the same underlying pathological processes and not a causal factor of dementia. If this is the case, disease reduction strategies targeting modifiable risk factors are unlikely to be successful.

A novel method for establishing causal relationships between exposures (e.g. modifiable risk factors) and outcomes (e.g. AD) is Mendelian randomization (MR). MR uses genetic variants as proxies for environmental exposures to provide an estimate of the causal association between an intermediate exposure and a disease outcome [6]. MR is similar to a 'genetic randomized control trial' due to the random allocation of genotypes from parents to offspring and is thus not affected by reverse causation and is independent of confounding factors that may influence disease outcomes [6]. MR analysis can be conducted using GWAS summary statistics, taking advantage of the increased samples sizes available in independent GWAS and the increasing number of genetic variants being discovered to increase statistical power [7].

In this study we used MR to establish causal relationships between modifiable risk factors and the AD phenome – AD status, AD age of onset survival (AAOS), amyloid-beta$_{42}$ (Aβ_{42}), tau and hyperphosphorylated tau (ptau$_{181}$) levels in cerebrospinal fluid (CSF), the neuropathological burden of neuritic plaques, neurofibrillary tangles and vascular brain injury, and hippocampal volume. Based on these analyses we identified a subset of modifiable risk factors that represent the most promising targets for public health initiatives to reduce AD burden in the population.

Methods

Data Sources

We obtained GWAS summary statistics (GWAS-SS) for each exposure and outcome of interest (Table 1). For the exposures these included: alcohol consumption [8], alcohol dependence [9],

100 Table 1: Genome-wide association studies used in this study

Study	Trait	Cohort / Consortium	N	Age	Females (%)
Exposures					
Liu et al 2019	Alcohol Consumption	GSCAN	537,349		
	Smoking Initiation	GSCAN	262,990		
	Cigarettes per Day	GSCAN	263,954		
Clarke et al 2017	Alcohol Use Disorder Test	UKBB	121,600	56.1	52.7
Walters et al 2018	Alcohol Dependence	PGC	46,568	-	-
NealLab	Oily Fish Intake	UKBB		56.5*	54.4*
NealLab	Hearing Problems	UKBB	346,635	56.5*	54.4*
Xue et al 2018	Type 2 Diabetes	DIAGRAM; UKBB; GERA	659,316	-	-
Yengo et al 2018	Body Mass Index	UKBB; GIANT	690,495	-	-
Willer et al 2013	Total Cholesterol	GLC	188,577	54.94	56.58

	LDL Cholesterol				
	HDL Cholesterol				
	Triglycerides				
Evangelou et al 2018	Diastolic Blood Pressure	UKBB; ICBP	757,601	-	-
	Systolic Blood Pressure				
	Pulse Pressure				
Howard et al 2018	Broad Depression Symptoms	UKBB	322,580	-	-
Wray et al 2018	Major Depression Disorder	PGC; deCODE; iPSYCH; GeneScotland; GERA; UKBB	480,359	-	-
Jansen et al 2018	Insomnia Symptoms	UKBB	386,533	56.7	54
Dashti et al 2019	Sleep Duration	UKBB	446,118	57.3	54.1
Day et al 2018	Social Isolation	UKBB	452,302	-	-
Lee et al 2018	Educational Attainment	UKBB; SSGAC	766,345	63.8	54.7

Outcomes

Lambert et al 2013	Late Onset Alzheimer's disease	IGAP	54,162	71	58.4
Kunkle et al 2019	Late Onset Alzheimer's disease	IGAP	63,926	72.6	58.5
Huang et al 2017	Alzheimer's Age of Onset Survival	IGAP	40,255	77.5	60.35
Deming et al 2017	CSF Ab_{42}	Knight-ADRC	3,146	71.8	49.57
	CSF $Ptau_{181}$				
	CSF Tau				
Hibar et al 2015	Hippocampal Volume	ENIGMA	13,688	39.9	51.8
Hibar et al 2017	Hippocampal Volume	ENIGMA; CHARGE	26,814	54.3	55.3
Beecham et al 2014	Neuritic Plaques	ADGC	4,914	74.7	65.4
	Neurofibrillary tangles				
	Vascular Brain Injury				

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the alcohol use disorder identification test (AUDIT) [10], moderate-vigorous physical activity (MVPA) [11], lipid traits [12], systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) [13], type 2 diabetes (T2D) [14], body mass index (BMI) [15], broad depression symptoms [16], major depression disorder (MDD) [17], Insomnia symptoms [18], sleep duration [19], social isolation [20], smoking initiation [8], cigarettes per day [8], and educational attainment [21]. We used unpublished summary statistics generated from the UK Biobank for oily fish intake, and hearing problems (http://www.nealelab.is/uk-biobank/), which have been implicated as risk factors for AD.

GWAS-SS for the AD phenome consisted of late-onset AD status [22], AAOS [23], CSF levels of $A\beta_{42}$, $ptau_{181}$ and total tau (Tau) [24], neuropathological burden of neuritic plaques, neurofibrillary tangle burden, and vascular brain injury [25], and hippocampal volume [26]. Due to data use restrictions, earlier GWAS for AD [27] and hippocampal volume [28] were used for estimating the causal effect of alcohol intake and educational attainment on these phenotypes.

GWAS-SS that were mapped to earlier human genome builds were lifted over to Human Genome Build 19 [29]. GWAS-SS were standardized using a pipeline (https://github.com/marcoralab/sumstats_munger), that 1) aligns effect alleles to the alternate allele on the forward strand of the human genome reference build and normalizes indels, 2) annotates variants with marker names using chromosome:position:ref:alt, 1000 Genomes rsIDs (phase 3), and dbSNP rsIDs (b151) 3) where allele frequencies are missing, annotates allele frequencies using non-Finnish Europeans from gnomAD (v2.1), and 4) converts summary statistics to VCF and TSV files.

Genetic Instruments

For each exposure, we constructed two instrumental variables (IV) using genome-wide significant ($p < 5 \times 10^{-8}$) and nominally significant ($p < 5 \times 10^{-6}$) loci. Increasing the number of loci in the instrumental variable increases the variance explained by the IV and improves power. However, this can increase the likelihood of bias due to variants violating the core MR assumptions and bias results towards the null by increasing weak instrument bias. To obtain independent SNPs, linkage disequilibrium (LD) clumping was performed by excluding SNPs that had an r2 > 0.001 with another variant with a smaller p-value association within a 1000kb window using PLINK [30]. For genetic variants that were not present in the outcome GWAS, PLINK was used to identify proxy SNPs that were in LD ($r^2 > 0.8$; EUR reference population). Finally, the exposure and outcome GWAS datasets were harmonized so that the effect size for the exposure and outcome correspond to the same effect alleles. Genetic variants that were palindromic with ambiguous allele frequencies (AF > 0.42) or incompatible alleles were removed. The proportion of variance in the phenotype explained by each instrument and F-statistic were calculated as previously described [31,32].

Mendelian Randomization Analysis

For each genetic variant, we calculated an instrumental variable ratio estimate by dividing the SNP-exposure by SNP-outcome and coefficients were combined in a fixed-effects meta-analysis using an inverse-variance weighted (IVW) approach to give an overall estimate of causal effect [7]. The IVW method assumes that all SNPs included in the causal estimate are valid instruments - that is they do not violate any of the underlying assumptions [7]. In order to account for potential violations of the assumptions underlying the IVW analysis, we conducted sensitivity analysis using alternative MR methods known to be more robust to horizontal pleiotropy, but at the cost of reduced statistical power. The alternative approaches included 1) Weighted Median Estimator (WME), which takes the median effect of all available variants,

allowing 50% of variants to exhibit horizontal pleiotropy [33]; 2) Weighted Mode Based Estimator (WMBE), which clusters variants into groups based on similarity of causal effects and reports the final causal effect based on the cluster with the largest number of variants [34]; and 3) MR-Egger regression, which allows all variants to be subject to direct effects [35].

The MR-Egger regression intercept was used to verify the absence of pleiotropic effects of the SNPs on the outcome [35]. To further confirm the absence of distortions in the causal effects due to heterogeneity or pleiotropy, we used the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test to detect and correct for horizontal pleiotropic outliers [36]. Where heterogeneity was detected (the MR-PRESSO Global Test) and significant outliers were detected (MR-PRESSO Outlier Test), the outliers were removed.

We report the IVW results for the p-value threshold model with the smallest p-value and where outliers were removed if detected. Where there was evidence of horizontal pleiotropy (MR-PRESSO Global Test $p < 0.05$ or an MR-Egger Intercept $p < 0.05$) we report the IVW results where the sensitivity analyses were also significant and the effect direction was concordant with the IVW results. To account for multiple testing burden, we report q-values, a false discovery rate-based measure of significance [37]. Power analyses were conducted using the non-centrality parameter based approach using the observed IVW coefficient [38].

All statistical analyses were conducted using R version 3.5.2 [39]. Mendelian randomization analysis was performed using the 'TwoSampleMR' package [40]. A Snakemake workflow was constructed that automates the MR analysis pipeline and allows for multiple exposure – outcomes datasets to be run in parallel [41].

177 The SNPs used in each instrument, their harmonized effects and, outliers are presented in S1
178 Table. The causal estimates for each p-value threshold, MR method and pre- and post-outlier
179 removal are presented in S2 Table. An R Shiny application is available to review the output of
180 the analysis pipeline (https://sjfandrews.shinyapps.io/MR_ADPhenome/). Code is available at
181 https://github.com/sjfandrews/MR_ADPhenome.
182

183 **Results**

184 We conducted a total of 405 tests – 9 outcomes, 23 exposures, and 2 P_t (alcohol dependence
185 was only run with a P_t < 5e-6) – and observed 18 tests that were significant at an FDR < 0.05
186 (Table 2; Figure 1). Of these 18 significant tests, 16 exposure-outcome pairs showed either no
187 evidence of horizontal pleiotropy, or in the presence of horizontal pleiotropy the additional MR
188 sensitivity analyses were significant. The PVE, F-statistics and power for each model are
189 presented in S2 Table.
190

191 **Alzheimer's disease**

192 Genetically predicted educational attainment was associated with significantly lower odds of AD
193 (OR [CI]: 0.7 [0.63, 0.78]). There was evidence of heterogeneity, but not of directional
194 pleiotropy, however, the associations were consistent in the MR-Egger, WMBE, and WME
195 sensitivity analyses. Genetically predicted higher DBP and SBP were associated with
196 significantly lower odds of developing AD after outlier removal (OR [CI]: 0.99 [0.98, 0.99], OR
197 [CI]: 0.99 [0.99, 1] respectively). For both exposures, there was evidence of heterogeneity, but
198 not of directional pleiotropy, however, the associations were consistent in the MR-Egger
199 sensitivity analysis. Genetically predicted increased MVPA was associated with significantly
200 increased odds of

201 Table 2: Causal association of potentially modifiable risk factors on Alzheimer's disease and Alzheimer's endophenotypes

Exposure	P_t	SNPs	Outliers	IVW		MR-Egger	WMBE	WME	MR-PRESSO Global	MR-Egger Intercept
		n	n	b (SE)	q-value	b (SE)	b (SE)	b (SE)	p	p
LOAD										
Diastolic Blood Pressure	5e-08	705	1	-0.013 (0.0038)	0.008	-0.025 (0.011)*	-0.009 (0.0062)	-0.016 (0.015)	<4e-05	0.23
Systolic Blood Pressure	5e-08	679	2	-0.0081 (0.0023)	0.008	-0.018 (0.0069)**	-0.0035 (0.0037)	0.0054 (0.0096)	<4e-05	0.11
Educational Attainment	5e-06	932	0	-0.36 (0.053)	4.07E-09	-0.87 (0.22)***	-0.38 (0.085)***	-0.74 (0.3)*	<2e-05	0.017
Moderate-to-vigorous PA	5e-08	26	7	0.91 (0.27)	0.01	1.6 (0.85).	0.83 (0.36)*	0.83 (0.59)	0.982	0.38
AAOS										
Diastolic Blood Pressure	5e-06	1180	0	0.0091 (0.0036)	0.081	0.002 (0.0094)	0.0069 (0.0063)	0.00073 (0.013)	0.0023	0.41

Educational Attainment	5e-06	957	0	-0.3 (0.049)	1.36E-07	0.03 (0.19)	-0.32 (0.078)***	-0.37 (0.23)	0.0154	0.071
BMI	5e-06	1462	2	0.12 (0.031)	0.002	-0.023 (0.092)	0.14 (0.052)**	0.13 (0.14)	0.0078	0.086
Type 2 Diabetes	5e-06	282	0	0.067 (0.016)	0.001	0.048 (0.042)	0.041 (0.031)	0.061 (0.035).	4.00E-04	0.6
CSF Ab_{42}										
Alcohol Consumption	5e-08	34	0	-0.15 (0.051)	0.024	-0.16 (0.16)	-0.12 (0.071).	-0.14 (0.097)	0.605	0.98
Moderate-to-vigorous PA	5e-08	18	0	0.25 (0.072)	0.01	0.55 (0.3).	0.27 (0.1)**	0.26 (0.16)	0.543	0.3
Depressive Symptoms	5e-08	15	1	0.5 (0.15)	0.011	2.2 (0.68)**	0.58 (0.21)**	-0.09 (0.46)	0.324	0.027
Major Depressive Disorder	5e-08	8	1	0.12 (0.032)	0.005	0.36 (0.16).	0.12 (0.043)**	0.14 (0.059).	0.446	0.19
CSF $Ptau_{181}$										

Depressive Symptoms	5e-06	105	0	-0.23 (0.088)	0.067	-0.65 (0.37).	-0.27 (0.13)*	-0.58 (0.33).	0.348	0.25
CSF Tau										
Diastolic Blood Pressure	5e-06	992	0	-0.0049 (0.0015)	0.01	-0.008 (0.0037)*	-0.0066 (0.0025)**	-0.011 (0.0057).	0.264	0.37
Neuritic Plaques										
LDL-Cholesterol	5e-08	81	0	0.62 (0.19)	0.01	0.66 (0.32)*	0.33 (0.31)	0.16 (0.44)	0.248	0.89
Total Cholesterol	5e-06	146	0	0.71 (0.17)	0.002	0.61 (0.3)*	0.75 (0.3)*	0.62 (0.46)	0.763	0.68
Neurofibrillary Tangles										
Total Cholesterol	5.00E-06	147	1	0.33 (0.11)	0.019	0.091 (0.22)	0.15 (0.19)	0.21 (0.21)	0.011	0.18
Vascular Brain Injury										

Diastolic Blood Pressure	5e-06	1135	0	0.048 (0.014)	0.01	0.08 (0.035)*	0.049 (0.023)*	0.056 (0.059)	0.6004	0.31
Systolic Blood Pressure	5e-06	1111	0	0.03 (0.0084)	0.008	0.066 (0.021)**	0.036 (0.014)*	0.032 (0.035)	0.6929	0.062
Pulse Pressure	5e-08	543	0	0.062 (0.015)	0.002	0.12 (0.041)**	0.055 (0.023)*	0.03 (0.068)	0.1518	0.12
Hippocampal Volume										
Total Cholesterol	5e-06	148	0	-0.065 (0.02)	0.011	-0.032 (0.038)	-0.076 (0.035)*	-0.056 (0.035)	0.0037	0.28
Hearing Problems	5e-06	110	0	0.39 (0.15)	0.075	0.9 (0.41)*	0.29 (0.22)	0.34 (0.56)	0.664	0.19
Insomnia Symptoms	5e-08	14	0	-0.2 (0.071)	0.038	0.044 (0.28)	-0.11 (0.1)	-0.061 (0.15)	0.1	0.38
Major Depressive Disorder	5e-08	8	0	0.18 (0.07)	0.081	0.84 (0.38).	0.23 (0.095)*	0.25 (0.14)	0.511	0.12

202 "." p < 0.1; * p < 0.05; ** p < 0.01; *** p < 0.001.; IVW = Inverse-variance weighted; WME = Weighted Median Estimator; WMBE =

203 Weighted Mode Based Estimator

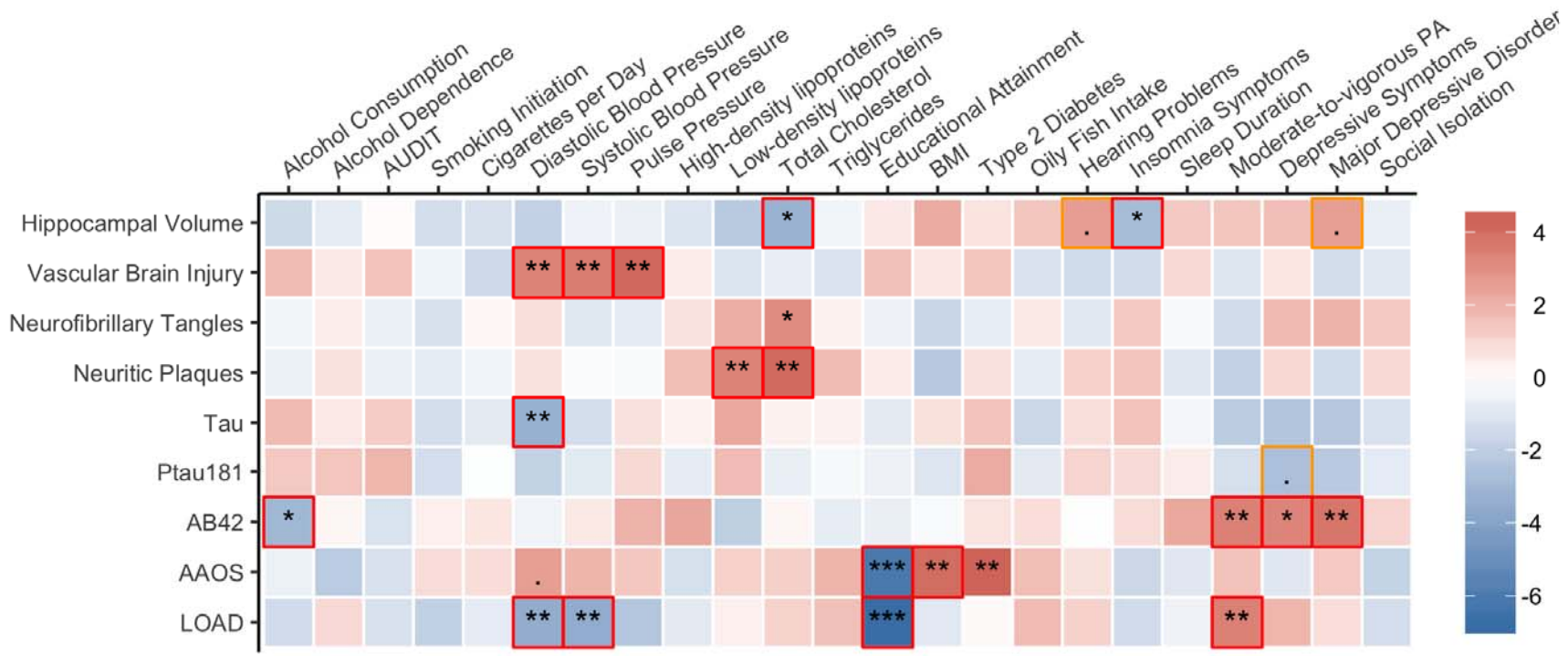

Figure 1: **Putative causal associations between modifiable risk factors and the AD phenome.** Shown are the best IVW results for each causal association, with colors representing the standardized effect sizes - for LOAD, NP, NFT, and AAOS red indicates increased risk / earlier onset and blue reduced risk / delayed onset, for CSF levels and Hippocampal volume, red indicates increased levels/volume and blue reduced levels/volume. "." FDR < 0.1; * FDR < 0.05; ** FDR < 0.01; *** FDR < 0.001. Causal estimates bracketed in red or orange indicate significant causal effects that showed no evidence for horizontal pleiotropy or where sensitivity analyses were also significant.

developing AD after outlier removal (OR [CI]: 2.5 [1.47, 4.23]), with no evidence of heterogeneity or directional pleiotropy observed.

Alzheimer's Age of Onset Survival

Genetically predicted higher BMI was associated with significantly earlier AAOS after outlier removal (HR [CI]: 1.13 [1.07, 1.2]). There was evidence of heterogeneity, but not of directional pleiotropy, however, the associations were consistent in the WME sensitivity analysis. Genetically predicted higher educational attainment was associated with significantly later age at onset of AD after outlier removal (HR [CI]: 0.74 [0.68, 0.82]). There was evidence of directional pleiotropy, but not of heterogeneity, however, the associations were consistent in the WME sensitivity analysis.

CSF $A\beta_{42}$, Tau, and $ptau_{181}$

Genetically predicted higher alcohol consumption was associated with significantly reduced CSF $A\beta_{42}$ (β [CI]: -0.15 [-0.25, -0.05]), with no evidence of heterogeneity or directional pleiotropy. Genetically predicted risk of depressive symptoms were associated with significantly increased $A\beta_{42}$ after outlier removal (β [CI]: 0.5 [0.2, 0.8]). There was evidence of directional pleiotropy, but not of heterogeneity, however, the associations were consistent in the MR-Egger and WME sensitivity analyses. Depressive symptoms were also nominally associated (FDR < 0.1) with reduced $ptau_{181}$. Additionally, genetically predicted risk of MDD was also associated with significantly increased $A\beta_{42}$ after outlier removal (β [CI]: 0.12 [0.05, 0.18]), with no evidence of heterogeneity or directional pleiotropy. Genetically predicted increased MVPA was associated with significantly increased $A\beta_{42}$ (β [CI]: 0.25 [0.1, 0.39]), with no evidence of heterogeneity or directional pleiotropy observed. Genetically predicted higher DBP was associated with

significantly reduced Tau (β [CI]: -0.005 [-0.007, -0.002]), with no evidence of heterogeneity or directional pleiotropy.

Vascular Brain Injury, Neuritic Plaque, and Neurofibrillary Tangle Burden

Genetically predicted higher DBP (OR [CI]: 1.05 [1.02, 1.08]), SBP (OR [CI]: 1.06 [1.03, 1.1]), and PP (OR [CI]: 1.03 [1.01, 1.05]) were associated with significantly increased odds of vascular brain injury. There was no evidence of heterogeneity or directional pleiotropy for these exposure-outcome pairs. Significantly increased odds of Neuritic plaque burden were observed for genetically predicted higher LDL-cholesterol (OR [CI]: 1.87 [1.3, 2.69]) and total cholesterol (OR [CI]: 2.03 [1.44, 2.85]), with no evidence of heterogeneity or directional pleiotropy observed. No robust associations were observed with Neurofibrillary Tangle burden.

Hippocampal Volume

Genetically predicted risk of insomnia symptoms were associated with significantly reduced hippocampal volume (β [CI]: -0.2 [-0.34, -0.06]), with no evidence of heterogeneity or directional pleiotropy. Genetically predicted higher total cholesterol was associated with significantly reduced hippocampal volume (β [CI]: -0.06 [-0.1, -0.03]). There was evidence of heterogeneity, but not of directional pleiotropy, however, the associations were consistent in the WME sensitivity analysis. Additionally, risk of hearing problems and risk MDD were nominally associated (FDR < 0.1) with increased hippocampal volume.

Discussion

Using genetic variants as proxies for modifiable risk factors, this MR analysis is the first to investigate the association of modifiable risk factors with the AD phenome. We found evidence of causal associations for educational attainment, BMI, blood pressure, lipid traits, insomnia symptoms, physical activity, depression, and alcohol consumption, on either AD or its associated endophenotypes.

Higher educational attainment was causally associated with a reduced risk of AD, which is consistent with previous MR analyses [42–44]. However, a multivariable MR analysis that accounted for intelligence in addition to educational attainment suggests that the relationship between education and AD is largely driven by intelligence [45]. Two of these studies [43,45] used a smaller GWAS of educational attainment that explained less phenotypic variance than the GWAS that was used in this analysis. One study used the same GWAS used in this analysis [46], while the other conducted a single sample MR analysis [44]. The MR analyses are also consistent with the observational literature [47]. We also observed a novel causal association between higher education and delayed AAOS, consistent with the cognitive reserve hypothesis. The observational literature, however, suggests that lower education is associated with delayed age at onset, however, this association is potentially confounded due to the symptoms of AD being recognized later among those with less education [48]. Additionally, we found no evidence that education is causally associated with AD neuropathology or CSF biomarkers, corroborating evidence from observational studies [49], and supports the hypothesis that education mitigates dementia risk via cognitive reserve rather than affecting AD pathogenesis [50].

BMI was causally associated with an earlier AAOS but was not associated with AD risk or other endophenotypes. Previous MR analyses have found that BMI is not a causal risk for AD [43,51,62,63] using smaller GWAS of BMI than used in our analysis, however, no previous MR study has evaluated the causal effect of BMI on age at onset. In contrast, observational studies

have observed that a higher midlife BMI is associated with increased risk of dementia, while late-life obesity has an apparent protective effect likely due to reverse causation [64–66]. Consistent with our AAOS results, midlife obesity is also associated with an earlier age of onset [67].

Increased DBP and SBP were causally associated with reduced AD risk, and, in addition to PP, associated with increased risk of VBI. Additionally, higher DBP was associated with reduced CSF tau levels. This corroborates the results from a previous MR analysis based on 24 variants but contradicts those of a more recent MR study that found no evidence of a causal association based on an instrument composed of 105 variants [43,51]. In contrast to these previous analyses, we selected instruments from a larger GWAS of blood pressure. Based on epidemiological research, high blood pressure in midlife is generally regarded as a risk factor for dementia in midlife while low blood pressure in late-life has been associated with an increased risk of dementia [5,52]. However, systematic reviews and meta-analysis of the role of hypertension in midlife, have observed that hypertension in midlife is associated with an increased risk of developing vascular dementia but not with AD [53–55]. Additionally, randomized control trials in elderly populations using either a pharmacotherapeutic or lifestyle change blood pressure lowering interventions did not significantly reduce the risk of dementia [56]. Our MR analysis also suggests that reducing blood pressure in late life may have limited utility in the prevention of AD, but may reduce the risk of vascular dementia by reducing the risk of VBI.

Increased LDL-cholesterol and total cholesterol were causally associated with an increased risk of neuritic plaques, corroborating previous observational studies that have reported associations between increased total plasma cholesterol levels and amyloid deposition [57,58]. We did not observe a causal association with AD risk, which is consistent with three previous MR analysis

of lipid traits on the risk of AD [43,51,59]. This study and the previous studies all used the same GWAS for selecting instruments associated with lipid traits. However, these results contrast with observational studies that have found higher midlife total cholesterol associated with an increased risk of AD and all-cause dementia, while higher late-life total cholesterol is not associated with all-cause dementia or dementia subtypes [60]. Furthermore, in cognitively intact individuals statins are associated with a reduced risk of all-cause dementia, AD, and MCI, but not VaD [61].

Insomnia symptoms were associated with reduced hippocampal volume but not with AD or other AD endophenotypes. Sleep duration was not causally associated with the AD phenome. Observational studies have indicated that sleep disturbances and problems are associated with an increased risk of all-cause dementia, AD and vascular dementia [68,69]. However, the association between insomnia and hippocampal volume is less established with studies either reporting a positive relationship [70,71] or no association [72]. The results of this study provide further support for insomnia being causally related to reduced hippocampal volumes and underlines the importance of sleep for brain health.

Increased MVPA was associated with increased CSF $A\beta_{42}$ levels, but also with an increased risk of AD. Increased physical activity is generally associated with a reduced risk of dementia [5], however, a recent meta-analysis found that the protective association with dementia was observed when physical activity was measured <10 years before dementia diagnosis, but when measured >10 years before dementia onset no association with dementia was observed [73]. Similarly, randomized control trials of single component physical activity interventions have not been shown to reduce the risk of dementia [74]. Less research has been conducted on the relationship between physical activity and AD Aβ biomarkers, however, increased physical activity has been associated with favorable AD $A\beta_{42}$ biomarker profiles [75–77].

Broad depressive symptoms and a clinical diagnosis of MDD were associated with increased CSF $A\beta_{42}$. Observational studies have indicated that depression is associated with a twofold increased risk of dementia, however, the late-life depressive symptoms may represent a prodromal phase of dementia while early life depression may be a risk factor for AD [78,79]. In cross-sectional observational studies, lower $A\beta_{42}$ levels are associated with depression [80]. In longitudinal studies elevated baseline $A\beta_{42}$ levels are associated with increased risk of developing depression, suggesting that emerging depressive symptoms are an early manifestation of AD [81–83].

Increased alcohol consumption was causally associated with lower CSF $A\beta_{42}$ levels. A previous MR study found no evidence of an association between alcohol consumption and AD risk, though this analysis was likely underpowered as the instrument only consisted of two SNPs [43]. Observational studies have indicated that light-moderate alcohol intake is associated with a decreased risk of AD while abstinence or heavy alcohol use is associated with an overall increased risk of AD [84]. The observational studies, however, are potentially confounded by selection bias, survivor bias, the inclusion of lifetime abstainers and former drinkers into control groups [84].

A suggestive causal association was observed between T2D and an earlier age of onset. However, there was evidence of heterogeneity, and the sensitivity analyses were non-significant. Previous MR analyses have not found evidence of a causal relationship between T2D, fasting glucose or fasting insulin with the risk of AD [43,51,85]. Conversely, observational studies have found an increased risk of dementia in patients with diabetes [86] and that metformin, a first line antihyperglycemic medication, prevents or delays the development of dementia in patients with diabetes [87].

There was no evidence of a causal association between smoking initiation or smoking quantity and the AD phenome. This contradicts two previous MR studies which found evidence of a protective effect of increased smoking quantity on AD risk [43,51], using only three SNPs selected from a smaller smoking GWAS [88]. These apparent protective effects may be due to survivor bias [89]. In contrast, the analysis presented here used data from the most recent GWAS on smoking behavior, using between 95 and 316 variants. Observational studies, however, implicate smoking as a risk factor for AD, with current smokers been at increased risk in comparison to never smokers [90].

There was no evidence of an association between increased oily fish consumption and the AD phenome. Observational studies have reported conflicting results for the association of fish consumption and risk of AD, with a systematic review focusing on dietary patterns finding limited evidence of an association [91,92], while a more recent analysis found an association with reduced risk [93].

A nominal association was observed between hearing problems and increased hippocampal volume, but that there was no evidence of a causal relationship with AD or AD endophenotypes. These results are contradictory to the observational literature which has found that hearing loss is associated with decreased total brain volume [94], accelerated brain atrophy in whole brain [95] and reduced hippocampal volume [96]. Similarly, age-related hearing loss is associated with cognitive decline, cognitive impairment, and all-cause dementia, though no association was observed for AD [97].

The results of this study should be interpreted in conjunction with knowledge of its limitations and those of MR in general. Firstly, inference of causality in MR analyses relies on the

388 assumption that the genetic variants used as instruments are strongly associated with the
389 exposure (the non-zero effect assumption). While we cannot exclude that our findings may be
390 affected by weak instrument bias, the F-statistics for all of the analyses were over 10 indicating
391 that the instrument strength was sufficient for MR analysis [32]. However, in Two-Sample MR
392 analyses, weak instrument bias is in the direction of the null, thus, we cannot exclude type II
393 error as an explanation for the null results that had limited power [98]. Second, we cannot
394 completely rule out violations of the independence and the exclusion restriction assumption,
395 particularly in regard to pleiotropy [99]. Nevertheless, we used several methods to identify
396 robust causal estimates, including outlier removal using MR-PRESSO and WMBE, WME and
397 MR-Egger sensitivity analyses. Thirdly, it is assumed that both samples used to generate the
398 GWAS summary statistics used in the MR model come from comparable populations. In
399 evaluating the demographics of the studies used in this analysis, the exposures have an
400 average age ranging from 56.1 – 63.8yrs while outcomes, with the exception of hippocampal
401 volume, have an average age ranging from 71 – 74.7yrs. As such, some of the results reported
402 here may be subject to survivor bias whereby mortality due to competing risks affects selection
403 into the target study [89]. Nevertheless, the bias introduced by survival effects is large for
404 exposures that strongly affect survival, however, when selection effects are weak or moderate,
405 selection bias does not adversely affect causal estimates [89]. Finally, these analyses were
406 conducted using GWAS from European populations, limiting their generalizability to other
407 populations. Replicating these findings in non-European populations where there is potentially
408 greater variability in the modifiable risk factors remains key.
409
410 Despite these limitations, this study has significant strengths. We assessed the causal effect
411 multiple potential modifiable risk factors on AD endophenotypes in addition to AD risk. In
412 addition, we selected genetic variants for the exposure that originated from the largest available
413 GWAS at the time of analysis, and also the most recent GWAS for AD. By utilizing larger GWAS

that previous MR analyses, we were able to include a larger number of instruments that explain a greater proportion of the phenotypic variance of the exposures, resulting in increased the statistical power for this analysis.

In conclusion, this study used large exposure and outcome GWAS in MR studies to evaluate the causal associations of modifiable risk factors with the AD phenome. We found evidence of causal associations of alcohol consumption, blood pressure, cholesterol traits, educational attainment BMI, hearing problems, insomnia symptoms, physical activity, and depressive symptoms on either AD or its associated endophenotypes. Around 29.5% of dementia cases can be attributed to educational attainment, hypertension, BMI, hearing loss and physical activity. Evidence of causal relationships on the AD phenome strongly supports that interventions targeting these modifiable risk factors could reduce the individual risk of developing AD and alter AD population prevalence.

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Conflicts of Interest

SJA has no conflicts of interest to declare.

EM has no conflicts of interest to declare.

AMG served on the scientific advisory board for Denali Therapeutics from 2015-2018. She has also served as a consultant for Biogen, AbbVie, Pfizer, GSK, Eisai and Illumina.

Data availability

This study used published summary results from published research papers, with the references for those studies provided in the main paper. S1 Table provides the harmonized SNP effects need to reproduce the results of this analysis.

Supplementary Tables

S1 Table: Harmonized exposure-outcome SNPs used in Mendelian randomization analysis

S2 Table: Mendelian Randomization causal estimates

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