

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

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

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5 Shea J Andrews^{a*}, Edoardo Marcora^a, Alison Goate^a

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7 ^a Ronald M. Loeb Center for Alzheimer's disease, Department of Neuroscience, Icahn School of
8 Medicine at Mount Sinai, New York, NY, USA

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10 *Correspondence to: Shea Andrews, The Icahn School of Medicine at Mount Sinai, 1 Gustave
11 L. Levy Place, New York, NY 10029.

12 Tel: +1-212-659-8632; E-mail: shea.andrews@mssm.edu

13 **Abstract**

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

19 **Methods and Findings:** Genetic instruments for the exposures were selected from genome
20 wide association studies (GWAS) for traits previously linked to AD in observational studies
21 including alcohol intake, physical activity, lipid traits, blood pressure traits, type 2 diabetes,
22 body-mass index (BMI), depression, sleep, social isolation, smoking, oily fish intake, and
23 educational attainment. The outcomes included AD status, AD age of onset survival (AAOS),
24 hippocampal volume, CSF levels of $A\beta_{42}$, tau, and ptau₁₈₁, and neuropathological burden of
25 neuritic plaques, neurofibrillary tangles, and vascular brain injury (VBI). MR estimates were
26 calculated using an inverse variance weighted approach. Genetically predicted educational
27 attainment (OR [CI]: 0.7 [0.63, 0.78]), diastolic blood pressure (DBP) (OR [CI]: 0.99 [0.98,
28 0.99]), systolic blood pressure (SBP) (OR [CI]: 0.99 [0.99, 1]) and physical activity (OR [CI]: 2.5
29 [1.47, 4.23]) were causally associated with AD risk. Genetically predicted BMI (HR [CI]: 1.13
30 [1.07, 1.2]) and educational attainment (HR [CI]: 0.74 [0.68, 0.82]) were causally associated with
31 AAOS. Genetically predicted alcohol consumption (β [CI]: -0.15 [-0.25, -0.05]), broad depressive
32 symptoms (β [CI]: 0.5 [0.2, 0.8]), major depressive disorder (β [CI]: 0.12 [0.05, 0.18]) and
33 physical activity were causally associated with CSF $A\beta_{42}$ (β [CI]: 0.25 [0.1, 0.39]). Genetically
34 predicted DBP was causally associated with CSF total Tau (β [CI]: -0.005 [-0.007, -0.002]).
35 Increased risk of VBI were observed for genetically predicted DBP (OR [CI]: 1.05 [1.02, 1.08]),
36 SBP (OR [CI]: 1.06 [1.03, 1.1]), and pulse pressure (OR [CI]: 1.03 [1.01, 1.05]). Increased risk of
37 neuritic plaque burden were observed for genetically predicted LDL-cholesterol (OR [CI]: 1.87

38 [1.3, 2.69]) and total cholesterol (OR [CI]: 2.03 [1.44, 2.85]). Genetically predicted insomnia
39 symptoms (β [CI]: -0.2 [-0.34, -0.06]) and total cholesterol were associated (β [CI]: -0.06 [-0.1, -
40 0.03]) with hippocampal volume. Potential limitations include weak instrument bias, non-
41 homogenous samples and other implicit limitations of MR analysis.

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

46

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

48

49

50 **Introduction**

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

56

57 In the absence of any pharmacotherapeutic intervention, the number of people living with
58 dementia – of which AD accounts for ~70% of cases – is expected to exceed 130 million by
59 2050 [3]. Observational studies have identified potentially modifiable risk factors that could be
60 targeted in intervention studies to reduce the risk of dementia [4]. From these studies it has
61 been estimated that 35% of AD cases may be attributable to preventable causes such as low
62 educational attainment, hearing loss, hypertension, obesity, smoking depression physical
63 inactivity, social isolation and diabetes [5]. This suggests that interventions that target modifiable
64 risk factors could significantly reduce the population prevalence of AD and related dementias.

65

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

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

78

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

88

89 In this study we used MR to establish causal relationships between modifiable risk factors and
90 the AD phenome – AD status, AD age of onset survival (AAOS), amyloid-beta₄₂ (A β ₄₂), tau and
91 hyperphosphorylated tau (ptau₁₈₁) levels in cerebrospinal fluid (CSF), the neuropathological
92 burden of neuritic plaques, neurofibrillary tangles and vascular brain injury, and hippocampal
93 volume. Based on these analyses we identified a subset of modifiable risk factors that represent
94 the most promising targets for public health initiatives to reduce AD burden in the population.

95

96 **Methods**

97 **Data Sources**

98 We obtained GWAS summary statistics (GWAS-SS) for each exposure and outcome of interest
99 (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 (%)
<u>Exposures</u>					
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				

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

110
111 GWAS-SS for the AD phenome consisted of late-onset AD status [22], AAOS [23], CSF levels
112 of $A\beta_{42}$, ptau₁₈₁ and total tau (Tau) [24], neuropathological burden of neuritic plaques,
113 neurofibrillary tangle burden, and vascular brain injury [25], and hippocampal volume [26]. Due
114 to data use restrictions, earlier GWAS for AD [27] and hippocampal volume [28] were used for
115 estimating the causal effect of alcohol intake and educational attainment on these phenotypes.

116
117 GWAS-SS that were mapped to earlier human genome builds were lifted over to Human
118 Genome Build 19 [29]. GWAS-SS were standardized using a pipeline
119 (https://github.com/marcoralab/sumstats_munger), that 1) aligns effect alleles to the alternate
120 allele on the forward strand of the human genome reference build and normalizes indels, 2)
121 annotates variants with marker names using chromosome:position:ref:alt, 1000 Genomes rsIDs
122 (phase 3), and dbSNP rsIDs (b151) 3) where allele frequencies are missing, annotates allele
123 frequencies using non-Finnish Europeans from gnomAD (v2.1), and 4) converts summary
124 statistics to VCF and TSV files.

125

126 **Genetic Instruments**

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

141

142 **Mendelian Randomization Analysis**

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

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

156

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

163

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

171

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

176

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 n	Outliers n	IVW		MR-Egger	WMBE	WME	MR-PRESSO Global	MR-Egger Intercept
				b (SE)	q-value	b (SE)	b (SE)	b (SE)	p	p
<u>LOAD</u>										
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
<u>AAOS</u>										
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
<u>CSF Ab₄₂</u>										
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 Ptau₁₈₁

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
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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
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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
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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
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Neurofibrillary Tangles

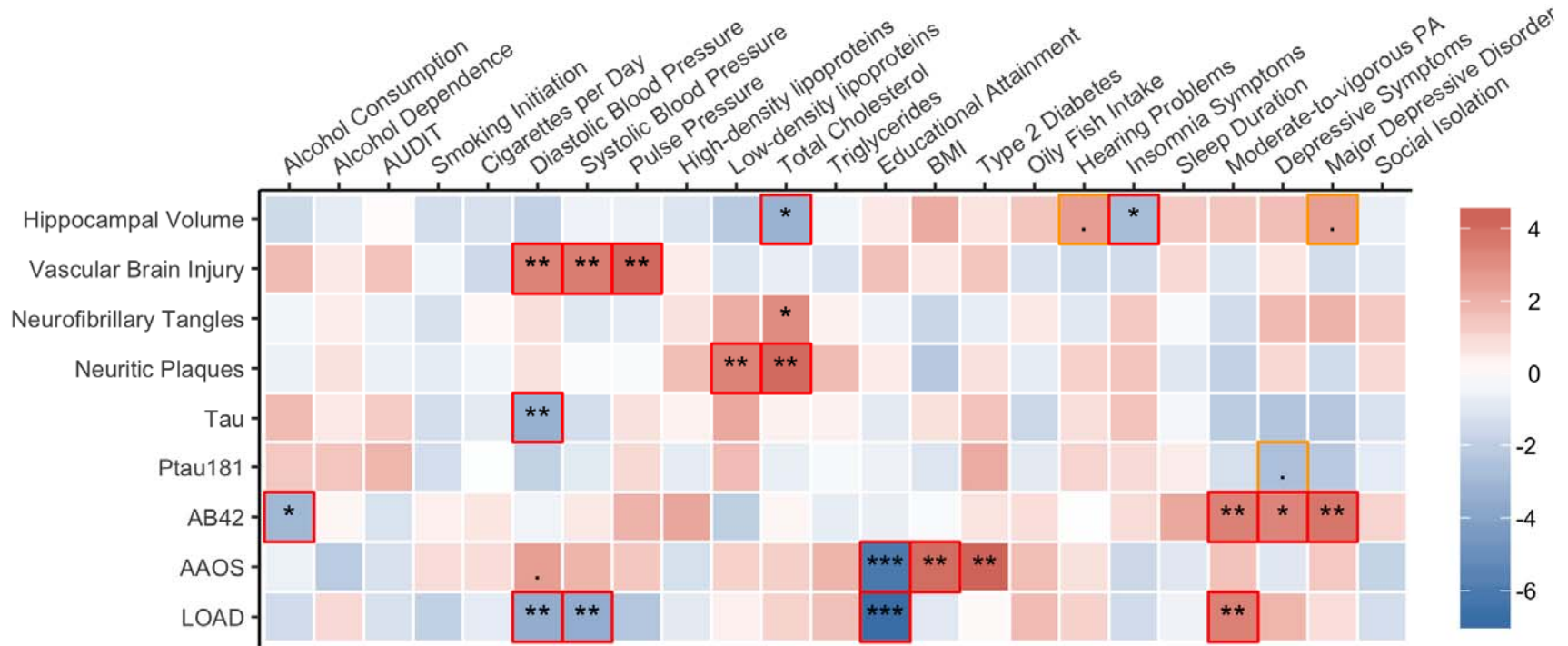
Total Cholesterol	5.00E-06	147	1	0.33 (0.11)	0.019	0.091 (0.22)	0.15 (0.19)	0.21 (0.21)	0.011	0.18
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Vascular Brain Injury

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
<u>Hippocampal Volume</u>										
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



204

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

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

212

213 **Alzheimer's Age of Onset Survival**

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

221

222

223 **CSF A β_{42} , Tau, and ptau $_{181}$**

224 Genetically predicted higher alcohol consumption was associated with significantly reduced
225 CSF A β_{42} (β [CI]: -0.15 [-0.25, -0.05]), with no evidence of heterogeneity or directional
226 pleiotropy. Genetically predicted risk of depressive symptoms were associated with significantly
227 increased A β_{42} after outlier removal (β [CI]: 0.5 [0.2, 0.8]). There was evidence of directional
228 pleiotropy, but not of heterogeneity, however, the associations were consistent in the MR-Egger
229 and WME sensitivity analyses. Depressive symptoms were also nominally associated (FDR <
230 0.1) with reduced ptau $_{181}$. Additionally, genetically predicted risk of MDD was also associated
231 with significantly increased A β_{42} after outlier removal (β [CI]: 0.12 [0.05, 0.18]), with no evidence
232 of heterogeneity or directional pleiotropy. Genetically predicted increased MVPA was associated
233 with significantly increased A β_{42} (β [CI]: 0.25 [0.1, 0.39]), with no evidence of heterogeneity or
234 directional pleiotropy observed. Genetically predicted higher DBP was associated with

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

237
238

239 **Vascular Brain Injury, Neuritic Plaque, and Neurofibrillary Tangle Burden**

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

247

248 **Hippocampal Volume**

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

256

257 **Discussion**

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

263
264 Higher educational attainment was causally associated with a reduced risk of AD, which is
265 consistent with previous MR analyses [42–44]. However, a multivariable MR analysis that
266 accounted for intelligence in addition to educational attainment suggests that the relationship
267 between education and AD is largely driven by intelligence [45]. Two of these studies [43,45]
268 used a smaller GWAS of educational attainment that explained less phenotypic variance than
269 the GWAS that was used in this analysis. One study used the same GWAS used in this analysis
270 [46], while the other conducted a single sample MR analysis [44]. The MR analyses are also
271 consistent with the observational literature [47]. We also observed a novel causal association
272 between higher education and delayed AAOS, consistent with the cognitive reserve hypothesis.
273 The observational literature, however, suggests that lower education is associated with delayed
274 age at onset, however, this association is potentially confounded due to the symptoms of AD
275 being recognized later among those with less education [48]. Additionally, we found no evidence
276 that education is causally associated with AD neuropathology or CSF biomarkers, corroborating
277 evidence from observational studies [49], and supports the hypothesis that education mitigates
278 dementia risk via cognitive reserve rather than affecting AD pathogenesis [50].

279
280 BMI was causally associated with an earlier AAOS but was not associated with AD risk or other
281 endophenotypes. Previous MR analyses have found that BMI is not a causal risk for AD
282 [43,51,62,63] using smaller GWAS of BMI than used in our analysis, however, no previous MR
283 study has evaluated the causal effect of BMI on age at onset. In contrast, observational studies

284 have observed that a higher midlife BMI is associated with increased risk of dementia, while
285 late-life obesity has an apparent protective effect likely due to reverse causation [64–66].
286 Consistent with our AOS results, midlife obesity is also associated with an earlier age of onset
287 [67].

288

289 Increased DBP and SBP were causally associated with reduced AD risk, and, in addition to PP,
290 associated with increased risk of VBI. Additionally, higher DBP was associated with reduced
291 CSF tau levels. This corroborates the results from a previous MR analysis based on 24 variants
292 but contradicts those of a more recent MR study that found no evidence of a causal association
293 based on an instrument composed of 105 variants [43,51]. In contrast to these previous
294 analyses, we selected instruments from a larger GWAS of blood pressure. Based on
295 epidemiological research, high blood pressure in midlife is generally regarded as a risk factor for
296 dementia in midlife while low blood pressure in late-life has been associated with an increased
297 risk of dementia [5,52]. However, systematic reviews and meta-analysis of the role of
298 hypertension in midlife, have observed that hypertension in midlife is associated with an
299 increased risk of developing vascular dementia but not with AD [53–55]. Additionally,
300 randomized control trials in elderly populations using either a pharmacotherapeutic or lifestyle
301 change blood pressure lowering interventions did not significantly reduce the risk of dementia
302 [56]. Our MR analysis also suggests that reducing blood pressure in late life may have limited
303 utility in the prevention of AD, but may reduce the risk of vascular dementia by reducing the risk
304 of VBI.

305

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

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

317

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

326

327 Increased MVPA was associated with increased CSF $A\beta_{42}$ levels, but also with an increased
328 risk of AD. Increased physical activity is generally associated with a reduced risk of dementia
329 [5], however, a recent meta-analysis found that the protective association with dementia was
330 observed when physical activity was measured <10 years before dementia diagnosis, but when
331 measured >10 years before dementia onset no association with dementia was observed [73].
332 Similarly, randomized control trials of single component physical activity interventions have not
333 been shown to reduce the risk of dementia [74]. Less research has been conducted on the
334 relationship between physical activity and AD $A\beta$ biomarkers, however, increased physical
335 activity has been associated with favorable AD $A\beta_{42}$ biomarker profiles [75–77].

336

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

345

346 Increased alcohol consumption was causally associated with lower CSF $A\beta_{42}$ levels. A previous
347 MR study found no evidence of an association between alcohol consumption and AD risk,
348 though this analysis was likely underpowered as the instrument only consisted of two SNPs
349 [43]. Observational studies have indicated that light-moderate alcohol intake is associated with a
350 decreased risk of AD while abstinence or heavy alcohol use is associated with an overall
351 increased risk of AD [84]. The observational studies, however, are potentially confounded by
352 selection bias, survivor bias, the inclusion of lifetime abstainers and former drinkers into control
353 groups [84].

354

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

362

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

371

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

377

378 A nominal association was observed between hearing problems and increased hippocampal
379 volume, but that there was no evidence of a causal relationship with AD or AD endophenotypes.
380 These results are contradictory to the observational literature which has found that hearing loss
381 is associated with decreased total brain volume [94], accelerated brain atrophy in whole brain
382 [95] and reduced hippocampal volume [96]. Similarly, age-related hearing loss is associated
383 with cognitive decline, cognitive impairment, and all-cause dementia, though no association was
384 observed for AD [97].

385

386 The results of this study should be interpreted in conjunction with knowledge of its limitations
387 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

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

417

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

427

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431

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436

437 **Conflicts of Interest**

438 SJA has no conflicts of interest to declare.

439 EM has no conflicts of interest to declare.

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

442

443 **Data availability**

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

447 **Supplementary Tables**

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

449 S2 Table: Mendelian Randomization causal estimates

450

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