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

Shea J Andrews, PhD^{1*}, Brian Fulton-Howard, PhD¹, Paul O'Reilly, PhD^{2,3}, Lindsay A Farrer, PhD⁴, Jonathan L Haines, PhD, ⁵, Richard Mayeux, MSc, MD⁶, Adam C Naj, PhD^{7,8}, Margaret A Pericak-Vance, PhD⁹, Gerard D Schellenberg PhD⁷, Li-San Wang PhD⁷, Edoardo Marcora, PhD¹, & Alison M Goate, PhD^{1,2}

¹ Ronald M. Loeb Center for Alzheimer's disease, Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA

² Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

³ MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.

⁴ Departments of Neurology, Biostatistics, Epidemiology, Medicine (Biomedical Genetics), Neurology, and Ophthalmology, Boston, MA, USA

⁵ Department of Population and Quantitative Health Sciences and Cleveland Institute for Computational Biology, Case Western Reserve University, Cleveland, OH, USA.

⁶ Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Department of Neurology, and Gertrude H. Sergievsky Center, Columbia University, New York, NY, USA

⁷ Department of Biostatistics, Epidemiology, and Informatics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

⁸ Penn Neurodegeneration Genomics Center, Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA

⁹ The John P. Hussman Institute for Human Genomics and Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL, USA

*Correspondence to:

Shea Andrews,

The Icahn School of Medicine at Mount Sinai,

1 Gustave L. Levy Place,

New York, NY 10029.

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

Abstract

Objective: To evaluate the causal association of 22 previously reported risk factors for Alzheimer's disease (AD) on the "AD phenome": AD, AD age of onset (AAOS), hippocampal volume, cortical surface area and thickness, cerebrospinal fluid (CSF) levels of A β ₄₂, tau, and ptau₁₈₁, and the neuropathological burden of neuritic plaques, neurofibrillary tangles, and vascular brain injury (VBI).

Methods: Polygenic risk scores (PRS) for the 22 risk factors were computed in 26,431 AD cases/controls and the association with AD was evaluated using logistic regression. Two-sample Mendelian randomization was used to evaluate the causal effect of risk factors on the AD phenome.

Results: PRS for increased education and diastolic blood pressure were associated with reduced risk for AD. PRS for increased total cholesterol and moderate-vigorous physical activity were associated with an increased risk of AD. MR indicated that only Education was causally associated with reduced risk of AD, delayed AAOS, and increased cortical surface area and thickness. Total- and LDL-cholesterol levels were causally associated with increased neuritic plaque burden, while diastolic blood pressure and pulse pressure are causally associated with increased risk of VBI. Furthermore, total cholesterol was associated with decreased hippocampal volume; smoking initiation and BMI with decreased cortical thickness; and sleep duration with increased cortical thickness.

Interpretation: Our comprehensive examination of the genetic evidence for the causal roles of previously reported risk factors in AD using PRS and MR, supports a causal role for education, blood pressure, cholesterol levels, smoking, and BMI with the AD phenome.

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

Introduction

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

In the absence of any disease-modifying therapies, the number of people living with dementia in the USA is expected to exceed 13.8 million by 2050 ¹. Observational studies have identified potentially modifiable risk factors that could be targeted in intervention studies to reduce the risk of dementia or delay its onset, thereby significantly reducing the population prevalence of AD and related dementias ². 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 ³. However, 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 due to potential confounding or reverse causation. Therefore, unless those modifiable factors specifically exacerbate disease progression, disease reduction strategies targeting them will not be successful.

Methods of causal inference that exploit genetic information, such as polygenic risk scores (PRS) and Mendelian randomization (MR), can overcome some of the limitations of observational studies. PRS are a measure of an individual's genetic propensity to a trait and can be used in cross-trait analyses to test whether genetic liability for one trait is associated with disease risk for a second ⁴. While this does not imply that the trait causally modifies disease

risk, since there are several alternative explanations, such a PRS-disease association would be expected if the trait were causal of disease, and thus PRS can be used to prioritize putative causal risk factors ⁴. 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. MR is akin to conducting a 'genetic randomized control trial', with the risk factors (genotypes) randomly allocated (from parents to offspring), independent of confounding factors that influence the risk factors and disease and unaffected by reverse causation ⁵. While MR can be used to directly assess causality between traits, it typically has lower statistical power than tests of PRS-disease associations ⁴.

In this study, we used PRS and MR to establish causal relationships between 22 modifiable risk factors and the AD phenome – AD status, AD age of onset survival (AAOS), CSF levels of amyloid-beta₄₂ (Aβ₄₂), tau and hyperphosphorylated tau (ptau₁₈₁), hippocampal volume, cortical surface area and thickness, and the neuropathological burden of neuritic plaques, neurofibrillary tangles and vascular brain injury (VBI). 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

Genome-wide association summary statistics

We obtained GWAS summary statistics (GWAS-SS) for each exposure and outcome of interest (Table 1). Exposures included: alcohol consumption ⁶, the alcohol use disorder identification test (AUDIT) ⁷, moderate-vigorous physical activity (MVPA) ⁸, lipid traits ⁹, systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) ¹⁰, type 2 diabetes (T2D) ¹¹, body mass index (BMI) ¹², meat-related diet and a fish- and plant-related diet ¹³, depression ¹⁴,

Insomnia symptoms ¹⁵, sleep duration ¹⁶, social isolation ¹⁷, smoking initiation ⁶, cigarettes per day ⁶, educational attainment ¹⁸, and hearing difficulty ¹⁹.

GWAS-SS for the AD phenome consisted of late-onset AD status ²⁰, AAOS ²¹, CSF levels of A β ₄₂, ptau₁₈₁ and total tau (Tau) ²², hippocampal volume ²³, cortical surface area and thickness ²⁴, neuropathological burden of neuritic plaques, neurofibrillary tangle burden and vascular brain injury ²⁵. Due to data use restrictions associated with evaluating alcohol intake and education phenotypes in the most recent GWAS of AD and hippocampal volume, we used an earlier GWAS for AD ²⁶ and hippocampal volume ²⁷ 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 ²⁸. GWAS-SS were standardized using a pipeline, 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) convert summary statistics to VCF and TSV files.

Alzheimer's Disease Genetics Consortium

Individual-level genetic and phenotypic data used to compute and test the association of polygenic risk scores were obtained from the Alzheimer's Disease Genetics Consortium (ADGC), a large multicenter project composed of 34 separate cohorts with the goal of performing genome-wide analyses of Alzheimer's Disease. The recruitment and genotyping of ADGC samples has been described in detail elsewhere ^{20,29}. Briefly, genotype data in each cohort underwent stringent quality control checks, with variants excluded if the call rate < 0.95,

not in Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$), and samples excluded if call rate was < 0.95 , discordant sex was reported based on X chromosome heterozygosity, cryptic relatedness, and non-European ancestry. Related individuals were determined within and across cohorts by identity-by-descent using KING³⁰, with individuals excluded based on a proportion of IBD < 0.1875 , corresponding to less than halfway between second- and third-degree relatives. Ancestry was determined empirically by projecting samples onto principal components from known ancestral populations in the 1000 Genomes Project, with samples determined to be European population outliers if they were ± 6 SD away from the EUR population mean on the first 10 principal components using PC-Air³¹ and PLINK³². SNPs that were not directly assayed were imputed on the Michigan Imputation Server individually for each of the cohorts or sub-cohorts using all ethnicities of the Haplotype Reference Consortium (HRC) 1.1 reference panel³³. Eagle was used for phasing and Minimac3 was used for imputation. Following imputation, poorly imputed ($r^2 < 0.8$) or rare (MAF < 0.01) variants were removed and the cohorts merged for joint analysis. Following this merger, variants with low call rate due to differential imputation ($< 95\%$) were removed, and then samples with low call rate ($< 95\%$) were removed. Within-ancestry principal components were created using PLINK to correct for residual population stratification within the European population subset. After sample QC, 26,431 participants were available (Table 2). Written informed consent was obtained from study participants or, for those with substantial cognitive impairment, from a caregiver, legal guardian, or other proxy, and the study protocols for all populations were reviewed and approved by the appropriate Institutional review boards (IRB's).

Polygenic Risk Scores

The software package *PRSice-2* was used to construct polygenic risk scores for each of the exposures of interest in ADGC³⁴. *PRSice* generates PRS as the sum of all alleles associated with the exposure of interest exceeding a given P -value threshold (P_i), weighted by their effect

size estimated in an independent GWAS on the trait. SNPs were clumped to obtain variants in linkage equilibrium with an $r^2 > 0.001$ within a 10MB window and PRS were constructed across a range of P_t ($P_t = 5e-8, 1e-6, 1e-5, 1e-4, 1e-3, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4$, and 0.5). The optimal P -value threshold was determined according to the results of a linear regression (PRSSice uses linear regression with binary traits to avoid issues of perfect separation during permutation) testing the association of the trait PRS and the AD outcome, adjusted for age, sex, *APOE* $\epsilon 4$ dose, and 10 principal components; the PRS P_t with the smallest P -value of association is selected for association analysis. To guard against overfitting, 1000 permutations were conducted to obtain an empirical P -value for each PRS-AD association. PRS were standardized to have a mean of 0 and SD of 1. After obtaining the optimal P_t the association between each exposure PRS and AD was evaluated using logistic regression adjusting for age, sex, *APOE* $\epsilon 4$ dose, and 10 principal components. The Benjamini & Hochberg false discovery rate was used to account for the multiple testing across the 22 different exposures.

Mendelian Randomization Analysis

Genetic Instruments

For each exposure, we constructed two different sets of instrumental variables (IV), corresponding to independent (1) genome-wide significant SNPs ($P < 5 \times 10^{-8}$) and (2) SNPs of at least borderline significance ($P < 5 \times 10^{-6}$). Increasing the number of SNPs used as IVs increases the phenotypic variance explained and, thus, has the potential to increase statistical power. However, if the additional variants included violate the core MR assumptions then they may instead reduce power, biasing the results towards the null by introducing weak instrument bias. To obtain independent SNPs, linkage disequilibrium (LD) clumping was performed by excluding SNPs that have an $r^2 > 0.001$ with another variant with a smaller p -value association within a 10MB window using PLINK³². 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 corresponded to the same effect alleles. Genetic variants that were palindromic with ambiguous allele frequencies ($AF > 0.42$), or that had incompatible alleles, were removed. Variants within the *APOE* region were excluded due to pleiotropy with AD. The proportion of variance in the phenotype explained by each instrument and F-statistic were calculated as previously described^{35,36}.

Statistical Analysis

For each genetic variant, we calculated an instrumental variable ratio estimate by dividing the SNP-exposure by SNP-outcome and the resulting coefficients were combined in a fixed-effects meta-analysis using an inverse-variance weighted (IVW) approach to give an overall estimate of causal effect⁵. The IVW method assumes that all SNPs included in the causal estimate are valid instruments - that is, that they do not violate any of the underlying MR assumptions, in particular horizontal pleiotropy, whereby genetic variants have direct effects on multiple phenotypes, could lead to false inference of causal associations⁵. In order to account for potential violations of the assumptions underlying the IVW analysis, we conducted sensitivity analyses using alternative MR methods known to be more robust to horizontal pleiotropy in particular, but at the cost of reduced statistical power. The alternative approaches included 1) Weighted Median Estimator (WME), which tests the median effect of all of the IV variants, allowing 50% of variants to exhibit horizontal pleiotropy⁵; 2) Weighted Mode Based Estimator (WMBE), which clusters variants into groups based on the similarity of causal effects and reports the final causal effect based on the cluster with the largest number of variants⁵; and 3) MR-Egger regression, which allows all variants to be subject to direct effects that bias the estimate in the same direction⁵.

The MR-Egger regression intercept was used to verify the absence of pleiotropic effects of the SNPs on the outcome ⁵. To further confirm the absence of distortions in the causal effects due to heterogeneity or horizontal pleiotropy, we used the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test to detect and correct for horizontal pleiotropic outliers ³⁷. 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 set of IV variants (*at* $P < 1 \times 10^{-8}$ or 1×10^{-6}) with the smallest p-value, outliers were removed if detected. Where there was evidence of horizontal pleiotropy or heterogeneity (MR-PRESSO Global Test $p < 0.05$ or an MR-Egger Intercept $p < 0.05$), we report the IVW results for which the sensitivity analyses were also significant and the effect direction was concordant with the IVW results. To account for multiple testing, we report q-values, a false discovery rate-based measure of significance ³⁸. Power analyses were conducted using the non-centrality parameter-based approach using the observed IVW coefficient ³⁹.

All statistical analyses were conducted using R version 3.5.2. Mendelian randomization analysis was performed using the 'TwoSampleMR' package ⁵. A Snakemake workflow was constructed that automates the PRS and MR analysis pipelines and allows for multiple exposure – outcomes datasets to be run in parallel ⁴⁰.

The SNPs used as IVs, their harmonized effects and outliers are presented in Supplementary Table 1. The causal estimates for each p-value threshold, MR method and pre- and post-outlier removal are presented in Supplementary Table 2.

Results

Polygenic Risk Score Analysis

We evaluated the association of 22 PRS for potentially modifiable risk factors with AD in ADGC. The P_t , number of SNPs and the association of each PRS with AD are presented in Table 3. After correction for multiple testing, a 1SD higher PRS for educational attainment increased risk of AD (OR [CI] 0.93 [0.91, 0.96]). Higher PRS for total cholesterol levels (OR [CI] 1.05 [1.02, 1.08]) and moderate-vigorous physical activity (OR [CI] 1.04 [1.01, 1.07]) were associated with an increased risk of AD. Using only genome-wide significant SNPs, only the PRS for educational attainment was significant after correction for multiple testing (Table 3).

Mendelian Randomization Analysis

We used Mendelian randomization to estimate the causal associations between 22 potentially modifiable risk factors and 11 AD outcomes, across two sets of IV variants corresponding to two different p-value thresholds. We observed 12 exposure-outcome pairs that were significant at an FDR < 0.05 and that either showed no evidence of heterogeneity or horizontal pleiotropy, or in the presence of heterogeneity or horizontal pleiotropy, the additional MR sensitivity analyses were significant (Figure 1; Table 4). The PVE, F-statistics and power for each model are presented in Supplementary Table 2.

Genetically predicted increased low-density lipoproteins (OR [CI]: 2.01 [1.39, 2.92]) and total cholesterol levels (OR [CI]: 1.99 [1.4, 2.84]) were associated with significantly increased risk of neuritic plaques. Genetically predicted higher diastolic blood pressure (OR [CI]: 1.08 [1.04, 1.11]) and pulse pressure (OR [CI]: 1.06 [1.02, 1.1]) were associated with significantly increased risk of vascular brain injury. Genetically predicted higher educational attainment was associated with significantly 1) lower risk of Alzheimer's disease (OR [CI]: 0.64 [0.56, 0.74]), 2) delayed AAOs (HR [CI]: 0.76 [0.67, 0.85]), 3) increased cortical surface area (β mm² [CI]: 4900 [4037.6,

5762.4]), and 4) increased cortical thickness (β mm [CI]: 0.01 [0, 0.02]). Genetically predicted longer sleep duration was associated with significantly increased cortical thickness after outlier removal (β mm [CI]: 0.02 [0.01, 0.02]). Genetically predicted smoking status was associated with significantly reduced cortical thickness (β mm [CI]: -0.02 [-0.03, -0.01]). Genetically predicted higher BMI was associated with significantly reduced cortical thickness after outlier removal (β mm [CI]: -0.01 [-0.01, 0]).

A further three risk factors, including AUDIT, diabetes, and insomnia, were causally associated with the AD phenome in the IVW analysis (Table 4; Figure 1), however, there was evidence of heterogeneity and the sensitivity analyses were non-significant suggesting that the observed associations were not robust to violations of MR underlying assumptions.

Discussion

Using genetic variants as proxies for modifiable risk factors, we applied PRS and MR analyses to investigate the association of putative modifiable risk factors with the AD phenome. PRS for higher educational attainment and diastolic blood pressure were observed to be associated with reduced risk for AD, while higher total cholesterol and increased moderate-vigorous physical activity were associated with an increased risk of AD. However, in the MR analysis, only higher educational attainment was causally associated with a reduced risk of AD. The lack of causal associations between modifiable risk factors and AD may reflect heterogeneity in the underlying pathogenesis that can lead to clinical phenotypes analogous to Alzheimer's disease.

An endophenotype is usually less genetically complex than the disorder it underlies due to the endophenotype being influenced by fewer genetic risk factors than the disease as a whole and reflecting a single pathophysiological pathway of the overall clinical disorder. As endophenotypes can be measured in both cases and controls there is greater power to detect

an association due to the effect allele influencing the endophenotype even in asymptomatic carriers. As such we expanded our MR analysis to evaluate the causal effect of modifiable risk factors on AD endophenotypes to evaluate how potential risk factors may influence the underlying pathophysiological pathways of AD. We observed 1) higher total-cholesterol and LDL-cholesterol levels to be causally associated with increased risk of neuritic plaque burden, 2) higher diastolic blood pressure and pulse pressure causally associated with increased risk of vascular brain injury, and 3) higher educational attainment causally associated with a delayed AAOS and increased cortical surface area and thickness. Furthermore, 1) higher total cholesterol was causally associated with decreased hippocampal volume, 2) smoking status and higher BMI were causally associated with reduced cortical thickness, and 3) longer sleep duration was causally associated with increased cortical thickness.

Observational studies have indicated that lifestyle interventions targeting modifiable risk factors can either prevent or delay the age of onset of dementia. In particular, low educational attainment, hearing loss, hypertension, obesity, smoking, depression, physical inactivity, social isolation and diabetes have been indicated to be key risk factors in the development of dementia³. However, with the exception of educational attainment, our analyses did not provide strong evidence of a causal association with these risk factors and AD or AAOS. The lack of a causal association between these risk factors and AD could be due to insufficient power in our analyses, but, alternatively, may be a result of confounding or reverse causation in observational studies. For instance, increased physical activity is generally associated with a reduced risk of dementia³, 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 – consistent with reverse causation driving the observed protective association⁴¹. Additionally, while these risk factors may not be associated with AD

pathogenesis, they may be associated with the pathogenesis of other dementia subtypes. For instance, the observed association between blood pressure and VBI suggests that while reducing blood pressure in late life may have limited utility in the prevention of AD, it may reduce the risk of vascular dementia by reducing the risk of VBI and therefore affect the risk for all-cause dementia, but not specifically affect the risk of AD.

The association of modifiable risk factor PRS with clinically diagnosed AD has not been extensively studied, though several studies have conducted phenome-wide scans to evaluate the association of AD PRS with a wide range of diseases and other traits. Using data from the UK Biobank ($n = 334,398$), Richardson and colleagues found that an AD PRS composed of 124 SNPs and inclusive of *APOE* ($P_t \leq 5e-05$) was associated with 72/551 traits ($FDR < 0.05$)⁴. In particular, a higher AD PRS was associated with lower diastolic blood pressure and BMI, reduced risk of self-reported diabetes, shorter sleep duration, increased risk of self-reported high cholesterol and increased amount of moderate-physical activity⁴. Similarly, a second study by Korologou-Linden and colleagues evaluated the association of an AD PRS composed of 18 SNPs, inclusive of *APOE*, ($P_t \leq 5e-08$) across 15,403 traits in the UK Biobank ($n = 334,968$)⁴². A higher AD PRS was associated with 165 traits and in particular, with lower diastolic blood pressure, lower BMI, increased total cholesterol, levels, reduced risk of self-reported diabetes, increased oily fish consumption, increased sleeplessness or insomnia, reduced sleep duration increased amount of moderate-physical activity and increased risk of self-reported depression⁴². In a follow-up MR analysis of these traits, only moderate-physical activity was observed to be causally associated with an increased risk of AD⁴².

Two earlier studies used MR to evaluate the association of potentially modifiable risk with AD cases-control status^{43,44}. First Østergaard and colleagues evaluated the association of 13 risk factors with AD and observed that higher systolic blood pressure, HDL-cholesterol and smoking

quantity were associated with a reduced risk of AD, while higher total cholesterol and LDL cholesterol were associated with increased risk ⁴⁴. No significant associations were observed for BMI, diabetes, insulin resistance, triglycerides, smoking initiation or education and after variants in the *APOE* locus were excluded from the analysis, the cholesterol levels were no longer significantly associated with AD risk ⁴⁴. Second, Larsson and colleagues evaluated the association of 22 risk factors with AD, finding that years of education, intelligence, and 25-hydroxyvitamin D were associated with a reduced risk of AD, while coffee consumption was associated with increased risk ⁴³. No significant associations were observed between alcohol consumption, serum folate, serum vitamin B₁₂, homocysteine, cardiometabolic factors or C reactive protein with Alzheimer's disease ⁴³.

The results of this study should be interpreted in conjunction with knowledge of its limitations and those of MR in general. First, while we cannot exclude that our findings may be affected by weak instrument bias, the F-statistics for all of the analyses were greater than 10, indicating that the instrument strength was sufficient for MR analysis ³⁶. However, in two-sample MR, weak instrument bias is in the direction of the null, thus, we cannot exclude low power as an explanation for the null results ⁴⁵. Second, we cannot completely rule out violations of the independence and the exclusion restriction assumption, particularly in regard to pleiotropy ⁴⁶. Nevertheless, we used several methods to identify robust causal estimates, including outlier removal using MR-PRESSO and WMBE, WME and MR-Egger sensitivity analyses. Finally, it is assumed that both samples used to generate the GWAS summary statistics used in the MR model come from comparable populations. In evaluating the demographics of the studies used in this analysis, the exposures have an average age of 56.1 – 63.8yrs, while outcomes, with the exception of hippocampal volume, have an average age of 71 – 74.7yrs. As such, some of the results reported here may be subject to survivor bias ⁴⁷. Nevertheless, the bias introduced by

survival effects is large for exposures that strongly affect survival. However, when selection effects are weak or moderate, selection bias does not adversely affect causal estimates ⁴⁷.

Despite these limitations, this study has significant strengths. We assessed the causal effect of multiple modifiable factors strongly hypothesized as affecting AD risk. In addition, we used the largest GWAS for AD and the exposure traits available at the time of analysis, allowing us to include the largest possible number of instruments for the exposures, resulting in increased statistical power. Finally, rather than limiting our analyses to AD case/control status, we expanded our MR analysis to include AD endophenotypes.

In conclusion, this study used large exposure and outcome GWAS to conduct PRS and MR analyses to evaluate the causal association of potentially modifiable risk factors with the AD phenome. The PRS analysis identified four traits for which a higher genetic predisposition influenced AD risk. In the follow-up MR analysis, only genetically predicted higher education was observed to have a causal association with reduced AD risk. Expanding our analysis to additional AD endophenotypes, we observed that higher genetically predicted cholesterol levels and blood pressure were associated with increased risk of neuritic plaque burden and vascular brain injury respectively, suggesting that these risk factors influence the development of neurodegenerative disease pathology.

Tables and Figures

Table 1: GWAS datasets utilized in this study

Study	Trait	Cohort/Consortium	N	Age	Females (%)
Exposures					
Liu et al 2019	Alcohol Consumption	GSCAN; 23andMe	941,280	-	-
	Smoking Initiation	GSCAN; 23andMe	1,232,091	-	-
	Cigarettes per Day	GSCAN; 23andMe	337,334	-	-
Sanchez-Roige et al 2019	Alcohol Use Disorder Test	UKBB; 23andMe	141,932	-	-
Wells et al 2019	Hearing Difficulty	UKBB	250,389	-	-
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 2019	Depression	UKBB; PGC; deCODE; iPSYCH; GeneScotland; GERA; 23andMe	807,553	-	-
Jansen et al 2018	Insomnia Symptoms	UKBB; 23andMe	1,331,010	-	-
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; 23andMe	1,131,881	63.8	54.7
Klimentidis et al 2018	Moderate-Vigorous Physical Activity	UKBB	377,234	-	-
Niarchou et al 2020	Meat-related diet	UKBB	335,576	-	54%
	Fish and plant related diet	UKBB	335,576	-	54%
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 A β 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
Grasby et al 2020	Cortical Surface Area	ENIGMA	33,709	45.9	51.9
	Cortical Thickness				
Beecham et al 2014	Neuritic Plaques	ADGC	4,914	74.7	65.4
	Neurofibrillary tangles				
	Vascular Brain Injury				

Table 2: Demographic characteristics of ADGC

Variable	Cases (n = 13,312)	Controls (n = 13,119)
Female	7,699 (57.8%)	7,785 (59.3%)
APOE e4+	7,690 (57.8%)	3,085 (23.5%)
Age	73.4 (8.3)	76.6 (8.3)

Table 3: Association of polygenic risk scores for potentially modifiable risk factors on Alzheimer's disease

Exposure	Genome-Wide Significant P_t				Best P_t				
	SNPs	b (se)	p	fdr	P_t	SNPs	b (se)	p	fdr
Educational Attainment	424	-0.066 (0.014)	3.80E-06	8.40E-05	1.00E-04	797	-0.07 (0.014)	8.00E-07	1.80E-05
Diastolic Blood Pressure	381	-0.03 (0.014)	0.032	0.354	0.001	863	-0.039 (0.014)	0.006	0.033
AUDIT	10	0.028 (0.014)	0.05	0.363	5.00E-08	10	0.028 (0.014)	0.05	0.139
Cigarettes per Day	39	-0.024 (0.014)	0.091	0.429	5.00E-08	39	-0.024 (0.014)	0.091	0.182
Systolic Blood Pressure	392	-0.023 (0.014)	0.098	0.429	1.00E-06	475	-0.025 (0.014)	0.081	0.182
Pulse Pressure	332	-0.022 (0.014)	0.124	0.456	5.00E-08	332	-0.022 (0.014)	0.124	0.228
Meat related diet	20	-0.015 (0.014)	0.283	0.742	1.00E-06	48	-0.032 (0.014)	0.024	0.105
Social Isolation	13	-0.014 (0.014)	0.309	0.742	0.01	858	0.028 (0.014)	0.047	0.139
BMI	450	-0.013 (0.014)	0.352	0.742	5.00E-08	450	-0.013 (0.014)	0.352	0.462
Depression	77	-0.013 (0.014)	0.357	0.742	5.00E-08	77	-0.013 (0.014)	0.357	0.462
Low-density lipoproteins	72	0.012 (0.015)	0.404	0.742	0.001	306	0.028 (0.015)	0.05	0.139
Smoking Initiation	307	-0.011 (0.014)	0.436	0.742	1.00E-06	419	-0.014 (0.014)	0.324	0.462
Fish and plant related diet	41	0.009 (0.014)	0.504	0.742	1.00E-06	81	-0.012 (0.014)	0.402	0.491
Hearing Difficulties	35	-0.009 (0.014)	0.504	0.742	1.00E-06	61	-0.024 (0.014)	0.083	0.182
Insomnia Symptoms	139	0.009 (0.014)	0.536	0.742	1.00E-04	515	-0.009 (0.014)	0.528	0.555
Alcohol Consumption	71	0.009 (0.014)	0.548	0.742	1.00E-06	123	0.018 (0.014)	0.2	0.338
High-density lipoproteins	85	-0.008 (0.014)	0.573	0.742	0.4	1398	0.009 (0.014)	0.513	0.555
Total Cholesterol	82	0.007 (0.014)	0.622	0.761	0.3	1344	0.045 (0.014)	0.002	0.02
Sleep Duration	58	0.004 (0.014)	0.761	0.842	0.001	593	0.009 (0.014)	0.53	0.555
Type 2 Diabetes	111	-0.004 (0.014)	0.783	0.842	1.00E-04	346	0.008 (0.014)	0.577	0.577
Moderate-to-vigorous PA	18	0.004 (0.014)	0.804	0.842	1.00E-04	234	0.039 (0.014)	0.005	0.033
Triglycerides	54	0.001 (0.014)	0.943	0.943	1.00E-05	105	0.018 (0.014)	0.215	0.338

Table 4: 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
				b (se)	q-value	b (se)	b (se)	b (se)	p	p
<u>LOAD</u>										
Educational Attainment	5e-8	478	0	-0.44 (0.071)	2.09E-07	-0.55 (0.27)*	-0.44 (0.11)***	-0.43 (0.37)	0.042	0.68
<u>AAOS</u>										
Educational Attainment	5e-6	716	0	-0.28 (0.058)	8.51E-05	-0.19 (0.21)	-0.31 (0.096)**	-0.28 (0.26)	0.002	0.62
Type 2 Diabetes	5.00E-06	218	0	0.072 (0.017)	0.001	0.035 (0.044)	0.045 (0.032)	0.067 (0.034).	5.00E-04	0.34
<u>Neuritic Plaques</u>										
Low-density lipoproteins	5e-8	74	0	0.7 (0.19)	0.007	0.67 (0.32)*	0.51 (0.33)	0.21 (0.45)	0.253	0.91
Total Cholesterol	5e-6	122	0	0.69 (0.18)	0.006	0.7 (0.31)*	0.8 (0.31)**	0.71 (0.44)	0.857	0.95
<u>Vascular Brain Injury</u>										
Diastolic Blood Pressure	5e-6	608	0	0.073 (0.017)	0.001	0.1 (0.041)*	0.068 (0.028)*	0.022 (0.054)	0.591	0.47
Pulse Pressure	5e-8	384	0	0.058 (0.017)	0.018	0.14 (0.045)**	0.055 (0.026)*	0.033 (0.068)	0.171	0.057
<u>Hippocampal Volume</u>										
Total Cholesterol	5e-6	125	0	-0.065 (0.02)	0.028	-0.034 (0.035)	-0.084 (0.034)*	-0.061 (0.031)*	0.106	0.26
<u>Cortical Surface Area</u>										
AUDIT	5e-6	51	1	5400 (1400)	0.004	-4800 (9900)	3400 (2300)	460 (4100)	0.0094	0.3
Educational Attainment	5e-6	707	3	4900 (440)	5.81E-26	3200 (1800).	3100 (690)***	350 (3100)	<4e-05	0.32
Type 2 Diabetes	5e-6	217	1	-460 (140)	0.016	-410 (400)	-360 (300)	-340 (310)	<1e-04	0.89
Insomnia Symptoms	5e-6	375	4	-4100 (1300)	0.029	-4900 (8200)	-2700 (2000)	700 (5700)	0.00188	0.92

<u>Cortical Thickness</u>										
Smoking Initiation	5e-6	554	1	-0.022 (0.0065)	0.012	-0.08 (0.029)**	-0.019 (0.01).	0.0083 (0.028)	<4e-05	0.04
Educational Attainment	5e-6	710	3	0.011 (0.0031)	0.01	0.013 (0.012)	0.011 (0.0051)*	0.013 (0.015)	<4e-05	0.87
BMI	5e-6	694	1	-0.0084 (0.0022)	0.005	-0.006 (0.0067)	-0.0078 (0.0038)*	-0.019 (0.01).	<4e-05	0.7
Sleep Duration	5e-6	191	2	0.016 (0.0045)	0.01	0.012 (0.02)	0.018 (0.007)*	0.036 (0.018).	0.0039	0.83

Figure 1: Putative causal associations between modifiable risk factors and the AD phenotype. 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.

Acknowledgments

SJA, BFH, EM and AMG were supported by the JPB Foundation (<http://www.jpbfoundation.org>) and by the National Institute of Health (U01AG052411 and U01AG058635; principal investigator Alison Goate). PFO was supported by funding from the UK Medical Research Council (MR/N015746/1) and the National Institute of Health (R01MH122866). Adam Naj was supported by funding from the National Institute of Aging (R01 AG054060 and RF1 AG061351). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. This analysis was possible due to the generous sharing of genome-wide association summary statistics. We would like to thank the research participants and employees of 23andMe for making this work possible.

ADGC: The Alzheimer's Disease Genetics Consortium supported collection and genotyping of samples used in this study through National Institute on Aging (NIA) grants U01AG032984 and RC2AG036528.

NCRAD: Samples from the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD), which receives government support under a cooperative agreement grant (U24 AG21886) awarded by the National Institute on Aging (NIA), were used in this study. We thank contributors who collected samples used in this study, as well as patients and their families, whose help and participation made this work possible.

NACC: The NACC database is funded by NIA/NIH Grant U01 AG016976. NACC data are contributed by the NIA-funded ADCs: P30 AG019610 (PI Eric Reiman, MD), P30 AG013846 (PI Neil Kowall, MD), P30 AG062428-01 (PI James Leverenz, MD) P50 AG008702 (PI Scott Small, MD), P50 AG025688 (PI Allan Levey, MD, PhD), P50 AG047266 (PI Todd Golde, MD, PhD), P30 AG010133 (PI Andrew Saykin, PsyD), P50 AG005146 (PI Marilyn Albert, PhD), P30

AG062421-01 (PI Bradley Hyman, MD, PhD), P30 AG062422-01 (PI Ronald Petersen, MD, PhD), P50 AG005138 (PI Mary Sano, PhD), P30 AG008051 (PI Thomas Wisniewski, MD), P30 AG013854 (PI Robert Vassar, PhD), P30 AG008017 (PI Jeffrey Kaye, MD), P30 AG010161 (PI David Bennett, MD), P50 AG047366 (PI Victor Henderson, MD, MS), P30 AG010129 (PI Charles DeCarli, MD), P50 AG016573 (PI Frank LaFerla, PhD), P30 AG062429-01 (PI James Brewer, MD, PhD), P50 AG023501 (PI Bruce Miller, MD), P30 AG035982 (PI Russell Swerdlow, MD), P30 AG028383 (PI Linda Van Eldik, PhD), P30 AG053760 (PI Henry Paulson, MD, PhD), P30 AG010124 (PI John Trojanowski, MD, PhD), P50 AG005133 (PI Oscar Lopez, MD), P50 AG005142 (PI Helena Chui, MD), P30 AG012300 (PI Roger Rosenberg, MD), P30 AG049638 (PI Suzanne Craft, PhD), P50 AG005136 (PI Thomas Grabowski, MD), P30 AG062715-01 (PI Sanjay Asthana, MD, FRCP), P50 AG005681 (PI John Morris, MD), P50 AG047270 (PI Stephen Strittmatter, MD, PhD).

NIAGADS: Data for this study were prepared, archived, and distributed by the National Institute on Aging Alzheimer's Disease Data Storage Site (NIAGADS) at the University of Pennsylvania (U24 AG041689).

Author Contributions

SJA, AMG, PFO, BFH, EM contributed to the conception and design of the study. SJA, BFH, EM. contributed to the acquisition and analysis of data. SJA, AMG, PFO, BFH, EM. contributed to drafting a significant portion of the manuscript or figures. LAF, JLH, RM, ACN, MAPV, GDS, LW contributed to the acquisition of data for the Alzheimer's Disease Genetics Consortium.

Potential Conflicts of Interest

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. SJA, BFH, EM and PO have no conflicts of interest to declare.

Data Availability

This study used published summary results from published research papers, with the references for those studies provided in the main paper. Supplementary Table 1 provides the harmonized SNP effects needed to reproduce the results of this analysis.

Supplementary Data

Supplementary Table 1: Harmonized SNP effects across exposures - outcomes

Supplementary Table 2: Mendelian Randomization results

References

1. 2020 Alzheimer's disease facts and figures. *Alzheimer's Dementia* 2020;16(3):391–460.
2. Anstey KJ, Ee N, Eramudugolla R, et al. A Systematic Review of Meta-Analyses that Evaluate Risk Factors for Dementia to Evaluate the Quantity, Quality, and Global Representativeness of Evidence. *J Alzheimer's Dis* 2019;1–21.
3. Livingston G, Sommerlad A, Orgeta V, et al. Dementia prevention, intervention, and care. *Lancet* 2017;390(10113):2673–2734.
4. Richardson TG, Harrison S, Hemani G, Smith GD. An atlas of polygenic risk score associations to highlight putative causal relationships across the human phenome. *Elife* 2019;8:e43657.
5. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. *Elife* 2018;7:e34408.
6. Liu M, Jiang Y, Wedow R, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat Genet* 2019;51(2):237–244.
7. Sanchez-Roige S, Palmer AA, Fontanillas P, et al. Genome-Wide Association Study Meta-Analysis of the Alcohol Use Disorders Identification Test (AUDIT) in Two Population-Based Cohorts. *Am J Psychiat* 2019;176(2):107–118.
8. Klimentidis YC, Raichlen DA, Bea J, et al. Genome-wide association study of habitual physical activity in over 377,000 UK Biobank participants identifies multiple variants including CADM2 and APOE. *Int J Obes* 2018;42(6):1161–1176.
9. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet* 2013;45(11):1274–83.
10. Evangelou E, Warren HR, Mosen-Ansorena D, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet* 2018;50(10):1412–1425.
11. Xue A, Wu Y, Zhu Z, et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nat Commun* 2018;9(1):2941.
12. Yengo L, Sidorenko J, Kemper KE, et al. Meta-analysis of genome-wide association studies for height and body mass index in 700,000 individuals of European ancestry. *Hum Mol Genet* 2018;27(20):3641–3649.
13. Niarchou M, Byrne EM, Trzaskowski M, et al. Genome-wide association study of dietary intake in the UK biobank study and its associations with schizophrenia and other traits. *Transl Psychiat* 2020;10(1):51.

14. Howard DM, Adams MJ, Clarke T-K, et al. Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions. *Nat Neurosci* 2019;22(3):343–352.
15. Jansen PR, Watanabe K, Stringer S, et al. Genome-wide analysis of insomnia in 1,331,010 individuals identifies new risk loci and functional pathways. *Nat Genet* 2019;51(3):394–403.
16. Dashti HS, Jones SE, Wood AR, et al. Genome-wide association study identifies genetic loci for self-reported habitual sleep duration supported by accelerometer-derived estimates. 2019;
17. Day FR, Ong KK, Perry JRB. Elucidating the genetic basis of social interaction and isolation. *Nat Commun* 2018;9(1):2457.
18. Lee JJ, Wedow R, Okbay A, et al. Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nat Genet* 2018;50(8):1112–1121.
19. Wells HRR, Freidin MB, Abidin FNZ, et al. GWAS Identifies 44 Independent Associated Genomic Loci for Self-Reported Adult Hearing Difficulty in UK Biobank. *Am J Hum Genetics* 2019;105(4):788–802.
20. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates A β , tau, immunity and lipid processing. *Nat Genet* 2019;51(3):414–430.
21. Huang K-L, Marcora E, Pimenova AA, et al. A common haplotype lowers PU.1 expression in myeloid cells and delays onset of Alzheimer's disease. *Nat Neurosci* 2017;20(8):1052–1061.
22. Deming Y, Li Z, Kapoor M, et al. Genome-wide association study identifies four novel loci associated with Alzheimer's endophenotypes and disease modifiers. *Acta Neuropathol* 2017;133(5):839–856.
23. Hibar DP, Adams HHH, Jahanshad N, et al. Novel genetic loci associated with hippocampal volume. *Nat Commun* 2017;8(1):13624.
24. Grasby KL, Jahanshad N, Painter JN, et al. The genetic architecture of the human cerebral cortex. *Science* 2020;367(6484):eaay6690.
25. Beecham GW, Hamilton K, Naj AC, et al. Genome-Wide Association Meta-analysis of Neuropathologic Features of Alzheimer's Disease and Related Dementias. *Plos Genet* 2014;10(9):e1004606.
26. Lambert J-C, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 2013;45(12):1452–1458.
27. Hibar DP, Stein JL, Renteria ME, et al. Common genetic variants influence human subcortical brain structures. *Nature* 2015;520(7546):224–229.

28. Kent WJ, Sugnet CW, Furey TS, et al. The Human Genome Browser at UCSC. *Genome Res* 2002;12(6):996–1006.
29. Naj AC, Jun G, Beecham GW, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* 2011;43(5):436–441.
30. Manichaikul A, Mychaleckyj JC, Rich SS, et al. Robust relationship inference in genome-wide association studies. *Bioinformatics* 2010;26(22):2867–2873.
31. Conomos MP, Miller MB, Thornton TA. Robust inference of population structure for ancestry prediction and correction of stratification in the presence of relatedness. *Genet Epidemiol* 2015;39(4):276–93.
32. Purcell S, Neale B, Todd-Brown K, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. *Am J Hum Genetics* 2007;81(3):559–575.
33. Consortium HR, McCarthy S, Das S, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet* 2016;48(10):ng.3643.
34. Choi SW, O'Reilly PF. PRSice-2: Polygenic Risk Score software for biobank-scale data. *Gigascience* 2019;8(7)
35. Shim H, Chasman DI, Smith JD, et al. A multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 Caucasians. *Plos One* 2015;10(4):e0120758.
36. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* 2011;40(3):755–764.
37. Verbanck M, Chen C-Y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* 2018;50(5):693–698.
38. Storey JD. A direct approach to false discovery rates. *J Royal Statistical Soc Ser B Statistical Methodol* 2002;64(3):479–498.
39. Brion M-JA, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. *Int J Epidemiol* 2013;42(5):1497–501.
40. Koster J, Rahmann S. Snakemake--a scalable bioinformatics workflow engine. *Bioinformatics* 2012;28(19):2520–2522.
41. Kivimäki M, Singh-Manoux A, Pentti J, et al. Physical inactivity, cardiometabolic disease, and risk of dementia: an individual-participant meta-analysis. *Bmj Clin Res Ed* 2019;365:l1495.
42. Korologou-Linden R, Anderson EL, Howe LD, et al. The causes and consequences of Alzheimer's disease: A Mendelian randomization analysis. *Medrxiv* 2019;2019.12.18.19013847.

43. Larsson SC, Traylor M, Malik R, et al. Modifiable pathways in Alzheimer's disease: Mendelian randomisation analysis. *Bmj* 2017;359:j5375.
44. Østergaard SD, Mukherjee S, Sharp SJ, et al. Associations between Potentially Modifiable Risk Factors and Alzheimer Disease: A Mendelian Randomization Study. *Plos Med* 2015;12(6):e1001841; discussion e1001841.
45. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol* 2013;178(7):1177–84.
46. Hemani G, Bowden J, Smith GD. Evaluating the potential role of pleiotropy in Mendelian randomization studies. *Hum Mol Genet* 2018;27(R2):R195–R208.
47. Gkatzionis A, Burgess S. Contextualizing selection bias in Mendelian randomization: how bad is it likely to be? *Int J Epidemiol* 2018;