

Leveraging DNA methylation quantitative-trait loci to characterize the relationship between metabolic traits and Alzheimer's disease

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Abstract

Background: The relationship between DNA methylation, common metabolic risk and Alzheimer's disease (AD) is not well understood.

Methods: Summary statistics integrating DNA methylation quantitative trait loci (mQTLs) and several genome-wide association study data were used. Network with bidirectional mendelian randomization (MR) analysis was performed to examine the causal association among metabolic traits, DNA methylation and AD.

Results: Our study showed that cis-mQTLs determined DNA methylation to higher total cholesterol (TC) was associated with higher AD risk (β [95% CI] =0.007 [0.002-0.013], $P=0.005$). The findings were robust in sensitivity analyses with different instrumental variables. We found no evidence to support causal associations of cis-mQTLs determined obesity and T2D with AD, and vice versa.

Conclusion: Overall, our study showed that the cis-mQTLs determined DNA methylation to higher TC was associated with higher AD risk, whereas the relation of cis-mQTLs determined AD and metabolic dysregulation was unlikely to be causal.

Background

Alzheimer's disease (AD), which is a disabling and fatal disease, is a large and growing public health problem due to population ageing and lack of effective therapies [1, 2]. AD is a heterogeneous neurodegenerative disease, whose pathogen results from a complex interplay between neuropathological, genetic and metabolic factors [3–6]. However, there are considerable gaps in our understanding of the nosology, and etiological complexity of AD.

DNA methylation, which is an epigenetic regulation mechanism, has been shown to play a key role in many biological processes and disease susceptibility [7, 8]. DNA methylation reflects at the molecular level a wide range of environmental exposures and genetic influences [9, 10], in such case, observational associations between complex traits and DNA methylation are prone to confounding and reverse causation. To address this limitation, a novel method of Mendelian randomization (MR) integrating DNA methylation quantitative trait loci (mQTL) and genome-wide association study (GWAS) data was widely used to explore the causal inference of DNA methylation on complex diseases [11–13]. The increasing evidence showed that DNA methylation were elucidated to mediate the influence of genetic variants on diseases, including AD and its related metabolic dysregulation [11, 12, 14, 15]. Increasing evidence suggests epidemiological and pathological links between AD and metabolic dysregulation, leading some to consider late-onset AD a "metabolic" disease [3, 16, 17]. However, the relationship between DNA methylation, common metabolic risk and AD has not been systematically studied.

Recently, a novel method, known as network mendelian randomization [18, 19], is performed to investigate more complex networks of relationships between variables, in particular where some of the effect of an exposure on the outcome may operate through a mediator. Since the causal relationship between metabolic dysregulation and AD remains unclear [17, 20, 21], we performed an analytical framework that integrates network MR and bidirectional MR to explore whether metabolic risk factors may act as potential mediators that lie in the pathway from aberrant DNA methylation to increased risk of AD (i.e., SNP→DNA methylation→Metabolic traits→AD) or the causal DNA methylation driving AD in turn leads to metabolic dysregulation(i.e., SNP→DNA methylation→AD→Metabolic traits). In our study, the metabolic traits include body mass index (BMI), waist-hip ratio (WHR), fasting glucose (FG), fasting insulin (FI), triglycerides (TG), low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), and total cholesterol (TC).

Methods

Summary of GWAS Data

Data included in this study were the GWAS summary statistics datasets from the International Genomics of Alzheimer Project (IGAP) for AD (<https://www.pasteur-lille.fr>); European Network for Genetic and Genomic Epidemiology (ENGAGE) 1000 Genome Consortium for BMI, WHR, glycemc traits including FG, FI, lipids, including HDL, LDL, TC and TG (http://diagram-consortium.org/2015_ENGAGE_1KG/). The summary of mQTL was available from MR-Base (<http://www.mrbase.org/>). All GWAS summary statistic data were based on European ancestry populations.

A brief summary of these data is presented in **Table S1**. The summary statistics of SNP-AD associations from the IGAP, comprised a total of 17,008 individuals with AD and 37,154 cognitively normal elderly controls. The summary statistics of SNP-BMI associations included 87,048 individuals and the summary statistics of SNP-WHR associations adjusted for BMI included 54,572 individuals as described in the previous study [22]. The summary statistics of SNP-FG associations included 46,694 individuals, and the summary statistics of SNP-FI associations adjusted for BMI included 24,245 individuals as described in the previous study [22]. The summary statistics of SNP-lipids associations included 62,166 individuals [23]. Detailed information can be obtained at http://diagram-consortium.org/2015_ENGAGE_1KG/ENGAGE_1KG.pdf. Summary statistics of cis-mQTL (i.e. SNPs within 1 MB distance of their associated CpG sites) were used ($n = 742$) as a DNA methylation of CpG site genetic instrument [24].

Study Design

The network MR study included two major components. First, we explored the casual DNA methylation for metabolic traits and AD and the cis-mQTLs determined DNA methylation as instrument variables (IVs) for metabolic traits and AD. Of note, the network MR exploring the causal pathway from DNA methylation to the outcome was proposed to use the cis-mQTLs determined DNA methylation as IVs for the mediator. Secondly, bidirectional association between metabolic traits and AD were taken forward for further analyses to better understand the relationship between DNA methylation, common metabolic risk and AD. As shown in Fig. 1, the framework of the network with bidirectional MR analysis consists of 3 different MR tests that are all described below (HIII).

First, the causal effects of cis-mQTL determined DNA methylation on metabolic risk factors and AD are estimated (I). Next, the causal effects of cis-mQTLs determined metabolic traits on AD are analyzed (II). Finally, the causal effects of cis-mQTLs determined AD on metabolic traits are estimated (III).

Statistical analysis

The first two-sample MR analyses was aimed to evaluate the potential causal relationship between DNA methylation at 41,760 CpG sites (The threshold of statistical significance was $P < 1 \times 10^{-10}$, as shown in **Figure S1**) and 9 complex traits. We only investigated CpG sites using cis-mQTL with the lowest P -value in order to improve the specificity of the IV for each CpG. Therefore, MR effect estimates were calculated using the Wald ratio. Then these cis-mQTLs corresponding to the casual DNA methylation for each mediator were as IVs removing the overlapping cis-mQTLs between AD and metabolic traits for further MR analysis.

The MR analysis required that the cis-mQTL SNPs in the instruments were not of horizontal pleiotropy (a genetic variant affects the outcome via a different biological pathway from the mediator under investigation) and in linkage equilibrium (LD). IVs for each mediator were composed of independent cis-mQTLs pruned at LD $r^2 < 0.1$. The LD proxies were defined using 1000 genomes European samples [25]. We conducted sensitivity analyses based on the cis-mQTL SNPs removing the overlapping cis-mQTLs among metabolic traits and based on the prior cis-mQTL SNPs pruned at LD $r^2 < 0.1$.

For forward MR, we used a conventional inverse-variance weighted (IVW) MR analysis, in which the SNPs-AD estimate was regressed on the SNPs-metabolic traits estimate with the intercept term set to zero, weighted by the inverse-variance of SNPs-AD estimate, and vice versa for reverse MR. The heterogeneity between SNPs was estimated by Cochran Q statistic. Random-effects IVW model was used if heterogeneity existed, otherwise fixed-effects IVW model was performed. In addition, the MR-Egger method was used to assess the robustness of estimates to potential violations of the standard IV assumptions attributing from the directional pleiotropy.

Data cleaning and statistical analysis was performed using R version 3.3.3 (<https://www.r-project.org/>) and PLINK 1.9 (<https://www.cog-genomics.org/plink/1.9/>). $P < 0.05$ was considered as suggestive of evidence for a potential association.

Results

As shown in **Table S2-10**, we identified 3597 causal CpGs for BMI, 2420 for WHR, 2307 for FG, 2157 for FI, 24 for HDL, 3200 for LDL, 4100 for TC, 3008 for TG, and 2806 for AD, were identified in this MR analysis. These identified cis-mQTLs determined DNA methylation could be potential IVs for metabolic traits and AD.

The causal CpGs that overlapped between the metabolic traits and AD is presented in Fig. 2. Of note, there were 291 causal CpGs that overlapped between BMI and AD, 143 (49.14%) of which changed in the same direction of BMI and AD (**Figure S2** and **Table S11**). There were 179 causal CpGs that overlapped between WHR and AD, 80 (44.69%) of which changed in the same direction of WHR and AD (**Figure S3** and **Table S12**). There were 152 causal CpGs that overlapped between FG and AD, 60 (40.79%) of which changed in the same direction of FG and AD (**Figure S4** and **Table S13**). There were 121 causal CpGs that overlapped between FI and AD, 58 (47.93%) of which changed in the same direction of FI and AD (**Figure S5** and **Table S14**). There was only one causal CpG that overlapped between HDL and AD, which performed the same direction of HDL and AD (**Figure S6** and **Table S15**). There were 264 causal CpGs that overlapped between LDL and AD, 151 (57.20%) of which changed in the same direction of LDL and AD (**Figure S7** and **Table S16**). There were 95 causal CpGs that overlapped between TC and AD, 45 (47.37%) of which changed in the same direction of TC and AD (**Figure S8** and **Table S17**). There were 234 causal CpGs that overlapped between TG and AD, 108 (46.15%) of which changed in the same direction of TG and AD (**Figure S9** and **Table S18**). There were 1803 causal CpGs that were specific to AD, accounting for 64.26% of the total.

We further performed a bidirectional MR to evaluate the causal associations between metabolic traits and AD. For forward MR analysis, we used the causal cis-mQTL SNPs associated with each metabolic trait as IVs removing the overlapping cis-mQTL between AD and metabolic traits for further MR analysis. We considered BMI ($N_{SNPs} = 2627$), WHR ($N_{SNPs} = 1820$), FG ($N_{SNPs} = 1674$), FI ($N_{SNPs} = 1582$), HDL-cholesterol ($N_{SNPs} = 17$), LDL-cholesterol ($N_{SNPs} = 2315$), TC ($N_{SNPs} = 2761$), and TG ($N_{SNPs} = 2187$). As shown in Table 1, the cis-mQTLs determined DNA methylation to higher TC was associated with higher AD risk (β [95%CI] = 0.007 [0.002–0.013], $P = 0.005$). In addition, MR-Egger regression showed no evidence of directional pleiotropy for the associations of TC with AD (β [intercept] = 0.000, $P = 0.417$). The findings were robust in sensitivity analyses with different IVs. The cis-mQTLs determined DNA methylation to higher BMI (β [95%CI] = 0.010 [0.002–0.016], $P = 0.010$) and HDL (β [95%CI] = 0.100 [0.022–0.177], $P = 0.011$) were associated with higher AD risk in sensitivity analyses, however, the findings were not robust with different IVs. In addition, MR-Egger regression showed evidence of directional pleiotropy for the associations of HDL with AD (β [intercept] = -0.001, $P = 0.017$). Therefore, the relationship of HDL and BMI with AD seemed to be less clear as compared with TC in our study. In addition, no evidence supported the causal associations of cis-mQTLs determined FG and FI with AD.

Table 1
Causal association between cis-mQTL determined metabolic traits and AD

Metabolic traits	Parameter	IVs1				IVs2				IVs3			
		SNP (n)	Effect (95%CI)	SE	P	SNP (n)	Effect (95%CI)	SE	P	SNP (n)	Effect (95%CI)	SE	P
BMI													
IVW	β	2627	0.004(-0.002-0.010)	0.003	0.207	1790	0.010(0.002-0.016)	0.004	0.010	1136	0.006(-0.003-0.015)	0.005	0.212
MR-Egger	β	2627	-0.014(-0.034-0.005)	0.010	0.147	1790	-0.007(-0.029-0.015)	0.011	0.522	1136	-0.004(-0.039-0.031)	0.018	0.821
	β (intercept)	2627	0.000(0.000-0.001)	0.000	0.053	1790	0.000(0.000-0.001)	0.000	0.104	1136	0.000(0.000-0.001)	0.000	0.530
WHR													
IVW	β	1820	0.004(-0.002-0.010)	0.003	0.196	1233	0.003(-0.005-0.010)	0.004	0.484	811	0.003(-0.006-0.011)	0.005	0.570
MR-Egger	β	1820	0.006(-0.014-0.026)	0.010	0.565	1233	0.001(-0.021-0.023)	0.011	0.928	811	0.008(-0.026-0.042)	0.017	0.648
	β (intercept)	1820	0.000(0.000-0.000)	0.000	0.856	1233	0.000(0.000-0.000)	0.000	0.884	811	0.000(-0.001-0.0001)	0.000	0.743
FG													
IVW	β	1674	0.003(-0.006-0.012)	0.005	0.544	1105	0.003(-0.008-0.014)	0.006	0.582	743	0.000(-0.014-0.014)	0.007	0.991
MR-Egger	β	1674	0.004(-0.025-0.032)	0.014	0.803	1105	0.018(-0.013-0.048)	0.015	0.252	743	0.018(-0.018-0.054)	0.018	0.330
	β (intercept)	1674	0.000(0.000-0.000)	0.000	0.988	1105	0.000(-0.001-0.000)	0.000	0.331	743	0.000(-0.001-0.000)	0.000	0.306
FI													
IVW	β	1582	0.006(-0.003-0.014)	0.004	0.205	1068	0.004(-0.007-0.014)	0.005	0.460	683	0.001(-0.013-0.014)	0.007	0.921
MR-Egger	β	1582	0.023(-0.005-0.050)	0.014	0.112	1068	0.014(-0.017-0.044)	0.015	0.378	683	-0.004(-0.053-0.045)	0.025	0.881
	β (intercept)	1582	0.000(-0.001-0.000)	0.000	0.225	1068	0.000(-0.001-0.000)	0.000	0.549	683	0.000(-0.001-0.001)	0.000	0.833
HDL													
IVW	β	17	0.019(-0.153-0.191)	0.088	0.828	7	0.067(-0.332-0.465)	0.203	0.743	7	0.067(-0.332-0.465)	0.203	0.743
MR-Egger	β	17	0.002(-0.019-0.023)	0.011	0.865	7	0.100(0.022-0.177)	0.039	0.011	7	0.100(0.022-0.177)	0.039	0.011
	β (intercept)	17	-0.001(-0.002-0.000)	0.000	0.191	7	-0.001(-0.003-0.000)	0.001	0.017	7	-0.001(-0.003-0.000)	0.001	0.017
LDL													
IVW	β	2315	0.000(-0.005-0.005)	0.002	0.884	1374	-0.005(-0.012-0.002)	0.004	0.152	964	-0.002(-0.011-0.007)	0.004	0.651
MR-Egger	β	2315	0.008(-0.003-0.018)	0.005	0.143	1374	0.018(-0.002-0.038)	0.010	0.083	964	0.009(-0.015-0.032)	0.012	0.483

IVs1 is based on cis-mQTLs corresponding to the casual DNA methylation for each metabolic trait removing the overlapping cis-mQTL between AD and metabolic traits.

IVs2 is based on cis-mQTLs corresponding to the casual DNA methylation for each metabolic trait removing the overlapping cis-mQTL among metabolic traits.

IVs3 is based on IVs2 pruned at LD $r^2 < 0.1$.

Beta is the estimated effect size.

AD indicates Alzheimer disease; BMI: body mass index; CI, confidence interval; FI, fasting insulin; FG, fasting glucose; HDL, high density lipoprotein cholesterol; IVW, inverse-variance weighted; IVs, instrument variables; LD: linkage equilibrium; LDL, low density lipoprotein cholesterol; mQTL: DNA methylation quantitative trait loci; MR mendelian randomization; SE, standard error; SNP, single-nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; WHR, waist-hip ratio.

Metabolic traits	Parameter	IVs1				IVs2				IVs3			
		SNP	Effect	SE	P	SNP	Effect (95%CI)	SE	P	SNP	Effect (95%CI)	SE	P
		(n)	(95%CI)			(n)				(n)			
	β (intercept)	2315	0.000(0.000–0.000)	0.000	0.110	1374	0.000(-0.001-0.000)	0.000	0.016	964	0.000(-0.001-0.000)	0.000	0.347
TC													
IVW	β	2761	0.007(0.002–0.013)	0.003	0.005	2470	0.008(0.002–0.014)	0.003	0.005	1353	0.007(-0.001-0.015)	0.004	0.081
MR-Egger	β	2761	0.007(-0.005-0.018)	0.006	0.266	2470	0.009(-0.003-0.022)	0.006	0.144	1353	0.019(0.002–0.037)	0.009	0.029
	β (intercept)	2761	0.000(0.000–0.000)	0.000	0.417	2470	0.000(0.000–0.000)	0.000	0.650	1353	0.000(-0.001-0.000)	0.000	0.230
TG													
IVW	β	2187	0.003(-0.003-0.009)	0.003	0.303	1327	-0.002(-0.010-0.006)	0.004	0.598	952	-0.001(-0.010-0.009)	0.005	0.842
MR-Egger	β	2187	0.006(-0.009-0.022)	0.008	0.440	1327	0.022(-0.010-0.054)	0.016	0.186	952	0.007(-0.028-0.041)	0.018	0.711
	β (intercept)	2187	0.000(0.000–0.000)	0.000	0.682	1327	0.000(-0.001-0.000)	0.000	0.113	952	0.000(-0.001-0.000)	0.000	0.664
IVs1 is based on cis-mQTLs corresponding to the casual DNA methylation for each metabolic trait removing the overlapping cis-mQTL between AD and metabolic traits.													
IVs2 is based on cis-mQTLs corresponding to the casual DNA methylation for each metabolic trait removing the overlapping cis-mQTL among metabolic traits.													
IVs3 is based on IVs2 pruned at LD $r^2 < 0.1$.													
Beta is the estimated effect size.													
AD indicates Alzheimer disease; BMI: body mass index; CI, confidence interval; FI, fasting insulin; FG, fasting glucose; HDL, high density lipoprotein cholesterol; IVW, inverse-variance weighted; IVs, instrument variables; LD: linkage equilibrium; LDL, low density lipoprotein cholesterol; mQTL: DNA methylation quantitative trait loci; MR mendelian randomization; SE, standard error; SNP, single-nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; WHR, waist-hip ratio.													

Taking these putative associations forward, we evaluated the potential for reverse causal relationships by performing MR of AD against metabolic traits. For reverse MR analysis, we used the casual cis-mQTL SNPs associated with AD as IVs removing the overlapping cis-mQTLs between AD and metabolic traits. Since we were using different databases, we couldn't extract all candidate cis-mQTL SNPs as IVs. Therefore, we considered AD to BMI ($N_{\text{SNPs}} = 1807$), AD to WHR ($N_{\text{SNPs}} = 2040$), AD to FG ($N_{\text{SNPs}} = 1930$), AD to FI ($N_{\text{SNPs}} = 1940$), AD to HDL-cholesterol ($N_{\text{SNPs}} = 1980$), AD to LDL-cholesterol ($N_{\text{SNPs}} = 1778$), AD to TC ($N_{\text{SNPs}} = 1953$), and AD to TG ($N_{\text{SNPs}} = 1784$). As shown in Table 2, the cis-mQTLs determined DNA methylation to higher AD risk was associated with higher BMI (β [95%CI] = 0.007 [0.003–0.012], $P = 0.002$) and higher HDL (β [95%CI] = 0.019 [0.005–0.032], $P = 0.007$). The findings were not robust in sensitivity analyses with different IVs. The cis-mQTLs determined DNA methylation to higher AD risk was associated with lower FG (β [95%CI] = -0.011 [-0.019–0.003] and lower TG (β [95%CI] = -0.019 [-0.023–0.014], $P < 0.001$); however, the findings were not robust in sensitivity analyses with different IVs. In addition, MR-Egger regression showed evidence of directional pleiotropy for the associations of AD with FG (β [intercept] = 0.000, $P < 0.001$) and TG (β [intercept] = 0.000, $P = 0.021$). Therefore, the result of reverse MR seemed to be less clear as compared with the result of forward MR in our study.

Table 2
Causal association between cis-mQTL determined AD and metabolic traits

Metabolic traits	Parameter	IVs1				IVs2				IVs3			
		SNP (n)	Effect (95%CI)	SE	P	SNP (n)	Effect (95%CI)	SE	P	SNP (n)	Effect (95%CI)	SE	P
BMI													
IVW	β	1807	0.003(-0.006-0.012)	0.005	0.520	1292	0.002(-0.008-0.013)	0.005	0.637	845	0.004(-0.040-0.047)	0.022	0.872
MR-Egger	β	1807	0.007(0.003-0.012)	0.002	0.002	1292	0.008(0.004-0.013)	0.002	< 0.001	845	0.033(-0.052-0.119)	0.044	0.445
	β (intercept)	1807	0.000(-0.001-0.000)	0.000	0.197	1292	0.000(-0.001-0.000)	0.000	0.031	845	0.000(-0.001-0.000)	0.000	0.396
WHR													
IVW	β	2040	-0.002(-0.011-0.007)	0.005	0.655	1232	0.005(-0.036-0.046)	0.021	0.806	845	0.010(-0.047-0.068)	0.029	0.721
MR-Egger	β	2040	-0.002(-0.011-0.007)	0.005	0.685	1232	0.004(-0.063-0.072)	0.035	0.897	845	0.050(-0.066-0.166)	0.059	0.397
	β (intercept)	2040	0.000(0.000-0.000)	0.000	0.868	1232	0.000(-0.001-0.001)	0.000	0.989	845	0.000(-0.001-0.001)	0.000	0.426
FG													
IVW	β	1930	0.014(-0.009-0.036)	0.011	0.228	1208	0.004(-0.027-0.035)	0.016	0.798	845	0.008(-0.029-0.045)	0.019	0.687
MR-Egger	β	1930	-0.011(-0.019-0.003)	0.004	0.005	1208	-0.062(-0.102-0.022)	0.020	0.002	845	-0.030(-0.105-0.046)	0.039	0.444
	β (intercept)	1930	0.000(0.00-0.001)	0.000	0.005	1208	0.001(0.000-0.001)	0.000	0.005	845	0.000(0.00-0.001)	0.000	0.306
FI													
IVW	β	1940	-0.003(-0.009-0.004)	0.003	0.436	1223	0.007(-0.031-0.044)	0.019	0.732	845	0.015(-0.030-0.061)	0.023	0.505
MR-Egger	β	1940	-0.002(-0.008-0.003)	0.003	0.380	1223	-0.006(-0.066-0.054)	0.031	0.841	845	0.039(-0.061-0.138)	0.051	0.447
	β (intercept)	1940	0.000(0.000-0.000)	0.000	0.836	1223	0.000(0.000-0.001)	0.000	0.445	845	0.000(-0.001-0.001)	0.000	0.666
HDL													
IVW	β	1980	0.019(0.005-0.032)	0.007	0.006	1294	0.006(-0.039-0.050)	0.023	0.806	903	0.014(-0.037-0.064)	0.026	0.594
MR-Egger	β	1980	0.016(0.006-0.027)	0.005	0.002	1294	-0.056(-0.192-0.080)	0.069	0.423	903	0.014(-0.091-0.119)	0.054	0.795
	β (intercept)	1980	0.000(0.000-0.001)	0.000	0.455	1294	0.000(-0.001-0.001)	0.001	0.458	903	0.000(-0.001-0.001)	0.000	0.889
LDL													
IVW	β	1778	0.000(-0.008-0.008)	0.004	0.953	1190	0.005(-0.040-0.050)	0.023	0.818	902	0.012(-0.040-0.064)	0.026	0.644
MR-Egger	β	1778	-0.003(-0.008-0.002)	0.003	0.257	1190	0.005(-0.094-0.105)	0.051	0.918	902	-0.002(-0.113-0.110)	0.057	0.979
	β (intercept)	1778	0.000(0.000-0.001)	0.000	0.264	1190	0.000(-0.001-0.001)	0.000	0.973	902	0.000(-0.001-0.001)	0.000	0.722

IVs1 is based on cis-mQTLs corresponding to the casual DNA methylation for AD removing the overlapping cis-mQTL between AD and metabolic traits.

IVs2 is based on cis-mQTLs corresponding to the casual DNA methylation for AD removing the overlapping cis-mQTL among metabolic traits.

IVs3 is based on IVs2 pruned at LD $r^2 < 0.1$.

Beta is the estimated effect size.

AD indicates Alzheimer disease; BMI: body mass index; CI, confidence interval; FI, fasting insulin; FG, fasting glucose; HDL, high density lipoprotein cholesterol; IVW, inverse-variance weighted; IVs, instrument variables; LD: linkage equilibrium; LDL, low density lipoprotein cholesterol; mQTL: DNA methylation quantitative trait loci. MR mendelian randomization; SE, standard error; SNP, single-nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; WHR, waist-hip ratio.

Metabolic traits	Parameter	IVs1				IVs2				IVs3			
		SNP	Effect	SE	P	SNP	Effect	SE	P	SNP	Effect	SE	P
		(n)	(95%CI)			(n)	(95%CI)			(n)	(95%CI)		
TC													
IVW	β	1953	0.013(-0.003-0.029)	0.008	0.100	1228	0.028(-0.024-0.080)	0.027	0.288	897	0.011(-0.043-0.065)	0.028	0.684
MR-Egger	β	1953	0.012(-0.004-0.028)	0.008	0.141	1228	0.079(-0.003-0.162)	0.042	0.060	897	0.011(-0.117-0.139)	0.065	0.865
	β (intercept)	1953	0.000(0.000-0.001)	0.000	0.336	1228	0.000(-0.001-0.000)	0.000	0.338	897	0.000(-0.001-0.001)	0.001	0.872
TG													
IVW	β	1784	0.017(-0.017-0.051)	0.018	0.334	1198	0.029(-0.017-0.075)	0.024	0.214	897	0.046(-0.008-0.101)	0.028	0.097
MR-Egger	β	1784	-0.019(-0.023--0.014)	0.002	< 0.001	1198	0.013(-0.081-0.106)	0.048	0.792	897	0.070(-0.042-0.182)	0.057	0.218
	β (intercept)	1784	0.000(0.000-0.001)	0.000	0.021	1198	0.000(-0.001-0.001)	0.000	0.633	897	0.000(-0.001-0.001)	0.000	0.686
IVs1 is based on cis-mQTLs corresponding to the casual DNA methylation for AD removing the overlapping cis-mQTL between AD and metabolic traits.													
IVs2 is based on cis-mQTLs corresponding to the casual DNA methylation for AD removing the overlapping cis-mQTL among metabolic traits.													
IVs3 is based on IVs2 pruned at LD $r^2 < 0.1$.													
Beta is the estimated effect size.													
AD indicates Alzheimer disease; BMI: body mass index; CI, confidence interval; FI, fasting insulin; FG, fasting glucose; HDL, high density lipoprotein cholesterol; IVW, inverse-variance weighted; IVs, instrument variables; LD: linkage equilibrium; LDL, low density lipoprotein cholesterol; mQTL: DNA methylation quantitative trait loci. MR mendelian randomization; SE, standard error; SNP, single-nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; WHR, waist-hip ratio.													

Discussion

To our knowledge, this is the first study to investigate the causal relationship between DNA methylation, metabolic traits, and AD using a network with bidirectional MR design integrating cis-mQTLs and summary GWAS data. Our study showed cis-mQTLs determined DNA methylation to higher TC was associated with higher AD risk, whereas the relation of the cis-mQTL determined AD and metabolic traits were unlikely to be causal.

The causal relationship between metabolic indicators and AD has been widely explored, and the findings showed that metabolic dysregulation related genetic risk factors did not predict AD risk [17, 20, 21, 26–28]. There are two possible explanations for these negative results. First, the findings might be faced with the bias of weak IVs due to the complexity of metabolic dysregulation and the small fraction of metabolic dysregulation variance accounting for the genetic variants [20, 21, 28, 29]. Completely ruling out an alternative direct causal pathway is a challenge for all MR analyses [30, 31], particularly for complex traits determined by both multiple genetic variants and complexly environmental exposures. Therefore, a promising approach was urgently needed to reduce the weak instrument bias and provide new clues to the common molecular mechanisms and biological processes of complex traits including AD and metabolic dysregulation. DNA methylation of CpGs is strongly associated with genetic loci, might explaining additional phenotypic variation in diseases besides genetic variants. Thus DNA methylation was considered as IVs (or intermediate phenotype) in a wide range of MR studies to infer the causal association between DNA methylation and complex diseases [11, 12, 14, 15], bridging the GWAS gap regarding SNPs to diseases. Therefore, we explored the casual DNA methylation for metabolic traits and AD with the cis-mQTLs determined DNA methylation as IVs. Incorporating the cis-mQTLs information into GWAS analyses, demonstrated a high potential to increase the power of GWAS in identifying loci associated with metabolic traits and AD, and improve the explanation of traits variance. The large number of SNPs was included as IVs for metabolic traits and AD; therefore, the sensitivity analysis was performed to rule out the issue of pleiotropy and linkage disequilibrium. The findings were robust in sensitivity analyses with different IVs. Secondly, these null findings suggest that the associations between metabolic factors and AD could attribute from the reverse causation bias [20]. Our bidirectional MR might reduce the bias, and the reverse MR analyses showed no significant association of cis-mQTLs determined AD with metabolic traits. The consistent causal direction indicated that the association of AD with the metabolic dysregulation were unlikely to be causal.

The genetic linkage and association studies have identified some AD susceptibility genes, a number of which are related to cholesterol metabolism or transport [3, 17, 32]. Lipid metabolism play an important pathway involved in the development of AD [32, 33]. However, contradictory evidence comes from epidemiological studies showing no or controversial association between dyslipidemia and AD risk [34–36]. In our study, cis-mQTLs determined DNA methylation to higher TG is associated with a higher AD risk (i.e., SNP→DNA methylation→higher TC→AD). Several studies reported that lipid-lowering medications-statins were of a protective effect against the development of AD [36, 37]. In addition, high cholesterol in late life was associated with decreased AD risk [35, 36], which may be explained by the timing of the cholesterol measurements in relationship to age and the clinical onset of AD. Taken together, inherited lifetime exposure to higher TC was associated with higher AD risk, however, the patients with higher TC and took lipid-lowering medications might reduce the risk of AD. Further analysis of AD outcomes in total cholesterol intervention trials is warranted. In addition, our findings suggest the imperative need for further investigation of the possibility that lipid-lowering medications might be of an independent effect on the prognosis of AD risk without dyslipidemia.

In the present study, we found no evidence to support causal associations of cis-mQTLs determined T2D and obesity with AD, and vice versa. The result was in line with the previously reported MR result [20, 28, 29]. For metabolic dysregulation, T2D was the only risk factor with convincing evidence for an association with AD [5, 6, 38]. The increasing evidence showed that obesity in midlife was associated with the risk of AD [5, 39, 40]. A wide range of studies suggest the genetic and pathological links between AD and T2D/obesity [17, 40, 41]. DNA methylation plays an important regulatory role in the pathogenesis of T2D, obesity and AD [11, 14]. In addition, our study identified that AD and T2D/obesity share several common genetic and epigenetic architectures. These findings indicated that the casual DNA methylation overlapped between AD and T2D/obesity might attribute to pleiotropy, shedding light on molecular mechanisms of DNA methylation underlying these comorbidities.

Almost half of the overlapped epigenetic architectures changing in AD and metabolic dysregulation were in the reverse direction. It is important to recognize that the relationship between AD and metabolic dysregulation is far from simple. These findings open the path toward a more detailed investigation of etiologic processes linking metabolic dysregulation and AD. The understanding of the epigenetic mechanisms of these diseases are likely only the tip of the iceberg. The potential downstream effects of DNA methylation (known as cis- gene expression- quantitative trait methylation analysis) on disease etiology still needs to be further verified. To the best of our knowledge, this is the first study to explore possible biological mechanisms in the causal pathway from DNA methylation to AD. There is considerable merit for using cis-mQTLs along with metabolic traits related cis-mQTLs to reveal much broader and more complex networks underlying genetic variant-AD associations. However, most causal DNA methylation of CpG-sites directly affect AD and are independent of metabolic dysregulation (SNP→DNA methylation→AD), which also suggests that AD is a highly heritable disease and DNA methylation is the critical the heritable epigenetic marks of the genome linked to AD.

Given the multifactorial etiology of AD, multidomain interventions that target several risk factors and mechanisms simultaneously might be necessary for an optimal preventive effect on AD. The previous study indicated that a third of AD cases might be attributable to modifiable factors such as diabetes mellitus, mid-life obesity and hypertension, physical activity, depression, smoking and low educational attainment [5, 6, 42]. Aside from metabolic traits, other modifiable factors also play important role in AD. Future researches are supposed to explore the association with DNA methylation, other modifiable factors and AD.

Limitation

The main strength of our study is the large sample size accrued from the GWAS summary statistics, enabling us to examine the causal relationship among DNA methylation, metabolic risk factors, and AD. However, some limitations should be considered when interpreting the findings. Since all participants in summary data are of European ancestry, population stratification is less likely to be a problem. However, the results of this study are of less necessarily to be validated in other ethnic groups. We only investigated CpG sites using cis-mQTL with the lowest *P*-value in order to improve the specificity of the IV for each CpG, which also could induce the bias of winner curse. Although the causal network is generally high-dimensional and unknown, the direction of potential causal relationships between layers of data can often be deduced from external biological knowledge. The putative causal associations reported in the network with bidirectional MR study are not definitive due to the cross-sectional observational nature of our study. Individual patient-level genotype and phenotype information are unavailable, which limit our exploration of other exposures. Our approach has the attractive advantage of enabling the interrogation of the potential epigenetic-complex trait interplay on a much wider scale by foregoing the requirement that “omic” data, environmental exposures and phenotypes are measured in the same sample for future study.

Conclusion

Taken together, we found that cis-mQTLs determined DNA methylation to higher TC was associated with higher AD risk. In contrast, the relation cis-mQTLs determined AD and metabolic dysregulation was unlikely to be causal. Further analysis of AD outcomes in total cholesterol intervention trials is warranted. In addition, our findings provided evidence that endeavors leveraging cis-mQTLs data could help to further characterize the complex networks of relationships between DNA methylation and complex traits. The continued research for additional risk factors for characterizing the relationship between DNA methylation and AD are still warranted.

Abbreviations

AD: Alzheimer's disease; BMI: body mass index; cis-mQTL: cis-DNA methylation quantitative trait loci; FI: fasting insulin; FG: fasting glucose; LD: linkage equilibrium; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; IVs: instrumental variables; IVW: inverse-variance weighted; GWAS: genome-wide association studies; MR: Mendelian randomization; mQTLs: DNA methylation quantitative trait locis; TC total cholesterol; TG: triglycerides; WHR: waist-hip ratio.

Declarations

Acknowledgments

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Disclosure

The authors have no conflict of interest to report.

Authors' contributions

DL, YW, and QM conceived the idea for the study. DL and WC obtained the genetic data. DL, ZY and WC performed the data analyses. DL and ZY interpreted the results of the data analyses. All authors wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Supplementary Figure Legends

Figure S1. Manhattan plot for the association between cis-mQTLs and DNA methylation.

Each point represents the association of a cis- mQTL SNP with CpG. In the plot, we only kept the cis- mQTL SNP having the smallest P-value for a CpG in the cases of multiple SNPs associated with the same CpG. The horizontal coordinate represents the location of a CpG within the chromosome. Points represent $-\log_{10}$ p values (y axis) for CpGs according to their location (x axis). Effects that survived the multiple-testing threshold in our analysis ($P < 1 \times 10^{-10}$) are above the red horizontal line.

Figure S2. Venn diagram for the casual DNA methylation of BMI and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between BMI and AD is shown. The consistency means that the causal CpGs overlapping between BMI and AD changes in the same direction.

AD indicates Alzheimer disease; BMI: body mass index.

Figure S3. Venn diagram for the casual DNA methylation of WHR and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between WHR and AD is shown. The consistency means that the causal CpGs overlapping between WHR and AD changes in the same direction.

AD indicates Alzheimer disease; WHR, waist-hip ratio.

Figure S4. Venn diagram for the casual DNA methylation of FG and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between FG and AD is shown. The consistency means that the causal CpGs overlapping between FG and AD changes in the same direction.

AD indicates Alzheimer disease; FG, fasting glucose.

Figure S5. Venn diagram for the casual DNA methylation of FI and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between FI and AD is shown. The consistency means that the causal CpGs overlapping between FI and AD changes in the same direction.

AD indicates Alzheimer disease; FI, fasting insulin.

Figure S6. Venn diagram for the casual DNA methylation of HDL and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between HDL and AD is shown. The consistency means that the causal CpGs overlapping between HDL and AD changes in the same direction.

AD indicates Alzheimer disease; HDL, high density lipoprotein cholesterol.

Figure S7. Venn diagram for the casual DNA methylation of LDL and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between LDL and AD is shown. The consistency means that the causal CpGs overlapping between LDL and AD changes in the same direction.

AD indicates Alzheimer disease; LDL, low density lipoprotein cholesterol.

Figure S8. Venn diagram for the casual DNA methylation of TC and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between TC and AD is shown. The consistency means that the causal CpGs overlapping between TC and AD changes in the same direction.

AD indicates Alzheimer disease; TC, total cholesterol.

Figure S9. Venn diagram for the casual DNA methylation of TG and AD.

The plot was performed by R package “VennDiagram”. The number of DNA methylation of CpG-sites overlapping between TG and AD is shown. The consistency means that the causal CpGs overlapping between TG and AD changes in the same direction.

AD indicates Alzheimer disease; TG, triglycerides.

Figures

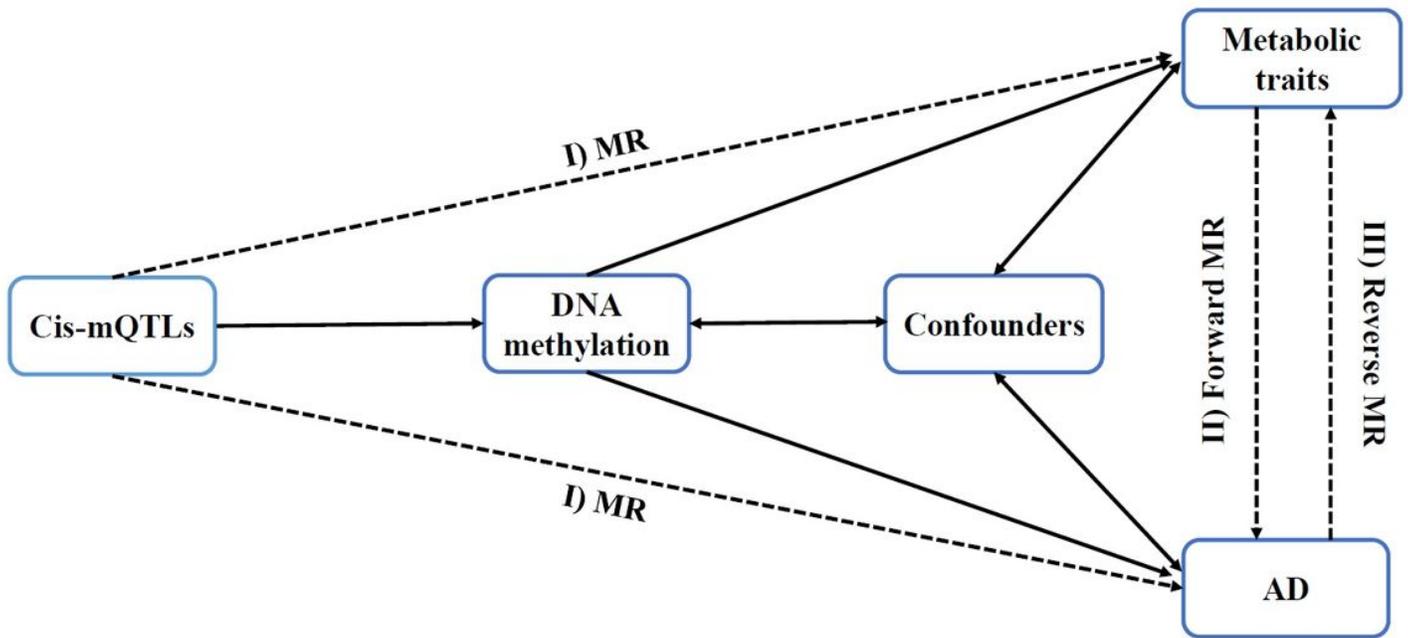


Figure 1
 Network with bidirectional Mendelian randomization analysis framework. The solid lines depict the true potential causal diagram. The dashed lines represent the parameters that need to be estimated. AD, Alzheimer disease; mQTL, DNA methylation quantitative trait loci; MR, mendelian randomization

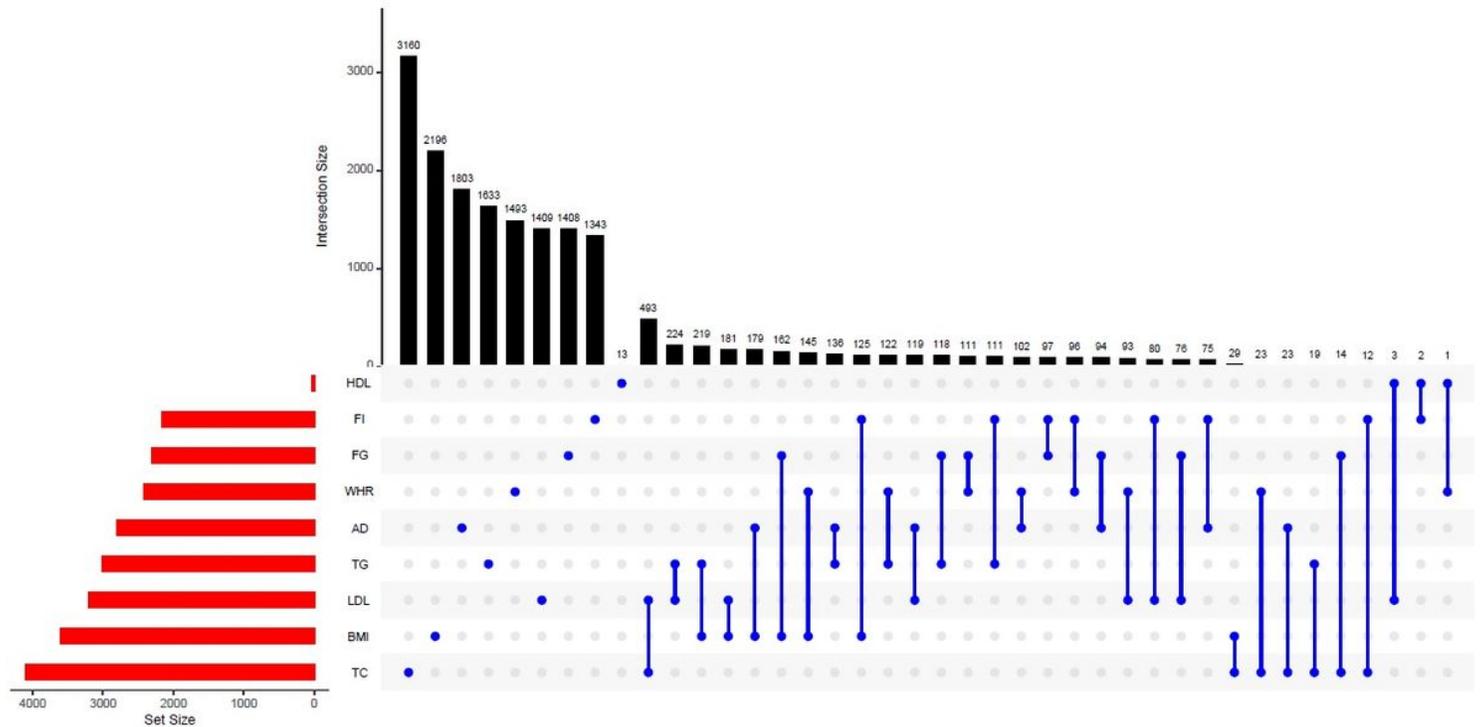


Figure 2
 Upset plot for the casual DNA methylation overlapping between metabolic traits and AD. The plot was performed by R package “UpSetR”. The number of DNA methylation of CpG-sites overlapping between metabolic traits and AD is shown. AD indicates Alzheimer disease; BMI: body mass index; FI, fasting insulin; FG, fasting glucose; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; WHR, waist-hip ratio.

Supplementary Files

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