TITLE: Mendelian randomization of blood metabolites suggests circulating glutamine protects against late-onset Alzheimer's disease

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ABSTRACT:

BACKGROUND: Late-onset Alzheimer's disease (LOAD) represents a growing health burden. Previous studies suggest that blood metabolite levels influence risk of LOAD.

OBJECTIVE: We used a genetics-based study design which may overcome limitations of other epidemiological studies to assess the influence of metabolite levels on LOAD risk.

METHODS: We applied Mendelian randomization (MR) to evaluate bidirectional causal effects using summary statistics from the largest genome-wide association studies (GWAS) of 249 blood metabolites (n=115,082) and GWAS of LOAD (n_{case}=21,982, $n_{control}$=41,944).

RESULTS: MR analysis of metabolites as exposures revealed a negative association of genetically-predicted glutamine levels with LOAD (Odds Ratio (OR)=0.83, 95%CI=0.73, 0.92) that was consistent in multiple sensitivity analyses. We also identified a positive association of genetically-predicted free cholesterol levels in small LDL (OR=1.79, 95%CI=1.36, 2.22) on LOAD. Using genetically-predicted LOAD as the exposure, we identified associations with phospholipids to total lipids ratio in large LDL (OR=0.96, 95%CI=0.94, 0.98), but not with glutamine, suggesting that the relationship between glutamine and LOAD is unidirectional.

CONCLUSIONS: Our findings support previous evidence that higher circulating levels of glutamine may be a target for protection against LOAD.

KEYWORDS: Alzheimer's Disease; Glutamine; Metabolites; Mendelian Randomization

1. INTRODUCTION:

Late Onset Alzheimer's Disease (LOAD) – a devastating age-related neurodegenerative disorder and the leading cause of dementia [1] – remains an urgent public health crisis. Today, the global prevalence of LOAD is 24 million and is expected to double by 2040, increasing the burden of disease on patients, families, and health-care systems [2]. Recent studies suggest that altered levels of circulating biomarkers in the blood, such as high-density lipoprotein cholesterol and glucose, may reduce the risk of LOAD [3]. With limited effective prevention or treatments for LOAD available [1,4], further investigation of the causal effect of blood metabolites on LOAD may yield insights for advancing prevention strategies, and informing public health interventions.

Blood metabolites (molecules <1,500 atomic mass units) are the end-products of biological processes. Due to their ability to cross the blood-brain-barrier, and their potential modification through diet and lifestyle interventions, metabolites may represent a promising target for prevention or treatment. Previous epidemiological studies suggest associations between metabolite levels such as lipids and branched chain amino acids with the incidence of LOAD [5–8]. However, these types of observational studies are susceptible to biases such as confounding and reverse causation, making it difficult to establish causal relationships.

Mendelian Randomization (MR) offers an alternative study design that uses single nucleotide polymorphisms (SNPs) in the DNA sequence as instrumental variables to assess causal inferences between an exposure and an outcome of interest [9]. Similar to randomized control trials (RCTs), MR studies leverage the random allocation of alleles at gametogenesis to assess causal effects. MR designs may therefore reduce susceptibility to reverse causation and confounding which would otherwise impede interpretation of traditional study designs. Recent

MR studies have explored the associations between blood metabolites and LOAD, suggesting that glutamine protects against LOAD [10,11]. Additional evidence from Lord et al. identified free cholesterol in extra-large high density lipoproteins as a protective factor against LOAD [11]. Previous MR studies have been based on GWAS of metabolites from a relatively small sample (n=24,925).

In this study, we performed MR of metabolite concentration with LOAD using much larger GWAS data on 249 blood metabolites (n=115,082). We aimed to identify causal relationships between metabolite concentrations and LOAD in the context of previous evidence, and further evaluate additional metabolomic exposures not included in previous analyses. We further examined bi-directional effects to assess the mutual exclusivity of causal relationships. A greater understanding of the etiology of LOAD may result in novel preventative and treatment strategies.

2. METHODS:

Study Design. We conducted bi-directional MR analyses of metabolites and LOAD. GWAS summary statistics were derived from non-overlapping samples to facilitate a two-sample MR study, which is less prone to weak instrument bias compared to one-sample MR [12]. We utilized the largest and most recent GWAS of metabolomic data from the UK Biobank (UKB) [13]. Briefly, the UKB is an ongoing prospective cohort study including 502,639 participants aged 37–70 in the United Kingdom. A random subset of participants were measured using targeted high-throughput NMR metabolomics on non-fasting baseline plasma samples (Nightingale Health Ltd; biomarker quantification version 2020). Metabolomic measures were quantified through 249 biomarkers, including lipids, amino acids, ketone bodies and glycolysis metabolites, on sample sizes between n=110,051 and n=115,082, after quality control procedures. Each metabolite was inverse-normalized to have a mean 0 and standard deviation of

1. GWAS summary statistics were adjusted for age, sex, fasting time, and genotyping chip, as previously described [14,15]. Summary statistics from these GWAS were obtained from the OpenGWAS platform (https://gwas.mrcieu.ac.uk/). Ethical approval of the UKB was provided by the Northwest Multicentre Research Ethics Committee, the National Information Governance Board for Health & Social Care, and the Community Health Index Advisory Group. No selection criteria were applied to sampling the entire UKB population. Details of the design of the UKB have been previously reported [16].

The GWAS for LOAD was taken from the recent meta-analysis by Kunkle et al. [17]. This study consisted of three stages, including a discovery phase (N=63,926), a replication phase (N=18,845), and a post-replication phase (N=11,666). We conducted parallel analyses using the previously released GWAS meta-analysis of LOAD from Lambert et al. [18]. This study consisted of two stages, including a discovery phase (N= 55,134), and a replication phase (N=19,884). It is important to note that the Lambert et al. and Kunkle et al. samples are not completely independent, as the sample used in Lambert et al. is a subset of the sample used in Kunkle et al. Both of these GWAS meta-analyses arose from the International Genomics of Alzheimer's Project, which includes four large consortia: Alzheimer's Disease Genetic Consortium (ADGC), the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE), the European Alzheimer's Disease Initiative (EADI), and the Genetics and Environmental Alzheimer's Disease Initiative (GERAD). Participants are non-Hispanic Whites, and cases are defined based on having a clinical diagnosis while controls are cognitively healthy, as previously described [18]. We utilized the stage one summary data from both studies for MR analyses. A flow diagram summarizing the methodology is detailed in Figure 1. Although there have been more recent larger GWAS meta-analyses of LOAD, we have opted not to use these in our primary analyses, because of the biases and reduced SNP heritability that have been demonstrated [19–21], which likely resulted from the use of proxy-cases, the inclusion of a very large number of young controls, among other potential factors. While recognizing these limitations, we performed an additional MR analysis of our main finding, glutamine, with the Alzheimer's disease and related dementias outcome from the more recent and larger Bellenguez et al. [22] GWAS meta-analysis, consisting of 85,934 cases and 401,577 controls.

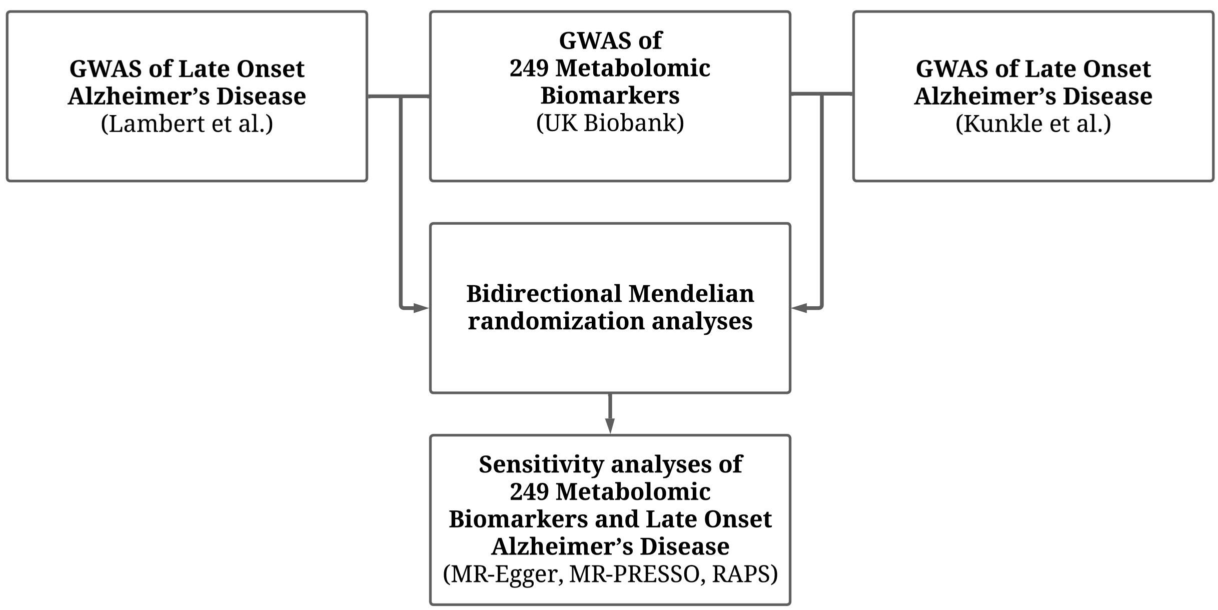

Figure 1. *Study Design. Flowchart describing sequence of analytical steps.*

2.1.Instrument selection:

Single nucleotide polymorphisms (SNP) were selected at the genome-wide significance threshold ($p<5.0 \times 10^{-8}$). SNPs in linkage disequilibrium were clumped to account for correlated genetic variants (clumping window of 10,000 kb, $r^2<0.001$), and the SNP with the lowest p-value is retained in a given genomic region. Proxies for genetic variants missing in outcome GWAS were identified using the 1,000 Genomes European sample data. Alleles were harmonized to ensure that the same allele was being referenced for exposure and outcome effect sizes. We attempted to infer the positive strand allele using allele frequencies for palindromic SNPs. We did not manually “prune” for genetic variants with suspected associations with outcome phenotypes as it risks the removal of only variants with a directional effect [9].

2.2. Analyses:

MR associations were first estimated using the inverse variance weighted (IVW) linear regression. Post hoc sensitivity analyses included the MR-Egger, weighted median, weighted mode, MR Robust Adjusted Profile Score (RAPS), and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) methods. Briefly, MR-Egger regression assumes linearity and homogeneity in the associations between the genetic variants, risk factor, and outcome. No assumption is made about the validity of the genetic variants. Egger regression provides an estimate of the causal effect that is consistent asymptotically even if all the genetic variants have pleiotropic effects on the outcome [23]. The weighted median and weighted mode derive causal estimates by assigning greater weight for variants with more precise ratio estimates. However, both mode- and median-based estimates are more vulnerable to horizontal pleiotropy [24]. MR-RAPS uses a profile-likelihood function to estimate the variance of the pleiotropic effect distribution, down-weighting outliers in causal estimation [25]. MR-PRESSO down-weights outliers by comparing the residual sum of squares against the expected distance under the null hypothesis of no pleiotropic effect [26]. Beta coefficients were reported as Odds Ratios (OR).

We initially applied a significance threshold of $p<0.05$ for bidirectional IVW estimates. To account for multiple testing, we subsequently applied an adjusted significance threshold of $p<2.0 \times 10^{-4}$, based on a conservative Bonferroni correction for the number of metabolites tested (n=249). At this type-1 error rate, we estimate [27] that we have 80% power to detect an OR as small as 1.2 with a sample size of 55,000, a case proportion of 30%, and a genetic instrument explaining 5% of the variance in metabolite level. The validity of MR estimates are dependent on three core assumptions: (1) the relevance assumption, meaning selected variants must be directly associated with the exposure; (2) the independence assumption that unmeasured confounders are not associated with the outcome; (3) and the exclusion restriction assumption that genetic variants are not pleiotropically associated with the exposure.

We performed additional sensitivity analyses to evaluate the MR assumptions for estimates meeting the adjusted threshold. Instrument strength was calculated using the F-statistic, with instruments exhibiting an F>10 considered sufficient to meet the first assumption. Next, heterogeneity was calculated using Cochran's Q to assess pleiotropic effects. We performed a visual assessment of IVW regression results, funnel plots and leave-one-out (LOO) analyses to investigate the influence of outlier SNPs on causal estimates. We applied the I^2_{GX} statistic to evaluate the reliability of the Egger regression results. We performed an additional Steiger filtering sensitivity analysis to remove genetic variants explaining greater variance of the exposure trait than of the outcome. Given its large effect on LOAD as well as effects on circulating lipid levels, we additionally conducted a separate analysis to evaluate the influence of the *APOE ε4* allele by removing IVs within 500kb of the rs429358 variant. Briefly, the rs429358 variant has been observed at lower frequencies among older participants in the UK Biobank, suggesting a potential selection bias for older participants with the risk allele to have longer, healthier lives [28]. All data extraction, processing and analyses were performed using the "TwoSampleMR" package in R version 3.6.3 (R Foundation for Statistical Computing) [29,30].

3. RESULTS:

Metabolomic Exposures. We identified 25 nominally significant (p<0.05) MR IVW associations between blood metabolite exposures and LOAD as an outcome (Supplementary Table 1). Using the Kunkle et al. GWAS, both glutamine (Odds Ratio (OR)=0.83, 95% CI=0.73, 0.92) and phospholipids to total lipids ratio in chylomicrons and extremely large VLDL (XXL.VLDL.PL.pct) (OR=0.74, 95%CI=0.50, 0.99) exhibited a protective effect on LOAD, while isoleucine (OR=1.53 ,95% CI=1.27, 1.79) and free cholesterol levels in LDL particles (LDL.FC) (OR=1.69, 95% CI=1.02, 2.83) were suggested to increase risk of LOAD. Phospholipids to total lipids ratio in very large HDL (XL.HDL.PL.pct) (OR=0.89, 95% CI=0.80, 0.98) and phospholipids to total lipids ratio in small HDL (S.HDL.PL.pct) (OR=0.54, 95% CI=0.04, 1.04) were additionally identified as potential protective markers against LOAD. We identified increased risk from glucose (OR=1.29, 95% CI=1.10, 1.47) and phospholipid concentration measures, including phospholipids in LDL (LDL.PL) (OR=1.69, 95% CI=1.21, 2.18), phospholipids in large LDL (L.LDL.PL) (OR=1.71, 95% CI=1.20, 2.22), and phospholipids in medium LDL (M.LDL.PL) (OR=1.68, 95% CI=1.20, 2.20). A complete list of identified associations is provided in Figure 2.

Using the LOAD summary statistics from the Lambert et al. GWAS, we identified 9 of the nominally significant associations in Kunkle et al. analyses, including protective effects from glutamine (OR=0.82, 95% CI=0.70, 0.94) and XXL.VLDL.PL.pct (OR=0.81, 95% CI=0.62, 0.99). Metabolomic exposures increasing risk of LOAD included isoleucine (OR=1.44, 95% CI=1.14, 1.74), and free cholesterol levels in large LDL (L.LDL.FC) (OR=1.08, 95%CI=1.02, 1.14), medium LDL (M.LDL.FC) (OR=1.08, 95% CI=1.02, 1.14), and small LDL (S.LDL.FC) (OR=1.09, 95% CI=1.02, 1.16). We further identified Cholesteryl esters to total lipids ratio in large LDL (L.LDL.CE.pct) as a risk factor for LOAD (OR=1.05, 95% CI=1.02, 1.08), which was not identified in the parallel analysis using the Kunkle et al. GWAS.

Figure 2. *Bi-directional Inverse Variance Weighted Mendelian Randomization Estimates for metabolites with at least one nominally significant association.*

After applying our corrected significance threshold, only glutamine was found to be a significant protective exposure using the Kunkle et al. GWAS. For glutamine as an exposure, we identified 32 genome-wide significant instruments ($p<5\times10^{-8}$) after clumping in the Kunkle et al GWAS (Supplementary Table 2). Sensitivity analyses including median, mode, MR-RAPS, and MR-PRESSO estimates yielded protective effects consistent with the IVW estimate (Figure 3). F-statistics suggested that all instruments were sufficiently associated with glutamine concentration (F >10, Supplementary Table 3). Cochran's Q (Supplementary Table 4) and the I^2_{GX} statistic (Supplementary Table 5) did not suggest statistically significant heterogeneity. MR-PRESSO analysis did not identify outlier variants (Supplementary Table 6). We did not identify directional changes for any associations after Steiger filtering (Supplementary Table 7). Inspection of forest plots and LOO analyses did not reveal heterogeneity or influential SNPs driving associations (Supplementary Figures 1 and 2). Additional sensitivity analyses for glutamine instruments did not identify SNPs within 500kb of the *APOE* gene. MR analyses of glutamine exposure using the Bellenguez et al. GWAS summary statistics for Alzheimer's disease and related dementias as our outcome, revealed slightly attenuated magnitudes of association as those obtained with LOAD GWAS outcomes (IVW OR=0.90, 95% CI=0.84, 0.95; see Supplementary Table 8).

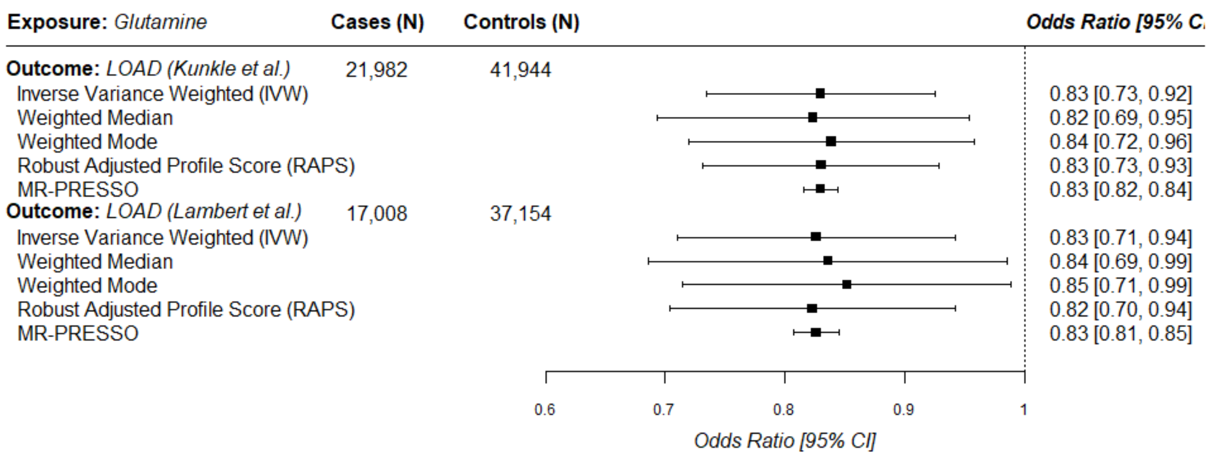

Figure 3. *Univariable Mendelian Randomization Estimates for Glutamine on LOAD Outcomes.*

Bidirectional analysis revealed 175 significant associations between LOAD exposures and blood metabolite outcomes at the p<0.05 threshold (Supplementary Table 9). Using the LOAD summary statistics from Lambert et al. we identified 98 significant associations, including 6 that were not identified using the Kunkle et al. GWAS. We subsequently identified 77 significant associations using the Kunkle et al. GWAS (Supplementary Table 9). Among the 25 associations initially identified using blood metabolites as our exposure, we identified a bidirectional effect among 22 measures, with the exceptions of glutamine, XXL.VLDL.PL.pct, and glucose (Figure 2).

After applying our adjusted significance threshold, we identified four associations using the GWAS from Kunkle et al. Using the GWAS from Lambert et al. as an exposure, we identified five additional non-overlapping associations with blood metabolite outcomes (see Figure 4 and Supplementary Table 9). Although all associations using the exposure GWAS from Lambert et al. – including cholesterol in very large HDL (XL.HDL.C), total lipids in very large HDL (XL.HDL.L), Cholesteryl esters in very large HDL (XL.HDL.CE), and Sphingomyelins – were nominally significant using the parallel GWAS from Kunkle et al., they did not suggest an association after applying our corrected significance threshold (see Figure 4). Similarly, all associations using the exposure GWAS from Kunkle et al. did not meet our corrected significance threshold in analyses using the LOAD GWAS from Lambert et al.

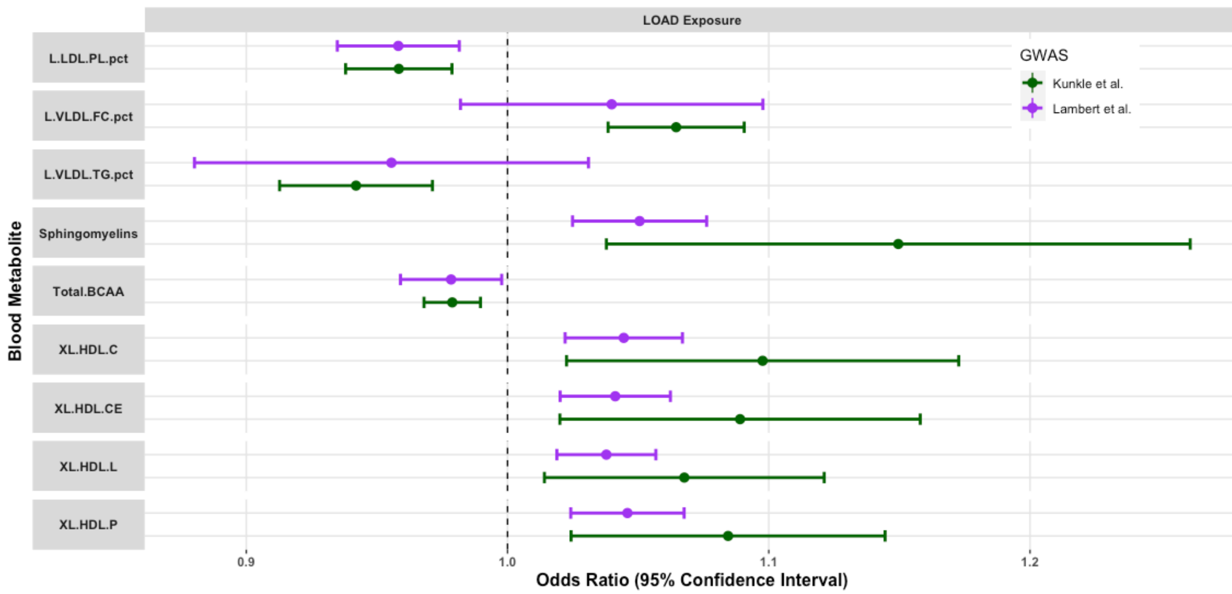

Figure 4. *Statistically Significant (at Bonferroni-Adjusted Threshold) Inverse Variance Weighted Mendelian Randomization Estimates for either Kunkle et al. or Lambert et al. LOAD on Blood Metabolites*

Sensitivity analyses for these measures including median, mode, MR-RAPS, and MR-PRESSO estimates yielded protective effects consistent with the IVW estimate. F-statistics suggested that all instruments were sufficiently associated with LOAD (F>10). Cochran's Q and the I^2_{GX} statistic did not suggest statistically significant heterogeneity. MR-PRESSO analysis did not identify outlier variants. Inspection of forest plots and LOO analyses did not reveal heterogeneity or influential SNPs driving associations. Additional sensitivity analyses removing SNPs within 500kb of the *APOE* gene were consistent with our primary analysis.

4. DISCUSSION:

In this study, we conducted bi-directional MR analyses on the largest set of GWAS summary statistics for blood metabolites and LOAD available to date. Across multiple sensitivity analyses, glutamine was shown to be a robust protective marker against LOAD. We additionally identified 24 metabolite exposures with nominal effects on LOAD, and 175 associations for LOAD on metabolite outcomes. We additionally identified nominal evidence suggesting an increased risk of LOAD from higher levels of both ‘phospholipids to total lipids ratio in chylomicrons and extremely large VLDL’ and circulating glucose. In our bi-directional analysis using LOAD as an exposure, we identified evidence suggesting associations with increased levels of ‘Concentration of very large HDL particles’ and decreased levels of ‘Phospholipids to total lipids ratio in large LDL’ and ‘Total BCAA’, but not with glutamine levels.

Previous epidemiological studies have suggested an association between blood metabolite levels and incidence of LOAD. In a recent analysis by Chen and Herrup [31], LOAD was suggested to directly reduce glutamine synthetase activity, as glutamine metabolism was shown to be compromised among LOAD patients. Our findings that genetically-predicted glutamine level is associated with lower risk of LOAD are consistent with previous traditional observational studies suggesting that higher circulating levels of glutamine are protective against LOAD [31]. A recent study from Buergel and colleagues identified creatinine, albumin and glutamine as predictive risk factors for all-cause dementia [32]. These findings have been further substantiated in two recent MR analyses, both using the same smaller metabolite GWAS than the one we used in our study, the two of which do not overlap [10,11]. Although we identified some evidence of MR associations for lipid metabolites, these typically displayed a bidirectional effect, leaving the causal direction of these markers uncertain. In contrast to previous MR studies, we found limited evidence of protective effects from higher concentrations of HDL particles. A recent study from Lorde and colleagues [33] identified protective effects against LOAD from free cholesterol in very large HDLs, total lipids in very large HDLs, phospholipids in very large HDLs, and concentration of very large HDL particles. However, we were unable to replicate these associations at any level of significance in our analyses, suggesting potentially limited generalizability of these previous findings.

Although it is not clear whether levels of glutamine in blood reflect levels in the brain [34], glutamine can be a precursor for the excitatory neurotransmitter, glutamate, and the inhibitory neurotransmitter gamma-amino butyric acid (GABA). Alterations in glutamate and GABA-ergic circuits may underlie neural network disruptions in LOAD [35]. Glutamine production in the brain, catalyzed by glutamine synthetase, is primary to the astrocytes and putatively controlled by feedback mechanisms based on the levels of ammonia and glutamate [36,37]. Glutamine synthetase in astrocytes converts glutamate and ammonia to glutamine and maintains homeostasis, which might be altered in LOAD [38]. Autophagy, a mechanism through which cells recycle and degrade, diminishes with age, which may further help long-living neurons remove protein remnants. Evidence supports that glutamine-deficient cells could reduce autophagy and may thus contribute to LOAD [31]. There is also evidence to suggest that glutamine is connected to inflammation and beta-amyloid levels [39]. Although most studies find a potential protective effect of glutamine on LOAD or related traits, some have found that glutamine levels are associated with increased risk, such as a study that found increased levels of glutamine in the cerebrospinal fluid of patients with Alzheimer’s disease [40], and a study that found higher levels of glutamine associated with an increased risk of dementia, Alzheimer’s disease, and lower cognitive function [41]. These conflicting results suggest the need for further study into the role of glutamine in neurodegenerative disease.

Our study has several strengths, including the use of the largest set of GWAS summary statistics for blood metabolites to date, which corroborate some and fail to support findings from previous studies on the effect of blood metabolites on LOAD. We performed analyses across two LOAD GWAS to identify consistent causal effects. Statistically significant associations were further required to meet an adjusted significance threshold ($p<2.0 \times 10^{-4}$) to reduce the odds of Type I error. We additionally applied a broad suite of sensitivity analyses and identified a consistent protective effect on LOAD from glutamine levels. Our study is also subject to several limitations. Although we used two LOAD GWAS in our main set of analyses, the samples used in these GWAS overlap, and thus cannot be considered to provide independent replication. Differences that we observed in the MR estimates between the two LOAD GWAS are therefore attributable to the additional samples included in the Kunkle et al GWAS. Second, we note that MR designs are inherently vulnerable to horizontal pleiotropy, which would violate the assumption of an independent effect on the exposure. Critically, sensitivity analyses did not identify a pleiotropic effect in analyses using glutamine as the exposure or outcome. We were additionally able to validate results across more conservative tests such as MR-EGGER, MR-RAPS, and MR-PRESSO, suggesting little evidence of directional pleiotropy biasing observed effects.

We have identified glutamine as a potential protective factor in the development of LOAD, confirming prior research. Further exploration of the mechanisms underlying this protection are warranted as they may enhance the clinical capacity to reduce the risk of cognitive decline and disease.

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DISCLOSURES:

No competing interest to declare.

DATA AVAILABILITY

Summary statistics from all GWAS used in this study are publicly available from the IEU OpenGWAS project (https://gwas.mrcieu.ac.uk/) from the Integrative Epidemiology Unit at the Medical Research Council (UK).

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Contents

Supplementary Table 1. Inverse variance weighted (IVW) Mendelian randomization estimates for blood metabolite exposures on LOAD outcomes for metabolites with at least one nominal ($p<0.05$) IVW association.

	Outcome: LOAD (Lambert et al.)				Outcome: LOAD (Kunkle et al.)			
Exposure	**SNPs (#)**	**Beta**	**SE**	***p***	**SNPs (#)**	**Beta**	**SE**	***p***
Glutamine	32	-0.19	0.06	0.00164	32	-0.19	0.05	0.00014
Glucose	18	0.24	0.12	0.05461	20	0.25	0.10	0.00846
IDL.FC.pct	32	0.16	0.08	0.05474	35	0.16	0.07	0.02308
Isoleucine	8	0.36	0.15	0.01826	8	0.43	0.13	0.00130
L.LDL.C	39	0.46	0.25	0.06813	41	0.50	0.25	0.04735
L.LDL.C.pct	46	0.47	0.24	0.05224	48	0.49	0.23	0.03417
L.LDL.FC	45	0.51	0.24	0.03585	47	0.51	0.23	0.02640
L.LDL.PL	38	0.48	0.26	0.06529	39	0.54	0.26	0.03964
LDL.C	38	0.47	0.26	0.06901	39	0.52	0.26	0.04606
LDL.FC	41	0.53	0.26	0.04033	43	0.55	0.24	0.02128
LDL.PL	39	0.48	0.25	0.05215	40	0.53	0.25	0.03289
LDL.size	26	0.79	0.40	0.04911	28	1.02	0.41	0.01178
M.LDL.FC	42	0.51	0.24	0.03510	43	0.55	0.24	0.02181
M.LDL.PL	38	0.46	0.25	0.06577	40	0.52	0.25	0.03480
M.LDL.PL.pct	25	0.28	0.15	0.05248	30	0.70	0.30	0.02018
S.HDL.PL.pct	43	-0.18	0.15	0.22664	47	-0.62	0.25	0.01455
S.LDL.C	40	0.47	0.25	0.05314	40	0.52	0.25	0.03804
S.LDL.FC	42	0.56	0.22	0.01298	44	0.58	0.22	0.00840
S.LDL.L	38	0.47	0.25	0.06013	40	0.52	0.24	0.03139
S.LDL.PL	41	0.47	0.22	0.03415	42	0.50	0.22	0.02275
S.VLDL.PL.pct	51	0.42	0.22	0.05837	58	0.40	0.20	0.04615
XL.HDL.PL.pct	50	-0.06	0.05	0.27523	49	-0.12	0.05	0.01542
XL.VLDL.PL.pct	39	0.62	0.48	0.19952	41	1.34	0.59	0.02362
XXL.VLDL.PL.pct	33	-0.22	0.10	0.02426	34	-0.30	0.12	0.01605

LOAD: Late Onset Alzheimer's Disease; SNPs: Single Nucleotide Polymorphisms; 95%CI: 95% Confidence Interval; LDL.size: Average diameter for LDL particles; Gln: Glutamine; L.LDL.FC: Free cholesterol in large LDL; Ile: Isoleucine; LDL.FC: Free cholesterol in LDL; S.LDL.PL: Phospholipids in small LDL; M.LDL.FC: Free cholesterol in medium LDL; L.LDL.CE.pct: Cholesteryl esters to total lipids ratio in large LD; XXL.VLDL.PL.pct: Phospholipids to total lipids ratio in chylomicrons and extremely large VLDL; S.LDL.FC: Free cholesterol in small LDL; M.LDL.PL.pct: Phospholipids to total lipids ratio in medium LDL; LDL.C: LDL cholesterol; XL.VLDL.PL.pct: Phospholipids to total lipids ratio in very large VLDL; L.LDL.PL: Phospholipids in large LDL; LDL.PL: Phospholipids in LDL; L.LDL.C.pct: Cholesterol to total lipids ratio in large LDL; S.VLDL.PL.pct: Phospholipids to total lipids ratio in small VLDL; M.LDL.PL: Phospholipids in medium LDL; XL.HDL.PL.pct: Phospholipids to total lipids ratio in very large HDL; S.LDL.C: Cholesterol in small LDL; S.HDL.PL.pct: Phospholipids to total lipids ratio in small HDL; S.LDL.L: Total lipids in small LDL; L.LDL.C: Cholesterol in large LDL; IDL.FC.pct: Free cholesterol to total lipids ratio in IDL

Supplementary Table 2. Association of genome-wide significant SNPs for glutamine with late-onset Alzheimer's Disease

SNP	Chr	Pos	EA	OA	N	EAF	BETA	SE	p	N	EAF	BETA	SE	p
						Exposure: *Glutamine*				Outcome: *Alzheimer's Disease (Lambert et al.)*				
rs10811663	9	22139220	A	G	115082	0.086	-0.047	0.008	<0.001	54162	--	-0.001	0.043	0.9888
rs113674212	12	47198856	A	C	115082	0.068	0.135	0.008	<0.001	54162	--	0.002	0.031	0.9377
rs117643180	17	7185779	A	C	115082	0.026	0.07	0.013	<0.001	54162	--	-0.017	0.057	0.7641
rs1260326	2	27730940	C	T	115082	0.604	0.079	0.004	<0.001	54162	--	0.001	0.016	0.9608
rs1274961	3	39191335	C	T	115082	0.783	-0.029	0.005	<0.001	54162	--	0.023	0.019	0.2377
rs13234131	7	73025975	G	A	115082	0.129	0.072	0.006	<0.001	54162	--	0.031	0.025	0.2135
rs147139486	5	128218645	A	G	115082	0.054	-0.051	0.009	<0.001	54162	--	0.05	0.033	0.129
rs1750768	10	22926227	T	C	115082	0.042	0.058	0.01	<0.001	54162	--	-0.039	0.04	0.3297
rs2039098	20	56112882	T	C	115082	0.597	-0.024	0.004	<0.001	54162	--	0.01	0.016	0.5166
rs2168101	11	8255408	A	C	115082	0.308	-0.067	0.005	<0.001	54162	--	0.006	0.023	0.8038
rs2657879	12	56865338	G	A	115082	0.183	-0.251	0.005	<0.001	54162	--	0.047	0.021	0.0242
rs28929474	14	94844947	T	C	115082	0.02	0.091	0.015	<0.001	54162	--	-0.06	0.118	0.6125
rs35261542	6	20675792	A	C	115082	0.261	0.03	0.005	<0.001	54162	--	-0.009	0.017	0.618
rs35752457	12	59945864	A	G	115082	0.164	0.066	0.006	<0.001	54162	--	-0.025	0.022	0.2636
rs3851365	3	160191236	A	C	115082	0.484	-0.042	0.004	<0.001	54162	--	0.029	0.015	0.0606
rs3988	4	18241903	G	A	115082	0.396	-0.022	0.004	<0.001	54162	--	0.024	0.016	0.1188
rs4128598	10	114785939	G	A	115082	0.467	-0.024	0.004	<0.001	54162	--	0.006	0.015	0.7103
rs4766214	12	4290249	G	A	115082	0.457	-0.029	0.004	<0.001	54162	--	-0.038	0.017	0.0251
rs56335308	8	17419461	A	G	115082	0.027	0.116	0.013	<0.001	54162	--	0.099	0.072	0.1718
rs58673065	7	1885600	G	A	115082	0.229	0.029	0.005	<0.001	54162	--	0.017	0.018	0.3608
rs62182473	2	191740996	T	C	115082	0.265	-0.071	0.005	<0.001	54162	--	0.005	0.018	0.7911
rs7078003	10	99359412	T	C	115082	0.178	0.104	0.005	<0.001	54162	--	-0.022	0.023	0.3351
rs7147721	14	75186010	G	A	115082	0.462	0.025	0.004	<0.001	54162	--	-0.041	0.016	0.0082
rs73186030	3	122013465	T	C	115082	0.13	0.032	0.006	<0.001	54162	--	-0.032	0.022	0.1505
rs738408	22	44324730	T	C	115082	0.217	0.033	0.005	<0.001	54162	--	-0.019	0.02	0.3458
rs78431863	11	74109553	T	C	115082	0.041	0.076	0.01	<0.001	54162	--	-0.029	0.037	0.4284
rs7925445	11	18398958	G	A	115082	0.556	0.028	0.004	<0.001	54162	--	-0.021	0.016	0.1844
rs7966322	12	121401846	T	C	115082	0.54	-0.025	0.004	<0.001	54162	--	0.022	0.018	0.2165
rs8074974	17	25595818	C	T	115082	0.45	0.027	0.004	<0.001	54162	--	0.008	0.016	0.6011
rs838737	2	234325052	A	G	115082	0.565	-0.024	0.004	<0.001	54162	--	0.029	0.016	0.063
rs9482770	6	127443092	C	T	115082	0.449	-0.033	0.004	<0.001	54162	--	0.02	0.016	0.2038
rs9687846	5	55861894	A	G	115082	0.201	-0.029	0.005	<0.001	54162	--	0.028	0.02	0.1602

							Exposure: *Glutamine*					Outcome: *Alzheimer's Disease (Kunkle et al.)*		
rs10811663	9	22139220	A	G	115082	0.086	-0.047	0.008	<0.001	63926	--	0.024	0.034	0.472
rs113674212	12	47198856	A	C	115082	0.068	0.135	0.008	<0.001	63926	--	-0.012	0.028	0.674
rs117643180	17	7185779	A	C	115082	0.026	0.07	0.013	<0.001	63926	--	0.046	0.047	0.325
rs1260326	2	27730940	C	T	115082	0.604	0.079	0.004	<0.001	63926	--	-0.015	0.014	0.31
rs1274961	3	39191335	C	T	115082	0.783	-0.029	0.005	<0.001	63926	--	0.022	0.018	0.209
rs13234131	7	73025975	G	A	115082	0.129	0.072	0.006	<0.001	63926	--	0.005	0.022	0.826
rs147139486	5	128218645	A	G	115082	0.054	-0.051	0.009	<0.001	63926	--	0.018	0.035	0.613
rs1750768	10	22926227	T	C	115082	0.042	0.058	0.01	<0.001	63926	--	-0.038	0.037	0.305
rs1998848	14	21492229	A	G	115082	0.011	0.167	0.021	<0.001	63926	--	-0.116	0.086	0.179
rs2039098	20	56112882	T	C	115082	0.597	-0.024	0.004	<0.001	63926	--	0.001	0.014	0.965
rs2168101	11	8255408	A	C	115082	0.308	-0.067	0.005	<0.001	63926	--	0.001	0.018	0.94
rs2657879	12	56865338	G	A	115082	0.183	-0.251	0.005	<0.001	63926	--	0.05	0.018	0.006
rs28929474	14	94844947	T	C	115082	0.02	0.091	0.015	<0.001	63926	--	-0.068	0.056	0.227
rs35261542	6	20675792	A	C	115082	0.261	0.03	0.005	<0.001	63926	--	-0.009	0.016	0.571
rs35752457	12	59945864	A	G	115082	0.164	0.066	0.006	<0.001	63926	--	-0.022	0.02	0.287
rs3851365	3	160191236	A	C	115082	0.484	-0.042	0.004	<0.001	63926	--	0.012	0.014	0.422
rs3988	4	18241903	G	A	115082	0.396	-0.022	0.004	<0.001	63926	--	0.022	0.014	0.138
rs4128598	10	114785939	G	A	115082	0.467	-0.024	0.004	<0.001	63926	--	0	0.014	0.991
rs4766214	12	4290249	G	A	115082	0.457	-0.029	0.004	<0.001	63926	--	-0.018	0.015	0.211
rs56335308	8	17419461	A	G	115082	0.027	0.116	0.013	<0.001	63926	--	0.048	0.056	0.398
rs58673065	7	1885600	G	A	115082	0.229	0.029	0.005	<0.001	63926	--	0.023	0.017	0.176
rs62182473	2	191740996	T	C	115082	0.265	-0.071	0.005	<0.001	63926	--	0.007	0.016	0.688
rs7078003	10	99359412	T	C	115082	0.178	0.104	0.005	<0.001	63926	--	-0.013	0.019	0.503
rs7147721	14	75186010	G	A	115082	0.462	0.025	0.004	<0.001	63926	--	-0.035	0.014	0.015
rs73186030	3	122013465	T	C	115082	0.13	0.032	0.006	<0.001	63926	--	-0.034	0.02	0.091
rs738408	22	44324730	T	C	115082	0.217	0.033	0.005	<0.001	63926	--	-0.012	0.018	0.514
rs78431863	11	74109553	T	C	115082	0.041	0.076	0.01	<0.001	63926	--	-0.031	0.036	0.382
rs7925445	11	18398958	G	A	115082	0.556	0.028	0.004	<0.001	63926	--	-0.012	0.014	0.405
rs8074974	17	25595818	C	T	115082	0.45	0.027	0.004	<0.001	63926	--	0.014	0.014	0.312
rs838737	2	234325052	A	G	115082	0.565	-0.024	0.004	<0.001	63926	--	0.017	0.014	0.236
rs9482770	6	127443092	C	T	115082	0.449	-0.033	0.004	<0.001	63926	--	0.011	0.014	0.462
rs9687846	5	55861894	A	G	115082	0.201	-0.029	0.005	<0.001	63926	--	0.019	0.018	0.284

Supplementary Table 3. F-Statistics for Mendelian Randomization Analyses for Glutamine and Alzheimer's Disease

Exposure	Outcome	SNPs (n)	Mean F	Range F
Glutamine	Alzheimer's Disease: Lambert et al.	36	153.44	27.86 -2208.96
Glutamine	Alzheimer's Disease: Kunkle et al.	37	154.42	27.86 -2208.96

Supplementary Table 4. Cochran's heterogeneity statistic for Inverse Variance Weighted Mendelian Randomization analyses for Glutamine and Alzheimer's Disease

Exposure	Outcome	SNPs (n)	Q Statistic	*p*
Glutamine	Alzheimer's Disease: Lambert et al.	36	37.71	0.189
Glutamine	Alzheimer's Disease: Kunkle et al.	37	25.69	0.736

Supplementary Table 5. I^2_{GX} statistic for Mendelian Randomization analyses of Glutamine and Alzheimer's Disease

Exposure	Outcome	SNPs (n)	I^2_{GX}
Glutamine	Alzheimer's Disease: Lambert et al.	36	0.985
Glutamine	Alzheimer's Disease: Kunkle et al.	37	0.986

Supplementary Table 6. MR-PRESSO Global and Distortion Analyses of Glutamine and Alzheimer's Disease

Exposure	Outcome	Global Test, *p*	Distortion Test, *p*
Glutamine	Alzheimer's Disease: Lambert et al.	0.762	--
Glutamine	Alzheimer's Disease: Kunkle et al.	0.257	--

Supplementary Table 7. Steiger Filtering of Mendelian Randomization Sensitivity Analyses of Glutamine and Alzheimer's Disease

Exposure	Outcome	Method	SNPs (n)	β	95%CI
Glutamine	Alzheimer's Disease: Lambert et al.	Inverse Variance Weighted	32	0.822	-0.317, -0.074
		Weighted Median	32	0.835	-0.328, -0.033
		Weighted Mode	32	0.847	-0.304, -0.03
		RAPS	32	0.819	-0.324, -0.076
		MR-PRESSO	32	0.823	-0.217, -0.174
Glutamine	Alzheimer's Disease: Kunkle et al.	Inverse Variance Weighted	32	0.826	-0.288, -0.093
		Weighted Median	32	0.823	-0.333, -0.057
		Weighted Mode	32	0.835	-0.303, -0.056
		RAPS	32	0.826	-0.292, -0.09
		MR-PRESSO	32	0.826	-0.207, -0.175

Supplementary Table 8. Mendelian randomization analyses of glutamine levels with Alzheimer's disease and related dementias using GWAS meta-analysis from Bellenguez et al., 2022 (n=85,934 cases, n=401,577 controls)

Exposure	Outcome	Method	SNPs (n)	OR	95% CI
Glutamine	Alzheimer's disease and related dementias: Bellenguez et al.	Inverse Variance Weighted	41	0.896	0.843, 0.952
		Weighted Median	41	0.873	0.809, 0.943
		MR-Egger	41	0.883	0.805, 0.968
		Simple Mode	41	0.794	0.652, 0.967
		Weighted Mode	41	0.858	0.797, 0.924

Supplementary Table 9. Inverse variance weighted (IVW) Mendelian randomization estimates for LOAD on blood metabolites for metabolites with at least one nominal (p<0.05) IVW association.

	Exposure: LOAD (Lambert et al.)				Exposure: LOAD (Kunkle et al.)			
Outcome	**SNPs (#)**	**Beta**	**SE**	***p***	**SNPs (#)**	**Beta**	**SE**	***p***
ApoA1	6	-0.02	0.02	0.31709	19	-0.04	0.02	0.02980
ApoB	6	0.09	0.04	0.02982	19	0.19	0.07	0.00775
ApoB.by.ApoA1	6	0.08	0.04	0.05980	19	0.18	0.07	0.00742
bOHbutyrate	6	0.02	0.01	0.24089	19	0.03	0.01	0.00444
Cholines	6	0.03	0.01	0.00240	19	0.05	0.02	0.03476
Clinical.LDL.C	6	0.08	0.03	0.01903	19	0.22	0.08	0.01020
Creatinine	6	0.00	0.01	0.74611	19	-0.02	0.01	0.03244
DHA	6	0.02	0.01	0.01266	19	0.04	0.01	0.01226
HDL.L	6	-0.02	0.02	0.37568	19	-0.03	0.02	0.03764
HDL.PL	6	-0.02	0.02	0.25796	19	-0.05	0.02	0.01086
HDL.TG	6	-0.01	0.01	0.25257	19	-0.09	0.04	0.03472
IDL.C	6	0.07	0.03	0.01853	19	0.20	0.08	0.01416
IDL.C.pct	6	0.02	0.01	0.08518	19	0.17	0.08	0.03380
IDL.CE	6	0.07	0.03	0.01936	19	0.21	0.09	0.01488
IDL.CE.pct	6	0.02	0.01	0.27272	19	0.17	0.09	0.04150
IDL.FC	6	0.07	0.03	0.01693	19	0.19	0.08	0.01213
IDL.FC.pct	6	0.02	0.01	0.11085	19	0.07	0.03	0.01999
IDL.L	6	0.07	0.03	0.02002	19	0.19	0.08	0.01245
IDL.P	6	0.08	0.04	0.03956	19	0.20	0.08	0.01287
IDL.PL	6	0.08	0.03	0.02202	19	0.18	0.07	0.01037
IDL.TG.pct	6	-0.04	0.02	0.00908	19	-0.17	0.08	0.02530
Ile	6	-0.01	0.01	0.13846	19	-0.02	0.01	0.00075
L.HDL.C.pct	6	0.05	0.02	0.00969	19	0.10	0.04	0.00970
L.HDL.CE.pct	6	0.04	0.02	0.04436	19	0.08	0.03	0.00974
L.HDL.FC.pct	6	0.07	0.03	0.01077	19	0.17	0.07	0.01189
L.HDL.PL.pct	6	-0.07	0.03	0.02398	19	-0.10	0.03	0.00067
L.HDL.TG	6	-0.01	0.01	0.22345	19	-0.09	0.04	0.03785
L.LDL.C	6	0.08	0.03	0.01162	19	0.21	0.08	0.00840
L.LDL.C.pct	6	0.06	0.02	0.00063	19	0.18	0.07	0.01027
L.LDL.CE	6	0.08	0.03	0.01205	19	0.21	0.08	0.00790
L.LDL.CE.pct	6	0.05	0.02	0.00225	19	0.10	0.03	0.00316
L.LDL.FC	6	0.08	0.03	0.01101	19	0.22	0.09	0.00968
L.LDL.FC.pct	6	0.02	0.01	0.03105	19	0.13	0.06	0.02917
L.LDL.L	6	0.08	0.03	0.01344	19	0.21	0.08	0.00830
L.LDL.P	6	0.09	0.04	0.02018	19	0.20	0.07	0.00625
L.LDL.PL	6	0.07	0.03	0.01936	19	0.21	0.08	0.01018
L.LDL.PL.pct	6	-0.04	0.01	0.00033	19	-0.04	0.01	0.00004
L.LDL.TG	6	0.03	0.02	0.09499	19	0.04	0.01	0.00163
L.LDL.TG.pct	6	-0.04	0.02	0.01132	19	-0.17	0.07	0.02052

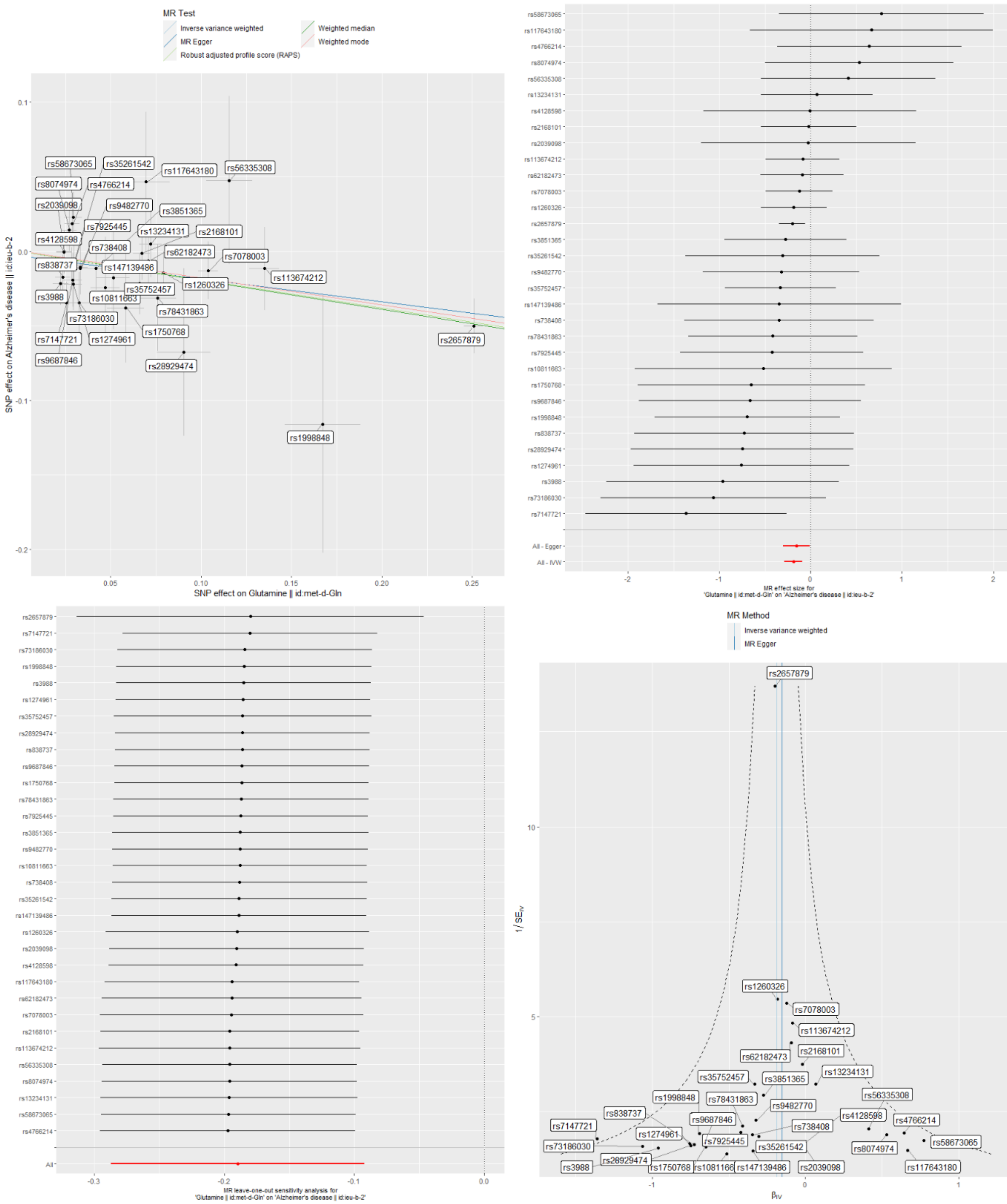
L.VLDL.C	6	0.05	0.03	0.09978	19	0.04	0.02	0.02311
L.VLDL.C.pct	6	0.04	0.03	0.21985	19	0.09	0.03	0.00382
L.VLDL.CE	6	0.05	0.03	0.08846	19	0.05	0.02	0.01354
L.VLDL.CE.pct	6	0.03	0.03	0.26767	19	0.08	0.03	0.01187
L.VLDL.FC.pct	6	0.04	0.03	0.18558	19	0.06	0.01	0.00000
L.VLDL.TG.pct	6	-0.05	0.04	0.23747	19	-0.06	0.01	0.00006
LA	6	0.06	0.02	0.00684	19	0.09	0.03	0.00344
LA.pct	6	0.03	0.02	0.04270	19	0.08	0.03	0.00600
LDL.C	6	0.08	0.03	0.01563	19	0.21	0.08	0.00833
LDL.CE	6	0.08	0.03	0.01706	19	0.20	0.07	0.00772
LDL.FC	6	0.08	0.03	0.01303	19	0.23	0.09	0.00976
LDL.L	6	0.08	0.03	0.01715	19	0.21	0.08	0.00803
LDL.P	6	0.09	0.04	0.02562	19	0.19	0.07	0.00693
LDL.PL	6	0.08	0.03	0.02066	19	0.22	0.08	0.00926
LDL.size	6	0.05	0.02	0.03073	19	0.10	0.03	0.00369
LDL.TG	6	0.03	0.02	0.10160	19	0.04	0.01	0.00459
Leu	6	-0.02	0.01	0.04746	19	-0.02	0.01	0.00164
M.HDL.C	6	-0.03	0.02	0.20973	19	-0.05	0.02	0.01435
M.HDL.C.pct	6	0.00	0.01	0.82608	19	0.07	0.03	0.02370
M.HDL.CE	6	-0.03	0.02	0.19449	19	-0.05	0.02	0.01232
M.HDL.CE.pct	6	0.00	0.02	0.96669	19	0.06	0.03	0.02552
M.HDL.FC	6	-0.02	0.02	0.30395	19	-0.03	0.01	0.04555
M.HDL.FC.pct	6	0.02	0.01	0.05746	19	0.08	0.04	0.02500
M.HDL.L	6	-0.03	0.02	0.15814	19	-0.07	0.03	0.00871
M.HDL.P	6	-0.03	0.02	0.19268	19	-0.06	0.02	0.01132
M.HDL.PL	6	-0.04	0.03	0.12755	19	-0.09	0.03	0.00665
M.HDL.PL.pct	6	-0.01	0.01	0.42489	19	-0.07	0.02	0.00381
M.HDL.TG	6	-0.02	0.01	0.04761	19	-0.11	0.05	0.02139
M.LDL.C	6	0.07	0.03	0.02642	19	0.19	0.07	0.00854
M.LDL.CE	6	0.07	0.03	0.03206	19	0.17	0.06	0.00828
M.LDL.CE.pct	6	0.00	0.01	0.89417	19	-0.08	0.03	0.00641
M.LDL.FC	6	0.08	0.03	0.01640	19	0.23	0.09	0.00920
M.LDL.FC.pct	6	0.02	0.01	0.20751	19	0.13	0.06	0.02510
M.LDL.L	6	0.07	0.03	0.02544	19	0.19	0.07	0.00763
M.LDL.P	6	0.07	0.03	0.04169	19	0.16	0.06	0.00911
M.LDL.PL	6	0.08	0.03	0.02260	19	0.21	0.08	0.00746
M.LDL.PL.pct	6	0.02	0.01	0.04987	19	0.13	0.05	0.01001
M.LDL.TG	6	0.03	0.02	0.09741	19	0.04	0.01	0.00556
M.LDL.TG.pct	6	-0.04	0.02	0.02494	19	-0.17	0.08	0.02646
M.VLDL.C	6	0.08	0.04	0.03829	19	0.16	0.06	0.00877
M.VLDL.C.pct	6	0.05	0.02	0.02319	19	0.15	0.06	0.01377
M.VLDL.CE	6	0.09	0.04	0.03607	19	0.18	0.07	0.00880
M.VLDL.CE.pct	6	0.04	0.02	0.02182	19	0.15	0.06	0.01406

M.VLDL.FC	6	0.07	0.04	0.04318	19	0.13	0.05	0.00842
M.VLDL.FC.pct	6	0.05	0.02	0.03068	19	0.15	0.06	0.01314
M.VLDL.L	6	0.06	0.03	0.05179	19	0.09	0.03	0.00799
M.VLDL.P	6	0.07	0.03	0.04416	19	0.11	0.04	0.00711
M.VLDL.PL	6	0.07	0.04	0.04518	19	0.12	0.04	0.00710
M.VLDL.PL.pct	6	0.06	0.03	0.03931	19	0.16	0.06	0.00768
M.VLDL.TG.pct	6	-0.05	0.02	0.02540	19	-0.15	0.06	0.01210
non.HDL.C	6	0.08	0.03	0.02229	19	0.19	0.07	0.00893
Omega.6	6	0.06	0.02	0.00535	19	0.09	0.03	0.00543
Omega.6.pct	6	0.01	0.01	0.70132	19	0.05	0.03	0.03700
PUFA	6	0.06	0.02	0.00244	19	0.08	0.03	0.00424
PUFA.pct	6	0.01	0.02	0.63391	19	0.05	0.02	0.01995
Remnant.C	6	0.08	0.04	0.03323	19	0.16	0.06	0.00982
S.HDL.C.pct	6	0.04	0.01	0.00772	19	0.17	0.06	0.00787
S.HDL.CE.pct	6	0.02	0.01	0.08056	19	0.12	0.05	0.01090
S.HDL.FC	6	0.00	0.01	0.88740	19	0.03	0.02	0.04337
S.HDL.FC.pct	6	0.06	0.02	0.00213	19	0.18	0.07	0.00511
S.HDL.L	6	-0.02	0.01	0.13124	19	-0.03	0.02	0.04235
S.HDL.PL	6	-0.03	0.02	0.08298	19	-0.06	0.02	0.01086
S.HDL.PL.pct	6	-0.06	0.03	0.02478	19	-0.20	0.06	0.00249
S.LDL.C	6	0.09	0.04	0.02226	19	0.21	0.08	0.00762
S.LDL.C.pct	6	0.02	0.02	0.19638	19	0.05	0.02	0.02370
S.LDL.CE	6	0.08	0.04	0.02376	19	0.18	0.07	0.00588
S.LDL.FC	6	0.09	0.04	0.01967	19	0.26	0.10	0.01101
S.LDL.FC.pct	6	0.01	0.01	0.27602	19	0.17	0.08	0.03117
S.LDL.L	6	0.09	0.04	0.02378	19	0.22	0.08	0.00756
S.LDL.P	6	0.08	0.04	0.03506	19	0.18	0.07	0.00717
S.LDL.PL	6	0.10	0.04	0.02435	19	0.26	0.10	0.00925
S.LDL.PL.pct	6	0.00	0.01	0.98362	19	0.10	0.05	0.04968
S.LDL.TG.pct	6	-0.03	0.01	0.01054	19	-0.17	0.08	0.02692
S.VLDL.C	6	0.07	0.04	0.06344	19	0.12	0.04	0.00404
S.VLDL.C.pct	6	0.06	0.03	0.03779	19	0.18	0.07	0.01014
S.VLDL.CE	6	0.06	0.04	0.07611	19	0.10	0.03	0.00271
S.VLDL.CE.pct	6	0.06	0.03	0.09451	19	0.15	0.05	0.00670
S.VLDL.FC	6	0.07	0.04	0.04543	19	0.14	0.05	0.00669
S.VLDL.FC.pct	6	0.05	0.02	0.00463	19	0.18	0.08	0.01597
S.VLDL.L	6	0.05	0.03	0.08116	19	0.06	0.02	0.00378
S.VLDL.P	6	0.05	0.03	0.08233	19	0.05	0.02	0.00366
S.VLDL.PL	6	0.07	0.03	0.05359	19	0.11	0.04	0.00459
S.VLDL.PL.pct	6	0.06	0.02	0.00509	19	0.19	0.08	0.01434
S.VLDL.TG.pct	6	-0.06	0.03	0.02307	19	-0.18	0.07	0.01124
SFA.pct	6	-0.04	0.03	0.14342	19	-0.04	0.01	0.00040
Sphingomyelins	6	0.05	0.01	0.00016	19	0.14	0.06	0.01448

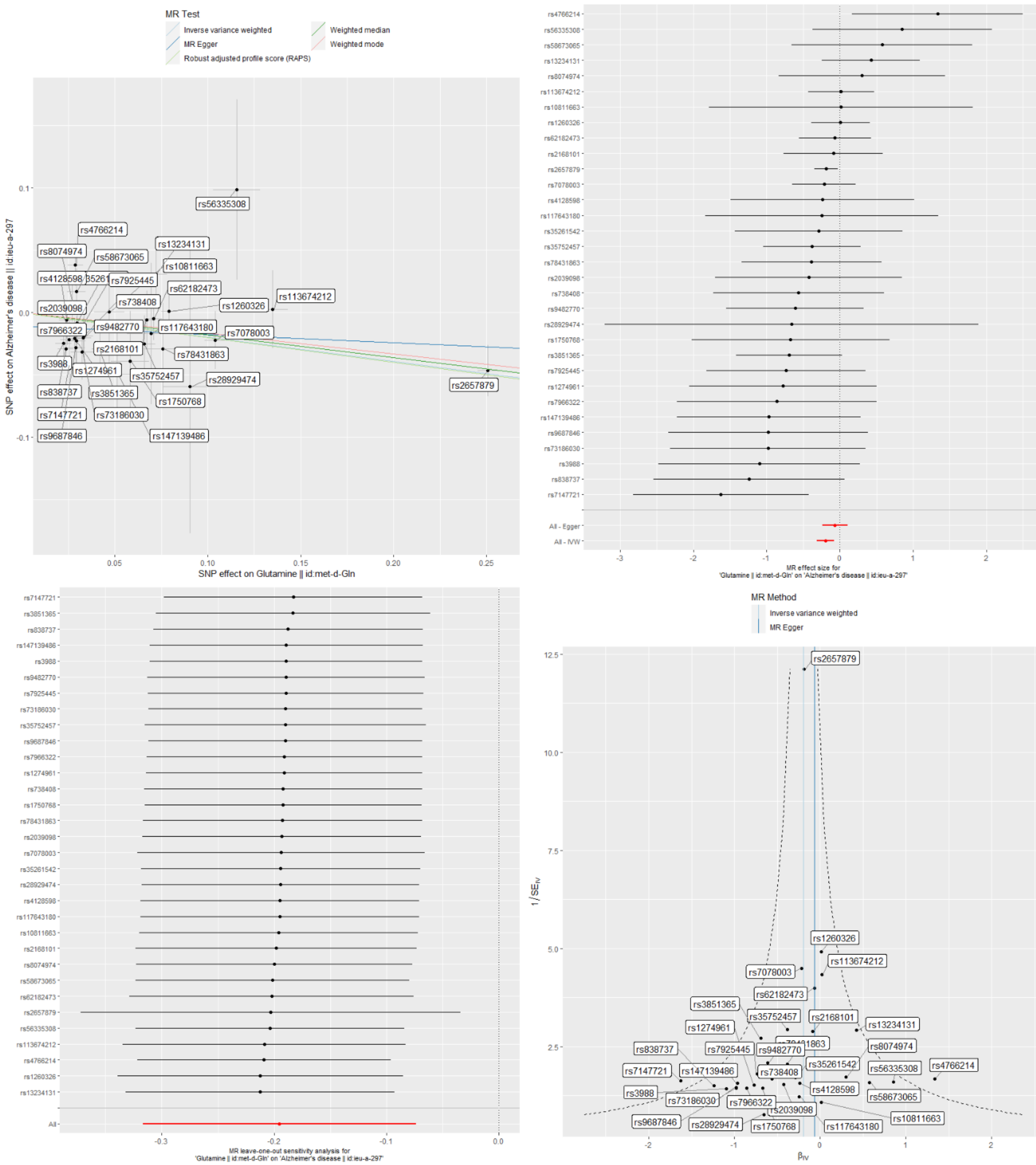
Total.BCAA	6	-0.02	0.01	0.02711	19	-0.02	0.01	0.00011
Total.C	6	0.07	0.03	0.00737	19	0.16	0.06	0.00975
Total.CE	6	0.07	0.02	0.00573	19	0.16	0.06	0.01002
Total.FA	6	0.04	0.02	0.02680	19	0.04	0.02	0.01361
Total.FC	6	0.07	0.03	0.01263	19	0.16	0.06	0.00911
Total.L	6	0.06	0.02	0.01306	19	0.11	0.04	0.00880
Total.PL	6	0.04	0.01	0.00195	19	0.07	0.03	0.01669
Tyr	6	-0.02	0.01	0.21523	19	-0.01	0.01	0.00918
Unsaturation	6	0.01	0.01	0.41172	19	0.04	0.02	0.01047
Val	6	-0.02	0.01	0.05153	19	-0.02	0.01	0.00133
VLDL.C	6	0.07	0.04	0.05834	19	0.10	0.03	0.00527
VLDL.CE	6	0.07	0.04	0.05574	19	0.11	0.04	0.00565
VLDL.FC	6	0.06	0.03	0.06735	19	0.07	0.03	0.00533
VLDL.L	6	0.05	0.03	0.10908	19	0.03	0.02	0.02628
VLDL.P	6	0.06	0.03	0.06738	19	0.08	0.03	0.00553
VLDL.PL	6	0.05	0.03	0.08227	19	0.05	0.02	0.00627
XL.HDL.C	6	0.04	0.01	0.00015	19	0.09	0.04	0.01498
XL.HDL.C.pct	6	0.01	0.01	0.26241	19	0.11	0.05	0.02182
XL.HDL.CE	6	0.04	0.01	0.00018	19	0.09	0.04	0.01538
XL.HDL.CE.pct	6	0.02	0.01	0.09454	19	0.12	0.05	0.01401
XL.HDL.FC	6	0.05	0.01	0.00031	19	0.12	0.05	0.01499
XL.HDL.L	6	0.04	0.01	0.00012	19	0.07	0.03	0.01654
XL.HDL.P	6	0.04	0.01	0.00005	19	0.08	0.03	0.00821
XL.HDL.PL	6	0.03	0.01	0.00150	19	0.04	0.02	0.02882
XL.HDL.PL.pct	6	-0.01	0.01	0.62914	19	-0.05	0.02	0.00622
XL.VLDL.C	6	0.05	0.03	0.10229	19	0.03	0.01	0.02041
XL.VLDL.C.pct	6	0.03	0.02	0.15394	19	0.10	0.04	0.01711
XL.VLDL.CE	6	0.06	0.03	0.08037	19	0.06	0.02	0.00542
XL.VLDL.CE.pct	6	0.02	0.02	0.19590	19	0.10	0.05	0.02100
XL.VLDL.FC.pct	6	0.05	0.03	0.09354	19	0.08	0.03	0.00362
XL.VLDL.PL.pct	6	0.07	0.05	0.14617	19	0.07	0.02	0.00031
XL.VLDL.TG.pct	6	-0.04	0.02	0.07992	19	-0.11	0.04	0.00901
XS.VLDL.C	6	0.07	0.03	0.05721	19	0.13	0.05	0.01409
XS.VLDL.C.pct	6	0.04	0.02	0.01244	19	0.15	0.07	0.02947
XS.VLDL.CE	6	0.07	0.03	0.05286	19	0.14	0.06	0.01522
XS.VLDL.CE.pct	6	0.04	0.01	0.01179	19	0.15	0.07	0.02880
XS.VLDL.FC	6	0.06	0.03	0.07167	19	0.10	0.04	0.01100
XS.VLDL.FC.pct	6	0.04	0.02	0.08222	19	0.11	0.06	0.04743
XS.VLDL.L	6	0.06	0.03	0.07169	19	0.09	0.03	0.00778
XS.VLDL.P	6	0.06	0.03	0.06694	19	0.11	0.04	0.00803
XS.VLDL.PL	6	0.06	0.03	0.08173	19	0.08	0.03	0.00620
XS.VLDL.TG.pct	6	-0.05	0.02	0.01654	19	-0.16	0.07	0.02772
XXL.VLDL.C.pct	6	0.01	0.03	0.72480	19	0.04	0.01	0.00347

XXL.VLDL.CE.pct	6	0.02	0.02	0.45387	19	0.05	0.02	0.00398

LDL.L: Total lipids in LDL; IDL.C: Cholesterol in IDL; NA: Phosphoglycerides; non.HDL.C: Total cholesterol minus HDL-C; S.VLDL.PL.pct: Phospholipids to total lipids ratio in small VLDL; M.VLDL.TG.pct: Triglycerides to total lipids ratio in medium VLDL; L.LDL.C: Cholesterol in large LDL; L.HDL.PL.pct: Phospholipids to total lipids ratio in large HDL; L.LDL.CE: Cholesteryl esters in large LDL; LDL.CE: Cholesteryl esters in LDL; L.LDL.C.pct: Cholesterol to total lipids ratio in large LDL; NA: Phosphatidylcholines; S.VLDL.TG.pct: Triglycerides to total lipids ratio in small VLDL; M.LDL.FC: Free cholesterol in medium LDL; NA: Omega-3 fatty acids; S.HDL.PL.pct: Phospholipids to total lipids ratio in small HDL; Sphingomyelins: Sphingomyelins; XL.HDL.FC: Free cholesterol in very large HDL; LA: Linoleic acid; L.LDL.L: Total lipids in large LDL; IDL.PL: Phospholipids in IDL; Total.CE: Total esterified cholesterol; XL.HDL.L: Total lipids in very large HDL; IDL.P: Concentration of IDL particles; Omega.6: Omega-6 fatty acids; Leu: Leucine; LA.pct: Ratio of linoleic acid to total fatty acids; XL.HDL.C: Cholesterol in very large HDL; Remnant.C: Remnant cholesterol (non-HDL, non-LDL -cholesterol); Total.BCAA: Total concentration of branched-chain amino acids (leucine + isoleucine + valine); M.LDL.L: Total lipids in medium LDL; ApoB: Apolipoprotein B; S.LDL.TG.pct: Triglycerides to total lipids ratio in small LDL; M.VLDL.FC.pct: Free cholesterol to total lipids ratio in medium VLDL; Total.PL: Total phospholipids in lipoprotein particles; M.LDL.P: Concentration of medium LDL particles; LDL.P: Concentration of LDL particles; L.LDL.CE.pct: Cholesteryl esters to total lipids ratio in large LDL; M.LDL.PL.pct: Phospholipids to total lipids ratio in medium LDL; LDL.PL: Phospholipids in LDL; Clinical.LDL.C: Clinical LDL cholesterol; Cholines: Total cholines; XL.HDL.PL: Phospholipids in very large HDL; M.VLDL.PL: Phospholipids in medium VLDL; S.VLDL.C.pct: Cholesterol to total lipids ratio in small VLDL; XL.HDL.CE: Cholesteryl esters in very large HDL; XS.VLDL.CE.pct: Cholesteryl esters to total lipids ratio in very small VLDL; M.VLDL.CE: Cholesteryl esters in medium VLDL; M.VLDL.CE.pct: Cholesteryl esters to total lipids ratio in medium VLDL; M.LDL.C: Cholesterol in medium LDL; L.LDL.TG.pct: Triglycerides to total lipids ratio in large LDL; DHA: Docosahexaenoic acid; S.LDL.FC: Free cholesterol in small LDL; LDL.FC: Free cholesterol in LDL; S.HDL.C.pct: Cholesterol to total lipids ratio in small HDL; M.HDL.TG: Triglycerides in medium HDL; NA: Triglycerides in very large HDL; S.LDL.L: Total lipids in small LDL; IDL.TG.pct: Triglycerides to total lipids ratio in IDL; XS.VLDL.C.pct: Cholesterol to total lipids ratio in very small VLDL; L.LDL.PL.pct: Phospholipids to total lipids ratio in large LDL; M.LDL.TG.pct: Triglycerides to total lipids ratio in medium LDL; S.LDL.PL: Phospholipids in small LDL; Total.FA: Total fatty acids; L.LDL.FC.pct: Free cholesterol to total lipids ratio in large LDL; S.LDL.C: Cholesterol in small LDL; LDL.C: LDL cholesterol; L.HDL.CE.pct: Cholesteryl esters to total lipids ratio in large HDL; S.LDL.CE: Cholesteryl esters in small LDL; LDL.size: Average diameter for LDL particles; L.LDL.PL: Phospholipids in large LDL; L.HDL.C.pct: Cholesterol to total lipids ratio in large HDL; Total.FC: Total free cholesterol; PUFA: Polyunsaturated fatty acids; Total.L: Total lipids in lipoprotein particles; S.VLDL.FC.pct: Free cholesterol to total lipids ratio in small VLDL; L.HDL.FC.pct: Free cholesterol to total lipids ratio in large HDL; bOHbutyrate: 3-Hydroxybutyrate; Total.C: Total cholesterol; M.VLDL.PL.pct: Phospholipids to total lipids ratio in medium VLDL; L.LDL.P: Concentration of large LDL particles; M.LDL.CE: Cholesteryl esters in medium LDL; M.VLDL.C: Cholesterol in medium VLDL; M.VLDL.C.pct: Cholesterol to total lipids ratio in medium VLDL; S.LDL.P: Concentration of small LDL particles; L.LDL.FC: Free cholesterol in large LDL; IDL.FC: Free cholesterol in IDL; S.VLDL.FC: Free cholesterol in small VLDL; S.HDL.FC.pct: Free cholesterol to total lipids ratio in small HDL; NA: Acetoacetate; XL.HDL.P: Concentration of very large HDL particles; M.LDL.PL: Phospholipids in medium LDL; NA: Acetone; M.VLDL.FC: Free cholesterol in medium VLDL; IDL.L: Total lipids in IDL; M.VLDL.P: Concentration of medium VLDL particles; XS.VLDL.TG.pct: Triglycerides to total lipids ratio in very small VLDL; IDL.CE: Cholesteryl esters in IDL; IDL.C.pct: Cholesterol to total lipids ratio in IDL; XS.VLDL.FC: Free cholesterol in very small VLDL; M.VLDL.L: Total lipids in medium VLDL; XS.VLDL.P: Concentration of very small VLDL particles; XL.HDL.PL.pct: Phospholipids to total lipids ratio in very large HDL; IDL.CE.pct: Cholesteryl esters to total lipids ratio in IDL; S.VLDL.PL: Phospholipids in small VLDL; XS.VLDL.C: Cholesterol in very small VLDL; XS.VLDL.PL: Phospholipids in very small VLDL; XS.VLDL.L: Total lipids in very small VLDL; XS.VLDL.CE: Cholesteryl esters in very small VLDL; L.VLDL.CE.pct: Cholesteryl esters to total lipids ratio in large VLDL; XXL.VLDL.C.pct: Cholesterol to total lipids ratio in chylomicrons and extremely large VLDL; ApoA1: Apolipoprotein A1; XS.VLDL.FC.pct: Free cholesterol to total lipids ratio in very small VLDL; ApoB.by.ApoA1: Ratio of apolipoprotein B to apolipoprotein A1; M.HDL.P: Concentration of medium HDL particles; M.HDL.PL: Phospholipids in medium HDL; M.LDL.TG: Triglycerides in medium LDL; VLDL.L: Total lipids in VLDL; M.HDL.CE.pct: Cholesteryl esters to total lipids ratio in medium HDL; VLDL.P: Concentration of VLDL particles; XL.VLDL.PL.pct: Phospholipids to total lipids ratio in very large VLDL; M.HDL.C.pct: Cholesterol to total lipids ratio in medium HDL; HDL.L: Total lipids in HDL; Tyr: Tyrosine; Creatinine: Creatinine; S.LDL.C.pct: Cholesterol to total lipids ratio in small LDL; M.HDL.FC.pct: Free cholesterol to total lipids ratio in medium HDL; S.LDL.FC.pct: Free cholesterol to total lipids ratio in small LDL; S.HDL.PL: Phospholipids in small HDL; XL.VLDL.CE: Cholesteryl esters in very large VLDL; XL.VLDL.FC.pct: Free cholesterol to total lipids ratio in very large VLDL; Omega.6.pct: Ratio of omega-6 fatty acids to total fatty acids; XL.VLDL.C: Cholesterol in very large VLDL; XL.VLDL.C.pct: Cholesterol to total lipids ratio in very large VLDL; XL.VLDL.CE.pct: Cholesteryl esters to total lipids ratio in very large VLDL; XL.HDL.C.pct: Cholesterol to total lipids ratio in very large HDL; M.HDL.CE: Cholesteryl esters in medium HDL; M.LDL.CE.pct: Cholesteryl esters to total lipids ratio in medium LDL; XL.VLDL.TG.pct: Triglycerides to total lipids ratio in very large VLDL; VLDL.CE: Cholesteryl esters in VLDL; L.VLDL.C: Cholesterol in large VLDL; PUFA.pct: Ratio of polyunsaturated fatty acids to total fatty acids; L.VLDL.C.pct: Cholesterol to total lipids ratio in large VLDL; XL.HDL.CE.pct: Cholesteryl esters to total lipids ratio in very large HDL; Unsaturation: Degree of unsaturation; LDL.TG: Triglycerides in LDL; L.LDL.TG: Triglycerides in large LDL; HDL.PL: Phospholipids in HDL; S.HDL.L: Total lipids in small HDL; VLDL.FC: Free cholesterol in VLDL; S.VLDL.CE.pct: Cholesteryl esters to total lipids ratio in small VLDL; S.HDL.FC: Free cholesterol in small HDL; Ile: Isoleucine; S.VLDL.C: Cholesterol in small VLDL; VLDL.C: VLDL cholesterol; M.LDL.FC.pct: Free cholesterol to total lipids ratio in medium LDL; S.VLDL.CE: Cholesteryl esters in small VLDL; M.HDL.L: Total lipids in medium HDL; M.HDL.C: Cholesterol in medium HDL; S.HDL.CE.pct: Cholesteryl esters to total lipids ratio in small HDL; M.HDL.FC: Free cholesterol in medium HDL; L.HDL.TG: Triglycerides in large HDL; IDL.FC.pct: Free cholesterol to total lipids ratio in IDL; XXL.VLDL.CE.pct: Cholesteryl esters to total lipids ratio in chylomicrons and extremely large VLDL; S.LDL.PL.pct: Phospholipids to total lipids ratio in small LDL; L.VLDL.TG.pct: Triglycerides to total lipids ratio in large VLDL; S.VLDL.L: Total lipids in small VLDL; Val: Valine; M.HDL.PL.pct: Phospholipids to total lipids ratio in medium HDL; VLDL.PL: Phospholipids in VLDL; L.VLDL.FC.pct: Free cholesterol to total lipids ratio in large VLDL; HDL.TG: Triglycerides in HDL; L.VLDL.CE: Cholesteryl esters in large VLDL; S.VLDL.P: Concentration of small VLDL particles; SFA.pct: Ratio of saturated fatty acids to total fatty acids



Supplementary Figure 1. Sensitivity analyses of Glutamine on Alzheimer's Disease (Kunkle et al.) including Method Comparison (top left), Forest plot (top right), Leave-one-out (bottom left) and Funnel Plot (bottom right)



Supplementary Figure 2. Sensitivity analyses of Glutamine on Alzheimer's Disease (Lambert et al.) including Method Comparison (top left), Forest plot (top right), Leave-one-out (bottom left) and Funnel Plot (bottom right)