
Article:
Şanlı, B.A., Whittaker, K.J., Motsi, G.K. et al. (3 more authors) (2022) Unbiased metabolome screen links serum urate to risk of Alzheimer's disease. Neurobiology of Aging, 120. pp. 167-176. ISSN: 0197-4580

https://doi.org/10.1016/j.neurobiolaging.2022.09.004

Neurobiology of Aging 120 (2022) 167–176

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging.org

Unbiased metabolome screen links serum urate to risk of Alzheimer's disease

Beyazıt Abdurrahman Şanlı[a], Katherine J. Whittaker[b], Gamuchirai K. Motsi[b], Emery Shen[b], Thomas H. Julian[c], Johnathan Cooper-Knock[b,*]

[a] Department of Agricultural Genetic Engineering, Ayhan Şahenk Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, Niğde, Turkey
[b] Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, UK
[c] Division of Evolution and Genomic Sciences, School of Biological Sciences, University of Manchester, Manchester, UK

ARTICLE INFO

Article history:
Received 4 June 2022
Revised 7 September 2022
Accepted 8 September 2022
Available online 15 September 2022

Keywords:
Alzheimer's Disease
Serum urate
Metabolome
Mendelian Randomization

ABSTRACT

Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disease caused by a combination of genetic and environmental risk factors. The serum metabolome refers to a set of small-molecules which are an important determinant of cellular health. We obtained genome-wide association study (GWAS) summary statistics for serum concentrations of 376 metabolites which were population matched with 2 GWAS studies of AD. For each metabolite we performed 2-sample MR (2SMR) using an inverse variance weighted (IVW) estimate for significance testing. After Bonferroni multiple testing correction one metabolite was causally linked to AD in both GWAS: serum urate. This result was supported by robust 2SMR measures and sensitivity analyses. We applied 2SMR to test for a causal relationship between serum urate and other neurodegenerative diseases: Parkinson disease (PD) and Amyotrophic lateral sclerosis (ALS). In ALS but not PD we identified a nominally significant link between serum urate and disease-risk, although in this case increased serum urate was protective. We conclude that serum urate is a modulator of risk for neurodegeneration. Our work has implications for the design of preventative interventions.

1. Introduction

Alzheimer's disease (AD) is an enormous burden on human health, and is caused by a complex interaction of genetic and environmental risk factors (Lord et al., 2021). Recognized environmental risk factors include the use of pesticide/industrial chemicals, inorganic/organic hazardous products, exposure to toxic molecules including aluminum and lead, and air pollutants (Yegambaram et al., 2015). Heritability for AD is estimated to be as high as 74% (Gatz et al., 1997) but only a small minority of individuals suffer monogenic disease. Our understanding of AD genetics is largely based on risk loci defined by genome-wide association studies (GWAS) (Wightman et al., 2021).

Metabolites which make up the metabolome are the intermediates and end products of cellular regulatory processes. Changes in the levels of metabolites are influenced by both genetic background and environmental stimuli (Bar et al., 2020). For example, concentration of a given metabolite may be lower if rate-limiting enzymes involved in its production are impaired by genetic variants altering their structure and function, or because of a limited supply of substrates from the diet. As a result, study of the metabolome is an ideal method for considering the convergence of genetic and environmental risk. Moreover, the metabolome is potentially modifiable making it an attractive therapeutic target (Jia et al., 2021)

Two-sample Mendelian randomization (2SMR) utilizes genetic information which is fixed at conception to form instrumental variables which are used to test whether a genetically associated "exposure" is causally linked to an "outcome" such as AD. The experiment is analogous to a randomized controlled trial where subjects are randomized to experimental or control groups

Abbreviations: AD, Alzheimer's disease; ALS, Amyotrophic lateral sclerosis; MR, Mendelian randomization; SNP, Single nucleotide polymorphism; GWAS, Genome-wide association study; 2SMR, Two-sample MR; IVW MR, Inverse variance weighted mendelian randomization; MRE, Multiplicative random effects, LD, linkage disequilibrium; MAF, Minor allele frequency, InSIDE, Instrument strength independent of direct effect; LOO, Leave-one-out.

* Corresponding author at: Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, 385A Glossop Road, Sheffield, S10 2HQ.
E-mail address: j.cooper-knock@sheffield.ac.uk (J. Cooper-Knock).

0197-4580/$ – see front matter
https://doi.org/10.1016/j.neurobiolaging.2022.09.004

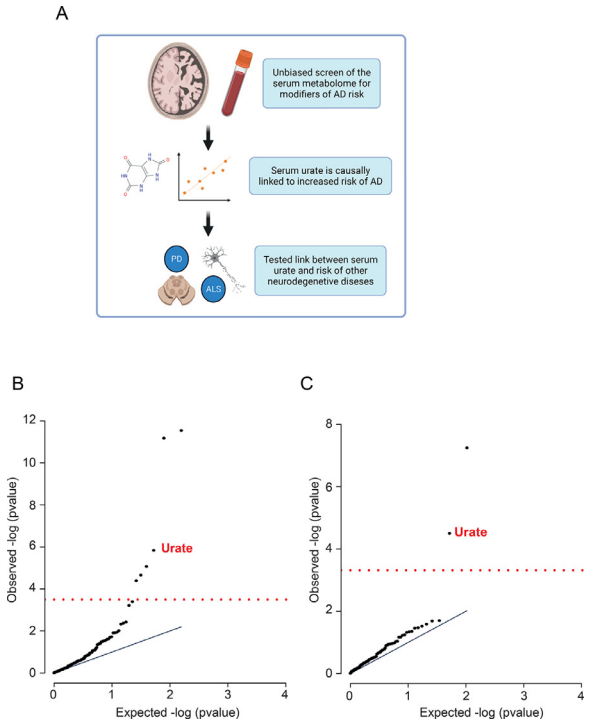

Fig. 1. Unbiased screen for the identification of potential metabolites related to AD. (A) Overview of our study plan. In order to identify metabolites causally linked to AD we performed an unbiased screen of 376 metabolites by 2SMR. After stringent Bonferroni multiple testing correction we identified a causal link between serum urate and AD. This result was further evaluated by robust 2SMR tests and sensitivity analyses. Finally we tested for a link between serum urate and other neurodegenerative diseases. (B–C) QQ-plots of IVW *p*-values demonstrate that six and two metabolites were significant after Bonferroni multiple testing correction (red line) in the larger (Kunkle et al., 2019) (B) and smaller (Lambert et al., 2013) (C) AD GWAS respectively.

(Hemani et al., 2018). This avoids much of the selection bias and reverse causation (Zhang et al., 2021) which can plague case-control studies and even prospective cohort studies. Standard epidemiological studies are often underpowered due to the requirement for measurement of both the exposure and disease in the same cohort (Julian et al., 2021), whereas 2SMR enables the use of separate cohorts to measure an exposure and outcome which makes larger well-powered studies more feasible.

We have developed methods for large-scale unbiased (by which we mean hypothesis-free or agnostic) application of 2SMR across large numbers of exposures including the entire metabolome (Boddy et al., 2022). Here, application of 2SMR in this way identified a causal effect of serum urate on risk for AD using 2 different GWAS. We have followed this up with robust measures, sensitivity analyses, and tests for reverse causation. We also show that there may be an effect of urate on risk of amyotrophic lateral sclerosis (ALS), another neurodegenerative disease. We offer important new insights into an area of controversy. Our approach is summarized in Fig. 1A.

2. Materials and methods

2.1. Exposure GWAS

Genetic instruments used to measure lifetime serum metabolite levels were derived from publicly available GWAS (Kettunen et al., 2016; Shin et al., 2014) for 376 metabolites. The 2 source studies differed in sample size and methodology: Kettunen et al. employed NMR spectroscopy to quantify metabolites in serum samples from up to 24,925 individuals (Kettunen et al., 2016); whereas Shin et al. employed liquid-phase and gas chromatography coupled with tandem mass spectroscopy in serum from 7824 individuals (Shin et al., 2014). The sample population for both studies was almost exclusively European; both studies included ~2000 individuals from the KORA cohort (Cooperative Health Research in the Region of Augsburg). The different methodologies employed were complementary: NMR is more easily applied in a high-throughput manner but sensitivity is lower than mass spectroscopy based quantification (Marshall and Powers, 2017). A total of 16 metabolites (4% of the total number) where measured in both source studies, not including serum urate.

Downstream analysis was supported by a GWAS specific to serum urate including 110,347 individuals of whom >90% were European (Köttgen et al., 2013). This study was a meta-analysis of >70 studies and included population controls together with a smaller number (~3%) of individuals who suffered gout, which is causally linked to elevated serum urate. Urate was quantified as uric acid via the "uricase method" in the large majority of cohorts; this relies on a measurable change in UV absorbance which occurs upon addition of uricase to a sample, and is dependent on the concentration of uric acid.

2.2. Outcome GWAS

Unbiased screening utilized 2 AD GWAS composed of 63,926 individuals (Kunkle et al., 2019) and 54,162 individuals (Lambert et al., 2013). Individuals within these studies were overlapping although the sample size and the precise population mix varied between studies. Both GWAS compared patients with a clinical or pathological diagnosis of AD to population controls and focused on Europeans. Only 434 controls from the KORA cohort were used in both the outcome and the exposure GWAS. Overlap between exposure and outcome GWAS can lead to bias but given the small proportion of overlapping individuals (<1%), in this instance we consider the effect to be negligible (Burgess et al., 2016).

2.3. Two-sample Mendelian Randomization (2SMR)

In our unbiased metabolome screen, we reported the multiplicative random effects inverse variance weighted (IVW) (Burgess et al., 2019) estimate of causal inference for all 2SMR tests because this carries the most statistical power and is more robust to heterogeneity than a fixed effects IVW (Julian et al., 2021). The unbiased screen for metabolites was performed as described previously (Boddy et al., 2022), including a Bonferroni correction for multiple testing, but with some modifications. Genetic instruments were initially selected with a conservative *p*-value cut-off ($p < 5E\text{-}8$). When the cut-off *p*-value is too stringent it can diminish power; however, if weak instruments are included this increases the risk of confounding pleiotropy or introduction on non-informative instruments which may bias toward the null (Julian et al., 2021). To avoid both of these scenarios we sequentially trialled larger *p*-value cut-offs (multiplied by a factor of 10) to determine the lowest *p*-value cut-off for which 5–50 SNPs were associated with the exposure after clumping. This was performed in an automated manner. Identified SNPs within a 10kb window were clumped for independence using a stringent cut-off of $R^2 \leq 0.001$ within a European reference panel; where SNPs were in linkage disequilibrium (LD) those with the lowest *p*-value were retained. Where an exposure SNP was unavailable in the outcome dataset, a proxy with a high degree of LD ($R^2 \geq 0.9$) was identified within a European reference population (Machiela and Chanock, 2015). The ef-

fects of SNPs on outcomes and exposures were harmonized in order to ensure that the beta values were signed with respect to the same alleles. For palindromic alleles, those with minor allele frequency (MAF) >0.42 were omitted from the analysis in order to reduce the risk of errors due to strand issues (Hartwig et al., 2016). After initial instrument selection we applied radial MR to detect and exclude outlier instruments (Bowden et al., 2018b). As before (Boddy et al., 2022), in the final step we excluded tests with too few or too many SNPs in order to minimize *p*-value inflation. Briefly: To ensure that we did not include false positive results in our unbiased screen we measured the inflation factor (λ) which is the ratio of the observed median *p*-value to the expected median *p*-value. Under the assumption that the majority of statistical tests will be non-significant then λ should ~1. It is expected that the majority of metabolites are unrelated to AD risk and therefore this is a reasonable assumption. The optimal number of instrumental SNPs was tuned to set λ as close as possible to 1. For both AD GWAS this resulted in a maximum number of instrumental SNPs of 15 per test. In the smaller AD GWAS (Lambert et al., 2013), we excluded tests including <7 instrumental SNPs and in the larger AD GWAS (Kunkle et al., 2019) we excluded tests including <5 instrumental SNPs. The final number of tests was used to determine the Bonferroni corrected *p*-value; in the smaller AD GWAS (Lambert et al., 2013) the number of tests was 104 and therefore the threshold *p*-value was 0.05/104 = 4.8E-4, and in the larger AD GWAS (Kunkle et al., 2019) the number of tests was 157 and therefore the threshold *p*-value was 0.05/157 = 3.2E–4.

Significance was calculated using a IVW estimate (Burgess et al., 2013) which is well powered providing there is not an excess of invalid SNPs (Bowden et al., 2016). To increase confidence in the IVW results from our unbiased screen, we performed a series of robust MR measures and sensitivity analyses. MR measures such as the weighted median (Bowden et al., 2016), weighted mode (Hartwig et al., 2017), and MR Egger (Slob and Burgess, n.d.) are relatively robust to the presence of invalid SNPs. The strength of instrumental variables associated with the exposure was evaluated using the F-statistic, except where we were unable to obtain effect allele frequencies (EAF) which prohibited this calculation. An F-statistic of >10 per instrumental SNP was taken to signify a sufficiently strong correlation with the exposure (Burgess et al., 2011). Cochran's Q test served to identify heterogeneity between instrumental SNPs (Bowden et al., 2018a). Radial MR was applied to detect and exclude outlier instruments (Bowden et al., 2018b). Finally, we performed a leave-one-out (LOO) analysis to determine if any single SNP(s) were exerting a disproportionate effect (Burgess et al., 2019). The MR-Egger intercept test determines whether there is directional horizontal pleiotropy. Finally, the presence of reverse causation, that is, an outcome which impacts an exposure, can violate assumptions underlying 2SMR and therefore we explicitly tested for this by reversing the direction of the 2SMR test (Richmond et al., 2014).

In this work, the entire MR analyses were performed through TwoSampleMR (version 0.5.6) and Mendelian Randomization (version 0.6.0), Radial MR (version 1.0) and R (version 4.1.3) packages, respectively.

3. Results

3.1. Unbiased 2SMR analysis highlights serum urate as a risk factor for AD

In our previous work, we have used an unbiased 2SMR approach to successfully identify and validate metabolites linked to risk for ALS (Boddy et al., 2022). We applied a similar approach here to identify metabolites linked to risk of AD. To increase confidence in our findings we applied our approach simultaneously in 2 different AD GWAS (Kunkle et al., 2019; Lambert et al., 2013) including a stringent Bonferroni multiple testing correction in both analyses (*Methods*). Only one metabolite was causally linked to AD in both analyses after multiple testing correction: serum urate (Supplementary Table 1, Supplementary Table 2, Fig. 1B–C).

In our unbiased screen, we derived instrumental SNPs associated with exposure to serum urate from a GWAS containing n = 7819 individuals (Shin et al., 2014). We selected 8 SNPs at a *p*-value threshold of <5E–7 (*Methods*). In the smaller AD GWAS containing n = 17,008 patients and n = 37,154 controls (Lambert et al., 2013) serum urate was causally linked to AD (IVW p = 3.14E-5, beta = 2.41, se = 0.58) (Table 1, Fig. 2A). In a larger AD GWAS containing n = 21,982 patients and n = 41,944 controls (Kunkle et al., 2019) serum urate was again significantly associated with risk for AD (IVW p = 1.44E–6, beta = 2.36, se = 0.49, Table 1, Fig. 2B).

3.2. Measurement of serum urate in a different GWAS confirms a causal link to AD

Our unbiased analysis has suggested a causal link between the serum urate and AD using 2 different AD GWAS. As a further confirmation we repeated the analysis using a different GWAS for serum urate to derive instrumental SNPs. This GWAS was larger (n = 110,347) but from a more heterogeneous population (Köttgen et al., 2013); we selected 21–25 SNPs at a *p*-value threshold of $< 5 \times 10^{-8}$ (*Methods*). We confirmed a causal association with AD in both the smaller AD GWAS (Lambert et al., 2013) (IVW p = 3.04E-2, beta = 0.092, se = 0.042, Table 1, Figure 2C) and the larger AD GWAS (Kunkle et al., 2019) (IVW p = 1.52E-4, beta = 0.137, se = 0.036, Table 1, Figure 2D).

3.3. Sensitivity analyses support the causative association of serum urate with AD

Our IVW 2SMR tests revealed the evidence of a causal effect of urate on AD which was consistent across multiple datasets. However, the IVW test is particularly vulnerable to pleiotropic instruments; therefore we performed a series of sensitivity analyses to check for confounding of the IVW test by SNP pleiotropy. The calculated F-statistic was >10 for each instrument suggesting that we had achieved adequate instrument strength (*Methods*) (Table 1), although it was not possible to calculate the F-statistic for instruments derived from the Köttgen et al. study (*Methods*). Cochran's Q test did not reveal the evidence of instrument heterogeneity (Table 1). Egger intercept *p*-values were greater than 0.05, suggesting that there was no statistically significant directional horizontal pleiotropy (Table 1). The LOO analysis revealed that the test of the larger urate GWAS (Shin et al., 2014) against the smaller AD GWAS (Lambert et al., 2013) was sensitive to removal of 4 different instrumental SNPs (Table 1) however, none of the other analyses were abrogated by removal of any single instrumental SNP, suggesting that this observation may be related to an underpowered test rather than instrument heterogeneity.

A causal effect of an outcome on an exposure can violate key assumptions underlying 2SMR (Julian et al., 2021). Using both AD and both serum urate GWAS we did not discover evidence that AD was causally linked to serum urate, taking AD as the exposure and serum urate as the outcome (Table 2, Fig. 3A–D); this is often referred to as bidirectional MR. An exception was the IVW test ultising the smallest AD GWAS (Lambert et al., 2013) and the smallest serum urate GWAS (Shin et al., 2014) (IVW p = 2.12E-3) however, this result was not significant in any of the robust measures.

Overall extensive sensitivity analyses and bidirectional MR are consistent with a true causal effect of urate on AD.

Table 1
Summary of robust MR measures and sensitivity analyses for 2SMR test of the causal effect of serum urate on AD. Odds ratios (OR) are derived from the IVW beta estimates

Test	Serum [urate] (Köttgen et al., 2013) vs. risk of AD (Lambert et al., 2013)	Serum [urate] (Köttgen et al., 2013) vs. risk of AD (Kunkle et al., 2019)	Serum [urate] (Shin et al., 2014) vs risk of AD (Lambert et al., 2013)	Serum [urate] (Shin et al., 2014) vs risk of AD (Kunkle et al., 2019)
IVW P value	3.04E-02	1.52E-04	3.14E-05	1.44E-06
MR Egger *p* value	3.35E-02	1.32E-02	3.10E-01	6.54E-01
Weighted median P value	7.73E-02	2.75E-03	1.01E-02	6.35E-03
Weighted mode P value	5.38E-02	4.05E-03	7.93E-02	6.40E-02
Number of SNPs F<10	NA	NA	0	0
Egger Cochrans's Q test P value	7.11E-01	9.00E-01	6.15E-01	7.10E-01
IVW Cochrans's Q test P value	6.53E-01	8.75E-01	7.20E-01	7.77E-01
Egger intercept test	1.80E-01	2.49E-01	8.30E-01	6.22E-01
I2	9.94E-01	9.94E-01	9.84E-01	9.84E-01
Number of SNPs LOO $p > 0.05$	4	0	0	0
Total Number of SNPs	25	21	8	8
p value	5.00E-08	5.00E-08	5.00E-07	5.00E-07
Outlier	No outlier	2 (rs1165161, rs653178)	No outlier	No outlier
Palindromic	2 (rs17632159, rs6830367)	2 (rs17632159, rs6830367)	1 (rs938554)	1 (rs938554)
95% OR_LCI	1.009	1.068	3.578	4.059
95% OR_UCI	1.190	1.230	34.577	27.694
OR	1.096	1.146	11.123	10.602
Beta IVW	0.092	0.137	2.409	2.361
SE IVW	0.042	0.036	0.579	0.490
Beta MR Egger	0.187	0.212	2.989	1.146
SE MR Egger	0.083	0.078	2.694	2.429
Beta Weighted median	0.114	0.177	2.468	2.452
SE Weighted median	0.062	0.060	0.965	0.888
Beta Weighted mode	0.114	0.182	2.637	2.469
SE Weighted mode	0.064	0.056	1.355	1.066

Table 2
Summary of robust MR measures and sensitivity analyses for 2SMR test of the causal effect of AD on serum urate

Test	Risk of AD (Lambert et al., 2013) vs. serum [urate] (Köttgen et al., 2013)	risk of AD (Kunkle et al., 2019) vs serum [urate] (Köttgen et al., 2013)	Risk of AD (Lambert et al., 2013) vs serum [urate] (Shin et al., 2014)	risk of AD (Kunkle et al., 2019) vs serum [urate] (Shin et al., 2014)
IVW *p* value	1.63E-01	1.36E-01	2.12E-03	4.97E-01
MR Egger *p* value	3.77E-01	6.31E-01	3.16E-01	3.32E-01
Weighted median *p* value	2.38E-01	1.80E-01	1.23E-01	3.21E-01
Weighted mode *p* value	4.61E-01	2.62E-01	2.96E-01	3.68E-01
Number of SNPs F<10	NA	NA	NA	NA
Egger Cochrans's Q test *p* value	7.31E-01	2.70E-01	8.16E-01	4.22E-01
IVW Cochrans's Q test *p* value	5.23E-01	3.08E-01	8.37E-01	4.19E-01
Egger intercept test	1.84E-01	4.74E-01	5.08E-01	3.72E-01
I2	9.96E-01	9.80E-01	9.97E-01	9.80E-01
Number of SNPs LOO $p > 0.05$	7	5	0	6
Total Number of SNPs	7	5	6	5
p value	5.00E-08	5.00E-08	5.00E-08	5.00E-08
Outlier	No outlier	No outlier	No outlier	1 (rs73223431)
Palindromic	2 (rs12972156, rs12977604)	0	2 (rs12972156, rs12977604)	0
Beta IVW	-2.40E-02	-3.80E-02	1.00E-02	4.00E-03

3.4. Robust 2SMR tests support the causative association of serum urate with AD

We have shown that the IVW test identifies a causal relationship between urate and AD and there is no evidence of confounding by reverse causation or instrument heterogeneity. To further increase confidence in our results we applied a series of robust MR measures which are less powerful than the IVW but less vulnerable to instrument heterogeneity.

The 2SMR test of association between the smaller urate GWAS (Shin et al., 2014) was significant in the weighted median analysis against AD with either the smaller (Lambert et al., 2013) (p = 1.01E-2, beta = 2.468, se = 0.965, Table 1, Fig. 2A) or the larger AD GWAS (Kunkle et al., 2019) (p = 6.35E-3, beta = 2.452 se = 0.888, Table 1, Fig. 2B). Moreover, the test of association between the larger urate GWAS (Köttgen et al., 2013) was also significant in the weighted median analysis (p = 2.75E-3, beta = 0.177, se = 0.060, Table 1, Fig. 2D) and the weighted mode analysis (p = 4.05E–3, beta = 0.182, se = 0.056, Table 1, Fig. 2D) against the larger AD GWAS (Kunkle et al., 2019); and in the MR Egger analysis (p = 3.35E-2, beta = 0.187, se = 0.083, Table 1, Fig. 2C) against the smaller AD GWAS (Lambert et al., 2013). These results are keeping with our IVW findings suggesting that there is a causal association of serum urate with increased risk of AD.

Not all robust measures were found to be significant in MR robust tests. However, this is not unexpected given the relatively poor power of these measures (Wang et al., 2020).

3.5. Evaluation of a causal effect of serum urate on risk of PD and ALS

After the validation of causal relation between urate and AD as supported by IVW and sensitivity/robust MR measures, we also tested whether serum urate was causally linked to other neurodegenerative diseases including PD or ALS by 2SMR (*Methods*). We

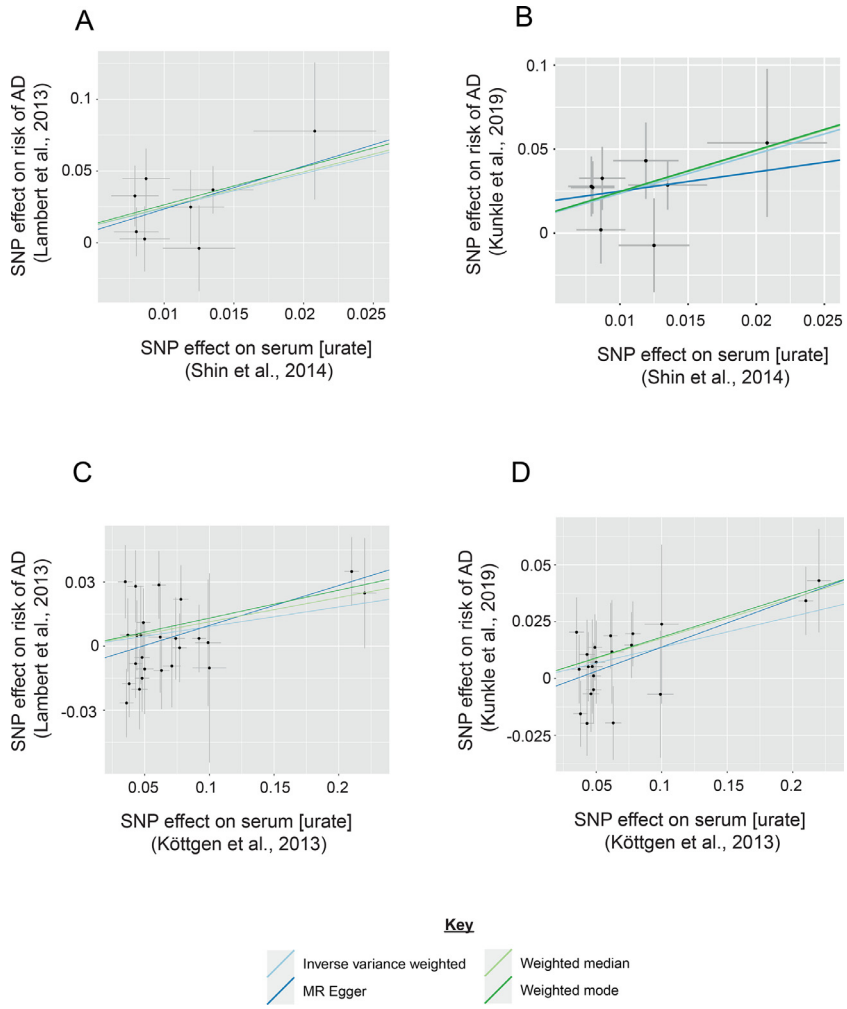

Fig. 2. 2SMR tests for a causal effect of serum urate on AD. (A–D) Scatter plots showing significant positive association of serum urate with increased risk of AD across two different AD (left and right panels) and serum urate (upper and lower panels) GWAS. In each case the IVW result was statistically significant: IVW p = 3.14e-05, beta = 2.409, se = 0.579 **(A)**; IVW p = 1.44e-06, beta = 2.361, se = 0.490 (**B**); IVW p = 3.04e-02, beta = 0.092, se = 0.042 **(C)**; IVW p = 1.52e-04, beta = 0.212, se = 0.078 (**D**). Points indicate effect size (β) and standard errors for each SNP-outcome relationship.

observed that IVW results were not significant for the analysis of either serum urate GWAS (Köttgen et al., 2013; Shin et al., 2014) against PD (Nalls et al., 2019) (IVW p = 7.78E-1, beta = -0.016, se = 0.057, Fig. 4C and IVW p = 8.00E-1, beta = -0.148, se = 0.586, Fig. 4A; Table 3). For ALS GWAS (Nicolas et al., 2018), there was some evidence of association between serum urate dataset measured using the smaller GWAS (Shin et al., 2014) (IVW p = 4.41E-3, beta = -0.072, se = 0.271, Table 4, Fig. 4B) and this test was also significant in the weighted median (p = 0.021, beta = -1.007, se = 0.436, Table 4). However, sensitivity analyses revealed that this result was invalidated by removal of a single instrumental SNP (Fig. 4B, Table 4) suggesting that it may be a false positive. There was no evidence of association between the serum urate and risk of ALS using the larger urate GWAS (Köttgen et al., 2013) (Fig. 4D, Table 4)

4. Discussion

In the present study, 2SMR was employed in an unbiased hypothesis-free fashion to explore the causal effect of serum metabolites on AD risk. Our results indicated that serum urate was causally associated with increased risk of AD. This result was supported by sensitivity analyses and robust MR measures. This result was significant in 2 different AD GWAS after stringent multiple testing correction even when urate was considered alongside hundreds of other metabolites. Interestingly, we observed some evidence of a causal relationship between serum urate and risk of another neurodegenerative disease, ALS. However, in this case the direction of association was reversed and the sensitivity analysis suggested that this may be a false positive. If this result is confirmed in future works then this could indicate that serum urate is linked to neurodegeneration more broadly.

In our analyses we did not observe a simple relationship between the effect size and sample size as might be expected if adding more patients linearly increased statistical power to detect an effect. However, we note that larger sample size also increases population heterogeneity which can confound 2SMR because of a reliance on LD-structure for the selection of consistent instrumental SNPs (Julian et al., 2021). Indeed we have previously discussed examples where the larger study does not necessarily provide the correct result (Julian et al., 2021). It should also be noted that in the analysis of the largest urate GWAS against the largest AD GWAS the causal relationship between serum urate and risk of AD achieved significance in the IVW and both the weighted median and weighted mode robust 2SMR measures.

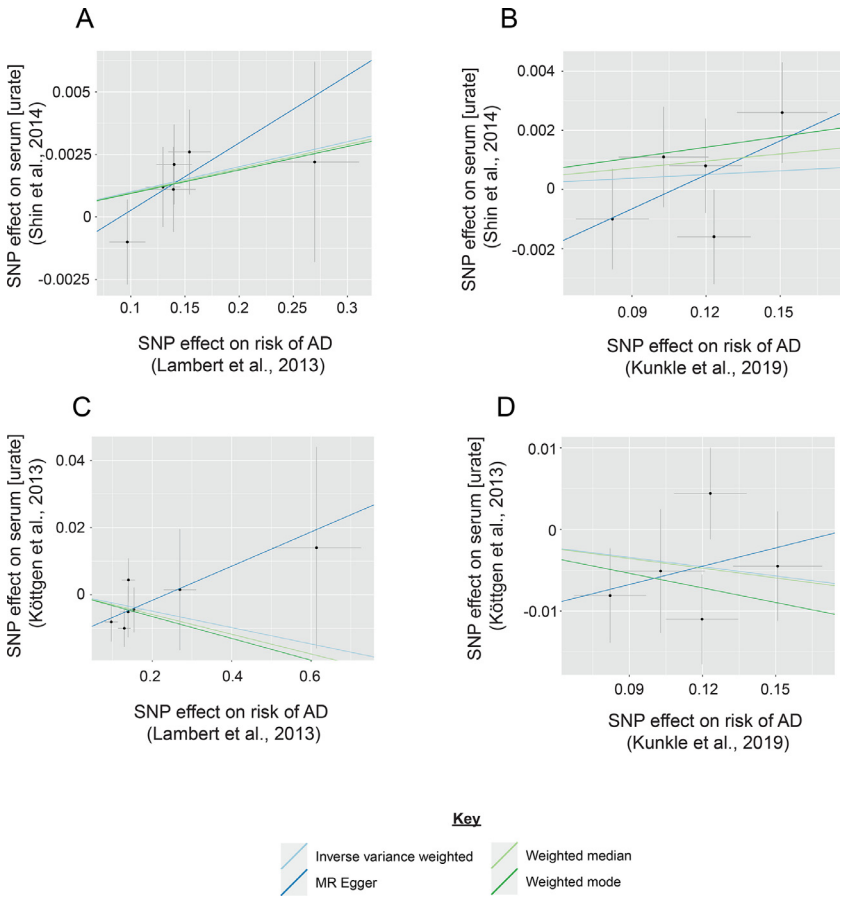

Fig. 3. Bidirectional 2SMR analysis of the effect of AD on serum urate (A–D) Scatter plots show no evidence of a significant association between AD on serum urate. Points indicate effect size (β) and standard errors for each SNP-outcome relationship.

Table 3
Summary of robust MR measures and sensitivity analyses for 2SMR test of the causal effect of serum urate on PD. Odds ratios (OR) are derived from the IVW beta estimates.

Test	Serum [urate] (Köttgen et al., 2013) vs. risk of PD	Serum [urate] (Shin et al., 2014) vs. risk of PD
IVW *p* value	7.78E-01	8.00E-01
MR Egger *p* value	1.41E-01	5.59E-01
Weighted median *p* value	4.71E-01	9.20E-01
Weighted mode *p* value	2.95E-01	9.76E-01
Number of SNPs F<10	NA	0
Egger Cochrans's Q test *p* value	5.72E-01	1.75E-01
IVW Cochrans's Q test *p* value	4.37E-01	1.78E-01
Egger intercept test	1.08E-01	3.99E-01
I2	9.94E-01	9.84E-01
Number of SNPs LOO $p > 0.05$	19	10
Total Number of SNPs	18	9
p value	5.00E-08	5.00E-07
Outlier	5 (rs10761587, rs1260326, rs1471633, rs2307394, rs729761)	No outlier
Palindromic	2 (rs17632159, rs6830367)	1 (rs938554)
95% OR_LCI	0.881	0.273
95% OR_UCI	1.100	2.719
OR	0.984	0.862
Beta IVW	-0.016	-0.148
SE IVW	0.057	0.586
Beta MR Egger	-0.149	0.661
SE MR Egger	0.096	1.079
Beta Weighted median	-0.056	0.054
SE Weighted median	0.078	0.559
Beta Weighted mode	-0.083	0.018
SE Weighted mode	0.081	0.526

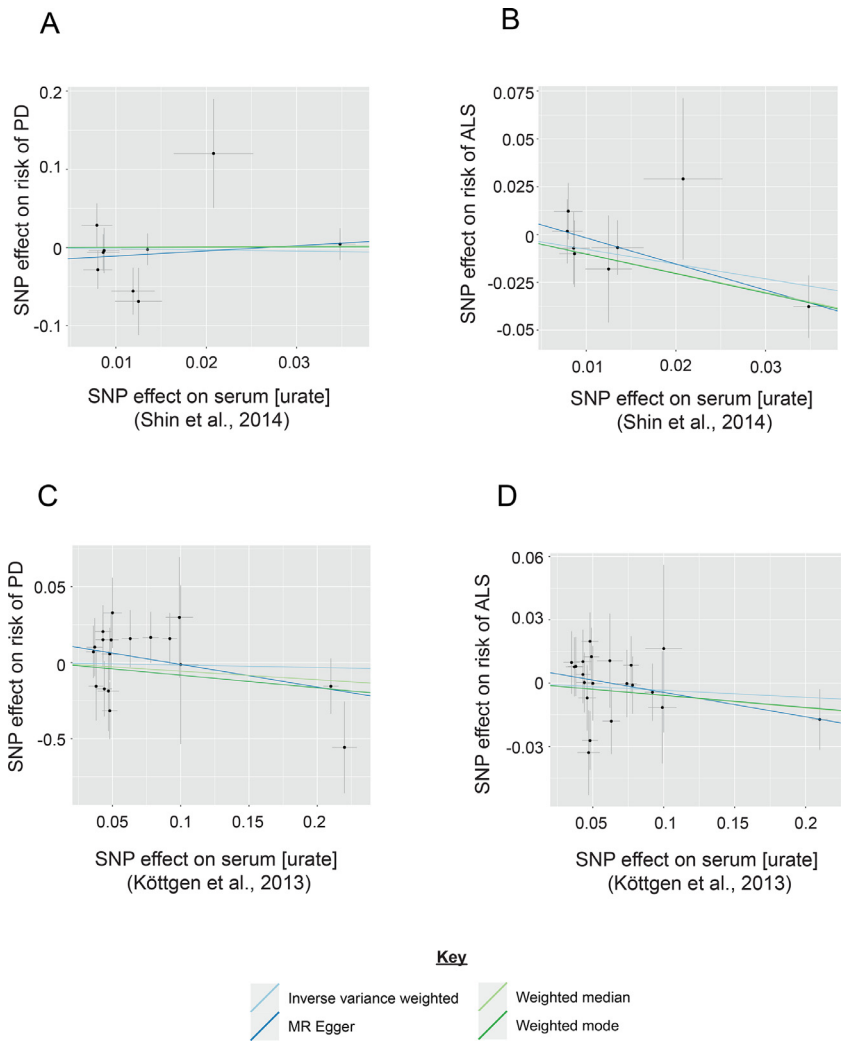

Fig. 4. 2SMR tests for a causal effect of serum urate on PD and ALS. (A–D) Scatter plots illustrate that there was no causal effect of serum urate on PD risk: IVW p = 8.00e-01, beta = -0.148, se = 0.586 **(A)**; IVW p = 7.78e-01, beta = -0.016, se = 0.057 (C). There was some evidence for a causal effect of serum urate on risk of ALS**:** IVW p = 4.41e-03, beta = -0.772, se= 0.271 (B) but not in the larger serum urate GWAS: IVW p = 3.81e-01, beta = -0.034, se = 0.038 (D). Points indicate effect size (β) and standard errors for each SNP-outcome relationship.

Urate is closely linked to uric acid (UA), a hydrophilic molecule which can scavenge free radicals including superoxide and singlet oxygen, that is essential for homeostasis within the CNS (Bowman et al., 2010). Despite this, there is controversy regarding the association of urate and UA with neurodegenerative disease. Lower serum UA levels have been associated with AD (Cankurtaran et al., 2013; Euser et al., 2009; Kim et al., 2006; Polidori and Mecocci, 2002) but another study found no significant differences in serum UA between aged healthy individuals and sufferers of AD or Parkinson's disease (PD) (Ahlskog et al., 1995). A meta-analysis of multiple studies also concluded that serum UA levels in AD patients are not significantly different from healthy controls (Chen et al., 2014). Higher serum UA has been proposed to reduce the rate of deterioration in cognitive function in individuals with mild cognitive impairment (Irizarry et al., 2009; Rinaldi et al., 2003) but another study suggested that even a mild increase in UA level might increase the risk of cognitive decline in elderly adults (Schretlen et al., 2007). Overall, controversy in the literature means that the role of urate and UA in AD suggests that there may be confounding, perhaps as a result of selection bias, which will not affect our 2SMR results (Williams et al., 2019).

Other literature is consistent with our finding that urate is causally linked to AD and suggests a mechanism whereby UA-induced oxidative stress can exacerbate inflammation and formation of β–amyloid pathology. UA induces the generation of hydrogen peroxide (Ko et al., 2019) and, causes the formation of superoxide anions when metabolized from xanthine in the UA biogenesis pathway (Vannorsdall et al., 2014) suggesting that urate may contribute to oxidative stress in the cell (Song and Zhao, 2018). This has been linked to core molecular defects underlying AD; a recent work concluded that long term exposure to serum urate might enhance the production of tumor necrosis factor–α (TNF-α) and β–amyloid peptide resulting from oxidative stress in rats (Tian et al., 2021).

A previous MR study of metabolites linked to risk of AD (Lord et al., 2021) concluded that a number of lipid traits were causally associated with AD. Of note plasma urate was not considered in this hypothesis-led study. Similarly, our relatively conservative method in which we excluded traits with a large number of instrumental SNPs due to the risk of instrument pleiotropy, removed the large majority of lipid traits considered by Lord et al. (2021).

Table 4
Summary of robust MR measures and sensitivity analyses for 2SMR test of the causal effect of serum urate on ALS. Odds ratios (OR) are derived from the IVW beta estimates

Test	Serum [urate] (Köttgen et al., 2013) vs. risk of ALS	Serum [urate] (Shin et al., 2014) vs. risk of ALS
IVW *p* value	3.81E-01	4.41E-03
MR Egger *p* value	1.92E-01	9.36E-02
Weighted median *p* value	3.34E-01	2.11E-02
Weighted mode *p* value	3.65E-01	5.82E-02
Number of SNPs F<10	NA	0
Egger Cochrans's Q test *p* value	8.28E-01	8.74E-01
IVW Cochrans's Q test *p* value	8.05E-01	8.32E-01
Egger intercept test	2.73E-01	3.38E-01
I2	9.93E-01	9.86E-01
Number of SNPs LOO $p > 0.05$	22	1
Total Number of SNPs	21	8
p value	5.00E-08	5.00E-07
Outlier	3 (rs2231142, rs3741414, rs653178)	1 (rs2231142)
Palindromic	2 (rs17632159, rs6830367)	1 (rs938554)
95% OR_LCI	0.897	0.271
95% OR_UCI	1.042	0.786
OR	0.967	0.462
Beta IVW	-0.034	-0.772
SE IVW	0.038	0.271
Beta MR Egger	-0.116	-1.365
SE MR Egger	0.086	0.685
Beta Weighted median	-0.059	-1.007
SE Weighted median	0.062	0.436
Beta Weighted mode	0.057	-1.022
SE Weighted mode	0.060	0.439

We propose that our findings could form the basis of therapeutic intervention, perhaps via dietary modification. A number of studies that have explored this, for example a longitudinal Taiwanese study concluded that risk of dementia in gout patients curated with urate-reducing agent was 30% less as compared to a control group or gout patients treated differently (Hong et al., 2015). Similarly, dietary interventions thought to be associated with reduced serum urate, such as the Mediterranean-type diet, Dietary Approach to Stop Hypertension (DASH) and Mediterranean-DASH diet Intervention for Neurodegenerative Delay (MIND), have achieved reduction both in cognitive decline and AD incidence (Solfrizzi et al., 2017). However, AD is associated with cardiovascular risk which may also have been modified by these dietary interventions.

We would like to draw attention to certain strengths and weaknesses of our work. Our analysis benefits from an unbiased data-driven approach to the serum metabolome, the use of multiple datasets, and our application of relatively stringent criteria (including robust tests and sensitivity analyses) for a positive result, all of which adds confidence to our findings. There is a degree of overlap in sample sets utilized by the 2 AD GWAS we employed (Kunkle et al., 2019; Lambert et al., 2013) although, it is informative that despite this, serum urate was the only exposure to be causally associated in both datasets after Bonferroni multiple testing correction. A weakness of our work is the lack of biological interpretation because we have not performed any follow-up functional analyses. However, the starting point of this study was the metabolome and not specifically serum urate, and thus experimental evaluation of serum urate is beyond the scope of this work. It is notable that our 2SMR study was performed at population-scale which makes it difficult to make individualized predictions regarding the effect of serum urate on AD risk. Future work will need to identify which specific individuals or genetic backgrounds are at risk of urate-induced AD; these individuals may benefit from urate-lowering interventions.

Disclosure statement

All authors report no conflict of interest.

Acknowledgement

This work was supported by a Wellcome Trust fellowship [216596/Z/19/Z to J.C.-K].

CRediT authorship contribution statement

Beyazıt Abdurrahman Şanlı: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Katherine J. Whittaker:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software. **Gamuchirai K. Motsi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization. **Emery Shen:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization. **Thomas H. Julian:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization. **Johnathan Cooper-Knock:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2022.09.004.

References

Ahlskog, J.E., Uitti, R.J., Low, P.A., Tyce, G.M., Nickander, K.K., Petersen, R.C., Kokmen, E., 1995. No evidence for systemic oxidant stress in Parkinson's or Alzheimer's disease. Mov. Disord. 10, 566–573.

Bar, N., Korem, T., Weissbrod, O., Zeevi, D., Rothschild, D., Leviatan, S., Kosower, N., Lotan-Pompan, M., Weinberger, A., Le Roy, C.I., Menni, C., Visconti, A., Falchi, M., Spector, T.D., Adamski, J., Franks, P.W., Pedersen, O., Segal, E.IMI DIRECT consortium, 2020. A reference map of potential determinants for the human serum metabolome. Nature 588, 135–140.

Boddy, S., Islam, M., Moll, T., Kurz, J., Burrows, D., McGown, A., Bhargava, A., Julian, T.H., Harvey, C., Marshall, J.N., Hall, B.P., Allen, S.P., Kenna, K.P., Sanderson, E., Zhang, S., Ramesh, T., Snyder, M.P., Shaw, P.J., McDermott, C., Cooper–Knock, J., 2022. Unbiased metabolome screen leads to personalized medicine strategy for amyotrophic lateral sclerosis. Brain Commun 4, fcac069.

Bowden, J., Davey Smith, G., Haycock, P.C., Burgess, S., 2016. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet. Epidemiol. 40, 304–314.

Bowden, J., Hemani, G., Davey Smith, G., 2018a. Invited Commentary: Detecting Individual and Global Horizontal Pleiotropy in Mendelian Randomization—A Job for the Humble Heterogeneity Statistic? Am. J. Epidemiol. 187, 2681–2685.

Bowden, J., Spiller, W., Del Greco, M, F., Sheehan, N., Thompson, J., Minelli, C., Davey Smith, G., 2018b. Improving the visualization, interpretation and analysis of two-sample summary data Mendelian randomization via the Radial plot and Radial regression. Int. J. Epidemiol. 47, 1264–1278.

Bowman, G.L., Shannon, J., Frei, B., Kaye, J.A., Quinn, J.F., 2010. Uric acid as a CNS antioxidant. J. Alzheimers. Dis. 19, 1331–1336.

Burgess, S., Butterworth, A., Thompson, S.G., 2013. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet. Epidemiol. 37, 658–665.

Burgess, S., Davey Smith, G., Davies, N.M., Dudbridge, F., Gill, D., Glymour, M.M., Hartwig, F.P., Holmes, M.V., Minelli, C., Relton, C.L., Theodoratou, E., 2019. Guidelines for performing Mendelian randomization investigations. Wellcome Open Res 4, 186.

Burgess, S., Davies, N.M., Thompson, S.G., 2016. Bias due to participant overlap in two-sample Mendelian randomization. Genet. Epidemiol. 40, 597–608.

Burgess, S., Thompson, S.G.CRP CHD Genetics Collaboration, 2011. Avoiding bias from weak instruments in Mendelian randomization studies. Int. J. Epidemiol. 40, 755–764.

Cankurtaran, M., Yesil, Y., Kuyumcu, M.E., Oztürk, Z.A., Yavuz, B.B., Halil, M., Ulger, Z., Cankurtaran, E.S., Arıoğul, S., 2013. Altered levels of homocysteine and serum natural antioxidants links oxidative damage to Alzheimer's disease. J. Alzheimers. Dis. 33, 1051–1058.

Chen, X., Guo, X., Huang, R., Chen, Y., Zheng, Z., Shang, H., 2014. Serum uric acid levels in patients with Alzheimer's disease: a meta-analysis. PLoS One 9, e94084.

Euser, S.M., Hofman, A., Westendorp, R.G.J., Breteler, M.M.B., 2009. Serum uric acid and cognitive function and dementia. Brain 132, 377–382.

Gatz, M., Pedersen, N.L., Berg, S., Johansson, B., Johansson, K., Mortimer, J.A., Posner, S.F., Viitanen, M., Winblad, B., Ahlbom, A., 1997. Heritability for Alzheimer's disease: the study of dementia in Swedish twins. J. Gerontol. A Biol. Sci. Med. Sci. 52, M117–M125.

Hartwig, F.P., Davey Smith, G., Bowden, J., 2017. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int. J. Epidemiol. 46, 1985–1998.

Hartwig, F.P., Davies, N.M., Hemani, G., Davey Smith, G., 2016. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. Int. J. Epidemiol. 45, 1717–1726.

Hemani, G., Zheng, J., Elsworth, B., Wade, K.H., Haberland, V., Baird, D., Laurin, C., Burgess, S., Bowden, J., Langdon, R., Tan, V.Y., Yarmolinsky, J., Shihab, H.A., Timpson, N.J., Evans, D.M., Relton, C., Martin, R.M., Davey Smith, G., Gaunt, T.R., Haycock, P.C., 2018. The MR-Base platform supports systematic causal inference across the human phenome. Elife 7. doi:10.7554/eLife.34408.

Hong, J.-Y., Lan, T.-Y., Tang, G.-J., Tang, C.-H., Chen, T.-J., Lin, H.-Y., 2015. Gout and the risk of dementia: a nationwide population-based cohort study. Arthritis Res. Ther. 17, 139.

Irizarry, M.C., Raman, R., Schwarzschild, M.A., Becerra, L.M., Thomas, R.G., Peterson, R.C., Ascherio, A., Aisen, P.S., 2009. Plasma urate and progression of mild cognitive impairment. Neurodegener. Dis. 6, 23–28.

Jia, L., Yang, J., Zhu, M., Pang, Y., Wang, Q., Wei, Q., Li, Y., Li, T., Li, F., Wang, Q., Li, Y., Wei, Y., 2021. A metabolite panel that differentiates Alzheimer's disease from other dementia types. Alzheimers. Dement. doi:10.1002/alz.12484.

Julian, T.H., Boddy, S., Islam, M., Kurz, J., Whittaker, K.J., Moll, T., Harvey, C., Zhang, S., Snyder, M.P., McDermott, C., Cooper-Knock, J., Shaw, P.J., 2021. A review of Mendelian randomization in amyotrophic lateral sclerosis. Brain doi:10.1093/brain/awab420.

Kettunen, J., Demirkan, A., Würtz, P., Draisma, H.H.M., Haller, T., Rawal, R., Vaarhorst, A., Kangas, A.J., Lyytikäinen, L.-P., Pirinen, M., Pool, R., Sarin, A.-P., Soininen, P., Tukiainen, T., Wang, Q., Tiainen, M., Tynkkynen, T., Amin, N., Zeller, T., Beekman, M., Deelen, J., van Dijk, K.W., Esko, T., Hottenga, J.-J., van Leeuwen, E.M., Lehtimäki, T., Mihailov, E., Rose, R.J., de Craen, A.J.M., Gieger, C., Kähönen, M., Perola, M., Blankenberg, S., Savolainen, M.J., Verhoeven, A., Viikari, J., Willemsen, G., Boomsma, D.I., van Duijn, C.M., Eriksson, J., Jula, A., Järvelin, M.-R., Kaprio, J., Metspalu, A., Raitakari, O., Salomaa, V., Slagboom, P.E., Waldenberger, M., Ripatti, S., Ala-Korpela, M., 2016. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nat. Commun. 7, 11122.

Kim, T.-S., Pae, C.-U., Yoon, S.-J., Jang, W.-Y., Lee, N.J., Kim, J.-J., Lee, S.-J., Lee, C., Paik, I.-H., Lee, C.-U., 2006. Decreased plasma antioxidants in patients with Alzheimer's disease. Int. J. Geriatr. Psychiatry 21, 344–348.

Ko, J., Kang, H.-J., Kim, D.-A., Kim, M.-J., Ryu, E.-S., Lee, S., Ryu, J.-H., Roncal, C., Johnson, R.J., Kang, D.-H., 2019. Uric acid induced the phenotype transition of vascular endothelial cells via induction of oxidative stress and glycocalyx shedding. FASEB J 33, 13334–13345.

Köttgen, A., Albrecht, E., Teumer, A., Vitart, V., Krumsiek, J., Hundertmark, C., Pistis, G., Ruggiero, D., O'Seaghdha, C.M., Haller, T., Yang, Q., Tanaka, T., Johnson, A.D., Kutalik, Z., Smith, A.V., Shi, J., Struchalin, M., Middelberg, R.P.S., Brown, M.J., Gaffo, A.L., Pirastu, N., Li, G., Hayward, C., Zemunik, T., Huffman, J., Yengo, L., Zhao, J.H., Demirkan, A., Feitosa, M.F., Liu, X., Malerba, G., Lopez, L.M., van der Harst, P., Li, X., Kleber, M.E., Hicks, A.A., Nolte, I.M., Johansson, A., Murgia, F., Wild, S.H., Bakker, S.J.L., Peden, J.F., Dehghan, A., Steri, M., Tenesa, A., Lagou, V., Salo, P., Mangino, M., Rose, L.M., Lehtimäki, T., Woodward, O.M., Okada, Y., Tin, A., Müller, C., Oldmeadow, C., Putku, M., Czamara, D., Kraft, P., Frogheri, L., Thun, G.A., Grotevendt, A., Gislason, G.K., Harris, T.B., Launer, L.J., McArdle, P., Shuldiner, A.R., Boerwinkle, E., Coresh, J., Schmidt, H., Schallert, M., Martin, N.G., Montgomery, G.W., Kubo, M., Nakamura, Y., Tanaka, T., Munroe, P.B., Samani, N.J., Jacobs, D.R., Jr, Liu, K., D'Adamo, P., Ulivi, S., Rotter, J.I., Psaty, B.M., Vollenweider, P., Waeber, G., Campbell, S., Devuyst, O., Navarro, P., Kolcic, I., Hastie, N., Balkau, B., Froguel, P., Esko, T., Salumets, A., Khaw, K.T., Langenberg, C., Wareham, N.J., Isaacs, A., Kraja, A., Zhang, Q., Wild, P.S., Scott, R.J., Holliday, E.G., Org, E., Viigimaa, M., Bandinelli, S., Metter, J.E., Lupo, A., Trabetti, E., Sorice, R., Döring, A., Lattka, E., Strauch, K., Theis, F., Waldenberger, M., Wichmann, H.-E., Davies, G., Gow, A.J., Bruinenberg, M., Stolk, R.P., Kooner, J.S., Zhang, W., Winkelmann, B.R., Boehm, B.O., Lucae, S., Penninx, B.W., Smit, J.H., Curhan, G., Mudgal, P., Plenge, R.M., Portas, L., Persico, I., Kirin, M., Wilson, J.F., Mateo Leach, I., van Gilst, W.H., Goel, A., Ongen, H., Hofman, A., Rivadeneira, F., Uitterlinden, A.G., Imboden, M., von Eckardstein, A., Cucca, F., Nagaraja, R., Piras, M.G., Nauck, M., Schurmann, C., Budde, K., Ernst, F., Farrington, S.M., Theodoratou, E., Prokopenko, I., Stumvoll, M., Jula, A., Perola, M., Salomaa, V., Shin, S.-Y., Spector, T.D., Sala, C., Ridker, P.M., Kähönen, M., Viikari, J., Hengstenberg, C., Nelson, C.P., Meschia, J.F., Nalls, M.A., Sharma, P., Singleton, A.B., Kamatani, N., Zeller, T., Burnier, M., Attia, J., Laan, M., Klopp, N., Hillege, H.L., Kloiber, S., Choi, H., Pirastu, M., Tore, S., Probst-Hensch, N.M., Völzke, H., Gudnason, V., Parsa, A., Schmidt, R., Whitfield, J.B., Fornage, M., Gasparini, P., Siscovick, D.S., Polašek, O., Campbell, H., Rudan, I., Bouatia-Naji, N., Metspalu, A., Loos, R.J.F., van Duijn, C.M., Borecki, I.B., Ferrucci, L., Gambaro, G., Deary, I.J., Wolffenbuttel, B.H.R., Chambers, J.C., März, W., Pramstaller, P.P., Snieder, H., Gyllensten, U., Wright, A.F., Navis, G., Watkins, H., Witteman, J.C.M., Sanna, S., Schipf, S., Dunlop, M.G., Tönjes, A., Ripatti, S., Soranzo, N., Toniolo, D., Chasman, D.I., Raitakari, O., Kao, W.H.L., Ciullo, M., Fox, C.S., Caulfield, M., Bochud, M., Gieger, C.MAGIC Consortium, 2013. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nat. Genet. 45, 145–154.

Kunkle, B.W., Grenier-Boley, B., Sims, R., Bis, J.C., Damotte, V., Naj, A.C., Boland, A., Vronskaya, M., van der Lee, S.J., Amlie-Wolf, A., Bellenguez, C., Frizatti, A., Chouraki, V., Martin, E.R., Sleegers, K., Badarinarayan, N., Jakobsdottir, J., Hamilton-Nelson, K.L., Moreno-Grau, S., Olaso, R., Raybould, R., Chen, Y., Kuzma, A.B., Hiltunen, M., Morgan, T., Ahmad, S., Vardarajan, B.N., Epelbaum, J., Hoffmann, P., Boada, M., Beecham, G.W., Garnier, J.-G., Harold, D., Fitzpatrick, A.L., Valladares, O., Moutet, M.-L., Gerrish, A., Smith, A.V., Qu, L., Bacq, D., Denning, N., Jian, X., Zhao, Y., Del Zompo, M., Fox, N.C., Choi, S.-H., Mateo, I., Hughes, J.T., Adams, H.H., Malamon, J., Sanchez-Garcia, F., Patel, Y., Brody, J.A., Dombroski, B.A., Naranjo, M.C.D., Daniilidou, M., Eiriksdottir, G., Mukherjee, S., Wallon, D., Uphill, J., Aspelund, T., Cantwell, L.B., Garzia, F., Galimberti, D., Hofer, E., Butkiewicz, M., Fin, B., Scarpini, E., Sarnowski, C., Bush, W.S., Meslage, S., Kornhuber, J., White, C.C., Song, Y., Barber, R.C., Engelborghs, S., Sordon, S., Voijnovic, D., Adams, P.M., Vandenberghe, R., Mayhaus, M., Cupples, L.A., Albert, M.S., De Deyn, P.P., Gu, W., Himali, J.J., Beekly, D., Squassina, A., Hartmann, A.M., Orellana, A., Blacker, D., Rodriguez-Rodriguez, E., Lovestone, S., Garcia, M.E., Doody, R.S., Munoz-Fernadez, C., Sussams, R., Lin, H., Fairchild, T.J., Benito, Y.A., Holmes, C., Karamujić-Čomić, H., Frosch, M.P., Thonberg, H., Maier, W., Roshchupkin, G., Ghetti, B., Giedraitis, V., Kawalia, A., Li, S., Huebinger, R.M., Kilander, L., Moebus, S., Hernández, I., Kamboh, M.I., Brundin, R., Turton, J., Yang, Q., Katz, M.J., Concari, L., Lord, J., Beiser, A.S., Keene, C.D., Helisalmi, S., Kloszewska, I., Kukull, W.A., Koivisto, A.M., Lynch, A., Tarraga, L., Larson, E.B., Haapasalo, A., Lawlor, B., Mosley, T.H., Lipton, R.B., Solfrizzi, V., Gill, M., Longstreth, W.T., Jr, Montine, T.J., Frisardi, V., Diez-Fairen, M., Rivadeneira, F., Petersen, R.C., Deramecourt, V., Alvarez, I., Salani, F., Ciaramella, A., Boerwinkle, E., Reiman, E.M., Fievet, N., Rotter, J.I., Reisch, J.S., Hanon, O., Cupidi, C., Andre Uitterlinden, A.G., Royall, D.R., Dufouil, C., Maletta, R.G., de Rojas, I., Sano, M., Brice, A., Cecchetti, R., George-Hyslop, P.S., Ritchie, K., Tsolaki, M., Tsuang, D.W., Dubois, B., Craig, D., Wu, C.-K., Soininen, H., Avramidou, D., Albin, R.L., Fratiglioni, L., Germanou, A., Apostolova, L.G., Keller, L., Koutroumani, M., Arnold, S.E., Panza, F., Gkatzima, O., Asthana, S., Hannequin, D., Whitehead, P., Atwood, C.S., Caffarra, P., Hampel, H., Quintela, I., Carracedo, Á., Lannfelt, L., Rubinsztein, D.C., Barnes, L.L., Pasquier, F., Frölich, L., Barral, S., McGuinness, B., Beach, T.G., Johnston, J.A., Becker, J.T., Passmore, P., Bigio, E.H., Schott, J.M., Bird, T.D., Warren, J.D., Boeve, B.F., Lupton, M.K., Bowen, J.D., Proitsi, P., Boxer, A., Powell, J.F., Burke, J.R., Kauwe, J.S.K., Burns, J.M., Mancuso, M., Buxbaum, J.D., Bonuccelli, U., Cairns, N.J., McQuillin, A., Cao, C., Livingston, G., Carlson, C.S., Bass, N.J., Carlsson, C.M., Hardy, J., Carney, R.M., Bras, J., Carrasquillo, M.M., Guerreiro, R., Allen, M., Chui, H.C., Fisher, E., Masullo, C., Crocco, E.A., DeCarli, C., Bisceglio, G., Dick, M., Ma, L., Duara, R., Graff-Radford, N.R., Evans, D.A., Hodges, A., Faber, K.M., Scherer, M., Fallon, K.B., Riemenschneider, M., Fardo, D.W., Heun, R., Farlow, M.R., Kölsch, H., Ferris, S., Leber, M., Foroud, T.M., Heuser, I., Galasko, D.R., Giegling, I., Gearing, M., Hüll, M., Geschwind, D.H., Gilbert, J.R., Morris, J., Green, R.C., Mayo, K., Growdon, J.H., Feulner, T., Hamilton, R.L., Harrell, L.E., Drichel, D., Honig, L.S., Cushion, T.D., Huentelman, M.J., Hollingworth, P., Hulette, C.M., Hyman, B.T., Marshall, R., Jarvik, G.P., Meggy, A., Abner, E., Menzies, G.E., Jin, L.-W., Leonenko, G., Real, L.M., Jun, G.R., Baldwin, C.T., Grozeva, D., Karydas, A., Russo, G., Kaye, J.A., Kim, R., Jessen, F., Kowall, N.W., Vellas, B., Kramer, J.H., Vardy, E., LaFerla, F.M., Jöckel, K.-H., Lah, J.J., Dichgans, M., Leverenz, J.B., Mann, D., Levey, A.I., Pickering-Brown, S., Lieberman, A.P., Klopp, N., Lunetta, K.L., Wichmann, H.-E., Lyketsos, C.G., Morgan, K., Marson, D.C., Brown, K., Martiniuk, F., Medway, C., Mash, D.C., Nöthen, M.M., Masliah, E., Hooper, N.M., McCormick, W.C., Daniele, A., McCurry, S.M., Bayer, A., McDavid, A.N., Gallacher, J., McKee, A.C., van den Bussche, H., Mesulam, M., Brayne, C., Miller, B.L., Riedel-Heller, S., Miller, C.A., Miller, J.W., Al-Chalabi, A., Morris, J.C., Shaw, C.E., Myers, A.J., Wiltfang, J., O'Bryant, S., Olichney, J.M., Alvarez, V., Parisi, J.E., Singleton, A.B., Paulson, H.L., Collinge, J., Perry, W.R., Mead, S., Peskind, E., Cribbs, D.H., Rossor, M., Pierce, A., Ryan, N.S., Poon, W.W., Nacmias, B., Potter, H., Sorbi, S., Quinn, J.F., Sacchinelli, E., Raj, A., Spalletta, G., Raskind, M., Caltagirone, C., Bossù, P., Orfei, M.D., Reisberg, B., Clarke, R., Reitz, C., Smith, A.D., Ringman, J.M., Warden, D., Roberson, E.D., Wilcock, G., Rogaeva, E., Bruni, A.C., Rosen, H.J., Gallo, M., Rosenberg, R.N., Ben-Shlomo, Y., Sager, M.A., Mecocci, P., Saykin, A.J., Pastor, P., Cuccaro, M.L., Vance, J.M., Schneider, J.A., Schneider, L.S., Slifer, S., Seeley, W.W., Smith, A.G., Sonnen, J.A., Spina, S., Stern, R.A., Swerdlow, R.H., Tang, M., Tanzi, R.E., Trojanowski, J.Q., Troncoso, J.C., Van Deerlin, V.M., Van Eldik, L.J., Vinters, H.V., Vonsattel, J.P., Weintraub, S., Welsh-Bohmer, K.A., Wilhelmsen, K.C., Williamson, J., Wingo, T.S., Woltjer, R.L., Wright, C.B., Yu, C.-E., Yu, L., Saba, Y., Pilotto, A., Bullido, M.J., Peters, O., Crane, P.K., Bennett, D., Bosco, P., Coto, E., Boccardi, V., De Jager, P.L., Lleo, A., Warner, N., Lopez, O.L., Ingelsson, M., Deloukas, P., Cruchaga, C., Graff, C., Gwilliam, R., Fornage, M., Goate, A.M., Sanchez-Juan, P., Kehoe, P.G., Amin, N., Ertekin-Taner, N., Berr, C., Debette, S., Love, S., Launer, L.J., Younkin, S.G., Dartigues, J.-F., Corcoran, C., Ikram, M.A., Dickson, D.W., Nicolas, G., Campion, D., Tschanz, J., Schmidt, H., Hakonarson, H., Clarimon, J., Munger, R., Schmidt, R., Farrer, L.A., Van Broeckhoven, C., C O'Donovan, M., DeStefano, A.L., Jones, L., Haines, J.L., Deleuze, J.-F.,

Owen, M.J., Gudnason, V., Mayeux, R., Escott-Price, V., Psaty, B.M., Ramirez, A., Wang, L.-S., Ruiz, A., van Duijn, C.M., Holmans, P.A., Seshadri, S., Williams, J., Amouyel, P., Schellenberg, G.D., Lambert, J.-C., Pericak-Vance, M.A.Polygenic and Environmental Risk for Alzheimer's Disease Consortium (GERAD/PERADES), 2019. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. Nat. Genet. 51, 414–430.

Lambert, J.C., Ibrahim-Verbaas, C.A., Harold, D., Naj, A.C., Sims, R., Bellenguez, C., DeStafano, A.L., Bis, J.C., Beecham, G.W., Grenier-Boley, B., Russo, G., Thorton-Wells, T.A., Jones, N., Smith, A.V., Chouraki, V., Thomas, C., Ikram, M.A., Zelenika, D., Vardarajan, B.N., Kamatani, Y., Lin, C.F., Gerrish, A., Schmidt, H., Kunkle, B., Dunstan, M.L., Ruiz, A., Bihoreau, M.T., Choi, S.H., Reitz, C., Pasquier, F., Cruchaga, C., Craig, D., Amin, N., Berr, C., Lopez, O.L., De Jager, P.L., Deramecourt, V., Johnston, J.A., Evans, D., Lovestone, S., Letenneur, L., Morón, F.J., Rubinsztein, D.C., Eiriksdottir, G., Sleegers, K., Goate, A.M., Fiévet, N., Huentelman, M.W., Gill, M., Brown, K., Kamboh, M.I., Keller, L., Barberger-Gateau, P., McGuiness, B., Larson, E.B., Green, R., Myers, A.J., Dufouil, C., Todd, S., Wallon, D., Love, S., Rogaeva, E., Gallacher, J., St George-Hyslop, P., Clarimon, J., Lleo, A., Bayer, A., Tsuang, D.W., Yu, L., Tsolaki, M., Bossù, P., Spalletta, G., Proitsi, P., Collinge, J., Sorbi, S., Sanchez-Garcia, F., Fox, N.C., Hardy, J., Deniz Naranjo, M.C., Bosco, P., Clarke, R., Brayne, C., Galimberti, D., Mancuso, M., Matthews, F., Moebus, S., Mecocci, P., Del Zompo, M., Maier, W., Hampel, H., Pilotto, A., Bullido, M., Panza, F., Caffarra, P., Nacmias, B., Gilbert, J.R., Mayhaus, M., Lannefelt, L., Hakonarson, H., Pichler, S., Carrasquillo, M.M., Ingelsson, M., Beekly, D., Alvarez, V., Zou, F., Valladares, O., Younkin, S.G., Coto, E., Hamilton-Nelson, K.L., Gu, W., Razquin, C., Pastor, P., Mateo, I., Owen, M.J., Faber, K.M., Jonsson, P.V., Combarros, O., O'Donovan, M.C., Cantwell, L.B., Soininen, H., Blacker, D., Mead, S., Mosley, T.H., Jr, Bennett, D.A., Harris, T.B., Fratiglioni, L., Holmes, C., de Bruijn, R.F., Passmore, P., Montine, T.J., Bettens, K., Rotter, J.I., Brice, A., Morgan, K., Foroud, T.M., Kukull, W.A., Hannequin, D., Powell, J.F., Nalls, M.A., Ritchie, K., Lunetta, K.L., Kauwe, J.S., Boerwinkle, E., Riemenschneider, M., Boada, M., Hiltuenen, M., Martin, E.R., Schmidt, R., Rujescu, D., Wang, L.S., Dartigues, J.F., Mayeux, R., Tzourio, C., Hofman, A., Nöthen, M.M., Graff, C., Psaty, B.M., Jones, L., Haines, J.L., Holmans, P.A., Lathrop, M., Pericak-Vance, M.A., Launer, L.J., Farrer, L.A., van Duijn, C.M., Van Broeckhoven, C., Moskvina, V., Seshadri, S., Williams, J., Schellenberg, G.D., Amouyel, P.Cohorts for Heart and Aging Research in Genomic Epidemiology, 2013. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat. Genet. 45, 1452–1458.

Lord, J., Jermy, B., Green, R., Wong, A., Xu, J., Legido-Quigley, C., Dobson, R., Richards, M., Proitsi, P., 2021. Mendelian randomization identifies blood metabolites previously linked to midlife cognition as causal candidates in Alzheimer's disease. Proc. Natl. Acad. Sci doi:10.1073/pnas.2009808118.

Machiela, M.J., Chanock, S.J., 2015. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics 31, 3555–3557.

Marshall, D.D., Powers, R., 2017. Beyond the paradigm: Combining mass spectrometry and nuclear magnetic resonance for metabolomics. Prog. Nucl. Magn. Reson. Spectrosc. 100, 1–16.

Nalls, M.A., Blauwendraat, C., Vallerga, C.L., Heilbron, K., Bandres-Ciga, S., Chang, D., Tan, M., Kia, D.A., Noyce, A.J., Xue, A., Bras, J., Young, E., von Coelln, R., Simón-Sánchez, J., Schulte, C., Sharma, M., Krohn, L., Pihlstrøm, L., Siitonen, A., Iwaki, H., Leonard, H., Faghri, F., Gibbs, J.R., Hernandez, D.G., Scholz, S.W., Botia, J.A., Martinez, M., Corvol, J.-C., Lesage, S., Jankovic, J., Shulman, L.M., Sutherland, M., Tienari, P., Majamaa, K., Toft, M., Andreassen, O.A., Bangale, T., Brice, A., Yang, J., Gan-Or, Z., Gasser, T., Heutink, P., Shulman, J.M., Wood, N.W., Hinds, D.A., Hardy, J.A., Morris, H.R., Gratten, J., Visscher, P.M., Graham, R.R., Singleton, A.B.International Parkinson's Disease Genomics Consortium, 2019. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet Neurol 18, 1091–1102.

Nicolas, A., Kenna, K.P., Renton, A.E., Ticozzi, N., Faghri, F., Chia, R., Dominov, J.A., Kenna, B.J., Nalls, M.A., Keagle, P., Rivera, A.M., van Rheenen, W., Murphy, N.A., van Vugt, J.J.F.A., Geiger, J.T., Van der Spek, R.A., Pliner, H.A., Shankaracharya, Smith, B.N., Marangi, G., Topp, S.D., Abramzon, Y., Gkazi, A.S., Eicher, J.D., Kenna, A., Mora, G., Calvo, A., Mazzini, L., Riva, N., Mandrioli, J., Caponnetto, C., Battistini, S., Volanti, P., La Bella, V., Conforti, F.L., Borghero, G., Messina, S., Simone, I.L., Trojsi, F., Salvi, F., Logullo, F.O., D'Alfonso, S., Corrado, L., Capasso, M., Ferrucci, L., Kamalakaran, S., Goldstein, D.B., Gitler, A.D., Harris, T., Myers, R.M., Phatnani, H., Musunuri, R.L., Evani, U.S., Abhyankar, A., Zody, M.C., Kaye, J., Finkbeiner, S., Wyman, S.K., LeNail, A., Lima, L., Fraenkel, E., Svendsen, C.N., Thompson, L.M., Van Eyk, J.E., Berry, J.D., Miller, T.M., Kolb, S.J., Cudkowicz, M., Baxi, E., Benatar, M., Taylor, J.P., Rampersaud, E., Wu, G., Wuu, J., Lauria, G., Verde, F., Fogh, I., Tiloca, C., Comi, G.P., Sorarù, G., Cereda, C., Consortium, French ALS, Corcia, P., Laaksovirta, H., Myllykangas, L., Jansson, L., Valori, M., Ealing, J., Hamdalla, H., Rollinson, S., Pickering-Brown, S., Orrell, R.W., Sidle, K.C., Malaspina, A., Hardy, J., Singleton, A.B., Johnson, J.O., Arepalli, S., Sapp, P.C., McKenna-Yasek, D., Polak, M., Asress, S., Al-Sarraj, S., King, A., Troakes, C., Vance, C., de Belleroche, J., Baas, F., Ten Asbroek, A.L.M.A., Muñoz-Blanco, J.L., Hernandez, D.G., Ding, J., Gibbs, J.R., Scholz, S.W., Floeter, M.K., Campbell, R.H., Landi, F., Bowser, R., Pulst, S.M., Ravits, J.M., MacGowan, D.J.L., Kirby, J., Pioro, E.P., Pamphlett, R., Broach, J., Gerhard, G., Dunckley, T.L., Brady, C.B., Kowall, N.W., Troncoso, J.C., Le Ber, I., Mouzat, K., Lumbroso, S., Heiman-Patterson, T.D., Kamel, F., Van Den Bosch, L., Baloh, R.H., Strom, T.M., Meitinger, T., Shatunov, A., Van Eijk, K.R., de Carvalho, M., Kooyman, M., Middelkoop, B., Moisse, M., McLaughlin, R.L., Van Es, M.A., Weber, M., Boylan, K.B., Van Blitterswijk, M., Rademakers, R., Morrison, K.E., Basak, A.N., Mora, J.S., Drory, V.E., Shaw, P.J., Turner, M.R., Talbot, K., Hardiman, O., Williams, K.L., Fifita, J.A., Nicholson, G.A., Blair, I.P., Rouleau, G.A., Esteban-Pérez, J., García-Redondo, A., Al-Chalabi, A., Rogaeva, E., Zinman, L., Ostrow, L.W., Maragakis, N.J., Rothstein, J.D., Simmons, Z., Cooper-Knock, J., Brice, A., Goutman, S.A., Feldman, E.L., Gibson, S.B., Taroni, F., Ratti, A., Gellera, C., Van Damme, P., Robberecht, W., Fratta, P., Sabatelli, M., Lunetta, C., Ludolph, A.C., Andersen, P.M., Weishaupt, J.H., Camu, W., Trojanowski, J.Q., Van Deerlin, V.M., Brown, R.H., Jr, van den Berg, L.H., Veldink, J.H., Harms, M.B., Glass, J.D., Stone, D.J., Tienari, P., Silani, V., Chiò, A., Shaw, C.E., Traynor, B.J., Landers, J.E.Project MinE ALS Sequencing Consortium, 2018. Genome-wide Analyses Identify KIF5A as a Novel ALS Gene. Neuron 97, 1268–1283 e6.

Polidori, M.C., Mecocci, P., 2002. Plasma susceptibility to free radical-induced antioxidant consumption and lipid peroxidation is increased in very old subjects with Alzheimer disease. J. Alzheimers. Dis. 4, 517–522.

Richmond, R.C., Davey Smith, G., Ness, A.R., den Hoed, M., McMahon, G., Timpson, N.J., 2014. Assessing causality in the association between child adiposity and physical activity levels: a Mendelian randomization analysis. PLoS Med 11, e1001618.

Rinaldi, P., Polidori, M.C., Metastasio, A., Mariani, E., Mattioli, P., Cherubini, A., Catani, M., Cecchetti, R., Senin, U., Mecocci, P., 2003. Plasma antioxidants are similarly depleted in mild cognitive impairment and in Alzheimer's disease. Neurobiol. Aging. doi:10.1016/s0197-4580(03)00031-9.

Schretlen, D.J., Inscore, A.B., Jinnah, H.A., Rao, V., Gordon, B., Pearlson, G.D., 2007. Serum uric acid and cognitive function in community-dwelling older adults. Neuropsychology 21, 136–140.

Shin, S.-Y., Fauman, E.B., Petersen, A.-K., Krumsiek, J., Santos, R., Huang, J., Arnold, M., Erte, I., Forgetta, V., Yang, T.-P., Walter, K., Menni, C., Chen, L., Vasquez, L., Valdes, A.M., Hyde, C.L., Wang, V., Ziemek, D., Roberts, P., Xi, L., Grundberg, E., Waldenberger, M., Richards, J.B., Mohney, R.P., Milburn, M.V., John, S.L., Trimmer, J., Theis, F.J., Overington, J.P., Suhre, K., Brosnan, M.J., Gieger, C., Kastenmüller, G., Spector, T.D., Soranzo, N.Multiple Tissue Human Expression Resource (MuTHER) Consortium, 2014. An atlas of genetic influences on human blood metabolites. Nat. Genet. 46, 543–550.

Slob, E.A.W., Burgess, S., n.d. A Comparison Of Robust Mendelian Randomization Methods Using Summary Data. https://doi.org/10.1101/577940

Solfrizzi, V., Custodero, C., Lozupone, M., Imbimbo, B.P., Valiani, V., Agosti, P., Schilardi, A., D'Introno, A., La Montagna, M., Calvani, M., Guerra, V., Sardone, R., Abbrescia, D.I., Bellomo, A., Greco, A., Daniele, A., Seripa, D., Logroscino, G., Sabbá, C., Panza, F., 2017. Relationships of Dietary Patterns, Foods, and Micro- and Macronutrients with Alzheimer's Disease and Late-Life Cognitive Disorders: A Systematic Review. J. Alzheimers Dis. doi:10.3233/jad-170248.

Song, C., Zhao, X., 2018. Uric acid promotes oxidative stress and enhances vascular endothelial cell apoptosis in rats with middle cerebral artery occlusion. Biosci. Rep. 38. doi:10.1042/BSR20170939.

Tian, T., Liu, X.-R., Li, T.-T., Nie, Z.-C., Li, S.-J., Tang, Y., Gu, C.-W., Xu, W.-D., Jia, H., 2021. Detrimental effects of long-term elevated serum uric acid on cognitive function in rats. Sci. Rep. 11, 6732.

Vannorsdall, T.D., Kueider, A.M., Carlson, M.C., Schretlen, D.J., 2014. Higher baseline serum uric acid is associated with poorer cognition but not rates of cognitive decline in women. Exp. Gerontol. 60, 136–139.

Wang, Z., Meng, L., Shen, L., Ji, H.-F., 2020. Impact of modifiable risk factors on Alzheimer's disease: A two-sample Mendelian randomization study. Neurobiol. Aging 91, 167.e11–167.e19.

Wightman, D.P., Jansen, I.E., Savage, J.E., Shadrin, A.A., Bahrami, S., Holland, D., Rongve, A., Børte, S., Winsvold, B.S., Drange, O.K., Martinsen, A.E., Skogholt, A.H., Willer, C., Bråthen, G., Bosnes, I., Nielsen, J.B., Fritsche, L.G., Thomas, L.F., Pedersen, L.M., Gabrielsen, M.E., Johnsen, M.B., Meisingset, T.W., Zhou, W., Proitsi, P., Hodges, A., Dobson, R., Velayudhan, L., Heilbron, K., Auton, A., Sealock, J.M., Davis, L.K., Pedersen, N.L., Reynolds, C.A., Karlsson, I.K., Magnusson, S., Stefansson, H., Thordardottir, S., Jonsson, P.V., Snaedal, J., Zettergren, A., Skoog, I., Kern, S., Waern, M., Zetterberg, H., Blennow, K., Stordal, E., Hveem, K., Zwart, J.-A., Athanasiu, L., Selnes, P., Saltvedt, I., Sando, S.B., Ulstein, I., Djurovic, S., Fladby, T., Aarsland, D., Selbæk, G., Ripke, S., Stefansson, K., Andreassen, O.A., Posthuma, D.23andMe Research Team, 2021. A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer's disease. Nat. Genet. 53, 1276–1282.

Williams, D.M., Hägg, S., Pedersen, N.L., 2019. Circulating antioxidants and Alzheimer disease prevention: a Mendelian randomization study. Am. J. Clin. Nutr. 109, 90–98.

Yegambaram, M., Manivannan, B., Beach, T.G., Halden, R.U., 2015. Role of environmental contaminants in the etiology of Alzheimer's disease: a review. Curr. Alzheimer Res. 12, 116–146.

Zhang, G., Zhang, L., Tang, L., Xia, K., Huang, T., Fan, D., 2021. Physical activity and amyotrophic lateral sclerosis: a Mendelian randomization study. Neurobiol. Aging 105, 374.e1–374.e4.