The relationship between glycaemia, cognitive function, structural brain outcomes and dementia: A Mendelian randomisation study in the UK Biobank

Victoria Garfield, PhD[1], Aliki-Eleni Farmaki, PhD[1], Ghazaleh Fatemifar, PhD[3, 5], Sophie V. Eastwood, MRCGP[1], Rohini Mathur, PhD[2], Christopher T. Rentsch, PhD[2], Spiros Denaxas, PhD[3,4.5], Krishnan Bhaskaran, PhD[2], Liam Smeeth, PhD[2], Nish Chaturvedi, MD[1]

[1]MRC Unit for Lifelong Health and Ageing at UCL, Institute of Cardiovascular Science, University College London, 1-19 Torrington Place, London, WC1E 7HB

[2]Department of Non-communicable Disease Epidemiology, London School of Hygiene & Tropical Medicine, Keppel Street, London, WC1E 7HT

[3]Institute of Health Informatics, University College London, 222 Euston Road, NW1 2DA

[4]The Alan Turing Institute, British Library, 96 Euston Road, London, NW1 2DB

[5]Health Data Research UK (HDR UK), 222 Euston Road, London, NW1 2DA

Corresponding author: Dr Victoria Garfield, 1-19 Torrington Place, London, WC1E 7HB, United Kingdom, Tel.: +44(0)2035495589, email: v.garfield@ucl.ac.uk

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Abstract

We investigated the relationship between glycaemia and cognitive function, brain structure and incident dementia using bidirectional Mendelian randomisation (MR). Data were from UK Biobank (n~500,000). Our exposures were genetic instruments for type-2 diabetes (157 variants) and HbA_{1c} (51 variants) and our outcomes were reaction time (RT), visual memory, hippocampal and white matter hyperintensity volumes, Alzheimer's dementia (AD). We also investigated associations between genetic variants for RT (43 variants) and, diabetes and HbA_{1c}. We used conventional inverse-variance weighted (IVW) MR, alongside MR sensitivity analyses. Using IVW, genetic liability to type-2 diabetes was not associated with reaction time (RT) (exponentiated ß=1.00, 95%CI=1.00; 1.00), visual memory (VM) (expß=1.00, 95%CI=0.99; 1.00), white matter hyperintensity volume (WMHV) (expß=0.99, 95%CI=0.97; 1.01), hippocampal volume (HV) (ß coefficient mm^3=-2.30, 95%CI=-12.39;7.78) or AD (OR 1.15, 95%CI=0.87;1.52). HbA_{1c} was not associated with RT (expß=1.00, 95%CI=0.99; 1.02), VM (expß=0.99, 95%CI=0.96;1.02), WMHV (expß=1.03, 95%CI=0.88; 1.22), HV (ß=-21.31, 95%CI=-82.96; 40.34), or risk of AD (OR 1.09, 95%CI=0.42; 2.83). IVW showed that reaction time was not associated with diabetes risk (OR 0.94, 95%CI=0.54; 1.65), or with HbA_{1c} (ß coefficient mmol/mol=-0.88, 95%CI=-1.88; 0.13) - after exclusion of a pleiotropic variant. Overall, we observed little evidence of causal association between genetic instruments for T2D or peripheral glycaemia and some measures of cognition and brain structure in midlife.

Observational evidence largely suggests that hyperglycaemia, diabetes and insulin resistance are associated with poorer brain health, including worse cognitive function, risk of cognitive decline and dementia (1–4). The exact mechanisms remain elusive (5), as well as how to best treat those with a diagnosis of both diabetes and cognitive dysfunction (6). These factors limit intervention attempts as it is unclear whether hyperglycaemia per se is the culprit or whether instead, vascular risk factors (e.g. hypertension, dyslipidaemia, inflammation) mediate the association between diabetes and poorer brain health outcomes. It is also unclear whether the associations between hyperglycaemic conditions and brain outcomes are causal in nature. Some evidence also supports a bidirectional relationship (5), implicating a vicious cycle whereby diabetes may result in dementia and dementia could then trigger further diabetes complications (7).

Mendelian randomisation (MR) overcomes some of the limitations of causal interpretation in observational studies. So far, MR studies have focussed solely on Alzheimer's dementia, with all three reporting no impact of diabetes (8–10). Pathways to cognitive decline and dementia involve a combination of vascular and neurocognitive mechanisms that may act either independently or in concert (11). Diabetes is more related to the vascular pathways but there is evidence that it also has neurotoxic consequences (12). There have been no previous MR studies that have investigated glycated haemoglobin (HbA_{1c}) and a range of brain health measures, such as cognitive function or structural brain abnormalities. No previous MR studies have investigated whether the bidirectional association may be causal in nature. Thus, the present study used i) genetic instruments for type-2 diabetes and HbA_{1c}, to examine the relationship with cognitive function, structural brain measures and Alzheimer's dementia (AD); and ii) where possible, genetic instruments for cognitive function to investigate whether the relationship with diabetes or HbA_{1c} might be bidirectional.

Research Design and Methods

Study design

Two-sample MR (a design that exploits genome-wide association summary statistics derived in non-overlapping samples) was used to mitigate biased results due to the 'winners' curse' (the over-estimation of genetic associations which are common in the one-sample MR setting) because it is neither necessary, nor desirable, that the genetic variants to be instrumented be derived from the same sample as the one under study (13). An important advantage of using two-sample MR is that it allows sensitivity analyses to identify unbalanced (directional) horizontal pleiotropy (described under *Statistical analyses*), which is crucial to satisfy MR assumptions. In our MR analyses, there was some sample overlap for diabetes and cognitive function (reaction time), but not for the HbA_{1c} genetic variants.

Sample

Full details of the UK Biobank (UKB) cohort have been described elsewhere (14). Briefly, UKB consists of 500,000 males and females from the general UK population, aged 40-69 years at baseline (2006-2010). There was a maximum of 349,326 participants of European ancestry with both genotype and all the phenotypes of interest in the present study (Figure 1).

Genotyping and quality control (QC) in UKB

487,409 UKB participants were genotyped using one of two customised genome-wide arrays that were imputed to a combination of the UK10K, 1000 Genomes Phase 3 and the Haplotype Reference Consortium (HRC) reference panels, which resulted in 93,095,623 autosomal variants (15). We then applied additional variant level QC and excluded genetic variants with: Fisher's exact test <0.3, minor allele frequency (MAF) <1% and a missing call rate of

≥5%. Individual-level QC meant that we excluded participants with: excessive or minimal heterozygosity, more than 10 putative third-degree relatives as per the kinship matrix, no consent to extract DNA, sex mismatches between self-reported and genetic sex, missing QC information and non-European ancestry (based on how individuals had self-reported their ancestry and the similarity with their genetic ancestry, as per a principal component analysis of their genotype).

Outcomes: baseline cognitive function, structural brain magnetic resonance imaging (MRI) and dementia

UKB administered five baseline cognitive assessments to all participants, via a computerised touch-screen interface, all of which are described in detail elsewhere (16). In the *visual memory* assessment, respondents were asked to correctly identify matches from six pairs of cards after they had memorised their positions. Then, the number of incorrect matches (number of attempts made to correctly identify the pairs) was recorded, with a greater number reflective of a poorer visual memory. Reaction time (in milliseconds) was recorded as the mean time taken by participants to correctly identify matches in a 12-round game of the card game 'Snap'. A higher score on this test indicated a slower (poorer) reaction time. Both of these variables were positively skewed and therefore, reaction time scores were transformed using the natural logarithmic function [ln(x)], whilst visual memory was transformed using [ln(x+1)].

Structural brain MRI scans were performed by UKB in a subsample of participants using standard protocols, as published previously (17). The post-processed measures derived by UKB and used in this study included: mean hippocampal volume (mm^3), and volume of white matter hyperintensities (WMH, mm^3). WMH volume was log-transformed as it was positively skewed. The number of participants with WMH volume was 32,506 and 32,407

with hippocampal volume data available (Figure 1). This was after excluding n=114 individuals who were outliers (+3SD from the mean) and who were not included in the genetic sample after QC. We checked whether there was any overlap between participants with AD and those with white matter hyperintensities, but as there was only n=1 with both AD and neuroimaging data, we did not consider this an issue for our analyses. We report results in mm^3 for hippocampal volume and exponentiated betas/percentages for WMH volume. UKB provided algorithmically defined Alzheimer's dementia. AD (2006-2017) was captured using ICD-10 codes (alphanumeric codes to classify symptoms, diseases, injuries, infections and disorders) in linked hospital episode statistics (HES) data, as well as from death certification, primary care, self-report and nurse interview. These algorithmically-defined outcomes were provided by UKB. Coded diagnoses were compared with clinical expert adjudication of full-text medical records. Details of ICD-10 and primary care Read codes are presented in ESM Tables 6-7 and more in-depth information on the algorithm by Wilkinson et al. can be found elsewhere (18).

Statistical analyses

Analyses were performed using a combination of the *mrrobust* package in STATA, version 15, the *MendelianRandomisation* R package, using RStudio version 1.1.456 and PLINK version 2.0.

Selection of genetic variants for exposures

For diabetes, 157 independent (via linkage disequilibrium clumping performed in PLINK, using r^2=0.2 and a 250kb window) genetic variants were chosen from the 2018 genome-wide association study (GWAS) by Mahajan et al. (19), in which they combined data across 32 studies, including 74,124 diabetes cases and 824,006 controls of European ancestry. In our

sample these variants had an F-statistic of 27.43 and explained ~1.5% (pseudo-R^2=0.015) of the variance in 14,010 diabetes cases [defined using a validated (against primary care data) algorithm of self-reported doctor diagnosis and/or medication(20)]. The 51 HbA_{1c} single nucleotide polymorphisms (SNPs) we used were from the latest trans-ancestry GWAS by Wheeler et al.(21). These variants explained 2.8% (R^2=0.028) of the variance in HbA_{1c} in our sample and had an F-statistic of 164.6. In UKB HbA_{1c} assays were performed using five Bio-Rad Variant II Turbo analysers(22)). LD clumping in PLINK confirmed that the 51 SNPs were independent (r^2=0.2, 250kb window). For bidirectional MR analyses we used 43 SNPs associated with reaction time (RT) from a recent GWAS (23) of 330,069 white European UKB participants with both phenotype and genotype data available. The RT variants explained 0.3% of the variance in RT in our study and the instrument had an F-statistic of 24.0. As with the diabetes and HbA_{1c} SNPs, the RT SNPs were also confirmed to be independent via clumping in PLINK. We harmonised genetic variants from the published GWAS with UKB by aligning the effect alleles. Full details of all the SNPs are in ESM Table 1. Our selection process for genetic instruments is detailed in Figure 2. Briefly, in relation to minor allele frequency (MAF), for T2DM and HbA_{1c}, the authors excluded any (rare) variants with a MAF <1%. For the reaction time variants, as we were uncertain of MAF filtering in the discovery GWAS we inspected the MAF for each SNP and found that one variant had a MAF of 0.3%. However, when we performed a leave-one-out analysis excluding this variant (rs141885450) our results remained identical (data not presented). More details on the discovery GWASs for our exposures can be found in the original papers (19,21,23).

Main analyses

We firstly performed linear/logistic regression to examine the associations between SNPs for HbA_{1c}/diabetes, and all of our outcomes in PLINK. Secondly, we fitted logistic/linear models to examine the associations between RT SNPs and diabetes, and HbA_{1c}. Then, inverse-variance weighted (IVW) MR was implemented as our main model. This approach calculates the effect of a given exposure (e.g. diabetes) on an outcome of interest (e.g. visual memory) by taking an average of the genetic variants' ratio of variant-outcome ($SNP \rightarrow Y$) to variant-exposure ($SNP \rightarrow X$) relationship estimated using the same principles as a fixed-effects meta-analysis (24). We also performed standard MR sensitivity analyses, including MR-Egger regression (which yields an intercept term which indicates the presence or absence of unbalanced horizontal pleiotropy)(25) and the weighted median estimator (WME – which can yield more robust estimates when up to 50% of the genetic variants are invalid)(26). Identical MR analyses were performed for diabetes (157 SNPs), HbA_{1c} (51 SNPs) and: reaction time, visual memory, white matter hyperintensity volume, hippocampal volume and AD. Additionally, for reaction time, visual memory, WMHV and hippocampal volume, we repeated the MR analyses using only the 16 glycaemic HbA_{1c} SNPs and then the 19 erythrocytic SNPs (16 SNPs were unclassified, as per the discovery GWAS). We did not perform these analyses for the AD outcome, due to the likelihood of imprecision because of a substantially reduced sample size. For bidirectional analyses, we used the reaction time SNPs to investigate associations with HbA_{1c} and diabetes. Results are presented as exponentiated ß coefficients (multiplicative effect size) for RT/visual memory/WMHV, AD risk and unit differences in hippocampal volume (mm^3), per unit increase in HbA_{1c} (mmol/mol) and 1-log-odds of diabetes. For bidirectional MR analyses, results are expressed as diabetes risk and unit differences in HbA_{1c} (mmol/mol) per unit increase in reaction time (milliseconds). To ensure that our results were not affected by residual population stratification we performed all

of our MR analyses with adjustment for 10 genetic principal components. These results were qualitatively identical to the main results and thus, we present them in ESM Table 9.

MR assumption checks

MR has three strict assumptions that must be met for study results to be valid:

I) The association between the genetic variants for the exposure and the exposure itself must be strong and robust (this means that these associations have usually been replicated and validated via genome-wide association studies – GWAS –). *This assumption was met because our genetic variants for diabetes, HbA_{1c} and reaction time (RT) were all from large-scale recently published GWAS. However, for the RT SNPs only, as there was some concern about weak instrument bias, we additionally included an MR-Egger Simulation Extrapolation (SIMEX)*(27) *sensitivity analysis, which we report in the Results section.*

II) The association between the genetic variants (for the exposure) and the outcome must only be via the exposure under study, otherwise this is known as unbalanced horizontal pleiotropy and may bias MR results. *This assumption was assessed using the methods detailed below, including MR-Egger.*

III) There should not be an association between the genetic variants (for the exposure) and common confounders of the relationship under study (e.g. the diabetes SNPs should not be associated with factors such as smoking). *We checked this assumption by regressing multiple confounders (BMI, deprivation, systolic blood pressure, total cholesterol, triglycerides, C-reactive protein – for which outliers >3 standard deviations were removed –), smoking and stroke) on the diabetes, HbA_{1c} and RT SNPs. We applied a Benjamini-Hochberg false discovery rate (BH-FDR) of 0.25 to account for multiple testing.*

Additional analyses to mitigate bias due to sample overlap for the diabetes instrument

As mentioned earlier, UKB contributed to the Mahajan et al., 2018 (19) diabetes GWAS and thus, we performed some analyses in addition to the main MR analyses to understand whether our diabetes and brain health results may be subject to 'Winner's curse' bias. Thus, we turned to the earlier 2014 GWAS by Mahajan and colleagues (28) as this study did not include UKB. We looked up the 157 diabetes SNPs used in our instrument and found 77 of them in the Mahajan et al. 2014 GWAS summary statistics (this reduced number of SNPs may be due to differences in coverage of imputation panels, i.e. the 2014 GWAS imputed to Phase II/III of HapMap and the 2018 GWAS used the Haplotype Reference Consortium – HRC -). We took the corresponding log(betas) and standard errors for this 77-SNP diabetes instrument (F-statistic=30.88) from the Mahajan et al. 2014 GWAS so, that the estimates would not include UKB. Third, we performed all of our MR analyses (IVW, MR-Egger and WME) with this instrument and as results were qualitatively the same as when we used the 157-SNP instrument, we present these in ESM Table 8.

Results

Sample characteristics

Sample characteristics are presented in ESM Table 2. In our sample 54% of participants were male and the mean age was 56.7 years; 27% participants reported ever smoking and 20% were in the most deprived group. Mean HbA_{1c} was 35.9 mmol/mol, mean reaction time was 554.6 milliseconds and the mean number of visual memory errors was 4.1. (number of incorrect matches – errors –). Mean hippocampal volume was 3830mm^3, while the median white matter hyperintensity volume was 2824mm^3. Mean SBP and BMI were 138.2mmHg and 27.3kg/m^2, respectively. Median values for triglycerides and CRP were 1.5 and 1.3 mmol/mol, respectively, while mean total cholesterol levels were 5.7 mmol/mol. There were

14010 participants with diabetes, 746 with AD and 6301 with stroke at baseline. On average, participants engaged in 3.6 days of moderate physical activity for more than 10 minutes.

MR results for diabetes/HbA_{1c} → reaction time and visual memory

Diabetes was not associated with reaction time or visual memory using IVW and these results were consistent with MR-Egger and WME approaches (Table 1). HbA_{1c} was not associated with reaction time using IVW, MR-Egger or the WME. However, the MR-Egger intercept p-value was <0.05; thus, we performed leave-one-out analyses and found that rs10774625 was pleiotropic. When we removed this SNP from the model the intercept p-value changed to >0.05 and results remained consistent. When restricted to the 16 glycaemic and subsequently 19 erythrocytic SNPs, there was no evidence of an association with RT (Table 1). Using all 51 SNPs, none of the three MR approaches used showed evidence of an association between HbA1c and visual memory (Table 1). When restricted to the 16 glycemic SNPs, there was also no association with visual memory (Table 1). Finally, when restricting to the 19 erythrocytic SNPs we also observed no associations with visual memory across all MR approaches (Table 1).

MR results for diabetes/HbA_{1c} → hippocampal volume, white matter hyperintensity volume and Alzheimer's dementia (AD)

Diabetes was not associated with hippocampal or white matter hyperintensity volume, or AD using IVW, MR-Egger, or WME approaches (Table 2). For HbA_{1c}, using the 51-SNP genetic instrument there was no evidence of associations with white matter hyperintensity volume (Table 2). When we restricted analyses to only the 16 glycaemic and the 19 erythrocytic SNPs we also saw no evidence of associations between HbA_{1c} and WMHV (Table 2). For HV, the 51-SNP HbA1c instrument showed no associations across the IVW, MR-Egger and

WME (Table 2). When analyses were restricted to the 16 glycaemic and subsequently, the 19 erythrocytic SNPs there was also no evidence of associations with hippocampal volume (Table 2). Both HbA_{1c} (using all 51 SNPs) and diabetes were not associated with AD using conventional IVW MR (OR (95% CI) 1.09 (0.42;2.83) and 1.15 (0.87;1.52), respectively).

Bidirectional MR results for: reaction time → diabetes/HbA_{1c}

In the bidirectional analyses, we observed no associations between RT and diabetes with all MR approaches producing consistent results (Table 3). However, our results suggested an association between RT and HbA1c, but the MR-Egger intercept p-value was <0.05 indicating unbalanced horizontal pleiotropy. Thus, we performed leave-one-out analyses and found that the pleiotropic SNP was rs10775404. The results for the WME after exclusion of this variant suggested an association such that slower RT was associated with lower HbA1c (Beta coefficient = -1.11 mmol/mol [95%CI -1.95;-0.28]). Also, the MR-Egger intercept p-value was >0.05 after exclusion of this variant.

Results from additional MR assumption checks

We performed MR-Egger SIMEX alongside the conventional IVW MR to address issues with weak instruments in relation to the reaction time SNPs. Our SIMEX analyses were consistent with all the other MR approaches for RT and diabetes (Table 3). However, for RT and HbA1c MR-Egger SIMEX suggested the presence of unbalanced horizontal pleiotropy and we decided to perform leave-one-out analyses. We identified rs10775404 as the pleiotropic SNP and after excluding this variant the MR-Egger SIMEX intercept p-value increased to >0.05. Additionally, we checked to see whether our genetic instruments for diabetes, HbA1c, and RT were associated with common confounders. When we regressed BMI, socioeconomic deprivation, systolic blood pressure, total cholesterol, smoking, stroke

at baseline, triglycerides, and C-reactive protein on the diabetes, HbA1c, and RT SNPs, we observed some associations between our genetic variants and confounders, using a Benjamini-Hochberg false discovery rate (BH-FDR) of 0.25 to account for multiple testing (Supplementary Tables 3–5).

Discussion

In the first comprehensive Mendelian randomisation study of HbA_{1c}/diabetes and brain health, we show that overall there is unlikely to be a causal relationship. In bidirectional MR analyses, we found no relationship between reaction time and diabetes or HbA_{1c}.

No previous studies have attempted to investigate, using MR, the association between HbA_{1c} and any of the outcomes reported here. Other approaches did not, nor did we find any association when the instrument was restricted to the glycaemic variants, providing little support for a true association. Bidirectional findings of RT and diabetes showed no evidence of causal relationships across IVW, MR-Egger and WME MR approaches. The IVW and MR-Egger also showed no associations between RT and HbA_{1c}. However, our WME result showed a marginal association, in an unexpected direction between RT and HbA_{1c}, such that slower RT was associated with lower HbA_{1c}. However, as this was inconsistent with the IVW (conventional MR) and MR-Egger we believe that this finding should be interpreted with utmost caution.

We are the first to investigate diabetes/HbA_{1c} and hippocampal and white matter hyperintensity volumes using an MR approach, but we observed no evidence of associations between these phenotypes. Although UKB has the largest brain imaging study in the world, perhaps a larger sample size would allow for more precise estimation of the relationships

with these structural brain outcomes. However, the weak association between diabetes and AD only is at least supported by previous MR studies, which reported no impact of diabetes on AD (8–10) and thus, taking all of this evidence together, it is likely that diabetes does not exert a causal influence on risk of AD. Additional support for these findings comes from a recent study which suggests that, using a polygenic risk score for diabetes, the association between diabetes and cognitive state shown by observational studies (2) may be explained by early life socioeconomic factors and childhood cognition, as well as educational attainment (29).

Our MR findings were validated by checking that we met all three core assumptions. Assumption I was met by ensuring that we selected the best available genetic variants for our exposures (diabetes, HbA_{1c} and reaction time) from the latest and most robust GWA studies. Assumption II, which relates to horizontal pleiotropy between exposure SNPs and the outcomes, was checked by performing standard sensitivity analyses under the MR-Egger and Weighted median estimator models. Where necessary (Egger intercept $p<0.05$), we performed additional leave-one-out analyses to exclude a SNP that was identified as pleiotropic and re-ran our MR analyses. Finally, we checked assumption 3 by performing linear/logistic regressions between our genetic instruments for diabetes, HbA_{1c}, and RT, and unobserved confounders. As we found evidence of associations with some confounders after applying a BH-FDR, we believe that these warrant further investigation but are beyond the scope of our study. The reasons for these associations could be firstly, related to the fact that these traits are all polygenic in nature, and/or secondly, it could also be that some of these associations indicate vertical, rather than horizontal pleiotropy. Future research could investigate whether any of these SNPs are vertically pleiotropic by performing MR mediation analyses, either using multivariable MR or two-step MR (30).

Our study design had some limitations in terms of the reaction time and diabetes genetic variants, as the GWAS from which we selected these SNPs both contained UKB in their samples. However, we also performed sensitivity analyses using a diabetes instrument and estimates from a previous GWAS that did not include UKB (28) and results remained qualitatively identical. For HbA_{1c} a two-sample MR design with no overlap was employed. We had lower precision for MR analyses with AD, hippocampal and white matter hyperintensity volumes and larger samples are required for more robust conclusions. It is also possible that the lack of evidence for causal relationships in the present study may indicate that other cognitive function and neuroimaging outcomes should be studied in future. Cognitive decline is also an important outcome that we did not investigate, but there would have been very few individuals for this analysis, as only a sub-sample underwent repeat cognitive testing. The time between tests is also not likely to be sufficient for cognitive decline to manifest (mean =6y for visual memory and 4y for reaction time), as participants were on average, aged 57 years at baseline. In relation to other exposures of interest, duration of diabetes, as well as other glycaemic exposures could be considered in future. However, there are currently no genetic variants for duration of diabetes and instruments for traits such as insulin resistance are not particularly strong (e.g. HOMA-IR has only two validated SNPs with small effect sizes). It would also be of value to test other mechanisms, as it is possible that the observational association between hyperglycaemia and brain health is not due to elevated peripheral glucose levels. Moreover, the UKB cognitive tests are novel and specific to this cohort and have thus, not been extensively validated (16). The AD diagnoses may be also be problematic, as accurate dementia diagnoses are extremely challenging to clinical experts, particularly amongst patients in the age range of UKB. However, previous UKB studies have used similar dementia diagnoses (31,32), although the algorithm we relied on

here additionally incorporates primary care data, alongside HES, mortality, self-report and nurse interview data. Our findings are unlikely to suffer from issues related to population stratification, as all of the individuals in our sample were of white European descent and in sensitivity analyses we adjusted for 10 principal components, which yielded the same results. However, MR studies should also be performed to investigate the associations we report here in other ethnic groups, particularly given that the SNPs we used were derived using trans-ethnic GWA approaches.

In conclusion, our Mendelian randomisation study of glycaemia and cognitive function, structural brain MRI measures and Alzheimer's dementia suggests that these associations are not likely to be causal. However, we observed that greater HbA_{1c} may worsen visual memory, but this finding, alongside all of the others we report, should be triangulated using other methods, in particular those relevant for causal inference.

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Author contributions

VG and NC conceived the idea and design of the study. VG performed all statistical analyses and wrote the manuscript. All authors contributed to the interpretation of the results, provided important intellectual input and approved the manuscript. VG guarantees the work carried out, had access to all of the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest statement

LS reports grants from BHF and Diabetes UK, during the conduct of the study; grants from Wellcome, grants from MRC, grants from NIHR, grants from GSK, grants from BHF, outside the submitted work and is a Trustee of the British Heart Foundation. NC reports grants from Diabetes UK, grants from British Heart Foundation, during the conduct of the study; personal fees from AstraZeneca, grants from Medical Research Council, outside the submitted work. The remaining authors declare that there are no conflicts of interest.

Data availability

The UK Biobank data are publicly available to all bona fide researchers at https://www.ukbiobank.ac.uk.

References

1. Ravona-Springer R, Luo X, Schmeidler J, Wysocki M, Lesser G, Rapp M, et al. Diabetes is associated with increased rate of cognitive decline in questionably demented elderly. Dement Geriatr Cogn Disord. 2010;29(1):68–74.
2. Rory J McCrimmon, Christopher M Ryan BMF. Diabetes and Cognitive Dysfunction. Lancet [Internet]. 2012;379:2291–9. Available from: http://dx.doi.org/10.1016/S0140-6736(12)60360-2
3. Biessels GJ, Despa F. Cognitive decline and dementia in diabetes mellitus: mechanisms and clinical implications. Nat Rev Endocrinol [Internet]. 2018; Available

from: http://dx.doi.org/10.1038/s41574-018-0048-7

4. Xue M, Xu W, Ou YN, Cao XP, Tan MS, Tan L, et al. Diabetes mellitus and risks of cognitive impairment and dementia: A systematic review and meta-analysis of 144 prospective studies. Ageing Res Rev. 2019;55(January):100944.
5. Biessels GJ, Nobili F, Teunissen CE, Simó R, Scheltens P. Understanding multifactorial brain changes in type 2 diabetes: a biomarker perspective. Lancet Neurol. 2020;19(8):699–710.
6. Srikanth V, Sinclair AJ, Hill-Briggs F, Moran C, Biessels GJ. Type 2 diabetes and cognitive dysfunction—towards effective management of both comorbidities. Lancet Diabetes Endocrinol [Internet]. 2020;8(6):535–45. Available from: http://dx.doi.org/10.1016/S2213-8587(20)30118-2
7. Ojo O, Brooke J. Evaluating the association between diabetes, cognitive decline and dementia. Int J Environ Res Public Health. 2015;12(7):8281–94.
8. Østergaard SD, Mukherjee S, Sharp SJ, Proitsi P, Lotta LA, Day F, et al. Associations between Potentially Modifiable Risk Factors and Alzheimer Disease: A Mendelian Randomization Study. PLoS Med. 2015;12(6):1–16.
9. Hagenaars SP, Gale CR, Deary IJ, Harris SE. Cognitive ability and physical health: A Mendelian randomization study. Sci Rep. 2017;7(1):1–7.
10. Larsson SC, Traylor M, Malik R, Dichgans M, Burgess S, Markus HS. Modifiable pathways in Alzheimer's disease: Mendelian randomisation analysis. BMJ. 2017;359:j5375.
11. Kisler K, Nelson AR, Montagne A, Zlokovic B V. Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease. Nat Rev Neurosci [Internet]. 2017;18(7):419–34. Available from: http://dx.doi.org/10.1038/nrn.2017.48
12. Fotuhi M, Do D, Jack C. Modifiable factors that alter the size of the hippocampus with ageing. Nat Rev Neurol [Internet]. 2012;8(4):189–202. Available from: http://dx.doi.org/10.1038/nrneurol.2012.27
13. Lawlor DA. Commentary: Two-sample Mendelian randomization: opportunities and challenges. Int J Epidemiol [Internet]. 2016;45(3):908–15. Available from: http://ije.oxfordjournals.org/lookup/doi/10.1093/ije/dyw127
14. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. PLoS Med. 2015;12(3):1–10.
15. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK

Biobank resource with deep phenotyping and genomic data. Nature. 2018 Oct;562(7726):203–9.

16. Lyall DM, Cullen B, Allerhand M, Smith DJ, Mackay D, Evans J, et al. Cognitive test scores in UK biobank: Data reduction in 480,416 participants and longitudinal stability in 20,346 participants. PLoS One. 2016;11(4):1–10.
17. Alfaro-almagro F, Jenkinson M, Bangerter NK, Andersson JLR, Sotiropoulos SN, Jbabdi S, et al. NeuroImage Image processing and Quality Control for the fi rst 10 , 000 brain imaging datasets from UK Biobank. 2018;166(April 2017):400–24.
18. Wilkinson T, Schnier C, Bush K, Rannikmäe K, Henshall DE, Lerpiniere C, et al. Identifying dementia outcomes in UK Biobank : a validation study of primary care , hospital admissions and mortality data. Eur J Epidemiol [Internet]. 2019;34(6):557–65. Available from: https://doi.org/10.1007/s10654-019-00499-1
19. Mahajan A, Taliun D, Thurner M, Robertson NR, Torres JM, Rayner NW, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nat Genet. 2018;50(11):1505–13.
20. Eastwood S V., Mathur R, Atkinson M, Brophy S, Sudlow C, Flaig R, et al. Algorithms for the capture and adjudication of prevalent and incident diabetes in UK Biobank. PLoS One. 2016;11(9).
21. Wheeler E, Leong A, Liu CT, Hivert MF, Strawbridge RJ, Podmore C, et al. Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis. PLoS Med. 2017;14(9):1–30.
22. Tierney A, Fry D, Almond R, Gordon M, Moffat S. UK Biobank Biomarker Enhancement Project Companion Document to Accompany HbA1c Biomarker Data. 2018;1–8. Available from: https://biobank.ndph.ox.ac.uk/showcase/showcase/docs/serum_hb1ac.pdf
23. Davies G, Lam M, Harris SE, Trampush JW, Luciano M, Hill WD, et al. Study of 300,486 individuals identifies 148 independent genetic loci influencing general cognitive function. Nat Commun [Internet]. 2018;9(1):2098. Available from: https://doi.org/10.1038/s41467-018-04362-x
24. Burgess S, Bowden J. Integrating summarized data from multiple genetic variants in Mendelian randomization: bias and coverage properties of inverse-variance weighted methods. 2015; Available from: http://arxiv.org/abs/1512.04486
25. Bowden J, Smith GD, Burgess S. Mendelian randomization with invalid instruments:

Effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512–25.

26. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 2016;40(4):304–14.
27. Bowden J, Del Greco M F, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. Int J Epidemiol [Internet]. 2016;1–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27616674
28. Consortium DiaGRAM (DIAGRAM), Consortium AGENT 2 D (AGEN-T, Consortium SAT 2 D (SAT2D), Consortium MAT 2 D (MAT2D), Consortium T 2 DGE by N sequencing in muylti-ES (T2D-G, Mahajan A, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet [Internet]. 2014/02/09. 2014 Mar;46(3):234–44. Available from: https://pubmed.ncbi.nlm.nih.gov/24509480
29. James, Sarah-naomi, Wong, Andrew, Tillin, Therese, Hardy, Rebecca, Chaturvedi, Nish, Richards M. The effect of mid-life insulin resistance and type 2 diabetes on older-age cognitive state : the explanatory role of early-life advantage. Diabetologia. 2019;61:1891–900.
30. Carter AR, Sanderson E, Hammerton G, Richmond RC, Davey Smith G, Heron J, et al. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. Eur J Epidemiol [Internet]. 2021;(0123456789). Available from: https://doi.org/10.1007/s10654-021-00757-1
31. Henry A, Katsoulis M, Masi S, Fatemifar G, Denaxas S, Acosta D, et al. The relationship between sleep duration , cognition and dementia : a Mendelian randomization study. 2019;1–12.
32. Llewellyn DJ, Lourida I, Hannon E, Littlejohns TJ, Langa KM, Hyppönen E, et al. Association of Lifestyle and Genetic Risk with Incidence of Dementia. JAMA - J Am Med Assoc. 2019;322(5):430–7.

Figure 1. Study design

Figure 2. Genetic instrument selection

*Note. *MAF filtering at 0.01 done by GWAS authors; **passed genetic QC and confirmed to be independent at LD clumping thresholds of r^2=0.2 and within a 250 kb window, using 1000 Genomes CEU data; ***we performed leave-one-out analyses for this SNP (rs141885450) and as results were identical we have not presented these.*

Table 1. MR results for the relationship between diabetes/HbA_{1c} and reaction time, and visual memory

	Outcome: Reaction time		Outcome: Visual memory	
Exposure: diabetes (157 SNPs)				
	exp(β) (95% CI)	Egger intercept p-value	exp(β) (95% CI)	Egger intercept p-value
IVW	1.00 (1.00;1.00)		1.00 (0.99;1.00)	
MR-Egger	1.00 (1.00;1.01)	0.170	1.00 (0.99;1.01)	0.570
WME	1.00 (1.00;1.00)		1.00 (0.99;1.01)	
Exposure: HbA_{1c} (All SNPs)				
	exp(β) (95% CI)	Egger intercept p-value	exp(β) (95% CI)	Egger intercept p-value
IVW	1.00 (0.99;1.02)		0.99 (0.96;1.02)	
MR-Egger	0.98 (0.96;1.01)	0.032*	1.00 (0.94;1.06)	0.675
WME	0.99 (0.98;1.00)		1.01 (0.97;1.05)	
Exposure: HbA_{1c} (16 glycaemic SNPs)				
	exp(β) (95% CI)	Egger intercept p-value	exp(β) (95% CI)	Egger intercept p-value
IVW	1.01 (0.99;1.03)		0.98 (0.91;1.04)	
MR-Egger	1.01 (0.97;1.05)	0.861	1.08 (0.91;1.28)	0.207
WME	1.00 (0.98;1.02)		1.01 (0.94;1.08)	
Exposure: HbA_{1c} (19 erythrocytic SNPs)				
	exp(β) (95% CI)	Egger intercept p-value	exp(β) (95% CI)	Egger intercept p-value
IVW	1.00 (0.98;1.02)		0.98 (0.94;1.02)	
MR-Egger	0.98 (0.95;1.01)	0.100	0.99 (0.93;1.05)	0.772
WME	0.99 (0.98;1.00)		1.00 0.95;1.05)	

Note. IVW= inverse-variance weighted, WME= weighted median estimator, exp(β)= exponentiated beta (after log transformation), 95%CI= 95% confidence interval, *after performing leave-one-out analysis SNP rs10774625 was found to be pleiotropic and when analyses were re-run the Egger intercept p-value changed to 0.098.

Table 2. MR results for the relationship between glycaemia and brain structure and Alzheimer's dementia

	Outcome: HV		Outcome: WMHV		Outcome: AD	
Exposure: HbA_{1c} (All SNPs)						
	β (95% CI)	Egger intercept p-value	exp(β) (95% CI)	Egger intercept p-value	OR (95% CI)	Egger intercept p-value
IVW	-21.31 (-82.96;40.34)		1.03 (0.88;1.22)		1.09 (0.42;2.83)	
MR-Egger	-81.68 (-195.96;32.61)	0.220	0.97 (0.70;1.32)	0.624	1.80 (0.30;10.80)	0.516
WME	-58.90 (159.26;41.45)		1.01 (0.97;1.05)		1.05 (0.24;4.57)	
Exposure: HbA_{1c} (16 glycaemic SNPs)						
IVW	60.85 (-57.25;157.45)		0.83 (0.59;1.18)			
MR-Egger	65.39 (-242.90;373.68)	0.846	0.69 (0.28;1.74)	0.812		
WME	54.04 (-100.79;208.86)		0.81 (0.55;1.19)			
Exposure: HbA_{1c} (19 erythrocytic SNPs)						
IVW	19.93 (-71.92;111.79)		1.03 (0.82;1.31)			
MR-Egger	-47.90 (-192.45;96.65)	0.237	0.94 (0.64;1.38)	0.521		
WME	-52.99 (-169.50;63.52)		1.11 (0.83;1.46)			
Exposure: Diabetes (157 SNPs)						
	β (95% CI)	Egger intercept p-value	exp(β) (95% CI)	Egger intercept p-value	OR (95% CI)	Egger intercept p-value
IVW	-2.30 (-12.39;7.78)		0.99 (0.97;1.01)		1.15 (0.87;1.52)	
MR-Egger	-6.69 (-29.37;15.99)	0.672	0.71 (0.92;1.12)	0.182	1.00 (0.54;1.86)	0.624
WME	-9.06 (-24.88;6.76)		0.99 (0.96;1.02)		1.03 (0.81;1.32)	

Note. IVW= inverse-variance weighted, β= beta coefficient (mm^3), exp(β)= exponentiated β (after log transformation), OR= odds ratio, HV= hippocampal volume, WMHV= white matter hyperintensity volume, AD= Alzheimer's dementia.

Table 3. MR results for the relationship between reaction time and HbA_{1c} and diabetes

	Outcome: diabetes		Outcome: HbA_{1c}	
	Exposure: reaction time (43 SNPs)			
	OR (95% CI)	Egger intercept p-value	β (95% CI)	Egger intercept p-value
IVW	0.94 (0.54;1.65)		-1.05 (-2.05;-0.05)	
MR-Egger	1.16 (0.01;112.17)	0.927	-10.51 (-18.16;-2.86)	0.0.015*
MR-Egger-SIMEX	1.22 (1.63E-07;9.25E+06))	0.972	-22.43 (-33.71;-11.16)	<0.001*
Weighted median	0.65 (0.34;1.23)		-1.16 (-1.95;-0.28)	

	Outcome: diabetes		Outcome: HbA_{1c}	
	Exposure: reaction time (43 SNPs)			
	OR (95% CI)	Egger intercept p-value	β (95% CI)	Egger intercept p-value
IVW	0.94 (0.54;1.65)		-1.05 (-2.05;-0.05)	
MR-Egger	1.16 (0.01;112.17)	0.927	-10.51 (-18.16;-2.86)	0.0.015*
MR-Egger-SIMEX	1.22 (1.63E-07;9.25E+06))	0.972	-22.43 (-33.71;-11.16)	<0.001*
Weighted median	0.65 (0.34;1.23)		-1.16 (-1.95;-0.28)	

Note. IVW= inverse-variance weighted, β= beta coefficient (mmol/mol), OR= odds ratio, MR-Egger-SIMEX= MR-Egger-Simulation extrapolation, *leave-one-out analyses excluding SNP rs10775404 changed the Egger intercept p-value to >0.05.