Impact of glucose on risk of dementia: Mendelian randomisation studies in 115,875 individuals

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Abstract

Aims/hypothesis Mendelian randomisation studies have not shown a clear causal effect of high plasma glucose on the risk of Alzheimer's disease. We tested the hypothesis that high plasma glucose caused by genetic variation has a causal effect on the risk of unspecified dementia, Alzheimer's disease and vascular dementia in the general population.

Methods A Mendelian randomisation design was used with data from 115,875 individuals from the Copenhagen General Population Study and the Copenhagen City Heart Study. Findings for Alzheimer's disease were validated in a two-sample Mendelian design with 455,258 individuals, including 71,880 individuals with Alzheimer's disease or a parent with Alzheimer's disease.

Results In observational multifactorial-adjusted analyses, HRs were 1.15 (95% CI 1.01, 1.32; $p = 0.039$) for unspecified dementia, 0.91 (95% CI 0.79, 1.06; $p = 0.22$) for Alzheimer's disease and 1.16 (95% CI 0.86, 1.55; $p = 0.34$) for vascular dementia in individuals with a glucose level higher than 7 vs 5–6 mmol/l. Corresponding HRs in individuals with vs without type 2 diabetes were 1.42 (95% CI 1.24, 1.63; $p < 0.001$), 1.11 (95% CI 0.95, 1.29; $p = 0.18$) and 1.73 (95% CI 1.31, 2.27; $p < 0.001$). In genetic causal analyses, a 1 mmol/l higher plasma glucose level had RRs of 2.40 (95% CI 1.18, 4.89; $p = 0.016$) for unspecified dementia, 1.41 (95% CI 0.82, 2.43; $p = 0.22$) for Alzheimer's disease and 1.20 (95% CI 0.82, 1.75; $p = 0.36$) for vascular dementia. Summary-level data from the Meta-Analyses of Glucose and Insulin-related Traits Consortium (MAGIC) combined with a consortium of the Alzheimer's Disease Sequencing Project (ADSP), the International Genomics of Alzheimer's Project (IGAP), the Alzheimer's Disease Working Group of the Psychiatric Genomics Consortium (PGC-ALZ) and the UK Biobank (UKB) gave an RR for Alzheimer's disease of 1.02 (95% CI 0.92, 1.13; $p = 0.42$), and this consortium combined with Copenhagen studies gave an RR for Alzheimer's disease of 1.03 (95% CI 0.93, 1.13; $p = 0.36$).

Conclusions/interpretation Observational and genetically high plasma glucose are causally related to the risk of unspecified dementia, but not to Alzheimer's disease or vascular dementia.

Keywords Alzheimer's disease · Diabetes mellitus · Glucose · Mendelian randomisation · Unspecified dementia · Vascular dementia

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Research in context

What is already known about this subject?

- Observational studies have reported an increased risk of dementia in individuals with diabetes
- Previous results from Mendelian randomisation studies on glycaemic traits and Alzheimer's disease are not clear, and unspecified dementia and vascular dementia have not been examined

What is the key question?

- Using Mendelian randomisation with data from 115,875 individuals from the Danish general population, does genetically high plasma glucose have a causal effect on the risk of dementia?

What are the new findings?

- A 1 mmol/l higher plasma glucose level had a causal effect with a 2.4-fold higher risk of unspecified dementia, but not a higher risk of Alzheimer's disease or vascular dementia

How might this impact on clinical practice in the foreseeable future?

- The finding that high plasma glucose is causally related to the risk of unspecified dementia underscores the importance of glycaemic control in patients with diabetes mellitus and potentially also in individuals with impaired glucose tolerance

Abbreviations

ADSP	Alzheimer's Disease Sequencing Project
IGAP	International Genomics of Alzheimer's Project
MAGIC	Meta-Analyses of Glucose and Insulin-related Traits Consortium
PGC-ALZ	Alzheimer's Disease Working Group of the Psychiatric Genomics Consortium
UKB	UK Biobank

Introduction

Observational studies have reported an association between raised plasma glucose concentrations and the risk of dementia in individuals both with and without diabetes [1]. However, patients with high plasma glucose concentrations and potentially type 2 diabetes often have several risk factors for dementia other than high plasma glucose (e.g. hypertension, hyperlipidaemia, being overweight, vascular disease), and it is not clear whether glucose per se has a causal effect on the risk of dementia, or whether the observed risks are due to confounding or reverse causation.

To investigate whether plasma glucose has a causal effect on the risk of unspecified dementia, Alzheimer's disease and vascular dementia, we used the Mendelian randomisation design, which aims to circumvent confounding and reverse causation through the use of genetic variation in populations [2–4]. The study design is very similar to that of a randomised clinical intervention trial using randomisation by genes

instead of treatment and, because of the random assortment of genetic variants at conception, variants with an effect on glucose are randomly distributed in relation to potential confounders. In addition, because genetic variants are determined at conception and remain constant throughout life, the Mendelian randomisation design is not influenced by reverse causation.

Previous Mendelian randomisation studies have examined the causal effects of type 2 diabetes, insulin sensitivity and fasting glucose on the risk of Alzheimer's disease but the results have not been clear, and the risks of unspecified dementia and vascular dementia have not been examined [5–7].

Using a Mendelian randomisation design in 115,875 individuals from two prospective general population studies, the Copenhagen General Population Study and the Copenhagen City Heart Study [8, 9], we tested the hypothesis that high plasma glucose caused by genetic variation is associated with a high risk of unspecified dementia, Alzheimer's disease and vascular dementia as an indication of causality.

We tested: (1) whether non-fasting plasma glucose and type 2 diabetes at baseline are observationally associated with dementia; (2) whether a weighted allele score of plasma glucose-increasing alleles is associated with high plasma glucose concentrations, as expected; (3) whether the weighted glucose-increasing alleles are associated with the risk of dementia, as an indication of a causal effect of high glucose on the risk of dementia; and (4) whether the causal effect of high plasma glucose is consistent with the corresponding observational associations using instrumental variable analysis (see electronic supplementary material [ESM] Fig. 1). For Alzheimer's disease, we also included a risk estimate using

summary-level data from the Meta-Analyses of Glucose and Insulin-related Traits Consortium (MAGIC), with data on 46,186 individuals without diabetes and of European ancestry [10–13], combined with the Alzheimer's Disease Working Group of the Psychiatric Genomics Consortium (PGC-ALZ), the International Genomics of Alzheimer's Project (IGAP) and the Alzheimer's Disease Sequencing Project (ADSP) comprising individuals with a clinical diagnosis of Alzheimer's disease and the Alzheimer's disease by proxy (parent with Alzheimer's disease) from the UK Biobank (UKB) (ADSP/IGAP/PGC-ALZ/UKB) [14] with 455,258 individuals, including 71,880 individuals with Alzheimer's disease (34% of participants) or with a parent with Alzheimer's disease (66% of participants), and examining the same genetic variants as in the Copenhagen studies.

Methods

Participants We included individuals from two similar prospective studies of the Danish general population: the Copenhagen General Population Study ($N=107,143$) and the Copenhagen City Heart Study ($N=18,732$) [8, 9]. Combining the two studies yielded a total of 125,875 individuals, including 115,958 with available genotypes of whom 2596 developed unspecified dementia, 2287 Alzheimer's disease and 493 vascular dementia during up to 43 years of follow-up with a median of 10 years. All individuals were white and of Danish descent, and none was included in more than one study. No individuals were lost to follow-up.

The Copenhagen General Population Study was initiated in 2003 and enrolment is ongoing [8, 9]. Individuals are selected based on the national Danish Civil Registration System to reflect the adult white Danish population at 20–100 years of age. Data are obtained from a questionnaire reviewed by the participant and an investigator on the day of attendance, a physical examination and blood samples including DNA extraction.

The Copenhagen City Heart Study is a prospective study of the Danish general population initiated in 1976–1978, with follow-up examinations in 1981–1983, 1991–1994 and 2001–2003 [8, 9]. Participants were recruited and examined exactly as in the Copenhagen General Population Study. Baseline was considered the first examination of an individual in which plasma glucose was measured.

Ethics approval Studies were approved by the Herlev and Gentofte Hospital and by Danish ethical committees (KF-100.2039/91, KF-01-144/01, H-KF-01-144/01). Written informed consent was obtained from participants.

Endpoints Diagnoses of endpoints according to ICD-8 (<http://www.wolffbane.com/icd/icd8.htm>) and ICD-10 (<http://apps.who.int/classifications/icd10/browse/2016/en>) codes were collected from 1977 to 13 December 2018 by reviewing all hospital admissions and diagnoses in the Danish National Patient Register and all causes of death in the national Danish Register of Causes of Death [15, 16]. Endpoints were defined as follows: unspecified dementia (ICD8: 290.18; ICD10: F03), Alzheimer's disease (ICD8: 290.10; ICD10: F00, G30) and vascular dementia (ICD10: F01), as done previously [15, 16]. In Denmark, these diseases are diagnosed by specialists in neurology, and a validation with full clinical work-up of register-based dementia diagnoses concluded that diagnostic validity in the Danish hospital registries is high [17]. In the present study, the validity of the register-based dementia diagnoses was further ensured by the presence of the well-known association with the *APOE* (encoding apolipoprotein E) $\epsilon 4$ allele with Alzheimer's disease in the Copenhagen General Population Study and the Copenhagen City Heart Study [16, 18].

Potential confounders and covariates Non-fasting plasma concentrations of glucose, total cholesterol, HDL-cholesterol and triacylglycerols were measured using standard hospital assays. LDL-cholesterol was calculated using the Friedewald equation if plasma triacylglycerols were ≤ 4.0 mmol/l, and measured by a direct enzymatic method at higher triacylglycerol concentrations (Konelab autoanalyser, Thermo Fisher Scientific, San Diego, CA, USA). BMI was measured-weight divided by measured-height squared. Hypertension was defined as systolic BP ≥ 140 mmHg (≥ 135 mmHg in individuals with diabetes mellitus), diastolic BP ≥ 90 mmHg (≥ 85 mmHg for diabetics) or use of antihypertensive medication prescribed specifically for hypertension [19]. Diabetes mellitus was self-reported or classified by non-fasting plasma glucose >11.0 mmol/l, medication prescribed for diabetes, and/or hospitalisation or death due to diabetes (ICD8: 249–250; ICD10: E10–11, E13–14) [20]. The use of cholesterol-lowering medication was self-reported, with $>97\%$ being statins. Participants also reported on smoking, coded as current smokers and non-smokers. Work and leisure time physical activity was coded as 'low' for 0–2 h moderate activity per week and 'intermediate and high' for more than 2 h moderate or vigorous activity per week during either work or leisure time. Information on alcohol consumption was summarised in units per week (1 unit = ~ 12 g alcohol) and coded as 'above recommended' if more than 14 or 21 units per week for women and men, respectively. Education was summarised as <8 or ≥ 8 years of completed education. For women, menopausal status was also recorded. Missing data on covariates varied from 0% to 1% for any individual variable. Individuals with missing covariates were excluded ($<1.3\%$ excluded) from observational analyses.

Genotyping An ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and TaqMan-based assays were used to genotype for the

glucose-associated genotypes rs560887 (*G6PC2*), rs4607517 (*GCK*), rs2191349 (*DGKB*), rs11708067 (*ADCY5*), rs7903146 (*TCF7L2*), and rs2383206 and rs10811661 (*CDKN2A/CDKN2B*). These genotypes have been reported to have the strongest effect on plasma glucose in genome-wide association studies, their functions are well known [12, 13] and pleiotropic effects have not been observed. A weighted allele score was constructed by summing the number of glucose-increasing alleles, weighted by frequency and effect size on plasma glucose, and divided into the following categories: 0–24th percentile, 25–49th percentile, 50–74th percentile, 75–94th percentile and 95–100th percentile by the weighted allele score. Individuals included in genetic analyses had complete data on age, sex, genotypes and endpoints.

Statistical analyses Data were analysed using StataIC 14.2 [21]. For genotypes, deviation from Hardy–Weinberg equilibrium was tested using a Pearson χ^2 test. To test whether high plasma glucose was observationally associated with the risk of disease (ESM Fig. 1, arrow 1), we used restricted cubic splines with 5 knots, selected using Akaike’s information criterion to balance best fit and overfitting [22], and a glucose concentration corresponding to the population median of 5.2 mmol/l as reference. To test whether type 2 diabetes mellitus was associated with disease, we used Cox regression models with age as the time scale and adjusted for sex and year of birth; and multifactorial models further adjusted for the potential confounders BMI, hypertension, smoking, physical activity, alcohol consumption, education and (for women) menopausal status. Adjustment for year of birth was performed to accommodate changes in diagnostic criteria and treatment over calendar time. To reduce the risk of bias due to confounding (factors with an effect on both exposure and outcome) and covariates (factors with an effect on the endpoint), multifactorial adjustment was included. Trends for significance across ordered categories of high plasma glucose concentrations and allele score (see below) were tested using the non-parametric Cuzick’s extension of a Wilcoxon rank-sum test.

Linear regression was used to test whether the weighted allele score was associated with a high plasma glucose concentration (ESM Fig. 1, arrow 2).

To test whether the weighted glucose allele score was associated with the risk of dementia, we used Cox regression models adjusted for age, sex and year of birth (ESM Fig. 1, arrow 3). The follow-up time for Cox regression analyses began at the first inclusion into a study and ended with censoring at the date of death, the occurrence of an event, emigration ($n = 853$) or on 13 December 2018 (corresponding to the date of register retrieval of diagnoses), whichever came first.

As a positive control of the genetic instrument and study power, the known association with ischaemic heart disease was included and, as a positive control on study power and endpoints, results on the known risk of Alzheimer’s disease

for *APOE* $\epsilon 34/\epsilon 44$ vs $\epsilon 33$ were included [16, 18]. As sensitivity analyses, associations were also examined excluding individuals using cholesterol-lowering medication and those with diabetes mellitus at baseline, in quintile categories and using non-Alzheimer’s disease as the endpoint.

Logistic regression was used to assess whether observational high plasma glucose, the weighted allele score, *APOE* $\epsilon 34/\epsilon 44$, Alzheimer’s disease, vascular dementia and unspecified dementia were associated with the potential confounders of age, sex, BMI, hypertension, smoking, physical activity, alcohol consumption, education and (for women) menopausal status.

Because genotypes are constant throughout life, and hence insensitive to reverse causation, a potential causal effect of genetically high plasma glucose on the risk of dementia was assessed using instrumental variable analysis (ESM Fig. 1, arrow 4) using the user-written *ivreg2* and *ivpois* commands in Stata [23, 24]. The strength of the instruments (i.e. the strength of the association of the genotypes with plasma glucose concentration) was confirmed by *F*-statistics of $F = 80.7$ from regressions using *ivreg2*, where $F > 10$ was considered acceptable [2].

Finally, we conducted a summary-level Mendelian randomisation analysis [4, 25] using the same genetic variants as in our own studies, but now with information on the effect on plasma glucose concentrations from the publicly available MAGIC (46,186 individuals without diabetes) data [10–13], and for each glucose-increasing variant estimated the risk of Alzheimer’s disease using the ADSP/IGAP/PGC-ALZ/UKB population (455,258 individuals, including 71,880 with Alzheimer’s disease or a parent with Alzheimer’s disease) [14]. For information on these study populations, please refer to ESM Methods. To verify the causal effect of high plasma glucose on the risk of Alzheimer’s disease from the summary-level data, we performed regression analysis by Egger Mendelian randomisation [26] using the user-written *mregger* command in Stata [27], and accounting for potential weak instruments and direct pleiotropic effects of the variants, respectively [26, 27]. Statistical power to detect a significant ($\alpha = 0.05$ and $\beta = 0.80$) effect of genetically determined glucose on the risk of dementia was calculated using $r^2 = 0.3\%$, a sample size of 110,000 individuals, and a frequency of endpoints of 1% and expressed as a fraction from 0 to 1.0 [28].

Results

Of 125,875 individuals included in the study, baseline non-fasting plasma glucose concentrations were <5.0 mmol/l in 35%, 5.0–5.9 mmol/l in 42%, 6.0–6.9 mmol/l in 14% and ≥ 7.0 mmol/l in 8.4% (Table 1). Individuals with a non-fasting plasma glucose level of ≥ 7.0 vs <6.0 mmol/l were older, more often men, had higher triacylglycerol

Table 1 Baseline characteristics of participants by plasma glucose categories

Characteristic	Plasma glucose, mmol/l				<i>p</i> _{trend}	All
	<5.0	5.0–5.9	6.0–6.9	≥7.0		
Individuals, <i>n</i> (%)	44,616 (35)	52,678 (42)	17,985 (14)	10,596 (8.4)		125,875 (100)
Age, years	54 (45–64)	58 (48–67)	59 (49–68)	60 (52–69)	<0.001	57 (47–66)
Women, <i>n</i> (%)	26,323 (59)	28,446 (54)	9352 (52)	4768 (45)	<0.001	69,231 (55)
Glucose, mmol/l	4.6 (4.4–4.8)	5.3 (5.1–5.6)	6.3 (6.1–6.6)	7.8 (7.3–8.8)	<0.001	5.2 (4.8–5.9)
LDL-cholesterol, mmol/l	3.2 (2.6–3.8)	3.3 (2.6–3.9)	3.3 (2.6–4.0)	3.1 (2.4–3.9)	<0.001	3.2 (2.6–3.9)
Triacylglycerols, mmol/l	1.2 (0.9–1.8)	1.4 (1.0–2.1)	1.6 (1.1–2.3)	1.7 (1.2–2.6)	<0.001	1.4 (1.0–2.1)
HDL-cholesterol, mmol/l	1.6 (1.3–2.0)	1.6 (1.2–1.9)	1.5 (1.2–1.9)	1.4 (1.1–1.8)	<0.001	1.6 (1.2–1.9)
BMI, kg/m ²	25.0 (22.7–27.8)	25.5 (23.1–28.3)	25.5 (23.1–28.5)	26.3 (23.6–29.5)	<0.001	25.4 (23.0–28.3)
Hypertension, <i>n</i> (%)	20,523 (46)	27,393 (52)	8633 (48)	5510 (52)	<0.001	61,679 (49)
Type 2 diabetes, <i>n</i> (%)	937 (2.1)	1949 (3.7)	1978 (11)	3603 (34)	<0.001	7930 (6.3)
Use of cholesterol-lowering medication, <i>n</i> (%)	3748 (8.4)	5795 (11)	1978 (11)	1801 (17)	<0.001	12,588 (10)
Current smoker, <i>n</i> (%)	25,431 (57)	28,973 (55)	8633 (48)	4980 (47)	<0.001	67,973 (54)
Physical activity, intermediate or high, <i>n</i> (%)	30,785 (69)	32,134 (61)	7734 (43)	4026 (38)	<0.001	74,266 (59)
Alcohol consumption above recommended, <i>n</i> (%)	8923 (20)	15,277 (29)	8453 (47)	5298 (50)	<0.001	37,763 (30)
Education <8 years, <i>n</i> (%)	3525 (7.9)	7375 (14)	4316 (24)	3073 (29)	<0.001	17,623 (14)
Postmenopausal (women only), <i>n</i> (%)	15,169 (34)	20,544 (39)	7554 (42)	4026 (38)	<0.001	47,833 (38)

Continuous values are median and interquartile range

To convert glucose values to mg/dl, multiply values in mmol/l by 18.0; to convert cholesterol values to mg/dl, multiply values in mmol/l by 38.6; and to convert triacylglycerol to mg/dl, multiply values in mmol/l by 88

concentrations and BMI, more often had diabetes (34% vs 2.1%), used cholesterol-lowering medication, had an alcohol intake above 21/14 units per week and had fewer than 8 years of education, were less physically active and were less often smokers (Table 1). Genotype distributions did not deviate from Hardy–Weinberg expectations overall or in individuals younger or older than age 70 years (all $p > 0.05$), and none of the variants were in linkage disequilibrium (all $r^2 < 0.4$). During up to 43 years of follow-up with a median follow-up of 10.0 years, 2596 individuals developed unspecified dementia (median age at diagnosis 83.0 years [interquartile range 77.1–88.2 years]), 2287 developed Alzheimer's disease (median age 81.3 years [interquartile range 76.1–85.9 years]) and 493 developed vascular dementia (median age 82.8 years [interquartile range 77.0–87.9 years]).

Characteristics of individuals with a diagnosis of dementia

Compared with individuals with a diagnosis of Alzheimer's disease or vascular dementia, those with a diagnosis of unspecified dementia had higher plasma glucose and LDL-cholesterol concentrations, more often had an alcohol intake above recommended, were less often current smokers, were less physically active, less often had hypertension and less often received statin treatment (all $p < 0.05$). Compared with individuals with vascular dementia, they were also more often

women and fewer individuals had had a previous stroke (both $p < 0.05$). Individuals with unspecified dementia and type 2 diabetes were less often treated with oral diabetes medication, compared with those with Alzheimer's disease or vascular dementia ($p < 0.001$) (ESM Table 1).

Non-fasting glucose and risk of dementia: observational estimates

In observational cubic spline analyses, glucose concentrations above the median of 5.2 mmol/l were associated with linear increases in the risk of unspecified dementia and vascular dementia, but not with the risk of Alzheimer's disease (Fig. 1). In observational multifactorial-adjusted analyses in individuals with a glucose concentration above 7 vs 5–6 mmol/l, the HRs were 1.15 (95% CI 1.01, 1.32; $p = 0.039$) for unspecified dementia, 0.91 (95% CI 0.79, 1.06; $p = 0.22$) for Alzheimer's disease and 1.16 (95% CI 0.86, 1.55; $p = 0.34$) for vascular dementia (data not shown). Multifactorial-adjusted HRs in individuals with vs without type 2 diabetes were 1.42 (95% CI 1.24, 1.63; $p < 0.001$) for unspecified dementia, 1.11 (95% CI 0.95, 1.29; $p = 0.18$) for Alzheimer's disease and 1.73 (95% CI 1.32, 2.27; $p < 0.001$) for vascular dementia (Fig. 1).

Genotypes, non-fasting plasma glucose and risk of dementia: genetic estimates

An increasing number of weighted glucose

Fig. 1 Prospective observational associations of plasma glucose concentration on a continuous scale and in individuals with vs without type 2 diabetes and risk of unspecified dementia (**a**), Alzheimer's disease (**b**) and vascular dementia (**c**) on a base 2 log scale, in 125,875 individuals from the general population, the Copenhagen General Population Study and the Copenhagen City Heart Study. Solid lines are multifactorial-adjusted HRs and dashed lines (blue area) indicate 95% CIs derived from restricted cubic spline Cox regression. p values are from tests for trend across increasing concentrations of glucose above the population median (5.2 mmol/l). Risk of dementia in individuals with vs without type 2 diabetes is by an age- and sex-adjusted (Model 1) and a multifactorial-adjusted (Model 2) Cox regression model. *** $p < 0.001$

alleles in percentile categories were associated with stepwise higher mean plasma glucose. Individuals in the 95–100th percentile of weighted glucose alleles had 5.1% higher plasma glucose levels compared with those in the 0–24th percentile. The weighted allele score explained 0.9% of the variation in non-fasting plasma glucose levels ($F = 80.7$) (Fig. 2).

HRs for unspecified dementia, adjusted for age, sex and year of birth, increased with an increasing number of glucose-increasing alleles (p_{trend} across percentile categories = 0.047) with an HR of 1.24 (95% CI 1.00, 1.53; $p = 0.05$) in individuals with a weighted glucose allele score in the 95–100th percentile vs the 0–24th percentile. The weighted allele score was not associated with Alzheimer's disease or vascular dementia (Fig. 2). Similar results were found when the analyses were restricted to

individuals not using cholesterol-lowering medication (ESM Fig. 2). Estimates were in the same direction in those without type 2 diabetes mellitus at baseline (ESM Fig. 3), and when using quintiles of the weighted allele score (ESM Fig. 4), although tests for trend across weighted alleles were not significant. Using non-Alzheimer's disease (i.e. all other types of dementia than Alzheimer's disease) as the endpoint gave results in the same direction as those for unspecified dementia; however, the test for trend across ordered categories was not significant (ESM Fig. 5). As dementia is mostly a disease of the old, survival bias may affect risk estimates; however, accounting for the competing risk by death by using a Fine and Gray model gave similar results (ESM Fig. 6). The *APOE* genotype, which was included as a positive control, showed the known association of the $\epsilon 43$ and

Fig. 2 Prospective risk of unspecified dementia, Alzheimer's disease and vascular dementia shown on a base 2 log scale as a function of percentile groups of a weighted genetic allele score (weighted on the effect on plasma glucose and allele frequency in the Copenhagen studies) adjusted for age, sex and birth year in 115,875 individuals from the general population. Estimates were by Cox regression, excluding individuals with an endpoint before study entry. As a positive control of study power and

ε44 alleles with the risk of unspecified dementia, Alzheimer's disease and vascular dementia (Fig. 2), confirming the validity of the endpoints used and the study's power to demonstrate positive associations with the endpoints.

Confounding factors Associations between potentially confounding factors and plasma glucose, genetic variants and diagnoses of dementia were tested. Age, sex, hypertension, smoking, physical activity, alcohol consumption, years of education and (for women) menopausal status were all strongly associated with plasma glucose and many were also associated with the risk of unspecified dementia, Alzheimer's disease and vascular dementia (ESM Fig. 7), and may therefore have confounded the observational associations. However, the glucose genotypes were not associated with any major changes in the potential confounders, suggesting that confounding and pleiotropic effects through any of the above factors are unlikely.

Causal effect of glucose on risk of dementia Observationally, a 1 mmol/l higher non-fasting plasma glucose level had a multifactorial-adjusted HR of 1.04 (95% CI 1.01, 1.08; $p = 0.011$) for unspecified dementia, 0.97 (95% CI 0.94, 1.01; $p = 0.16$) for Alzheimer's disease and 1.04 (0.97, 1.12; $p = 0.24$) for vascular dementia (Fig. 3).

In genetic, causal analyses, a 1 mmol/l higher non-fasting plasma glucose level had an RR of 2.40 (1.18, 4.89; $p = 0.016$) for unspecified dementia, 1.41 (95% CI 0.82, 2.43; $p = 0.22$) for Alzheimer's disease and 1.20 (95% CI 0.82, 1.75; $p = 0.36$) for vascular dementia.

endpoints, risk of dementia is also shown as a function of the *APOE* ε43 and ε44 genotypes compared with the common ε33 genotype. Arrows denote a point estimate and 95% CI out of scale. The number of individuals varies slightly due to the availability of genotype data. p values are from tests for trend of HRs across ordered categories. To convert glucose values to mg/dl, multiply values in mmol/l by 18.02

Using summary-level data from MAGIC combined with the ADSP/IGAP/PGC-ALZ/UKB, the RR for Alzheimer's disease was 1.02 (95% CI 0.92, 1.13; $p = 0.42$). The RR for Alzheimer's disease using the Copenhagen studies combined with MAGIC + ADSP/IGAP/PGC-ALZ/UKB was 1.03 (95% CI 0.93, 1.13; $p = 0.36$) (Fig. 3). In the MAGIC + ADSP/IGAP/PGC-ALZ/UKB studies, the genetic variants showed no indication of directional pleiotropy for variants with low precision (ESM Fig. 8), assumptions for using Egger regression were not violated (ESM Figs 8, 9) and the estimate was not driven by a single genetic variant (ESM Fig. 10).

The power to detect a significant ($\alpha = 0.05$ and $\beta = 0.80$) effect of glucose on the risk of dementia was 1.0 for all endpoints. The risk of ischaemic heart disease, which was included as a positive control for the effect of the genetic instrument, showed the known association of non-fasting plasma glucose levels with the risk of ischaemic heart disease both observationally and genetically (Fig. 3) [9], confirming the validity of the glucose genetic instruments and study power to demonstrate positive associations using these instruments.

Discussion

In 115,875 individuals from the Copenhagen general population, we found a causal effect of genetically high non-fasting plasma glucose concentrations on the risk of unspecified dementia. Causal genetic effects were not observed for the risk of Alzheimer's disease or of vascular dementia in the

Fig. 3 Risk of unspecified dementia, Alzheimer's disease and vascular dementia for a 1 mmol/l higher observational and causal, genetically determined plasma glucose concentration. HRs with 95% CIs for a 1 mmol/l higher observational plasma glucose concentration were calculated using Cox regression and RRs for genetically higher plasma glucose were derived from instrumental variable analyses. Risk estimates are shown on a base 2 log scale. For Alzheimer's disease, risk was also estimated using summary risk estimates from the PGC-ALZ, IGAP and ADSP, and Alzheimer's disease by proxy (parent with Alzheimer's disease) from the UKB (ADSP/IGAP/PGC-ALZ/UKB) on the same

genetic variants, but tested in MAGIC using Egger Mendelian randomisation. Estimates for the known observational and causal genetic associations of glucose with the risk of ischaemic heart disease are included as a positive control of the validity of the weighted genetic allele score and study power. *p* values are for the significance of HRs and RRs. ^aBecause the observational risks of dementia as a function of glucose concentrations were only linear at glucose concentrations above the population median of 5.2 mmol/l (Fig. 1), estimates for the observational risk of disease for a 1 mmol/l higher glucose were limited to glucose concentrations above the median

Copenhagen populations or in the MAGIC + ADSP/IGAP/PGC-ALZ/UKB participants either alone or combined.

Several studies have examined the observational association between diabetes mellitus and risk of dementia [29–32], and a large meta-analysis [33] found an increased risk of dementia in individuals with vs without diabetes mellitus, with RRs of 1.73 (95% CI 1.65, 1.82) for all dementia, 1.53 (95% CI 1.42, 1.63) for Alzheimer's disease and 2.27 (95% CI 1.94, 2.66) for vascular dementia. In addition, one study has examined the observational association between plasma glucose concentrations and risk of dementia, and reported an increased risk of unspecified dementia and Alzheimer's disease combined [1]. In our study, the statistically significant increased risk was observed specifically for unspecified dementia.

A large systematic review has summarised the existing literature on the association between markers of poor glycaemic control (i.e. plasma glucose, HbA_{1c}, glucose variability, hypoglycaemic events and fasting insulin) and cognitive function and dementia in patients with diabetes mellitus, and found good evidence for a modest association between

poor glycaemic control and worse cognitive function, low evidence for associations with the risk of dementia and some evidence that poor glycaemic control is associated with brain abnormalities on MRI [34]. The study also concluded that there are limited data on the effects of glucose-lowering treatment on long-term cerebral outcomes.

The observations in the present study are in concordance with two previous Mendelian randomisation studies of modifiable risk factors for Alzheimer's disease using data from the IGAP, which reported no causal effects of type 2 diabetes, fasting glucose or insulin resistance on the risk of Alzheimer's disease [5, 6]. However, these studies addressed Alzheimer's disease only and did not include data on vascular and unspecified dementia, or observational data.

Elevated plasma glucose concentrations are obviously linked to the presence of diabetes mellitus, and even using the Mendelian randomisation design it is difficult to separate the effect of glucose on the risk of dementia from the multiple effects of diabetes mellitus. In the present study, the genetic variants were associated with lifelong elevated glucose concentrations via known mechanisms, but not with increased

BMI, hypertension or hyperlipidaemia, confounding factors often associated with diabetes. Excluding individuals with diabetes mellitus at baseline from the causal genetic analyses gave similar results, suggesting that glucose per se has a causal effect on the risk of unspecified dementia, but not on the risk of Alzheimer's disease.

The finding of a causal effect of glucose on the risk of unspecified dementia but not on Alzheimer's dementia is interesting. Evidence from brain autopsy studies has shown that the key features of Alzheimer's disease, such as extracellular deposits of amyloid β and intraneuronal aggregates of hyperphosphorylated tau, are not more common in patients with vs without type 2 diabetes [35]. In addition, type 2 diabetes is not associated with cerebrospinal or positron emission tomography biomarkers of increased deposition of cerebral amyloid β or tau pathology [36, 37]. This supports the suggestion that high glucose concentrations and diabetes do not contribute directly to the pathology of Alzheimer's disease.

In contrast, there is firm evidence that type 2 diabetes is associated with brain atrophy [38], widespread changes in the microstructure of the white matter and connectivity often related to cognitive dysfunction [38, 39], and an increased burden of cerebrovascular lesions, especially lacunes, but not with an increased burden of large artery infarcts or microinfarcts [40, 41]. These factors may contribute to cognitive dysfunction and unspecified dementia. This is supported by the present study, which observed that individuals with unspecified dementia, compared with individuals with Alzheimer's disease and vascular dementia, had higher plasma glucose concentrations and, in the presence of type 2 diabetes, were less often treated with oral glucose-lowering drugs (15% vs 33% and 22%, respectively), suggesting that poor glycaemic control might have contributed to disease risk in these individuals and that improved diabetes diagnosis and management may reduce this risk.

An important limitation of this study is that the endpoints were based on ICD registry codes from hospitals and death certificates from routine clinical practice, and the study therefore only captured individuals in contact with hospitals. This is in contrast to research studies in which all individuals living in one area have been examined and diagnosed using standardised instruments, trained staff and standardised diagnostic methods [42]. Validation of the Danish registry diagnoses has shown that clinical use of the Alzheimer's disease diagnosis is largely limited to individuals fulfilling the diagnostic criteria for this disease, while the diagnoses of vascular dementia and unspecified dementia include individuals with cognitive impairment of mixed causes, including age-related structural brain changes, and those with a previous stroke, atrophy, and large- and small-vessel disease [16–18].

As a sensitivity analysis, we used non-Alzheimer's disease (all other types of dementia than Alzheimer's disease) as an endpoint and obtained results in the same direction as the

result for unspecified dementia, although much attenuated and the test for trend across ordered categories was not significant. This suggests that glucose may have a different or no effect in different types of dementia, although our population study was not designed to examine this in further detail. Despite this, data from the ADSP/IGAP/PGC-ALZ/UKB consortium, examining individuals or relatives who have been carefully screened for dementia, showed similar results for Alzheimer's disease as the Danish studies using registry diagnoses. Another potential limitation is the uncertainty of age at onset for dementia diseases. It is commonly accepted that dementia prodromal phases can last for decades. Hence, we cannot exclude that some of the participants receiving a dementia diagnosis during our follow-up time already had dementia pathology at baseline.

As dementia is a disease with onset usually late in life, individuals may die of other causes before developing dementia. This may introduce ascertainment bias, since you cannot participate in a study if you are dead [43]. This is most often an issue in cross-sectional studies. However, in the present prospective study, individuals were followed for up to 43 years with a median of 10 years and diagnoses were retrieved from registers, reducing the risk of low participation because of pre-existing conditions. Late onset of disease may also introduce survivor bias; however, genotypes used in the present study were in Hardy–Weinberg equilibrium, suggesting that the genetic instrument was not invalidated by the preferential death of individuals with specific genotypes. Furthermore, using a Fine and Gray model taking the competing risk of death into account did not change the results of the association between weighted glucose alleles and the risk of dementia. If survivor bias was present in our study, it may have reduced the strength of the genetic instrument and resulted in an underestimate of the causal effect in the individual-level analyses of the Mendelian randomisation analysis [44, 45], which could pose a problem for the Alzheimer's disease result. However, the presence of Hardy–Weinberg equilibrium as well as robust supporting results from Fine and Gray models underscore that no strong survivor bias affected our data.

Strengths of this study include our examination of a large, homogenous general population cohort, access to individual participant data of high validity, no losses to follow-up and the use of the Mendelian randomisation design. This approach allowed us to examine potential causal effects of high plasma glucose concentrations, largely without known confounding or reverse causation. The finding of a similar result for the risk of Alzheimer's disease in a two-sample Mendelian randomisation study combining the MAGIC with the ADSP/IGAP/PGC-ALZ/UKB data suggest that the results may apply to other populations, although the fact that 66% of participants in the ADSP/IGAP/PGC-ALZ/UKB had self-reported disease in a parent and not their own diagnosed disease is a limitation.

Conclusions and future research In the present study, we used genetic variants that increase plasma glucose concentrations to examine the causal effect of lifelong higher plasma glucose on the risk of unspecified dementia, Alzheimer's disease and vascular dementia in individuals from the general population, and found a causal effect of glucose on the risk of unspecified dementia, but not on that of Alzheimer's disease or vascular dementia. Our finding that high plasma glucose has a causal effect on the risk of unspecified dementia underscores the importance of glycaemic control in patients with diabetes mellitus and potentially also in individuals with impaired glucose tolerance. Future research should address the causal role and potential disease mechanisms of plasma glucose on the risk of unspecified dementia.

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Data availability Due to participant confidentiality, only data presented in the paper and the ESM are available.

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