

# Genetic Variation in Targets of Antidiabetic Drugs and Alzheimer Disease Risk

## A Mendelian Randomization Study

Bowen Tang, MSc, Yunzhang Wang, PhD, Xia Jiang, PhD, Madhav Thambisetty, MD, PhD, Luigi Ferrucci, MD, PhD, Kristina Johnell, PhD, and Sara Hägg, PhD

*Neurology*® 2022;99:e650-e659. doi:10.1212/WNL.0000000000200771

### Correspondence

Dr. Hägg  
sara.hagg@ki.se

### RELATED ARTICLE

#### Editorial

The Potential of Genetic Data for Prioritizing Drug Repurposing Efforts

Page 267

## Abstract

### Background and Objectives

Previous studies have highlighted antidiabetic drugs as repurposing candidates for Alzheimer disease (AD), but the disease-modifying effects are still unclear.

### Methods

A 2-sample mendelian randomization study design was applied to examine the association between genetic variation in the targets of 4 antidiabetic drug classes and AD risk. Genetic summary statistics for blood glucose were analyzed using UK Biobank data of 326,885 participants, whereas summary statistics for AD were retrieved from previous genome-wide association studies comprising 24,087 clinically diagnosed AD cases and 55,058 controls. Positive control analysis on type 2 diabetes mellitus (T2DM), insulin secretion, insulin resistance, and obesity-related traits was conducted to validate the selection of instrumental variables.

### Results

In the positive control analysis, genetic variation in sulfonylurea targets was associated with higher insulin secretion, a lower risk of T2DM, and an increment in body mass index, waist circumference, and hip circumference, consistent with drug mechanistic actions and previous trial evidence. In the primary analysis, genetic variation in sulfonylurea targets was associated with a lower risk of AD (odds ratio [OR] = 0.38 per 1 mmol/L decrement in blood glucose, 95% CI 0.19–0.72,  $p = 0.0034$ ). These results for sulfonylureas were largely unchanged in the sensitivity analysis using a genetic variant, *rs757110*, that has been validated to modulate the target proteins of sulfonylureas (OR = 0.35 per 1 mmol/L decrement in blood glucose, 95% CI 0.15–0.82,  $p = 0.016$ ). An association between genetic variations in the glucagon-like peptide 1 (GLP-1) analogue target and a lower risk of AD was also observed (OR = 0.32 per 1 mmol/L decrement in blood glucose, 95% CI 0.13–0.79,  $p = 0.014$ ). However, this result should be interpreted with caution because the positive control analyses for GLP-1 analogues did not comply with a weight-loss effect as shown in previous clinical trials. Results regarding other drug classes were inconclusive.

### Discussion

Genetic variation in sulfonylurea targets was associated with a lower risk of AD, and future studies are warranted to clarify the underlying mechanistic pathways between sulfonylureas and AD.

From the Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm (B.T., Y.W., K.J., S.H.); Department of Clinical Neuroscience, Karolinska Institutet, Stockholm (X.J.); Brain Aging and Behavior Section, National Institute on Aging (M.T.); and Longitudinal Studies Section (L.F.), National Institute on Aging.

Go to [Neurology.org/N](https://www.neurology.org/N) for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## Glossary

**AD** = Alzheimer disease; **BMI** = body mass index; **DPP-4** = dipeptidyl peptidase 4; **GLP-1** = glucagon-like peptide 1; **GWAS** = genome-wide association study; **HIP** = hip circumference; **IVs** = instrumental variables; **IVW** = inverse variance-weighted method; **MR** = mendelian randomization; **PNDM** = permanent neonatal diabetes mellitus; **RCTs** = randomized clinical trials; **SGLT2** = sodium-glucose cotransporter 2; **SNV** = single-nucleotide variation; **T2DM** = type 2 diabetes mellitus; **TZD** = thiazolidinediones; **UKB** = UK Biobank; **WC** = waist circumference.

Alzheimer disease (AD) is a neurodegenerative disease, characterized by aberrant protein aggregation and neuronal loss in the brain that leads to cognitive decline, memory loss, and ultimately death.<sup>1</sup> However, currently, only a few agents or drugs that can improve symptoms have been approved for AD, whereas their neuroprotective effects remain uncertain.<sup>2</sup> Developing new drugs for AD is imperative but also extremely challenging with more than 400 candidates recently failed in phase III trials.<sup>3</sup> Drug repurposing or repositioning, where approved drugs are tested for a novel indication, has been proposed as a more rapid and cost-effective strategy to identify potential AD treatments because approved drugs possess well-documented information for mechanism of actions and comprehensive safety profiles.<sup>4</sup>

AD and type 2 diabetes mellitus (T2DM) are 2 of the most prevalent diseases in the aged population. A meta-analysis of 1,746,777 participants reported a 53% higher risk of developing AD in patients with T2DM.<sup>5</sup> Besides, AD has been proposed as “type 3 diabetes” with insulin resistance and impaired glucose control in the brain.<sup>6</sup> Antidiabetic drugs, based on their original intention of enhancing insulin signaling and regulating glucose metabolism, have been highlighted as repurposing candidates for AD.<sup>7</sup> Several randomized clinical trials (RCTs) have been conducted in patients with early or mild-to-moderate AD to investigate the disease-modifying effects of antidiabetic drugs, but the evidence to date was inconclusive.<sup>8</sup> Given the long prodromal phase of AD, clinical trials targeting early or mild-to-moderate AD have been considered belated, whereas primary intervention in pre-clinical AD or even earlier may offer the best opportunity of therapeutic success.<sup>9</sup> However, such primary prevention trials are challenging because they require considerably large sample sizes and long-duration intervention.

Mendelian randomization (MR) is a statistical tool using genetic variants as instrumental variables (IVs) to make causal inference between exposure(s) and outcome(s). Because genetic variants are assigned randomly at conception and before disease onset, MR is considered as a “natural” RCT, which can minimize confounding and reverse causation.<sup>10</sup> Particularly for the genetic variants within the genes that encode drug target proteins, such variants may influence the expression of genes, modulate the function of encoded proteins, and thereby closely proxy drug mechanism actions. In the spirit of natural RCTs, MR studies leveraging such druggable variants are useful in identifying drug repurposing

opportunities and predicting side effects.<sup>11</sup> An MR study used a variant on the *HMGCR* gene to proxy statin use and found that genetically mimicked statin use was associated with a higher risk of T2DM, consistent with the evidence from an RCT of 129,170 participants.<sup>12</sup> Apart from controlling for confounding and reverse causality, MR also provides the possibility to emulate primary prevention trials that comprise large sample sizes (recent genetic discoveries are usually based on hundreds of thousands of participants) and long intervention duration (genetically instrumented exposure occurs before the outcome and is lifelong). Hence, we conducted an MR study to examine the effects of genetic variation in antidiabetic drug targets on AD risk.

## Methods

### Study Design

The current study was conducted using a 2-sample MR design, which extracted exposure and outcome data from 2 independent nonoverlapping populations. Genetic variants within the genes that encode protein targets of antidiabetic drugs (*cis*-variants) were identified in a genome-wide association study (GWAS) summary dataset for blood glucose and used as proxy for antidiabetic drug use. Lowering blood glucose is an established physiologic response to antidiabetic drug treatment, and hence, blood glucose is the biomarker of interest in our study. To retain the validity of causal estimation, 3 MR model assumptions are essential, which are (1) a robust association between IVs and target proteins (relevance), (2) independence of IVs on confounders (exchangeability), and (3) no direct effects of IVs on AD risk other than through the drug targets (exclusion restriction). A framework of our study design is presented in eFigure 1, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91).

### Blood Glucose GWAS Data

IV-exposure associations were extracted from a GWAS of blood glucose analyzed on participants of European ancestry from UK Biobank (UKB).<sup>13</sup> Individuals with a diagnosis of diabetes in the inpatient registry (defined as E10-14 in *ICD-10* and 2500-2529 in *ICD-9*) or with self-reported diabetes in questionnaires were excluded from the analysis. In the association testing, a mixed linear model-based method was used to control for population stratification by principle components and relatedness by a genetic relationship matrix.<sup>14</sup> Finally, 326,885 participants were analyzed. Details about the GWAS are provided in eAppendix 1, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91).

## AD GWAS Data

The AD summary statistics (IV-outcome associations) were extracted from a previously conducted GWAS.<sup>15</sup> In phase 1, the data from Alzheimer's disease working group of the Psychiatric Genomics Consortium, the International Genomics of Alzheimer's Project, and the Alzheimer's Disease Sequencing Project were meta-analyzed, totaling 24,087 clinically diagnosed late-onset AD cases and 55,058 controls. In phase 3, 47,793 AD-by-proxy cases and 328,320 controls from UKB were additionally meta-analyzed on top of the phase 1 stage, resulting in 71,880 AD/AD-by-proxy cases and 383,378 controls. We used the dataset that only contains clinically diagnosed AD cases in primary analysis and the dataset that contains AD/AD-by-proxy cases in sensitivity analysis.

## Instrument Selection

Seven major classes of antidiabetic drugs were initially identified, including metformin, dipeptidyl peptidase 4 (DPP-4) inhibitor, sodium-glucose cotransporter 2 (SGLT2) inhibitor, insulin/insulin analogues, glucagon-like peptide 1 (GLP-1) analogues, sulfonylureas, and thiazolidinediones (TZD).<sup>16</sup> Information regarding the pharmacologically active protein targets and corresponding encoding genes was retrieved from the DrugBank and the ChEMBL databases separately (Table 1).<sup>17,18</sup> Because the protein targets of metformin differed in the 2 databases and the molecules underlying metformin's physiologic effects remain largely unknown,<sup>19</sup> metformin was excluded from further analysis.

Next, we identified the *cis*-variants within each encoding gene ( $\pm 2,500$  base pairs of the gene location) and retained the

variants associated with blood glucose at a false discovery rate of  $<0.05$ . None of the variants for SGLT2 inhibitors survived the selection, hence being excluded from further analysis. Palindromic single-nucleotide variations (SNVs [formerly SNPs]; SNVs with the same pair of purine pyrimidine bases on forward and reverse strands) were excluded to avoid ambiguity in the identification of effect alleles. The 2 variants for DPP4 inhibitors are both palindromic (no high-LD proxies were found), so the drug was excluded from further analysis. The variants that remained for each drug class were then clumped with a  $R^2$  of 0.01 and a window size of 500 kB, complemented with a sensitivity analysis clumping with  $R^2$  from 0.01 to 0.50 to gain greater precision by including a larger number of partially independent variants.<sup>11</sup> The process of instrument selection is displayed in Figure 1. In addition, we consulted the literature and identified 1 additional variant, *rs757110*, for sulfonylureas, which has been validated as a strong proxy in *in vitro* and population studies.<sup>20,21</sup>

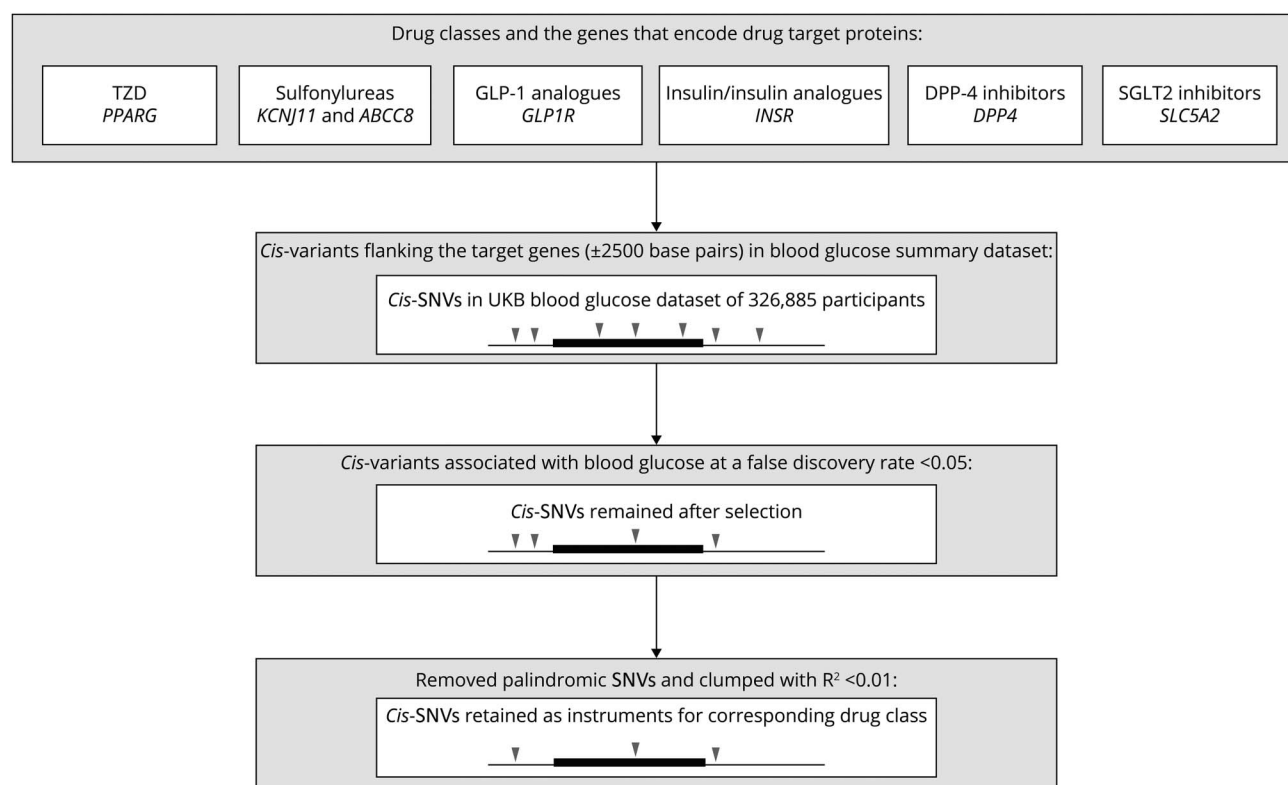
## Positive Control Analysis

To validate our selection of IVs, positive control analysis was performed with T2DM, insulin secretion, insulin resistance, and obesity-related traits as outcomes. T2DM is the original indication of antidiabetic drugs, whereas sulfonylureas and GLP-1 analogues promote insulin secretion and TZD increases insulin sensitivity.<sup>16</sup> IVs for insulin/insulin analogues were selected within *INSR* that encodes the insulin receptor, so it would be expected to alter the function of the insulin

**Table 1** Summary Information of Antidiabetic Drug Classes, Targets, and Encoding Genes

Drug class	Drug targets		Encoding genes		Included in analysis	Gene region (in GRCh37 from Ensembl)
	DrugBank	ChEMBL	DrugBank	ChEMBL		
<b>Metformin</b>	5'-AMP-activated protein kinase subunit beta-1	Mitochondrial complex I (NADH dehydrogenase)	<i>PRKAB1</i>	58 encoding genes	No	Not included
	Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial	Mitochondrial glycerol-3-phosphate dehydrogenase	<i>ETFDH</i>	<i>GPD2</i>		
<b>Dipeptidyl peptidase 4 (DPP-IV) inhibitor</b>	Dipeptidyl peptidase IV	Dipeptidyl peptidase IV	<i>DPP4</i>	<i>DPP4</i>	No	Chr2: 162,848,755-162,930,904
<b>Sodium-glucose cotransporter 2 (SGLT2) inhibitor</b>	Sodium/glucose cotransporter 2	Sodium/glucose cotransporter 2	<i>SLC5A2</i>	<i>SLC5A2</i>	No	Chr16: 31,494,323-31,502,181
<b>Insulin/insulin analogues</b>	Insulin receptor	Insulin receptor	<i>INSR</i>	<i>INSR</i>	Yes	Chr19: 7,112,266-7,294,425
<b>Glucagon-like peptide 1 (GLP-1) analogues</b>	Glucagon-like peptide 1 receptor	Glucagon-like peptide 1 receptor	<i>GLP1R</i>	<i>GLP1R</i>	Yes	Chr6: 39,016,557-39,059,079
<b>Sulfonylureas (SU)</b>	ATP-sensitive potassium channel	ATP-sensitive potassium channel	<i>KCNJ11</i>	<i>KCNJ11</i>	Yes	Chr11: 17,386,719-17,410,878
			<i>ABCC8</i>	<i>ABCC8</i>		Chr11: 17,414,045-17,498,441
<b>Thiazolidinediones (TZD)</b>	Peroxisome proliferator-activated receptor gamma	Peroxisome proliferator-activated receptor gamma	<i>PPARG</i>	<i>PPARG</i>	Yes	Chr3: 12,328,867-12,475,855

**Figure 1** Instrument Selection for Antidiabetic Drug Classes



Black line represents DNA strand, and raised box represents the target gene region. Wedges represent specific genetic variants (SNVs). Disappearing of wedges indicates the exclusion of SNVs. DPP-4 inhibitors = dipeptidyl peptidase 4 inhibitors; GLP-1 analogues = glucagon-like peptide 1 analogues; SGLT2 inhibitors = sodium-glucose cotransporter 2 inhibitors; SNVs = single-nucleotide variations; TZD = thiazolidinediones; UKB = UK Biobank.

receptor and reduce insulin resistance. Obesity is another phenotype influenced by antidiabetic drugs. A meta-analysis of clinical trials suggests that insulin analogues, sulfonylureas, and TZD contribute to weight gain, and GLP-1 analogues cause weight loss.<sup>22</sup> Hence, 3 obesity-related traits, including body mass index (BMI), waist circumference (WC), and hip circumference (HIP), were used as outcomes.

Because the GWAS datasets for insulin secretion, insulin resistance, WC, and HIP (outcome datasets) contain less SNVs than the UKB blood glucose GWAS (exposure dataset), we retained nonpalindromic SNVs available in both datasets and performed clumping to obtain IVs for the positive control analyses ( $R^2 < 0.01$ ). Genetically predicted drug effects that showed directional consistency with clinical trial evidence/drug mechanisms were considered to pass the positive control analysis.

In addition to the positive control analyses, we also explored the association between genetic variation in antidiabetic drug targets and cardiovascular diseases/hippocampal volume despite rather inconclusive evidence given by previous clinical trials.<sup>23–28</sup> The details about these analyses and results are provided in eAppendix 2, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91). Information for the GWAS datasets used in our study is summarized in eTable 1, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91).<sup>29–33</sup> Characteristics

of the SNVs retained as IVs for each analysis are described in eTables 2 and 3, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91).

## Standard Protocol Approvals, Registrations, and Patient Consents

Our analysis of UKB data has been conducted under application number “22224.” The summary statistics for AD, BMI, and T2DM do not contain any personal information, and the GWAS have obtained ethical approval from relevant ethics review boards.

## Statistical Analysis

First, the IV-exposure association from the blood glucose GWAS dataset and the IV-outcome association from the outcome GWAS dataset were merged. The causal association was estimated with the Wald ratio test for 1 single IV and with the random-effects inverse variance-weighted (IVW) method for multiple IVs.<sup>10</sup> The IVW method provides unbiased causal estimation when all IVs are valid or if overall pleiotropy is balanced to be zero.<sup>10</sup> The Cochran’s Q test was performed to test heterogeneity within IVs and to detect any types of pleiotropy, balanced or unbalanced ( $p$  for heterogeneity  $<0.05$ ).<sup>10</sup> To deal with unbalanced pleiotropy, the MR-Egger regression was applied. MR-Egger provides reliable causal estimation even if all IVs are invalid and indicates the presence



of unbalanced pleiotropy at  $p$  for intercept  $<0.05$ .<sup>10</sup> Strength of the IVs was tested by F statistics and indicates weak instruments when F statistics  $<11$ . All analyses were conducted using the “TwoSampleMR” package in R software version 3.6.0.<sup>34</sup> To resemble the lowering effects of blood glucose by antidiabetic drugs, all estimations were scaled to per 1 mmol/L decrement in blood glucose. A Bonferroni-corrected significance level of  $p$  value  $<0.012$  ( $0.05/4$ ) was used to adjust for multiple testing of 5 drug classes. For the genetically instrumented drug class confirmed to be associated with AD, we further investigated the colocalization between blood glucose and AD within the target gene region using the “coloc” package.<sup>35</sup>

## Data Availability

Genetic datasets for AD/AD-by-proxy, T2DM, insulin secretion, insulin resistance, BMI, WC, and HIP are publicly available through the link listed in eTable1, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91). The UKB data are available through application. Scripts are available on personal request (email: [bowen.tang@ki.se](mailto:bowen.tang@ki.se)).

## Results

### Positive Control Analyses

As shown in Figure 2, panel A, genetic variation in the targets of TZD, sulfonylureas, insulin analogues, and GLP-1 analogues was associated with a decreased risk of T2DM. When looking into insulin secretion and insulin resistance (Figure 2, panels B and C), genetic variation in the targets of sulfonylureas and GLP-1 analogues was associated with increased insulin secretion, whereas insulin/insulin analogues and TZD were associated with decreased insulin resistance, consistent with the drug mechanism of actions. For obesity-related traits (Figure 2, panels D–F), the estimates for TZD and sulfonylureas suggested increment in BMI, WC, and HIP, consistent with evidence from the meta-analysis of clinical trials. However, the estimates for insulin/insulin analogues and GLP-1 analogues were varying across the 3 obesity-related traits.

### Effects of Genetic Variation in Antidiabetic Drug Targets on AD Risk

The results from our primary analysis using a GWAS dataset that contains 24,087 clinically diagnosed late-onset AD cases are shown in Figure 3. Generally, genetic variation in sulfonylurea targets was associated with a reduced risk of AD at the Bonferroni-corrected threshold (odds ratio [OR] = 0.38 per 1 mmol/L decrement in blood glucose, 95% CI 0.19–0.72,  $p = 0.0034$ ). The association was consistent in the analysis using the validated proxy, *rs757110* (OR = 0.35, 95% CI 0.15–0.82,  $p = 0.016$ ). Meanwhile, the estimates for sulfonylureas in the weighted median method (OR = 0.38, 95% CI 0.17–0.84) and MR-Egger regression (OR = 0.42, 95% CI 0.05–3.79) also confirmed a reduced risk of AD, although with a wider CI as the MR-Egger regression produces larger standard errors than the conventional IVW method (eTable 4, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91)). Besides, a suggestive association between genetic variation in the GLP-1 analogue target and lower risk of

AD was observed across different MR methods (Figure 3 and eTable 4, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91), IVW: OR = 0.32, 95% CI 0.13–0.79,  $p = 0.014$ ; weighted median: OR = 0.34, 95% CI 0.11–1.07,  $p = 0.066$ ; MR-Egger: OR = 0.35, 95% CI 0.05–2.52,  $p = 0.49$ ). For all the estimates, no heterogeneity within IVs or substantial pleiotropy was detected (Figure 3,  $p$  for heterogeneity  $>0.05$ ,  $p$  for pleiotropy  $>0.05$ ).

In the sensitivity analysis using a GWAS dataset comprising 71,880 AD/AD-by-proxy cases (eFigure 2, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91)), genetic variation in the targets of sulfonylureas and GLP-1 analogues showed protective effects (sulfonylureas: OR = 0.78, 95% CI 0.58–1.05; GLP-1 analogues: OR = 0.53, 95% CI 0.37–0.77), although the effect sizes attenuated toward the null possibly because of the dilution effects by AD-by-proxy cases. Considering the possible uncertainty arising from instrumenting each drug class with a relatively small number of IVs, we conducted a sensitivity analysis retaining more partially independent IVs by clumping the variants with relaxing  $R^2$ . The estimates for sulfonylureas and GLP-1 analogues stabilized throughout the  $R^2$  ranging from 0.001 to 0.50; meanwhile, the standard error decreased with more IVs being included. However, the estimates insulin analogue and TZD varied across different  $R^2$  and included the null (Figure 4).

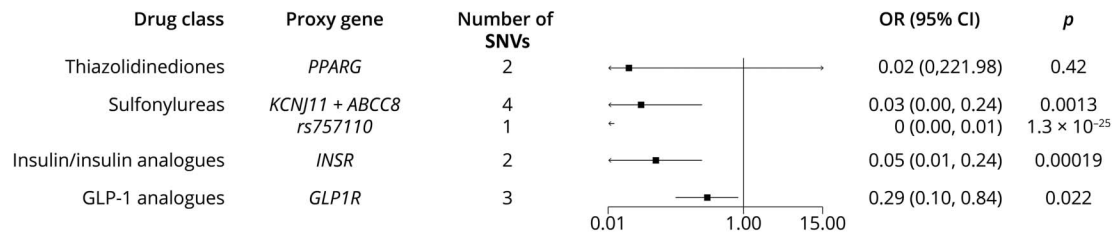
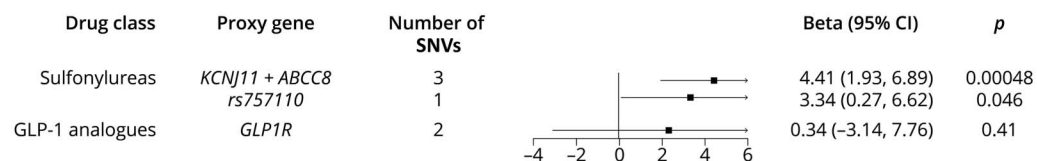
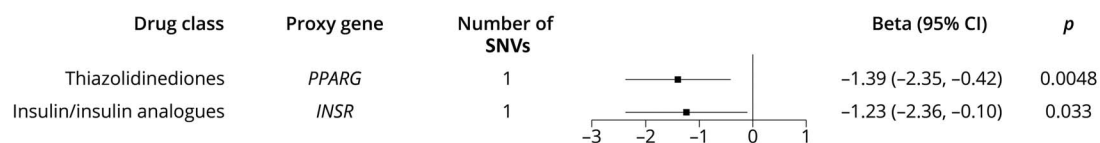
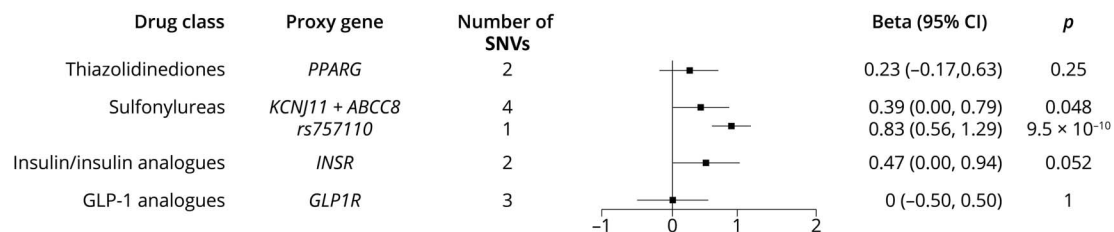
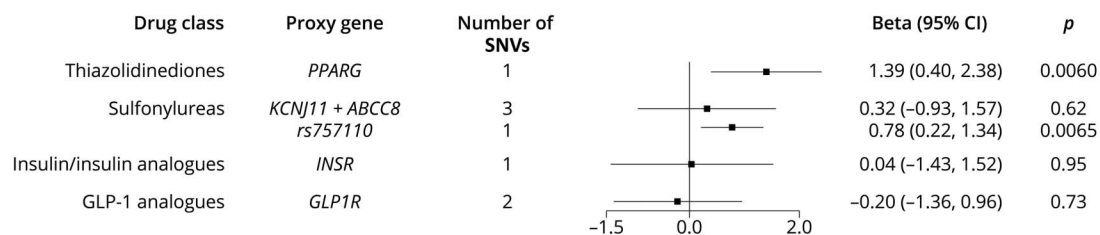
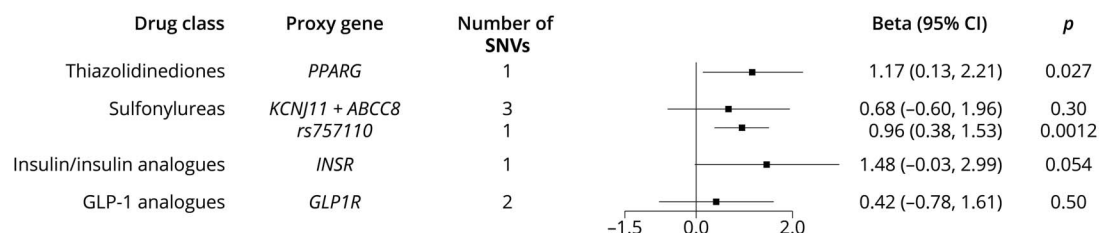
### Colocalization Analysis for Sulfonylureas and GLP-1 Analogues

Colocalization analysis was performed for sulfonylureas and GLP-1 analogues within the drug target encoding genes ( $\pm 2,500$  base pairs of *KCNJ11* and *ABCC8* for sulfonylureas and of *GLP1R* for GLP-1 analogues). The results are shown in eTable 5, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91). Generally, we did not observe strong evidence suggesting colocalization between blood glucose and AD within the 2 gene regions for sulfonylureas (probability for sharing 1 common causal variant: 2.5% in *KCNJ11* and 3.9% in *ABCC8*); however, when looking into the regional association plots (eFigure 3, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91)), a trend of colocalization was noted. For GLP-1 analogues, we did not find any evidence of either sharing a common variant or a distinct trend of colocalization within *GLP1R* (eTable 5 and eFigure 4, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91)). For more details regarding the colocalization analyses and results, please see eAppendix 3, [links.lww.com/WNL/C91](https://links.lww.com/WNL/C91).

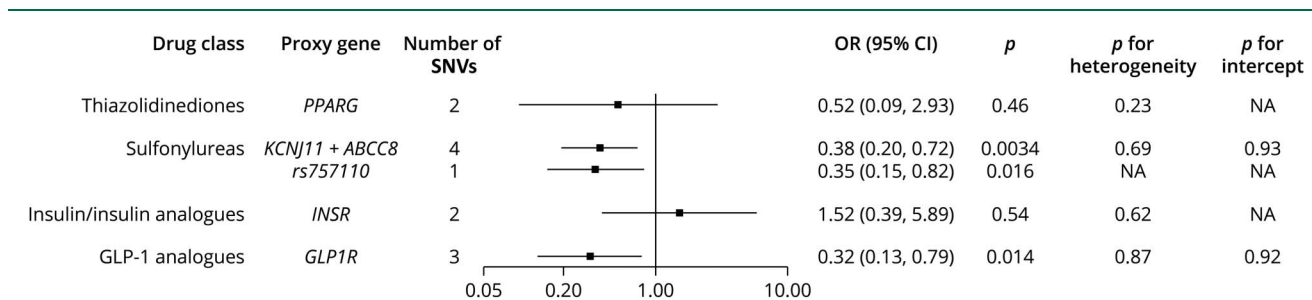
## Discussion

We investigated the effects of genetic variation in antidiabetic drug targets on AD risk using a combination of genetic datasets for blood glucose ( $\sim 300,000$  participants) and AD (24,087 AD cases and 55,058 controls). We found evidence to support that genetic variation in sulfonylurea targets was associated with a lower risk of AD.

A handful of observational studies have investigated the relationship of sulfonylureas with AD, but the results are rather

**Figure 2** Estimated Effects of Genetic Variation in Antidiabetic Drug Targets on Glucose Metabolism–Related Traits (Panel A–C) and Obesity-Related Traits (Panel D–F)**A. Genetic variation in antidiabetic drug targets on T2DM****B. Genetic variation in antidiabetic drug targets on insulin secretion****C. Genetic variation in antidiabetic drug targets on insulin resistance****D. Genetic variation in antidiabetic drug targets on BMI****E. Genetic variation in antidiabetic drug targets on WC****F. Genetic variation in antidiabetic drug targets on HIP**

Proxy gene is the gene that encodes the drug target protein, and *rs757110* is the variant that has been validated to modulate the protein target of sulfonylureas. All the estimates were scaled to per 1 mmol/L decrement in blood glucose. The estimated beta coefficients on BMI, WC, and HIP are expected to be in agreement with the evidence from clinical trials, that is, taking insulin analogues, sulfonylureas, and TZD contributes to weight gain, and taking GLP-1 analogues causes weight loss. BMI = body mass index; GLP-1 analogues = glucagon-like peptide 1 analogues; HIP = hip circumference; IVs = instrumental variables; OR = odds ratio; SNVs = single-nucleotide variations; T2DM = type 2 diabetes mellitus; TZD = thiazolidinediones; WC = waist circumference.

**Figure 3** Estimated Effects of Genetic Variation in Antidiabetic Drug Targets on Alzheimer Disease and Results From the Primary Analysis Using a GWAS Dataset Comprising 24,087 Clinically Diagnosed Late-Onset AD Cases

Proxy gene is the gene that encodes the drug target proteins, and rs757110 is the variant that have been validated to modulate the protein targets of sulfonylureas.  $p < 0.01$  indicates statistical significance, and  $0.01 < p < 0.05$  indicates suggestive significance.  $p$  for heterogeneity  $< 0.05$  indicates possible pleiotropy, whereas  $p$  for intercept  $< 0.05$  indicates substantial bias from pleiotropy. All the ORs were scaled to per 1 mmol/L decrement in blood glucose. NA indicates that the Cochran's Q test (heterogeneity test) or the MR-Egger regression (intercept test) is not available because of the limited number of IVs. AD = Alzheimer disease; GLP-1 analogues = glucagon-like peptide 1 analogues; GWAS = genome-wide association study; IVs = instrumental variables; MR = mendelian randomization; OR = odds ratio; SNVs = single-nucleotide variations; TZD = thiazolidinediones.

inconclusive.<sup>36-39</sup> In a prospective study following 127,209 dementia-free individuals for 7 years, a marginal protective effect was observed in diabetic patients (sulfonylureas vs not taking any antidiabetic drugs: HR = 0.85, 95% CI 0.71–1.01).<sup>38</sup> Conversely, in a case-control study of 7,086 AD cases, sulfonylureas were not associated with the development of AD (users vs nonusers: OR = 1.01, 95% CI 0.72–1.42).<sup>37</sup> The present MR study differs from these observational studies in 3 key aspects. First, the observational studies were conducted in diabetic patients and hence were unable to separate the true drug effect from that of diabetes, i.e., confounding by indication. Meanwhile, the present MR study investigated the genetically predicted drug effects in a nondiabetic general population. Second, the observational studies measured drug use at baseline, but the existence of poor monotherapy adherence may cause contamination. A study on veterans showed that around 40% of diabetic patients who initiated sulfonylureas added or switched to metformin afterward.<sup>39</sup> Drug adherence is less of a concern in our MR models because the genetically instrumented exposure is lifelong. Finally, unmeasured confounding could also be an issue in observational studies. Although the observational studies have controlled for confounders available in their datasets, these are difficult to measure, or even unknown, and may linger and induce bias. Conversely, taking advantage of the random allocation of genetic variants at conception, MR is expected to be less affected by confounding.<sup>10</sup>

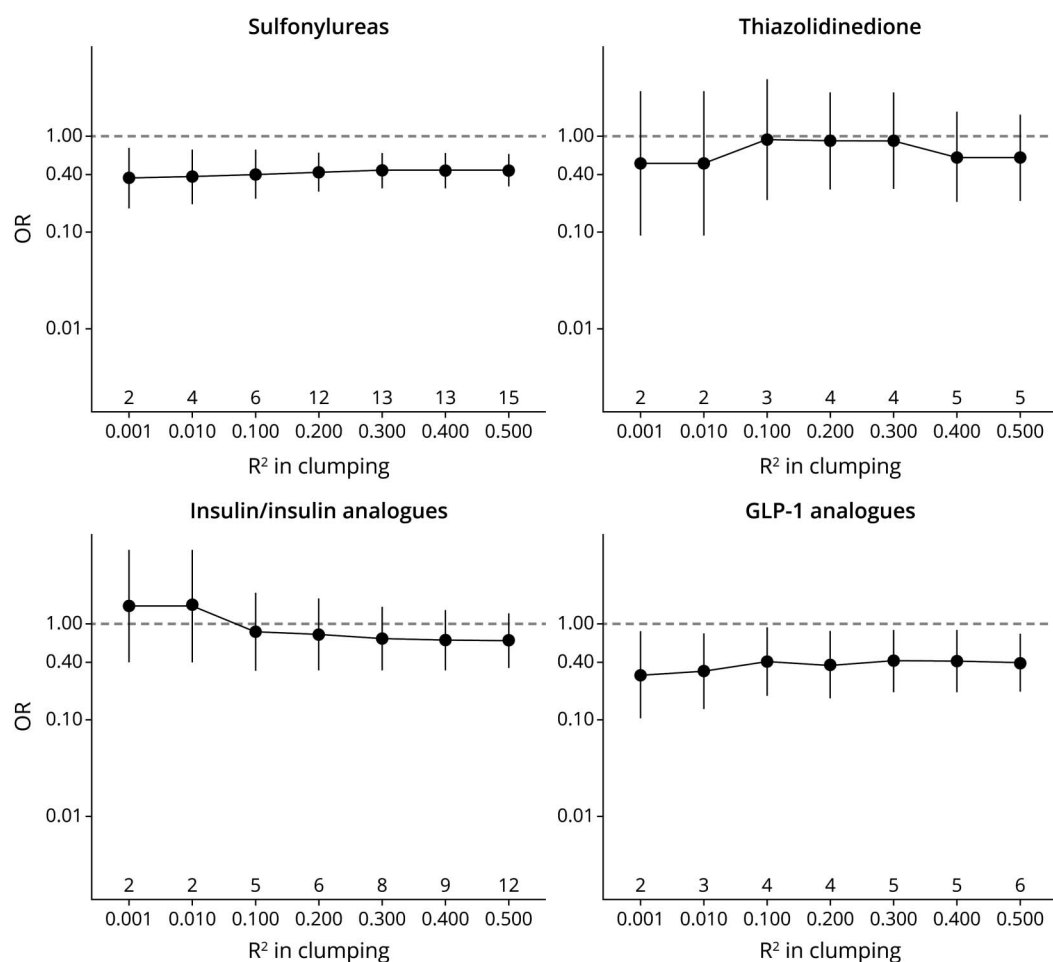
Sulfonylureas lower blood glucose by blocking the  $K_{ATP}$  channel on the membrane of pancreatic beta cells. Physiologically, the pancreatic  $K_{ATP}$  channel closes as a response to blood glucose increase, subsequently producing a membrane depolarization and stimulating insulin secretion.<sup>20</sup> Therefore, sulfonylurea use might relate to AD partly through its regulatory effects on glucose metabolism. Evidence from a previous MR study showed that increased fasting blood glucose (OR = 1.33, 95% CI 1.04–1.68) and  $\beta$ -cell dysfunction (OR = 1.92, 95% CI 1.15–3.21) contributed to a higher risk of AD.<sup>40</sup> Particularly, AD has been proposed as a brain-specific form of diabetes, a “type 3 diabetes.”<sup>6</sup> A study by An et al.<sup>41</sup> found that

abnormalities in brain glucose homeostasis, characterized by higher brain blood glucose levels, reduced glycolytic flux, and lower neuronal glucose transporter 3 levels, were intrinsic to AD pathogenesis. Moreover, periphery glucose regulation might affect the glucose homeostasis in the CNS. The same study showed that longitudinal increases in fasting plasma glucose levels were associated with higher brain tissue glucose concentrations.<sup>41</sup>

The  $K_{ATP}$  channel is also abundantly expressed in the brain.<sup>42</sup> Although the exact role of  $K_{ATP}$  channels in the brain is largely unknown, it has been found to modulate neurotransmitter release and to mediate the action of memantine in the mouse hippocampus.<sup>42-44</sup> Besides, the brain  $K_{ATP}$  channel has been evident to affect CNS function by clinical investigations. A study compared neuropsychological features between adults with permanent neonatal diabetes mellitus (PNDM) caused by *KCNJ11* variations and by *INS* variations (the gene encodes insulin; its variations influence insulin synthesis). Patients with *KCNJ11* variations were more likely to have abnormal CNS features, such as learning difficulties, reduced IQ, and motor deficits, whereas patients with *INS* variations rarely showed neurologic abnormalities.<sup>45</sup> Furthermore, Clark et al.<sup>46</sup> have confirmed that such neurologic abnormalities originate from the CNS. Analogous to the genetic variations in patients with PNDM, our IVs selected within the *KCNJ11* and *ABCC8* genes may simultaneously capture the modulation of the  $K_{ATP}$  channel in the brain and pancreas. This synthesis would incorporate the downstream effects, either through neurologic alterations or blood glucose regulation, in our MR models. Given the poor penetration of the sulfonylurea across the blood-brain barrier,<sup>47</sup> caution is warranted in repurposing the existing sulfonylurea agents for AD, especially until the elucidation of the underlying mechanistic pathways, pharmacokinetics, and pharmacodynamics.

A suggestive association between genetic variation in GLP-1 analogue targets and lower risk of AD was also observed in our study. GLP-1 lowers blood glucose by binding to GLP-1

**Figure 4** Estimated Effects of Genetic Variation in Antidiabetic Drug Targets on Alzheimer Disease and Results From the Sensitivity Analysis Using a GWAS Dataset Comprising 24,087 Clinically Diagnosed Late-Onset AD Cases and Clumping the *cis*-Variants With  $R^2$  From 0.001 to 0.50



The numbers below the x axis are the value of  $R^2$ , and the numbers above the x axis are the number of IVs after clumping with the corresponding  $R^2$ . AD = Alzheimer disease; GLP-1 analogues = glucagon-like peptide 1 analogues; GWAS = genome-wide association study; IVs = instrumental variables; OR = odds ratio.

receptors on pancreatic  $\beta$ -cells and stimulating insulin production and secretion.<sup>48</sup> GLP-1 crosses the blood-brain barrier, and GLP-1 receptors are also found in the CNS.<sup>48</sup> In vivo studies have found that GLP-1 analogues increased hippocampal neuronal density, protected against synaptic dysfunction, and reduced hyperphosphorylated tau.<sup>49</sup> In an RCT following 38 patients with AD for 26 weeks, liraglutide, a GLP-1 analogue, has been found to prevent the decline of brain glucose metabolism compared with placebo but produce no effect on cognition or A $\beta$  load, although the study was underpowered to detect such changes.<sup>50</sup> Besides, 2 ongoing RCTs (phase 2 and phase 3) are planning to recruit patients with early AD to examine the effects of GLP-1 analogues (liraglutide and semaglutide, respectively) on cerebral glucose metabolism, cognitive performance, and pathologic biomarkers (ClinicalTrials.gov Identifier: NCT01843075 and NCT04777396), which is expected to provide more convincing evidence to this topic.

The present MR study has several strengths. First, the participants were restricted to people of European ancestry,

which minimized the possible bias arising from population stratification. Second, the IVs were selected from the *cis*-variants within the coding genes for drug targets ( $\pm 2.5$  kB), which controlled the likelihood of pleiotropy by IVs tagging other genes. Also, no heterogeneity within the IVs was detected in our MR analysis, further substantiating the absence of pleiotropy that would bias our estimations. Third, we conducted a set of positive control analyses to validate the strength of our IVs. The results for sulfonylureas, instrumented either by the 4 clumped IVs or by the validated proxy *rs757110*, were in line with the evidence from prior meta-analyses of clinical trials. This strongly supported these IVs as appropriate proxies. Then, a reduced risk in AD was observed, consistent in these 2 sets of IVs, which further lends support for a putative causal relationship between sulfonylureas and AD.

The study has several limitations. First, our study can only predict the on-target effects of antidiabetic drugs because only the well-documented protein targets were included in our analysis. Drug effects that are not exerted through these



protein targets (off-target effects) cannot be captured in our MR models. Second, the genetically predicted drug effects may be somewhat different from therapeutic practice. An exposure instrumented by genetic variants is present from birth and lasts for a lifetime. Our analyses may therefore be interpreted as assessing long-term modulation effects of drug target proteins. Moreover, given that genetic effects are life-long, our estimates cannot reflect the effects of exposure to antidiabetic drugs during a certain period of life. Third, metformin, SGLT2 inhibitors, and DPP4 inhibitors were excluded from the analysis because of yet unclear mechanism of actions or lack of proper IVs. Also, our results regarding insulin/insulin analogues, TZD, and GLP-1 analogues are inconclusive, likely because of the uncertainty by the small number of IVs. Future studies are warranted to reexamine these drug classes when more or stronger IVs are available. Besides, because we only used the genetic summary data restricted to the population of European ancestry, the generalizability of our results would be confined in European ancestral populations. Finally, we did not find strong colocalization evidence for blood glucose and AD within *KCNJ11* and *ABCC8* for sulfonylureas, which might be due to weak variant-AD association in the region. Therefore, our findings regarding sulfonylureas warranted further examinations when a larger AD GWAS is available.

In conclusion, this study provides supportive evidence for genetic variation of sulfonylurea targets, through the modulation of the  $K_{ATP}$  channel, which was associated with a lower risk of AD. Future studies should be conducted to clarify the underlying mechanistic pathways between sulfonylureas and AD. The study also exemplifies how the MR design may be a promising tool for finding new indications for approved drugs. The method allows testing interventions that are otherwise costly, time-consuming, or, in other ways, impractical and should be considered a screening instrument in the drug development phase.

## Acknowledgment

The analyses on UK Biobank genotypes were enabled by resources provided by the Swedish National Infrastructure for Computing (SNIC) at UPPMAX partially funded by the Swedish Research Council through grant agreement no. 2018-05973. The authors thank the participants of the UK Biobank and the investigators who contributed to the data collection. Summary statistics for the genetic associations with AD, T2DM, insulin secretion, insulin resistance, BMI, WC, and HIP were obtained from the GWAS previously published. The authors thank all investigators for sharing the genome-wide summary statistics.

## Study Funding

The work was conducted with support from the Swedish Research Council (2015-03255, 2019-01272, 2020-06101), Karolinska Institutet Joint NIH-grant for doctoral studies, Karolinska Institutet Foundation, Karolinska Institutet Strategic Research Program in Epidemiology, and King Gustaf V

and Queen Victoria's Foundation of Freemasons. This work was supported by the NIH R01AG067996 to Sara Hägg.

## Disclosure

The authors report no disclosures relevant to the manuscript. Go to [Neurology.org/N](https://www.neurology.org/N) for full disclosures.

## Publication History

Received by *Neurology* October 11, 2021. Accepted in final form April 8, 2022. Submitted and externally peer reviewed. The handling editors were Rawan Tarawneh, MD, and Brad Worrall, MD, MSc, FAAN.

## Appendix Authors

Name	Location	Contribution
<b>Bowen Tang, MSc</b>	Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm	Analysis or interpretation of data; drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and study concept or design
<b>Yunzhang Wang, PhD</b>	Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
<b>Xia Jiang, PhD</b>	Department of Clinical Neuroscience, Karolinska Institutet, Stockholm	Drafting/revision of the manuscript for content, including medical writing for content
<b>Madhav Thambisetty, MD, PhD</b>	Brain Aging and Behavior Section, National Institute on Aging	Drafting/revision of the manuscript for content, including medical writing for content
<b>Luigi Ferrucci, MD, PhD</b>	Longitudinal Studies Section, National Institute on Aging	Drafting/revision of the manuscript for content, including medical writing for content
<b>Kristina Johnell, PhD</b>	Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm	Drafting/revision of the manuscript for content, including medical writing for content
<b>Sara Hägg, PhD</b>	Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm	Analysis or interpretation of data; drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and study concept or design

## References

1. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement J Alzheimers Assoc*. 2007;3(3):186-191. doi: 10.1016/j.jalz.2007.04.381
2. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M. Alzheimer's disease: clinical trials and drug development. *Lancet Neurol*. 2010;9(7):702-716. doi: 10.1016/S1474-4422(10)70119-8
3. Mehta D, Jackson R, Paul G, Shi J, Sabbagh M. Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015 *Expert Opin Investig Drugs*. 2017;26(6):735-739. doi: 10.1080/13543784.2017.1323868

4. Corbett A, Pickett J, Burns A, et al. Drug repositioning for Alzheimer's disease. *Nat Rev Drug Discov*. 2012;11(11):833-846. doi: 10.1038/nrd3869
5. Zhang J, Chen C, Hua S, et al. An updated meta-analysis of cohort studies: diabetes and risk of Alzheimer's disease. *Diabetes Res Clin Pract*. 2017;124:41-47. doi: 10.1016/j.diabres.2016.10.024
6. Kandimala R, Thirumala V, Reddy PH. Is Alzheimer's disease a type 3 diabetes? A critical appraisal. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863(5):1078-1089. doi: 10.1016/j.bbdis.2016.08.018
7. Boccardi V, Murasecco I, Mecocci P. Diabetes drugs in the fight against Alzheimer's disease. *Ageing Res Rev*. 2019;54:100936. doi: 10.1016/j.arr.2019.100936
8. Muñoz-Jiménez M, Zaarkti A, García-Arnés JA, García-Casares N. Antidiabetic drugs in Alzheimer's disease and mild cognitive impairment: a systematic review. *Demen Geriatr Cogn Disord*. 2020;49(5):423-434. doi: 10.1159/000510677
9. Dubois B, Hampel H, Feldman HH, et al. Proceedings of the Meeting of the International Working Group IWG and the American Alzheimer's Association on "The Preclinical State of AD"; July 23, 2015; Washington DC, USA. Preclinical Alzheimer's disease: definition, natural history, and diagnostic criteria. *Alzheimers Dement*. 2016;12(3):292-323. doi: 10.1016/j.jalz.2016.02.002
10. Hemani G, Bowden J, Davey Smith G. Evaluating the potential role of pleiotropy in Mendelian randomization studies. *Hum Mol Genet*. 2018;27(R2):R195-R208. doi: 10.1093/hmg/ddy163
11. Schmidt AF, Finan C, Gordillo-Marañón M, et al. Genetic drug target validation using Mendelian randomisation. *Nat Commun*. 2020;11(1):3255. doi: 10.1038/s41467-020-16969-0
12. Swerdlow DI, Preiss D, Kuchenbaecker KB, et al. HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *Lancet*. 2015;385(9965):351-361. doi: 10.1016/s0140-6736(14)61183-1
13. Allen NE, Sudlow C, Peakman T, Collins R, UK Biobank. UK Biobank data: come and get it. *Sci translational Med*. 2014;6(224):224ed4. doi: 10.1126/scitranslmed.3008601
14. Jiang L, Zheng Z, Qi T, et al. A resource-efficient tool for mixed model association analysis of large-scale data. *Nat Genet*. 2019;51(12):1749-1755. doi: 10.1038/s41588-019-0530-8
15. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51(3):404-413. doi: 10.1038/s41588-018-0311-9
16. Chaudhury A, Duvoor C, Reddy Dendi VS, et al. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. *Mini Rev Front Endocrinol*. 2017;8:6. doi: 10.3389/fendo.2017.00006
17. Wishart DS, Feunang YD, Guo AC, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res*. 2018;46(D1):D1074-D1082. doi: 10.1093/nar/gkx1037
18. Gaulton A, Hersey A, Nowotka M, et al. The ChEMBL database in 2017. *Nucleic Acids Res*. 2017;45(D1):D945-D954. doi: 10.1093/nar/gkw1074
19. Rena G, Hardie DG, Pearson ER. The mechanisms of action of metformin. *Diabetologia*. 2017;60(9):1577-1585. doi: 10.1007/s00125-017-4342-z
20. Hamming KSC, Soliman D, Matemiz LC, et al. Coexpression of the type 2 diabetes susceptibility gene variants KCNJ11 E23K and ABCC8 S1369A alter the ATP and sulfonylurea sensitivities of the ATP-sensitive K(+) channel. *Diabetes*. 2009;58(10):2419-2424. doi: 10.2337/db09-0143
21. Emdin CA, Klarin D, Natarajan P, et al. Genetic variation at the sulfonylurea receptor, type 2 diabetes, and coronary heart disease. *Diabetes*. 2017;66(8):2310-2315. doi: 10.2337/db17-0149
22. Liu S-C, Tu Y-K, Chien M-N, Chien K-L. Effect of antidiabetic agents added to metformin on glycaemic control, hypoglycaemia and weight change in patients with type 2 diabetes: a network meta-analysis. *Diabetes Obes Metab*. 2012;14(9):810-820. doi: 10.1111/j.1463-1326.2012.01606.x
23. Hibar DP, Adams HHH, Jahanshad N, et al. Novel genetic loci associated with hippocampal volume. *Nat Commun*. 2017;8(1):13624. doi: 10.1038/ncomms13624
24. Nikpay M, Goel A, Won HH, et al. A comprehensive 1,000 genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet*. 2015;47(10):1121-1130. doi: 10.1038/ng.3396
25. Shah S, Henry A, Roselli C, et al. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun*. 2020;11(1):163. doi: 10.1038/s41467-019-13690-5
26. Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50(4):524-537. doi: 10.1038/s41588-018-0058-3
27. Younk LM, Lamos EM, Davis SN. The cardiovascular effects of insulin. *Expert Opin Drug Saf*. 2014;13(7):955-966. doi: 10.1517/14740338.2014.919256
28. Savarese G, Butler J, Lund LH, Bhatt DL, Anker SD. Cardiovascular effects of non-insulin glucose-lowering agents: a comprehensive review of trial evidence and potential cardioprotective mechanisms. *Cardiovasc Res*. 2021;cvab271. doi: 10.1093/cvr/cvab271
29. Prokopenko I, Poon W, Mägi R, et al. A central role for GRB10 in regulation of islet function in man. *PLoS Genet*. 2014;10(4):e1004235. doi: 10.1371/journal.pgen.1004235
30. Manning AK, Hivert MF, Scott RA, et al. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nat Genet*. 2012;44(6):659-669. doi: 10.1038/ng.2274
31. Mahajan A, Taliun D, Thurner M, 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):1505-1513. doi: 10.1038/s41588-018-0241-6
32. Pulit SL, Stoneman C, Morris AP, et al. Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Hum Mol Genet*. 2019;28(1):166-174. doi: 10.1093/hmg/ddy327
33. Shungin D, Winkler TW, Croteau-Chonka DC, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature*. 2015;518(7538):187-196. doi: 10.1038/nature14132
34. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenotype. *eLife*. 2018;7:e34408. doi: 10.7554/eLife.34408
35. Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet*. 2014;10(5):e1004383. doi: 10.1371/journal.pgen.1004383
36. Cheng C, Lin C-H, Tsai Y-W, Tsai C-J, Chou P-H, Lan T-H. Type 2 diabetes and antidiabetic medications in relation to dementia diagnosis. *J Gerontol Ser A Biol Sci Med Sci*. 2014;69(10):1299-1305. doi: 10.1093/gerona/glu073
37. Imfeld P, Bodmer M, Jick SS, Meier CR. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. *J Am Geriatr Soc*. 2012;60(5):916-921. doi: 10.1111/j.1532-5415.2012.03916.x
38. Hsu C-C, Wahlqvist ML, Lee M-S, Tsai H-N. Incidence of dementia is increased in type 2 diabetes and reduced by the use of sulfonylureas and metformin. *J Alzheimers Dis*. 2011;24(3):485-493. doi: 10.3233/JAD-2011-101524
39. Scherrer JE, Salas J, Floyd JS, Farr SA, Morley JE, Dublin S. Metformin and sulfonylurea use and risk of incident dementia. *Mayo Clin Proc*. 2019;94(8):1444-1456. doi: 10.1016/j.mayocp.2019.01.004
40. Pan Y, Chen W, Yan H, Wang M, Xiang X. Glycemic traits and Alzheimer's disease: a Mendelian randomization study. *Aging (Albany NY)*. 2020;12(22):22688-22699. doi: 10.18632/aging.103887
41. An Y, Varma VR, Varma S, et al. Evidence for brain glucose dysregulation in Alzheimer's disease. *Alzheimers Dement*. 2018;14(3):318-329. doi: 10.1016/j.jalz.2017.09.011
42. Avshalumov MV, Rice ME. Activation of ATP-sensitive K(+) (K(ATP)) channels by H2O2 underlies glutamate-dependent inhibition of striatal dopamine release. *Proc Natl Acad Sci U S A*. 2003;100(20):11729-11734. doi: 10.1073/pnas.1834314100
43. Shi X-R, Chang J, Ding J-H, Fan Y, Sun X-L, Hu G. Kir6.2 knockout alters neurotransmitter release in mouse striatum: an in vivo microdialysis study. *Neurosci Lett*. 2008;439(3):230-234. doi: 10.1016/j.neulet.2008.05.024
44. Moriguchi S, Ishizuka T, Yabuki Y, et al. Blockade of the K(ATP) channel Kir6.2 by memantine represents a novel mechanism relevant to Alzheimer's disease therapy. *Mol Psychiatry*. 2018;23(2):211-221. doi: 10.1038/mp.2016.187
45. Bowman P, Day J, Torrens L, et al. Cognitive, neurological, and behavioral features in adults with KCNJ11 neonatal diabetes. *Diabetes Care*. 2019;42(2):215-224. doi: 10.2337/dc18-1060
46. Clark RH, McTaggart JS, Webster R, et al. Muscle dysfunction caused by a K(ATP) channel mutation in neonatal diabetes is neuronal in origin. *Science*. 2010;329(5990):458-461. doi: 10.1126/science.1186146
47. Lahmann C, Kramer HB, Ashcroft FM. Systemic administration of glibenclamide fails to achieve therapeutic levels in the brain and cerebrospinal fluid of rodents. *PLoS One*. 2015;10(7):e0134476. doi: 10.1371/journal.pone.0134476
48. Ferreira ST. Brain insulin, insulin-like growth factor 1 and glucagon-like peptide 1 signalling in Alzheimer's disease. *J Neuroendocrinology*. 2021;33(4):e12959. doi: 10.1111/jne.12959
49. Kellar D, Craft S. Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. *Lancet Neurol*. 2020;19(9):758-766. doi: 10.1016/s1474-4422(20)30231-3
50. Gejl M, Gjedde A, Egefford L, et al. In Alzheimer's disease, 6-month treatment with GLP-1 analog prevents decline of brain glucose metabolism: randomized, placebo-controlled, double-blind clinical trial. *Front Aging Neurosci*. 2016;8:108. doi: 10.3389/fnagi.2016.00108