ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Nutritional Epidemiology and Public Health

Insulin Receptor Genetic Variants Causal Association with Type 2 Diabetes: A Mendelian Randomization Study

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ABSTRACT

Background: Type 2 diabetes (T2D) is a prevalent chronic disease associated with several comorbidities.

Objectives: This study investigated whether the risk of T2D varied with genetically predicted insulin (INS), insulin receptor (INS-R), or insulin-like growth factor 1 receptor (IGF-1R) using genetic variants in a Mendelian randomization (MR) study.

Methods: A 2-sample MR study was conducted using summary statistics from 2 genome-wide association studies (GWASs). Genetic predictors of the exposures (INS, INS-R, and IGF-1R) were obtained from a publicly available proteomics GWAS of the INTERVAL randomized controlled trial of blood donation in the United Kingdom. For T2D, the study leveraged the DIAbetes Meta-ANalysis of Trans-Ethnic association studies (DIAMANTE) consortium. The estimated associations of INS, INS-R, and IGF-1R proteins with T2D were based on independent single nucleotide polymorphisms (SNPs) strongly ($P < 5 \times 10^{-6}$) predicting each exposure. These SNPs were applied to publicly available genetic associations with T2D from the DIAMANTE case ($n = 74{,}124$) and control ($n = 824{,}006$) study of people of European descent. SNP-specific Wald estimates were meta-analyzed using inverse variance weighting with multiplicative random effects. Sensitivity analysis was conducted using the weighted median (WM) and MR-Egger.

Results: INS-R (based on 13 SNPs) was associated with a lower risk of T2D (OR: 0.95 per effect size; 95% CI: 0.92, 0.98; $P = 0.001$), with similar estimates from the WM and MR-Egger. Insulin (8 SNPs) and IGF-1R (10 SNPs) were not associated with T2D. However, 1 of the SNPs for INS-R was from the *ABO* blood group gene.

Conclusions: This study is consistent with a causally protective association of the INS-R with T2D. INS-R in RBCs regulates glycolysis and thus may affect their functionality and integrity. However, a pleiotropic effect via the blood group *ABO* gene cannot be excluded. The INS-R may be a target for intervention by repurposing existing therapeutics or otherwise to reduce the risk of T2D. *Curr Dev Nutr* 2022;6:nzac044.

Keywords: Insulin receptor (INS-R), insulin (INS), insulin-like growth factor-1 receptor (IGF-1R), genome-wide association studies (GWAS), inverse variance weighted (IVW), single nucleotide polymorphism (SNP), weighted median (WM), type 2 diabetes (T2D), mean corpuscular hemoglobin concentration (MCHC)

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

The data were obtained from the publicly available summary aggregate data for DIAMANTE, DIAGRAM, and the UK Biobank. MR-Base online was used for the Mendelian randomization using insulin receptor as the exposure and mean corpuscular hemoglobin concentration as an outcome.

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Abbreviations used: DIAMANTE, DIAbetes Meta-ANalysis of Trans-Ethnic association studies (DIAMANTE) consortium study; GWAS, genome-wide association study; HbA1c, glycosylated hemoglobin; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; INS, insulin; INS-R, insulin receptor; IVW, inverse variance weighted; MCHC, mean corpuscular hemoglobin concentration; MR, Mendelian randomization; mTORC, mechanistic target of rapamycin complex; PKB/AKT, phosphatidylinositol-3-kinase and serine/threonine protein kinase B; RCT, randomized clinical trial; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; VSMC, vascular smooth muscle cell; WM, weighted median.

Introduction

Diabetes mellitus is one of the most prevalent chronic diseases globally and in the United States. According to the CDC, in 2017, the cost of diagnosed diabetes in the United States was $327 billion (1, 2). In 2018, it was estimated that 34.2 million people in the United States had diabetes (10.5% of the population), mainly type 2 diabetes (T2D), in parallel with obesity-related comorbidities (3). In addition, it is estimated that 33.9% of US adults have prediabetes or are insulin resistant. Thus, there is an urgent need for new prevention strategies, early detection, diagnosis, and T2D interventions.

T2D is a complex disease with multiple risk factors and possibly gene–environment interactions. However, while both the obesogenic environment and other genetic components play a role in T2D pathogenesis, the exact causes of disease onset remain elusive. T2D is characterized by hyperglycemia due to 2 metabolic defects: increased

resistance to insulin action in target tissues (muscle, adipose tissue, and liver) and decreased insulin secretion by pancreatic β cells (4). Insulin resistance is characterized by a defect in insulin-mediated glucose control in peripheral tissues. Insulin binds to the insulin receptor (INS-R) to initiate the insulin pathway signaling cascade via insulin receptor substrate (IRS) and phosphatidylinositol-3-kinase and serine/threonine protein kinase B (PKB/AKT), which is phosphorylated at serine 473 by the mechanistic target of rapamycin complex (mTORC) 2 (mTORC2), and activates an array of downstream targets including mTORC1 to initiate protein synthesis (5–11). In concert, mTORC1 integrates inputs from the insulin receptor and nutrient and growth factors and coordinates cellular growth and metabolism (12–14). Aberrant mTOR complexes signaling has been reported in T2D (15–22). For example, hyperactivity of mTORC1 driven by insulin and excess glucose may lead to insulin resistance (17). Furthermore, the inactivation of mTORC1 was reported to ameliorate the T2D phenotype in animal models (18, 20) and humans (19, 23, 24). mTORC2 also plays a role in glucose uptake in skeletal muscles in T2D animal models and thereby may regulate insulin resistance (22). As such, the insulin receptor impact on T2D could be transmitted and amplified via mTORC1 and mTORC2 (25–31). In addition, glucose-transporter protein (GLUT4) increases glucose uptake. It also inhibits glycogen synthase kinase-3 (GSK3) to increase glycogen synthesis as well as adenosine monophosphate-activated protein kinase (AMPK) and acetyl CoA carboxylase (ACC) to increase lipid synthesis and decrease lipolysis (4).

The rationale for this study is that recent evidence supports the notion that causes of insulin resistance are heterogeneous and may involve gene–environment interactions (4). Several factors contribute to the pathogenesis of T2D, including genetic susceptibility, lifestyle, and the environmental exposome (32–34). Nonmodifiable risk encompasses genetic variants, family history, and age. The environmental exposome refers to the totality of exposures across the lifespan and their health effects (35). As such, the external exposome embodies the built environment, social and physico-chemical environment, and food and lifestyle environments, and they all can play a role in diabetes development. Together, all of these factors contribute to the complexity of T2D pathogenesis.

Several functions of insulin and insulin-like growth factor (IGF) 1 receptors overlap, leading to a built-in redundancy between both pathways (36–39). However, the insulin and IGF-1 receptors are also tissue specific, which adds to the complexity of insulin-mediated regulation of glucose metabolism and T2D (40–43). Thus, we sought to determine whether the insulin and/or IGF-1 receptors play a causal role in the development of T2D. We hypothesized that the insulin receptor but not IGF receptor or insulin hormone has a protective effect on T2D. Unlike other observational studies, the Mendelian randomization (MR) approach allows us to determine causality and thereby can differentiate between components of the insulin signaling pathway. To investigate further, we used a 2-sample MR study because, by taking advantage of existing genome-wide association studies (GWASs), it can provide unconfounded estimates even when no study including both exposure and outcome exists. Previous MR studies have suggested that IGF-binding protein 2 may play a protective role in diabetes (44), while IGF is positively associated with diabetes (45). Therefore, we investigated whether the risk of T2D varied with genetically predicted insulin (INS), insulin receptor (INS-R), or IGF-1 receptor (IGF-1R) protein concentrations using an MR study.

Methods

MR takes advantage of the random allocation of genetic material at conception to obtain less confounded estimates without conducting costly randomized clinical trials (RCTs) that could have unanticipated side effects. An MR study is an instrumental variable analysis that utilizes genetic proxies of exposure from the wealth of GWASs (46). As such, MR can inform the susceptibility to and etiology of T2D, as shown in **Figure 1**. As an instrumental variable analysis with a genetic instrument, MR must fulfill the 3 assumptions of instrumental variable analysis: *1*) relevance, *2*) independence, and *3*) exclusion restriction. To meet the assumption of relevance, we only used single nucleotide polymorphisms (SNPs) as instruments that were independently ($r^2 < 0.05$) associated with the exposures at P values $<5 \times 10^{-6}$. To meet the exclusion-restriction assumption, we assessed whether the selected SNPs could affect the outcomes directly by identifying possible pleiotropic associations from a comprehensive, curated genotype-to-phenotype cross-references PhenoScanner (47, 48).

Study design and MR assumptions

This is a 2-sample MR study using summary statistics from 2 separate GWASs. First, we obtained genetic predictors of the exposures (INS, INS-R, and IGF-1R) from a publicly available proteomics GWAS of the INTERVAL RCT of blood donation in the United Kingdom (49). A total of 3301 individuals were included in the final study, with a mean age of 44 y; 51% were men. Participants were generally in good health because blood donation criteria excluded people with a history of major diseases. Proteins were measured using a multiplexed, aptamer-based approach. Genotyping used 1000 genomes with phase 3 imputation and gave 87 million variants. Genetic associations with proteins were adjusted for age, sex, blood draw to processing time, and the first 3 ancestry components. For T2D health outcomes, we leveraged the DIAbetes Meta-Analysis of TransEthnic study (DIAMANTE) GWAS available at https://kp4cd.org/node/169 (50). The European DIAMANTE study compiled GWAS data from approximately 900,000 individuals of European descent. The DIAMANTE investigators meta-analyzed estimates from 32 studies to generate genetic associations with T2D. We used estimates that were not adjusted for BMI. These GWASs included participants from the UK Biobank, Framingham Heart Study, Finland-United States Investigation of NIDDM, the Health Professionals Follow-Up Study, and the Nurses' Health Study, as shown in the flow diagram in **Figure 2**. For replication, we also used FINNGEN (E4_DM2_STRICT).

Genetic instruments for the exposures

We obtained independent SNPs that were strongly ($P < 5 \times 10^{-6}$) and independently ($r^2 < 0.05$) associated with each exposure, giving insulin (8 SNPs), INS-R (13 SNPs), and IGF-1R (10 SNPs). We calculated the F-statistics for instrument strength using an established approximation (51, 52); an F-statistic >10 is usually taken as indicating adequate strength.

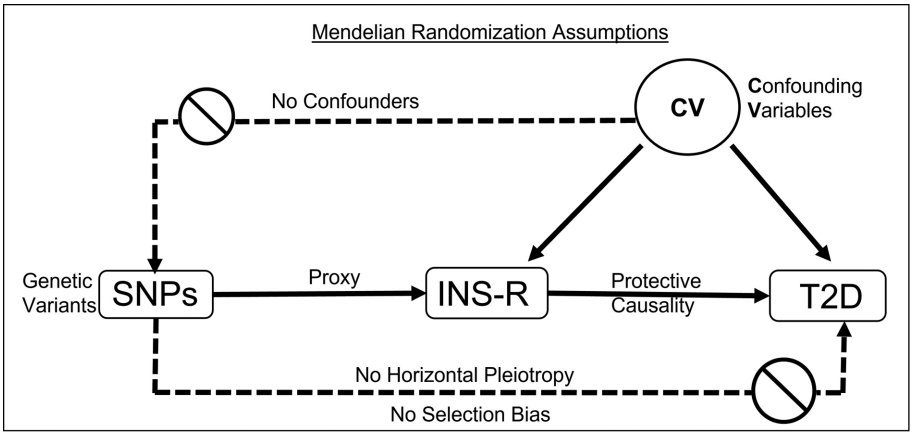

FIGURE 1 MR assumptions for unbiased causal associations. MR is an instrumental variable (IV) approach using genetic variants single nucleotide polymorphism (SNPs) as instrumental variables. The SNPs serve as a proxy of the exposure.To fulfill the MR assumptions, the SNPs must be associated only with the exposure but not the confounders. As an instrumental variable analysis with a genetic instrument, MR must fulfill the 3 assumptions: *1*) relevance, *2*) independence, and *3*) exclusion restriction. CV, confounding variables; INS-R, insulin receptor; MR, Mendelian randomization; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

Health outcomes

We used publicly available summary genetic associations with diabetes from the DIAMANTE T2D GWAS (cases, n = 74,124; controls, n = 824,006) (http://diagram-consortium.org/http://www.type2diabetesgenetics.org/). The mean age was 57.4 y, cases comprised 41.7% women and controls comprised 53% women. In addition, to validate the findings in other populations, we also used genetic summary statistics for T2D from another study of European ancestry, FINNGEN (cases = 11,006, controls = 82,655).

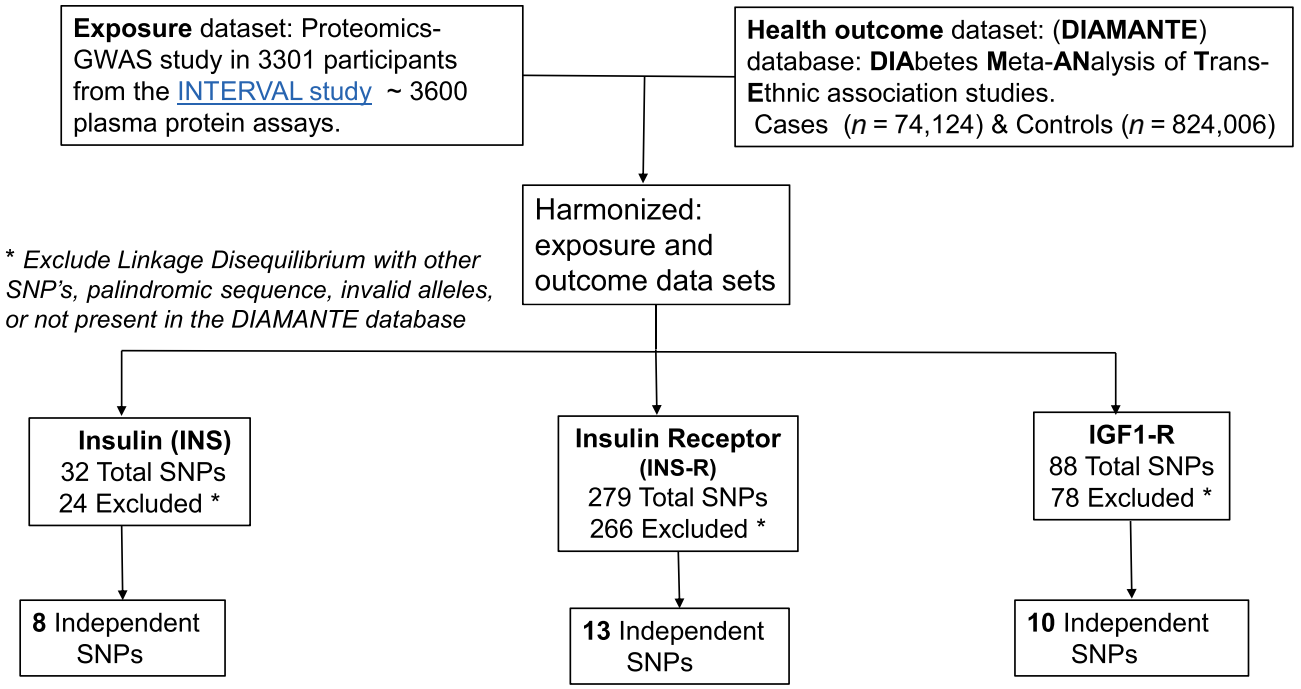

FIGURE 2 Flowchart of the MR study design. The 2-sample MR study used summary statistics from 2 separate GWASs. Genetic predictors of the exposures (INS, INS-R, and IGF-1R) were from a publicly available proteomics GWAS of the INTERVAL randomized controlled trial of blood donation in the United Kingdom (49). For T2D health outcomes, the DIAMANTE GWAS (50) was utilized. This GWAS included participants from the UK Biobank, Framingham Heart Study, Finland–United States Investigation of NIDDM, the Health Professionals Follow-Up Study, and the Nurses' Health Study, as shown in the flow diagram. The exposure and health outcome T2D data were harmonized. The independent SNPs for insulin were 8 SNPs; for INS-R, 13 SNPs; and for IGF-1R, were 10 SNPs. GWAS, genome-wide association study; IGF-1R, insulin-like growth factor 1 receptor; INS, insulin; INS-R, insulin receptor; MR, Mendelian randomization; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

TABLE 1 MR estimates for the association of INS-R (based on 13 independent SNPs with a *P* value of 5×10^{-6}), insulin (based on 8 independent SNPs with a *P* value of 5×10^{-6}), and IGF-R (based on 10 independent SNPs with a *P* value of 5×10^{-6}) with type 2 diabetes[1]

Exposure	MR method	OR	95% CI	*P*	Cochran's *Q* statistic (*P* value)	MR-Egger intercept *P* value	I^2
Insulin Effect Size	Inverse variance weighted	1.01	0.97, 1.05	0.58	5.15 (0.64)	—	—
	Weighted median	1.01	0.96, 1.06	0.82	—	—	—
	MR-Egger	1.01	0.92, 1.10	0.89	5.13 (0.52)	0.92	0.0%
INS-R Effect Size	Inverse variance weighted	0.95	0.92, 0.98	0.001	26.2 (0.01)	—	—
	Weighted median	0.92	0.89, 0.94	2×10^{-9}	—	—	—
	MR-Egger	0.91	0.87, 0.96	0.0004	20.3 (0.04)	0.075	93.72%
IGF-1R Effect Size	Inverse variance weighted	0.97	0.89, 1.05	0.47	59.7 (0.0)	—	—
	Weighted median	0.97	0.89, 1.05	0.47	—	—	—
	MR-Egger	0.95	0.72, 1.24	0.70	59.5 (0.0)	0.048	0.0%

[1]The data source for exposure is the human plasma proteomics–GWAS interval study participants ($n = 3301$) from publicly available aggregate summary data (49). The source for diabetes health outcomes is DIAMANTE. DIAbetes Meta-ANalysis of Trans-Ethnic association studies (DIAMANTE) consortium; GWAS, genome-wide association study; IGF-R, insulin-like growth factor receptor; IGF-1R, insulin-like growth factor 1 receptor; INS-R, insulin receptor; MR, Mendelian randomization; SNP, single nucleotide polymorphism.

Statistical analysis

We aligned the SNPs on the same effect allele for both exposure and outcome; palindromic SNPs were aligned on the effect allele or dropped if they could not be unambiguously aligned. We meta-analyzed SNP-specific Wald estimates (SNP on outcome divided by SNP on exposure) using inverse variance weighting (IVW) with fixed effects for 3 or fewer SNPs and multiplicative random effects for 4 or more SNPs. As a sensitivity analysis, we repeated the analysis using methods with different assumptions. First, the weighted median (WM) estimate is valid as long as >50% of the weight comes from valid instruments. Second, MR-Egger detects unknown genetic pleiotropy as long as the instrument strength independent of the direct effect assumption is satisfied (51, 53–55). To minimize pleiotropy, we also excluded SNPs with known potential pleiotropic effects.

Data management

We used R 4.1.2 and the Mendelian Randomization package (version 0.3.6) to conduct MR analysis using summary genetic associations from publicly available published data (56, 57). Both R and Mendelian Randomization packages are released under General Public Licenses (GPL-2, GPL-3).

Ethical considerations

We conducted secondary analysis from publicly available aggregate summary data with no involvement of the participants in the primary studies. No original data were generated from this manuscript. Ethical approval of each of the studies used is available in the original publications. There is no required Institutional Review Board approval for the secondary analysis of summary data. This study follows the ethical guidelines of the Declaration of Helsinki 1975.

Results

Of the 9 SNPs selected to predict INS, 8 SNPs were available for T2D in DIAMANTE, and of the 15 SNPs for INS-R, 13 were available, and all 10 SNPs were available for IGF-1R. The F-statistics were all greater than 10 (INS, based on 8 SNPs: mean F-statistic = 23.6; INS-R, based on 13 SNPs: mean F-statistic = 51.4; IGF-1R, based on 10 SNPs: mean F-statistic = 26.0). The independent SNPs for each exposure and outcome, including the chromosome number and position, β and SE for exposure, effect allele and the other allele, the *P* value, and Wald estimators are summarized in **Table 1**.

INS-R was associated with a lower risk of T2D (OR: 0.95 per effect size; 95% CI: 0.92, 0.98; $P = 0.001$), with similar estimates from the WM and MR-Egger (Table 1), and similarly using UK Biobank and FINNGEN (OR: 0.94; 95% CI: 0.89, 0.99; $P = 0.03$). A summary of the harmonized merged SNPs for exposure and outcome files is shown in **Table 2**.

Sensitivity analysis did not indicate pleiotropic effects. INS was not associated with T2D using IVW (OR: 1.01 per effect size; 95% CI: 0.97, 1.05; $P = 0.58$); sensitivity analysis gave similar estimates. IGF-1R was not associated with T2D using IVW (OR: 0.97; 95% CI: 0.89, 1.05; $P = 0.47$); sensitivity analysis gave similar estimates. Replication using FINNGEN gave a similar interpretation (data not shown). Potentially pleiotropic effects obtained from PhenoScanner are shown in **Supplemental Table 1**.

Although sensitivity analysis did not indicate pleiotropic effects, 1 of the selected SNPs for INS-R (rs507666) is in the pleiotropic *ABO* blood group gene. **Figure 3** shows the leave-one-out analysis for each exposure, excluding that SNP gave a null estimate (IVW OR: 0.98; 95% CI: 0.96, 1.02). Similarly, replication using FINNGEN, but excluding rs507666, gave a null association. To determine the causal link of blood groups with INS-R, we investigated the association of INS-R with the RBC attributes [mean corpuscular hemoglobin concentration (MCHC)] because it might affect glycosylated hemoglobin (HbA1c), and hence the diagnosis of T2D. We found that INS-R was associated with higher MCHC using an IVW estimate in the UK Biobank (http://www.nealelab.is/uk-biobank; $\beta = 0.012$; 95% CI: 0.003, 0.021; $P = 0.01$) (**Figure 4**).

Discussion

We found that the INS-R protein, but not insulin or IGF-1R protein, was associated with a lower risk of T2D, consistent with the complex

TABLE 2 Summary of the harmonized merged data of the exposure and outcome[1]

		Outcome data						Exposure data						
		Outcome			Allele			Exposure		Allele				
Protein	SNP	Chromosome position	β	SE	Effect	Other	*P* value Outcome	β	SE	Effect	Other	*P* value Ex posure	Wald	Variable Waldvar
INS														
1	rs147704083	1:203727055	−0.011	0.0097	A	G	0.24	0.175	0.0371	A	g	2.40×10^{-6}	−0.21714	0.01727
2	rs11597148	10:70129138	0.002	0.0088	T	G	0.82	0.1481	0.0322	T	g	4.17×10^{-6}	0.039163	0.02207
3	rs61933752	12:93220961	0	0.0068	A	G	1	−0.1252	0.0264	A	g	2.14×10^{-6}	0.013578	0.01633
4	rs797992	17:33411428	−0.0074	0.0068	T	C	0.28	−0.1165	0.025	T	c	3.16×10^{-6}	0.013734	0.01658
5	rs116860573	19:53046951	0.026	0.018	A	G	0.24	0.3666	0.0679	A	g	6.61×10^{-8}	0.020458	0.01441
6	rs143354263[2]	22:18536175	na	na	T	C	na	−0.5325	0.1103	T	c	1.38×10^{-6}	0.035681	0.02857
7	rs67137445	22:43610753	−0.0086	0.014	T	C	0.54	−0.2385	0.0489	T	c	1.10×10^{-6}	−0.03606	0.01914
8	rs62411666	4:20755950	6.00×10^{-4}	0.019	A	G	0.97	−0.3506	0.0722	A	g	1.20×10^{-6}	0.059897	0.01953
9	rs17548793	5:161492577	0.013	0.019	T	C	0.5	−0.3563	0.0723	T	c	8.51×10^{-7}	0.053326	0.01525
INS-R														
1	rs12740482	1:214595963	0.0007	0.0130	T	C	0.9	0.2383	0.0497	T	c	1.62×10^{-6}	0.205623	0.01692
2	rs140626119[2]	12:130741260	na	na	A	G	na	−0.6894	0.1384	A	g	6.31×10^{-7}	−0.00232	0.00304
3	rs13338928	16:31339903	−0.0007	0.0075	T	C	0.93	0.1353	0.0292	T	c	3.63×10^{-6}	−0.04065	0.0177
4	rs57090015	17:48384221	0.0004	0.0066	T	C	0.95	0.1269	0.0255	T	c	6.31×10^{-7}	−0.26793	0.0159
5	rs6751433	2:240757266	−0.0097	0.011	A	C	0.28	0.1621	0.0347	A	c	2.88×10^{-6}	0.135719	0.01842
6	rs73128888	20:50104490	0.01	0.0066	T	C	0.36	0.2165	0.0467	T	c	3.47×10^{-6}	−0.194	0.01794
7	rs760460	21:46328820	−0.0011	0.0077	T	C	0.87	0.1326	0.0249	T	c	9.77×10^{-8}	−0.01207	0.01456
8	rs11153713	6:118344588	−0.01	0.02	A	G	0.19	0.1396	0.0301	A	g	3.47×10^{-6}	−0.09312	0.01663
9	rs62436839	6:162106954	−0.018	0.0086	T	C	0.38	−0.371	0.0751	T	c	7.94×10^{-7}	0.09434	0.0189
10	rs67937303	7:20727925	−0.004	0.0064	T	C	0.64	0.1498	0.0324	T	c	3.89×10^{-6}	−0.25367	0.01783
11	rs2039184	9:136047393	−0.0082	0.011	A	G	0.2	0.1212	0.0253	A	g	1.62×10^{-6}	−0.20627	0.01532
12	rs8176751	9:136131022	0.019	0.0082	T	C	0.077	0.2441	0.0432	T	c	1.62×10^{-8}	−0.04916	0.00967
13	rs507666	9:136149399	0.05	0.011	A	G	9.30×10^{-10}	−0.5786	0.0305	A	g	4.79×10^{-8}	−0.12098	0.00108
14	rs79158370	9:136182159	0.025	0.011	A	G	0.024	−0.2957	0.0438	A	g	1.41×10^{-11}	−0.19953	0.00715
15	rs181552334[2]	9:16907646	na	na	A	G	na	0.1321	0.0268	A	g	8.51×10^{-7}	−0.1514	0.01857
IGF-R														
1	rs75771313	1:195326794	−0.017	0.0075	A	G	0.026	−0.1417	0.0289	A	g	9.77×10^{-7}	−0.02399	0.01439
2	rs7911360	10:122805547	-9.00×10^{-4}	0.0067	A	G	0.89	−0.1385	0.0274	A	g	4.27×10^{-7}	−0.00722	0.01173
3	rs117074452	11:84247210	0.0013	0.022	T	C	0.95	−0.4128	0.0792	T	c	1.91×10^{-7}	0.133236	0.01352
4	rs9524857	13:95890809	−0.015	0.007	A	G	0.037	−0.1288	0.0277	A	g	3.31×10^{-6}	0.010093	0.01356
5	rs7173285	15:63673411	0.0051	0.0096	T	C	0.59	0.1694	0.0365	T	c	3.55×10^{-6}	0.051358	0.01687
6	rs59944838	16:5534782	−0.002	0.0076	T	G	0.79	0.1377	0.0289	T	g	1.86×10^{-6}	−0.38489	0.01709
7	rs9897044	17:17563659	−0.024	0.0064	A	G	0.00017	0.1136	0.0249	A	g	4.79×10^{-6}	−0.25528	0.01984
8	rs75180877	2:62999792	−0.036	0.026	A	C	0.16	−0.4345	0.0887	A	c	9.77×10^{-7}	0.096663	0.01844
9	rs13060961	3:22923867	-5.00×10^{-4}	0.0083	A	C	0.95	−0.1632	0.032	A	c	3.31×10^{-7}	−0.11642	0.01355
10	rs635634	9:136155000	0.05	0.0082	T	C	8.00×10^{-10}	−0.2179	0.0321	T	c	1.15×10^{-11}	−0.33043	0.0076

[1]Association between genetically determined insulin, insulin receptor, and IGF-1R targets with type 2 diabetes health outcome. A 2-sample MR study was conducted using summary statistics from 2 separate GWASs. The data source for exposure is the human plasma proteomics–GWAS interval study participants ($n = 3301$) from publicly available aggregate summary data (49). The source for diabetes health outcomes is DIAMANTE. SNP-specific Wald estimates were meta-analyzed using IVW with multiplicative random effects. DIAMANTE, DIAbetes Meta-ANalysis of Trans-Ethnic association studies consortium; GWAS, genome-wide association study; IGF-1R, insulin-like growth factor 1 receptor; IGF-R, insulin-like growth factor receptor; INS, insulin; INS-R, insulin receptor; MR, Mendelian randomization; na, not applicable; SNP, single nucleotide polymorphism.
[2]Indicates that this SNP was included in the DIAGRAM database but not in the DIAMANTE database.

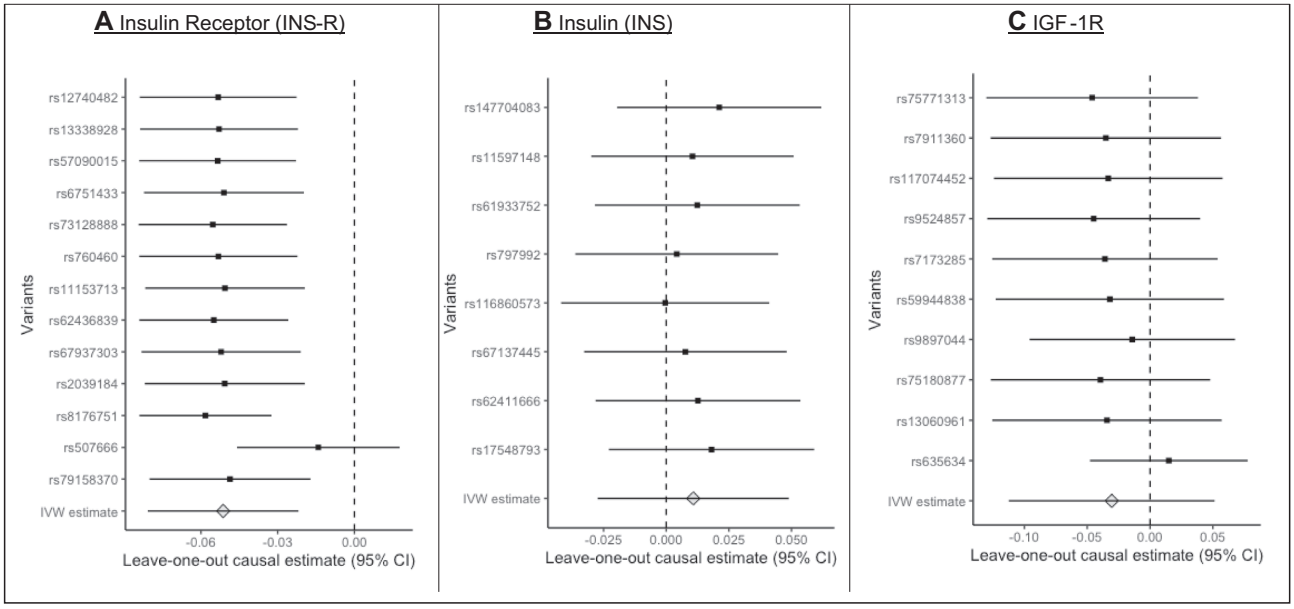

FIGURE 3 Leave one-out sensitivity test for the INS, INS-R, and IGF-1R exposures on T2D outcome. As a sensitivity analysis, we repeated the analysis using methods with different assumptions**.** First, the weighted median, valid as long as >50% of the weight comes from valid instruments. Second, MR-Egger detects unknown genetic pleiotropy as long as the instrument strength, independent of the direct effect assumption, is satisfied (51, 53–55). Third, leave-one-out analysis was applied to determine if 1 SNP drove the effect. Finally, SNPs with known potential pleiotropic effects were excluded to minimize pleiotropy. IGF-1R, insulin-like growth factor 1 receptor; INS, insulin; INS-R, insulin receptor; IVW, inverse variance weighted; MR, Mendelian randomization; SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

role of insulin in health (58). The association for INS-R was driven by 1 SNP from the *ABO* gene. As such, the insulin receptor may mediate its effect via the *ABO* gene variant rs507666. Furthermore, intracellular internalization of glucose by INS-R could prevent excess blood glucose from glycosylating RBCs and thus decrease HbA1c. We also found that INS-R increased MCHC (Figure 4), which measures the RBCs' oxygen-carrying capacity, possibly via rs507666 and other genetic instruments. Thus, the *ABO* gene could mediate any protective effect on T2D via INS-R. Additionally, INS-R increases MCHC, which could be protective as it elevates the oxygen-carrying capacity of RBCs and thereby delivers more oxygen and nutrients to peripheral tissues such as adipose tissue and muscle, thereby reducing the insulin resistance of such organs. However, the alternative explanation of a pleiotropic effect of the *ABO* gene cannot be ruled out.

Our findings support the concept that INS-R and its signaling pathway internalize glucose via glucose transporters and thus reduce circulating glucose available for RBC glycation and formation of HbA1C. INS-R binds with high affinity to RBCs with thousands of insulin-binding sites per erythrocyte (59). Genetic studies in mice showed that deletion of the insulin receptor in the vascular smooth muscle cells (VSMCs), but not IGF-1R, leads to decreased VSMC proliferation, indicating that the insulin receptor mediates intimal hyperplasia and VSMC proliferation after intimal injury in insulin resistance and T2D (60). Myocardial infarction and cardiovascular diseases are major comorbidities associated with insulin resistance and T2D (61). The insulin receptor could mediate its protective effect on T2D, at least partially, via reducing RBC glycation. Given that the insulin receptor facilitates glucose uptake in peripheral tissues such as adipose tissue and muscle and activates the nutrient-sensing pathway mTORC1, the INS-R causality findings are a gateway to precision nutrition interventions by shedding light on the mechanisms of interindividual variability in responses to food and carbohydrate intake. The results also have applications in developing and validating personalized nutrition algorithms that predict what individuals might eat to promote optimal health.

T2D is a heterogeneous multifactorial disease, which is also impacted by gene–environment interplay (62). As such, a constellation of factors within the insulin-signaling cascade, insulin resistance, and environmental factors may be relevant to T2D. Current literature has downplayed a role for the proximal canonical insulin signaling, which begins with the binding of insulin hormone to its membrane INS-R, which becomes glycosylated, dimerizes, and creates a tetramer composed of 2 extracellular α subunits and 2 transmembrane β subunits ($\alpha_2\beta_2$) and phosphorylates insulin receptor substrate in developing insulin resistance (63–67). Researchers have argued that the distal insulin components downstream of AKT are the only key players in T2D pathophysiology and that phosphorylation of these components at multiple sites leads to insulin resistance in adipose tissue and muscles (4, 68). However, some investigators have disputed the notion of sparing insulin receptors (69, 70). Our MR study using SNPs as genetic instrumental variables found that genetically

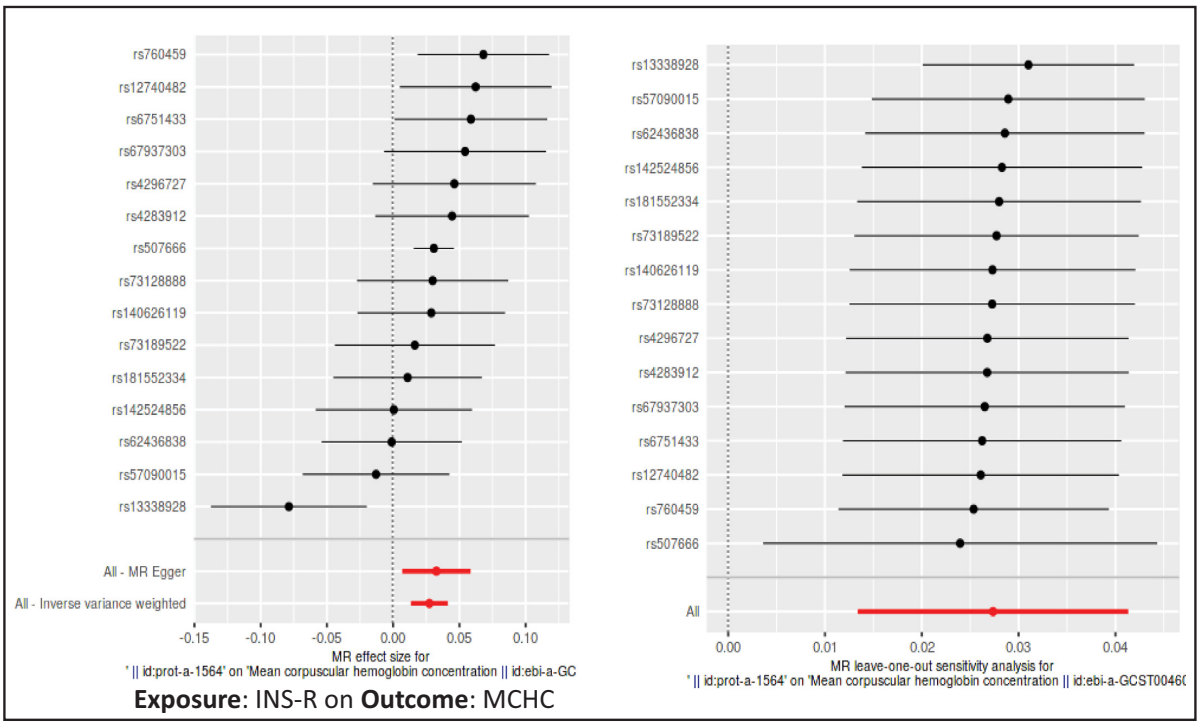

FIGURE 4 Two-sample MR with exposure as INS-R and outcome as MCHC. To determine the causal link of blood groups with INS-R, we investigated the association of INS-R with the RBC attributes (MCHC) because it might affect HbA1c, and hence the diagnosis of T2D. We found that INS-R was associated with higher MCHC using an IVW estimate in the UK Biobank (http://www.nealelab.is/uk-biobank). HbA1c, glycosylated hemoglobin; INS-R, insulin receptor; IVW, inverse variance weighted; MCHC, mean corpuscular hemoglobin concentration; MR, Mendelian randomization; T2D, type 2 diabetes.

determined INS-R proteins could have a causal protective association with T2D.

Limitations

While MR is robust in addressing bias from residual or unmeasured confounders, the use of MR could potentially be associated with some limitations. To address the MR assumption of relevance, we only used SNPs with an F-statistic >10. However, currently available protein GWASs are quite small, so we were not able to use genome-wide significance as a criterion for instrument selection, and it is possible that the instruments available do not capture the relevant phenotypes well. Larger GWASs of proteins might provide stronger instruments. To address exclusion restriction, we assessed whether the genetic predictors had possible pleiotropic effects and found that 1 variant for INS-R was in the highly pleiotropic *ABO* gene. A leave-one-out analysis clearly showed that the SNP from *ABO* was driving the association of INS-R with T2D. We also used the WM estimator and MR-Egger regression to detect potential bias, as described by Bowden et al. (51, 54, 55, 71). Finally, given the limited size of the protein GWASs, we cannot rule out the possibility that the null results for insulin and IGF-1R are due to lack of power.

Future directions will be guided by the MR determination of the causality of the mTOR complexes network downstream of the insulin signaling pathway on T2D. It is possible that the causal association of insulin receptors with T2D could be transmitted and amplified via mTORC1 and mTORC2. In the future, we will confirm findings related to mTOR genetic variants obtained by MR by testing T2D human biospecimen and patient-derived organoids obtained from the National Disease Research Exchange (NDRI), Human Tissue, and Organ for Research Resource (https://ndriresource.org/for-researchers/request-tissue).

Conclusions

This MR study is consistent with a causally protective association of INS-R with T2D. Insulin receptors in RBCs regulate glycolysis and thus may affect their functionality and integrity, as well as increase oxygen-carrying capacity, although a pleiotropic effect via *ABO* cannot be excluded. INS-R may be a target for intervention by repurposing existing therapeutics to reduce the risk of T2D.

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The authors' responsibilities were as follows—GAS and CMS: contributed to the study design, execution of the studies, data analysis, review of the manuscript, and final content; GAS: wrote the first draft of the manuscript; and both authors: read and approved the final manuscript.

Data Availability

All of the data and R codes will be shared with any research investigator upon written request.

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