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Article

Polygenic Scores for Type 2 Diabetes Causally Impact Vitamin D Levels Across Diverse Populations

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Abstract

Background: Vitamin D (25(OH)D) deficiency affects over one billion people globally and is associated with type 2 diabetes (T2D) and cardiometabolic diseases. However, causal relationships remain unclear, as vitamin D supplementation have shown limited benefit in reducing T2D risk. Genetic studies have identified variants influencing circulating 25(OH)D levels, but whether genetically determined vitamin D status predicts cardiometabolic outcomes is still uncertain. We therefore used bidirectional Mendelian randomization with genome-wide polygenic scores to evaluate the causal relationship and directionality between vitamin D status and T2D. **Methods and Results:** We analyzed multi-ethnic populations from the UK Biobank (N = 471,861), and 3,486 participants from the Asian Indian Diabetic Heart Study/Sikh Diabetes Study with serum 25(OH)D measures and genome-wide genotype data. A global polygenic score of vitamin D-raising alleles did not significantly reduce the risk of T2D, coronary artery disease, stroke, or other cardiometabolic risk factors. In contrast, a higher T2D polygenic risk score (PRS) was strongly associated with increased risk for 25(OH)D deficiency (<50 nmol/L). Genetically instrumented analyses showed per SD increase in T2D PRS significantly reduced circulating 25(OH)D levels ($\beta = -8.9$ nmol/L; 95% CI: -9.3 to -8.5; $p = 3.6 \times 10^{-28}$). **Conclusions:** Our findings suggest low circulating vitamin D levels are unlikely to causally predict T2D risk but may serve as a marker for secondary prevention in endocrine and cardiovascular health. Instead, genetic susceptibility to T2D appears to contribute to reduced vitamin D levels. Further studies are needed to clarify the mechanisms underlying vitamin D deficiency in diabetes.

Keywords: vitamin D deficiency; type 2 diabetes; cardiometabolic risk; mendelian randomization; polygenic scores

1. Introduction

Vitamin D (25(OH)D) deficiency has emerged as a major global public health crisis, affecting 30-50% of the world's population [1,2]. Vitamin D plays a crucial role in metabolic pathways and in the regulation of gene transcription across various tissues [3]. As a primary regulator of key components in metabolism, its deficiency is closely associated with the development of various health conditions, including cardiovascular disease (CVD), hypertension, and certain types of cancer [4-7]. The reciprocal relationship of circulating 25(OH)D levels with type 2 diabetes (T2D), obesity, and CVD is well established through multiple clinical and observational studies performed in diverse cohorts of

European (EU), Australian, Asian, and American populations [8–12]. However, a causal association and the exact biological mechanism between low 25(OH)D levels and cardiometabolic disease risk remain unknown [13]. Clinical trials have also shown that vitamin D supplementation does not reduce the risk of T2D [14,15] even though the environmental and genetic factors account for 50%–80% of the variability in 25(OH)D levels [16,17].

Genome-wide association studies (GWAS) have identified multiple loci associated with circulating 25(OH)D levels and, interestingly, most robustly associated gene variants (identified mainly in large GWAS originating from EUs) map near genes involved in vitamin D synthesis, transport, or metabolism, such as *DHCR7/NADSYN1*, *CYP2R1*, *GC*, and *CYP24A1* [18–20]. In addition to the contribution of these pathway genes, studies have identified the genome-wide common variant associations and ancestry-specific associations that regulate circulating 25(OH)D, exhibiting a positive and significant association between 25(OH)D-raising alleles (as polygenic scores or PGS) and circulating 25(OH)D levels [21–23]. However, it is still unclear if the genetically increased 25(OH)D by genome-wide variants can predict the cardiometabolic outcomes.

Mendelian randomization (MR) studies provide a framework to assess the causal relationship between 25(OH)D levels and cardiometabolic disease outcomes, while minimizing confounding factors such as geographic location, diet, and vitamin D supplementation, and accounting for reverse causation. Several studies have identified causal links between genetically determined 25(OH)D levels and the risk of conditions including psoriasis, atopic dermatitis, vitiligo, Graves' disease, cataracts, and esophageal cancer in EU populations [24,25]. However, studies investigating the genetic relationship between 25(OH)D levels and T2D, including potential reverse effects, have produced inconsistent results [26]. To explore if the 25(OH)D insufficiency predisposes people to T2D, we previously performed a candidate-gene targeted bidirectional MR study and meta-analysis using 3 candidate gene variants of T2D (*IGF2BP2*, *TCF7L2*, and *KCNQ1*) and 3 GWAS-variants from vitamin D pathway genes (*GC*, *CYP2R1*, and *DHCR7*). Our MR study could not detect any causal evidence between T2D and 25(OH)D using eight multiethnic study cohorts comprising 59,890 individuals [27].

This study aimed to further investigate the putative role of vitamin D insufficiency in predicting endocrine and cardiometabolic health risk through genome-wide polygenic score analysis and identify the directional effects by utilizing distinct datasets. The first dataset consists of 471,861 individuals from the UK Biobank (UKBB), which includes 459,143 EU, 9372 South Asians (SA), and 3346 Africans (AF). The second population comprises a well-characterized cohort of 3,486 individuals from the Asian Indian Diabetic Heart Study/Sikh Diabetes Study (AIDHS/SDS), both available with serum 25(OH)D levels and genome-wide genotypes.

2. Methodology

2.1. Study Subjects

A total of 475,347 individuals with 25(OH)D levels from the UKBB and AIDHS/SDS were used for this study. We used genome-wide genotype and phenotype data from the UKBB in individuals of EU (n=459,143), SA (n=9372), and AF (n=3346), following the approval (Application #78635) described previously [28,29]. Serum 25(OH)D levels were measured by chemiluminescence immunoassay (DiaSorin LIAISON XL, Italy) [30]. T2D was characterized by physician-diagnosed disease phenotype and glycated hemoglobin (HbA1c) levels. Coronary artery disease (CAD) was classified based on physician-diagnosed vascular/heart problems. Additionally, we studied 3486 subjects from the AIDHS/SDS who were available with genome-wide genotype data and serum 25(OH)D levels [10,22,27,31]. The Sikh population is a relatively homogenous endogamous community from Northern India. Sikhs are primarily non-smokers, and ~ 50% of them are vegetarians. However, the incidence of cardiometabolic diseases in Sikhs and SAs has markedly increased over the past two decades [32,33]. T2D was diagnosed based on their medical records, including symptoms and use of antidiabetic medications, and in accordance with the American

Diabetes Association guidelines described earlier [34,35]. Non-diabetic controls were selected based on a fasting blood glucose (FBG) < 100.8 mg/dL (5.6 mmol/L) or a 2-hour glucose < 141.0 mg/dL (7.8 mmol/L) as previously described [31,35,36]. CAD was considered if there was use of nitrate medication (nitroglycerine), electrocardiographic evidence of angina pain, coronary angiographic evidence of severe (greater than 50%) stenosis, or echocardiographic evidence of myocardial infarction. The diagnosis was based on the date of coronary artery bypass graft (CABG) or angioplasty and medication usage obtained from patient records, as described previously [22,35]. Body mass index (BMI) was calculated as [weight (kg)/height (m²)]. A tape measure of the waist and hip circumferences at the abdomen and the hip, respectively, were recorded. For using BMI thresholds for obesity, we used the World Health Organization's (WHO) guidelines [37]. Blood pressure (BP) was measured twice after a 5-minute seated rest period with the participant's feet flat on the floor. Serum lipids [total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)] were measured using standard enzymatic methods (Roche, Basel, Switzerland) as described previously [22,35,38–40]. Vitamin D levels were measured using 10 µL serum with standard monoclonal antibody-based ELISA kits from ALPCO Diagnostics (Salem, NH, USA) in the entire AIDHS/SDS cohort, as described previously [10]. A standard curve was used across a range of concentrations (2-fold dilutions), and any sample that fell out of range was repeated. All participants in this study were recruited after providing written informed consent, and the study was approved by the institutional review boards (IRBs). All AIDHS/SDS protocols and consent documents were reviewed and approved by the University of Oklahoma Health Science Center's IRB and by the Human Subject Protection (Ethics) Committees at the participating hospitals and institutes in India, as described previously [41–43]. All human studies reported in this manuscript abide by the Declaration of Helsinki principles.

2.2. Genotyping, Imputation, and Quality Controls

For genetic analysis, we used imputed data released by the UKBB for EU, AF, and SA subjects and excluded outliers based on heterozygosity or genotype missingness (missing rate > 0.2) and ambiguous SNPs (MAF > 0.44). Participants with inconsistent reports and genotypic inferred sex inconsistencies or withdrawn consent were removed, as explained previously [44].

For the AIDHS/SDS, samples were genotyped using the Illumina 660W Quad BeadChip, Illumina Global Screening Arrays (GSA), and GSA with multi-disease content (GSA+) arrays as described previously [22,36,45]. Samples with genotyping call rate < 95%, cryptic relatedness, population outliers, departures from Hardy-Weinberg equilibrium (HWE) ($p < 10^{-7}$), or MAF < 5% were excluded before association testing. To increase genome coverage, data were imputed using Minimac4 [46] (<https://imputationserver.sph.umich.edu/>) with TOPMED r3 multiethnic reference panel in NCBI Build 38 (hg38) coordinates as reported previously [29,47]. Of a total of 23,739,260 variants, we removed variants with an imputation certainty info score < 0.5, MAF < 0.001, and with HWE in controls ($p < 1 \times 10^{-6}$) before further analysis. The genetic principal components (PCs) were estimated from our Sikh population, as the existing HapMap2, HapMap3, and 1000 Genomes data do not include data from Punjabi Sikhs, as described previously [35,48].

2.3. Genome-Wide Genetic Score Construction and Analysis

Ancestry-specific PGS for 25(OH) D-raising alleles were constructed using candidate variants derived from genome-wide genotypes of UKBB and AIDHS/SDS. To construct EU-ancestry PGS, we used summary statistics from Revez et al. (2020) [3] comprising 6,098,063 variants from Sunlight Consortium. To test the associations of SNPs with circulating 25(OH)D levels, linear regression and an additive genetic model were used, with the natural-log-transformed 25(OH)D level adjusted for age, gender, BMI, 10 genetic PCs, and T2D status. We excluded INDELs, duplicate and multiallelic SNPs, and SNPs with info score < 0.80, and included SNPs with MAF > 0.01 and MAF < 0.45. After regression analysis, SNPs with $p < 10^{-4}$ were chosen. After linkage disequilibrium (LD) clumping using $R^2 \leq 0.25$ and 500 Kb distance, a total of 2179 SNPs were used for the construction of the PGS. The

EU-derived PGS performed poorly in SAs from AIDHS/SDS and the UK. We constructed ancestry-specific PGS using ~15 million variants from the AIDHS/SDS tested and trained using discovery (n=1616) and validation (n= 1870) cohorts of the same Punjabi ethnicity and 9372 SAs from UKBB. A total of 2051 SNPs were chosen for the construction of the PGS using the same selection criteria and LD clumping and regression analysis model as described for EU. For the construction of the AF PGS, we used GWAS summary statistics of 23,615,737 SNPs derived from African ancestry from Wang et al. 2023 [49] for vitamin D. The individual-level regression coefficients were multiplied by the number of risk alleles to compute the PGS as described previously [27,29,47]. The weighted PGS was calculated using the following equation 1:

$$PGS_j = \sum_i^N \beta_i * dosage_{ij}$$

where N is the number of SNPs in the score, β_i is the effect size (or beta) of variant i, and dosage is the number of copies of SNP in the genotype of individual j [50]. The polygenic risk score (PRS) for T2D was constructed using the summary statistics data from O'Connor et al., [51] which was derived from 312,646 individuals of EU ancestry with a similar methodology as described above for all ethnic groups.

2.4. Statistical Analysis

The clinical and demographic variables were summarized as means for continuous variables and percentages for categorical variables, using SPSS version 31 (IBM, New York City, USA). Multivariate linear regression analyses were performed to assess the impact of 25(OH)D PGS on T2D, CAD, acute ischemic stroke (AIS), and other cardiometabolic risk factors (e.g., waist, waist-to-hip ratio (WHR), and glucose) after adjusting for covariates such as age, sex, and BMI. To evaluate the discrimination capability at the extreme tail of the genetic score, we divided the PGS into quartiles. We then compared the extreme scores in the 4th quartile with those in the 1st quartile to assess the protective effects of genetically raised 25(OH)D on T2D, CAD, and AIS. Additionally, we also assessed the effects of genetically enhanced diabetes risk (T2D PRS) for modulating 25(OH)D levels in UKBB(EU/AF/SA) and AIDHS/SDS cohorts.

We used genetic instrumental variable methods to obtain estimates of the causal association between circulating vitamin D levels and T2D and determined the direction of causality by performing a bidirectional MR study [27,36]. The associations between the exposure (T2D) and the outcome (25(OH)D) levels and vice versa are estimated from different cohorts, mainly UKBB (EU, SA, and AF), and AIDHS/SDS. For conducting MR analysis, three fundamental hypotheses were considered: a. Cumulative genetic instrument variables (IVs) should be strongly linked to the exposure. b. IVs must not directly correlate with the outcome, influencing it solely through the exposure. c. IVs need to be independent of potential confounders. The combined estimates were calculated using the conventional MR method [52,53]. In sensitivity analyses, we used the two-stage least squares (2SLS) method to validate the causal effect and the strength of the association since the allelic score methods were used for the MR [53,54]. In stage 1, the exposure of interest is regressed on the polygenic score (controlling for covariates of age, gender, BMI, and ancestry) to obtain predicted values of the exposure. The stage 2 estimates the causal effect by regressing the predicted values of the exposure obtained from the first stage [55] and F values > 10 were considered for confirming for causal effect. All analyses were performed using PLINK 2.0 [56], SVS version 8.9.1 (Golden Helix, Bozeman, MT, USA), and SPSS version 31 (IBM, New York City, USA), and R (version 4.3.3).

3. Results

The clinical and demographic characteristics of the UKBB and AIDHS/SDS study participants are presented in **Table 1**. The AIDHS/SDS individuals showed higher levels of most clinical risk traits than the UKBB cohort. For instance, the mean WHR was significantly higher for AIDHS/SDS (0.94±0.08) compared to UKBB EU (0.87±0.09) (p= 2.5x10⁻²²⁵). Similarly, the average blood glucose

levels were significantly higher in AIDHS/SDS (134.78±63.72) than in UKBBEU (80.30±36.89; $p=4.65\times10^{-325}$) and UKBBSA (97.66±33.94; $p=2.07\times10^{-196}$). Similarly, triglycerides were significantly higher in AIDHS/SDS (169.87±112.60) in comparison to UKBB (EU, and AF) individuals (**Table 1**). The participant flow chart and study workflow is presented in **Supplementary Figure 1**.

Table 1. Clinical characteristics of the UKBB and AIDHS/SDS individuals.

Trait	Europeans (N =459,143)	AIDHS/SDS (N = 3486)	South Asians (N = 9372)	Africans (N=3346)
Males (%)	46	55	54	51
Age (years)	56.77±8.03	51.98±13.27*	53.30±8.45^	51.00±7.94
BMI (kg/m²)	27.40±4.77	26.56±4.79*	27.16±4.40^	29.68±5.14
Waist (cm)	90.26±13.51	92.23±11.94*	91.45±11.86^	94.16±11.63
Waist-to-hip ratio	0.87±0.09	0.94±0.08*	0.90±0.09^	0.88±0.08
Systolic BP (mmHg)	137.98±18.64	137.07±28.83	129.90±29.73^	138.66±18.87
Diastolic BP (mmHg)	82.18±10.12	82.51±12.43	79.45±17.47^	84.92±10.83
Blood glucose (mg/dL)	80.30±36.89	134.78±63.72*	97.66±33.94^	91.99±27.23
Triglycerides (mg/dL)	147.89±94.52	169.87±112.60*	173.98±103.35	107.29±67.55
HDL-C (mg/dL)	49.03±23.28	40.53±14.80*	48.79±12.48^	53.84±13.84
LDL-C (mg/dL)	131.31±44.17	112.79±39.04*	129.34±32.96^	123.96±32.19
Total Cholesterol (mg/dL)	212.05±61.18	184.06±61.74*	205.38±43.62^	198.73±42.34
Vitamin D levels (nmol/L)	41.23±26.13	35.05±26.69*	21.36±17.81^	27.67±19.04
T2D (%)	5	51*	21^	11
CAD (%)	5	19*	31^	6

Values are displayed in mean±SD; * Comparison between UKBB Europeans and AIDHS/SDS ($p<0.001$); ^Comparison between UKBB South Asians and AIDHS/SDS ($p<0.001$); AIDHS/SDS: Asian Indian Diabetic Heart Study/Sikh Diabetes Study; BMI: Body mass index; BP: Blood Pressure; CAD: Coronary artery disease; HDL-C: High-density lipoproteins-Cholesterol; LDL-C: Low-density lipoproteins-Cholesterol; T2D: Type 2 diabetes; UKBB: UK Biobank.

The assessment of cumulative effects of vitamin D-raising alleles as PGS for each ancestry showed a stronger association with 25(OH)D levels, as indicated by larger effect sizes. As shown in **Figure 1A** the AIDHS/SDS showed the strongest allelic effect for increasing 25(OH)D levels ($\beta=0.76$ (95% CI 0.61-0.91; $p=9.5\times10^{-178}$), and a similar trend was observed in AF $\beta=0.29$ (95% CI 0.25-0.33; $p=4.4\times10^{-56}$), SA $\beta=0.28$ (95% CI 0.24-0.31; $p=4.6\times10^{-129}$), and EU $\beta=0.09$ (95% CI 0.08-0.10; $p=2.5\times10^{-350}$) (**Figure 1A**). Combining all cohorts, the overall meta-analysis showed a strong effect of vitamin D PGS on increasing 25(OH)D levels, $\beta=0.38$ (95% CI 0.14-0.62; $p=4.5\times10^{-440}$) (**Figure 1A**). However, the reverse effects of vitamin D-raising alleles (25(OH)D PGS) on reducing the risk for T2D were not observed in these cohorts (**Supplementary 2b**). To further evaluate if genetically increased vitamin D lowers T2D susceptibility, we divided the vitamin D-PGS into quartiles. When comparing the individuals in the 4th quartile (high 25(OH)D levels) vs. the 1st quartile (25(OH)D deficiency group), there was a marginal non-significant decrease in the risk for T2D in EU, showing a protective odds ratio (OR) of 0.97 (95%CI 0.93-1.00; $p=0.08$). Similarly, a protective but non-significant association was observed in AIDHS/SDS OR 0.74 (95%CI 0.54-0.95; $p=0.19$), and in AF OR 0.76 (95%CI 0.45-1.08; $p=0.09$), showing the reduced risk for T2D with increased 25(OH)D PGS (**Figure 2A**). Similarly, comparing the extreme quartiles of vitamin D PGS, the individuals with genetically enhanced 25(OH)D levels reduced the risk of CAD with marginal significance in EU (OR 0.96 (95%CI 0.92-1.00; $p=0.05$). A similar but non-significant trend was observed in AIDHS/SDS and AF, showing the decreased CAD risk with increased 25(OH)D (**Supplementary Table 1**). However, it was interesting to observe that the extreme quartiles (4th vs 1st) of the vitamin D-raising PGS showed a significantly decreased risk of AIS in EU, with the OR of 0.95 (95%CI 0.92-0.98; $p=6.9\times10^{-4}$). The same but non-significant pattern for decreased AIS risk was observed in all cohorts: AIDHS/SDS (OR 0.65 (95%CI

0.06-1.24; $p=0.15$), SA (OR 0.83 (95%CI 0.68-0.98; $p=0.18$)), and AF (OR 0.81 (95%CI 0.54-1.08; $p=0.15$)) (Supplementary Table 1).

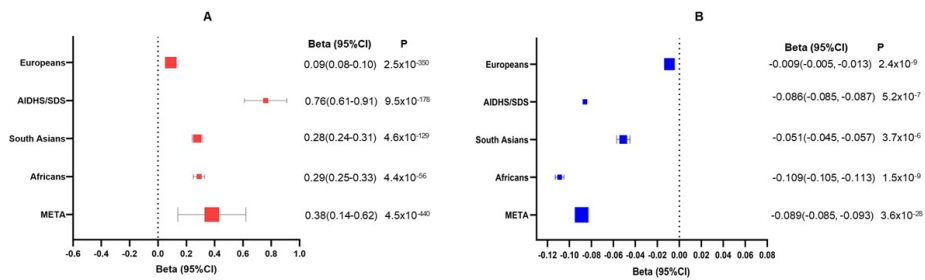


Figure 1. Forest plots showing effect sizes and confidence intervals of (A) Vitamin D PGS effect on vitamin D levels. Allele score regression using two-stage least squares (2-SLS) showed a mean $F = 5563.31$; $p = 3.62 \times 10^{-338}$; % covariance = 1.172. (B) T2D PRS effect on vitamin D levels in UK Biobank and AIDHS/SDS cohorts. Allele score regression showed a mean $F = 228.02$; $p = 1.66 \times 10^{-51}$; % covariance = 0.049. AIDHS/SDS: Asian Indian Diabetic Heart Study/Sikh Diabetes Study; PGS: Polygenic score; PRS: Polygenic risk scores; T2D: Type 2 diabetes.

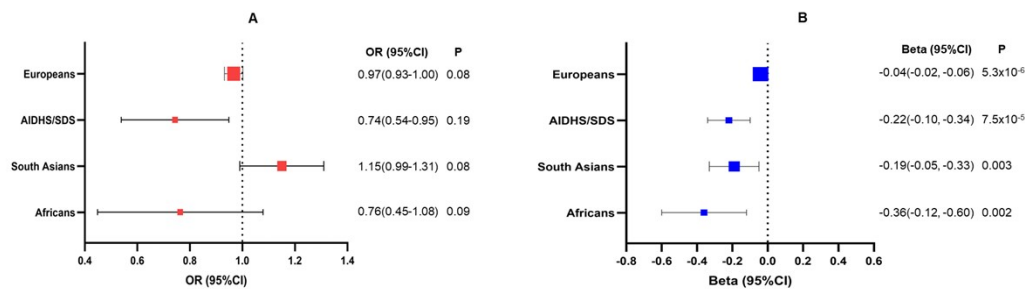


Figure 2. Forest plots showing effect sizes and confidence intervals of individuals with extreme PGS in the 4th quartile compared with those in the 1st quartile to determine the risk for (A) Vitamin D PGS effect on T2D (B) T2D PRS effect on vitamin D levels in UK Biobank and AIDHS/SDS cohorts. AIDHS/SDS: Asian Indian Diabetic Heart Study/Sikh Diabetes Study; PGS: Polygenic score; PRS: Polygenic risk scores; T2D: Type 2 diabetes.

Conversely, the cumulative PRS score for T2D exhibited significantly lowered 25(OH)D levels across all ethnic groups. People with an increased T2D PRS had consistently lower 25(OH)D levels when compared with those with lower T2D PRS ($\beta = -0.086$; 95% CI, -0.085, -0.087; $p = 5.2 \times 10^{-7}$) in AIDHS/SDS and UKBB cohort EU ($\beta = -0.009$; 95% CI, -0.005, -0.013; $p = 2.4 \times 10^{-9}$), SA ($\beta = -0.051$; 95% CI -0.045, -0.057; $p = 3.7 \times 10^{-6}$), and AF ($\beta = -0.109$; 95% CI -0.105, -0.113; $p = 1.5 \times 10^{-9}$), respectively (Figure 1B). The combined meta-analysis of all cohorts showed significantly reduced 25(OH)D levels ($\beta = -0.089$; 95% CI -0.085, -0.093; $p = 3.6 \times 10^{-28}$) with increased T2D PRS (Figure 1B).

Next, we compared the extreme 4th quartile (high T2D risk) vs. the 1st quartile (low T2D risk) PRS. In the EU, 25(OH)D levels were significantly decreased ($\beta = -0.04$, 95% CI = -0.02, -0.06; $p = 5.3 \times 10^{-6}$) in individuals with high T2D PRS. In AIDHS/SDS, 25(OH)D levels were also significantly reduced ($\beta = -0.22$, 95% CI = -0.10, -0.34; $p = 7.5 \times 10^{-5}$). Similarly, SA and AF showed a significantly decreased 25(OH)D levels in the group with the extremely high T2D PRS ($\beta = -0.19$; 95% CI -0.05, -0.33; $p = 0.003$) and ($\beta = -0.36$; 95% CI -0.12, -0.60; $p = 0.002$), respectively (Figure 2B).

We further studied the association between ancestry-derived vitamin D PGS effects on cardiometabolic traits, which revealed a significantly negative association with WHR in UKBB EU ($\beta \pm SE = -0.008 \pm 0.001$; $p = 8.9 \times 10^{-11}$). Similarly, individuals with increased vitamin D PGS had a significant reduction in waist circumference in SA ($\beta \pm SE = -0.009 \pm 0.001$; $p = 1.5 \times 10^{-13}$) (Supplementary Table 2a) and, also had a significantly lowered glucose levels ($\beta \pm SE = -0.12 \pm 0.06$;

p=0.03) (**Supplementary Table 2a**) and T2D risk (OR 0.91 (95%CI 0.85-0.98; p=0.009)) in the AF (**Supplementary Table 2b**).

We also analyzed individual variants from bonafide/established candidate genes involved in vitamin D synthesis, transport, and metabolism such as *GC*, *DHCR7*, *CYP24A1*, *CYP2R1*, *LIPC*, *CETP*, *HAL*, and *CRX*, that also showed GWAS-level significance for 25(OH)D levels in this study. We found that individuals with and without vitamin D supplements had a similar association between these variants and vitamin D levels (**Supplementary Table 3a**). However, no significant association of these variants was observed with other cardiometabolic risk factors in the UKBB EU (**Supplementary Tables 3b-f**).

Next, we constructed PGS of these candidate genes (*GC*, *DHCR7*, *CYP24A1*, *CYP2R1*, *LIPC*, *CETP*, *HAL*, and *CRX*) involved in vitamin D synthesis, transport, and metabolism. As expected, the targeted genetic score of candidate genes using GWAS-level significant variants revealed strong association for increasing 25(OH)D levels in each ethnicity (**Supplementary Table 4a**). However, the targeted vitamin D-raising PGS was ineffective in predicting protection against T2D or CAD risk (**Supplementary Table 4b**).

We further investigated the relationship between individual SNPs (T2D risk alleles) that are part of the T2D PRS for their effect on vitamin D levels. We found that some of these T2D risk gene variants were associated with a reduction in 25(OH)D levels; however, the significance of these associations was either marginal or not significant across all cohorts (**Supplementary Table 5**).

We also observed that circulating 25(OH)D levels did differ by T2D onset in UKBB EU decreasing significantly from (41.38±23.83) nmol/L at 17-30 years to (38.51±22.05) nmol/L at 31-50 years and then increased from (41.12±22.75) nmol/L at 51-60 years to (44.63±22.63) nmol/L at 61-70 years (F = 79.99; p = 1.79×10⁻⁵¹). But the 25(OH)D levels did not differ for the duration of T2D in UKBB EU (F = 0.72; p = 0.54) (**Supplementary Table 6a**). In AIDHS/SDS the average 25(OH)D levels showed a significant linear increase from (34.07±39.12) nmol/L to (45.21±45.43) nmol/L for age of T2D onset at 13 years to 92 years (F = 6.68; p = 1.75×10⁻⁰⁴), while vitamin D levels decreased significantly from (44.26±45.18) nmol/L at the fresh T2D onset (0 year duration) to (28.66±33.03) nmol/L with >11 years of T2D duration (F = 15.54; p = 5.58×10⁻¹⁰) (**Supplementary Table 6b**).

Additionally, as expected, the T2D PRS was also associated with an increased risk for CAD in all cohorts. UKBB EU ($\beta \pm SE = 0.018 \pm 0.003$; p = 4.9×10⁻¹⁰), AIDHS/SDS ($\beta \pm SE = 10.773 \pm 3.066$; p = 4.4×10⁻⁰⁴), UKBB SA ($\beta \pm SE = 0.238 \pm 0.046$; p = 1.7×10⁻⁰⁷), and UKBB AF ($\beta \pm SE = 0.955 \pm 0.325$; p = 0.003) (**Supplementary Table 7**). Likewise, the T2D PRS was linked to a significantly increased risk of stroke across all UK Biobank cohorts: EU ($\beta \pm SE = 0.091 \pm 0.002$; p = 3.1×10⁻³⁰⁸), SA ($\beta \pm SE = 1.178 \pm 0.050$; p = 4.9×10⁻¹¹⁸), and AF ($\beta \pm SE = 4.503 \pm 0.211$; p = 1.2×10⁻⁹⁴). Although the effect of T2D PRS on AIS was in the same direction, it was not significant in AIDHS/SDS ($\beta \pm SE = 1.349 \pm 0.795$; p = 0.84), perhaps because of the small number of stroke cases (n=111) in this cohort. (**Supplementary Table 7**).

4. Discussion

This study aimed to determine the critical role of vitamin D insufficiency in predicting the risk of cardiometabolic diseases, utilizing diverse ethnic cohorts and advanced genome-wide approaches. Using candidate gene variants and cumulative genome-wide polygenic scores, our findings could not confirm whether vitamin D insufficiency would predispose people to the development of T2D and other cardiovascular complications; rather, it suggests that vitamin D may only be considered as a marker for secondary prevention for endocrine and cardiometabolic health. Although ancestry-derived 25(OH)D PGS were strongly associated with circulating 25(OH)D levels in this multi-ethnic study, they did not translate into predictive value for significant protection against cardiometabolic risk. This pattern was consistent across multiple analytical frameworks, including continuous and quartile-based analysis. On the other hand, our MR findings suggest that people with T2D would be more likely to develop vitamin D deficiency and subsequent cardiovascular complications.

Vitamin D deficiency is an established risk factor for T2D, CAD, and other cardiometabolic diseases [57,58]. We earlier reported a very high prevalence of vitamin D deficiency in this Punjabi

diabetic cohort (AIDHS/SDS), showing a strong association of reduced 25(OH)D with T2D and other cardiometabolic risk traits [10,22,59]. In a bidirectional MR study conducted by our group, using GWAS variants from 3 T2D candidate genes (*IGF2BP2*, *TCF7L2*, *KCNQ1*) and three vitamin D pathway genes (*GC*, *CYP2R1*, *DHCR7*) across 59,890 individuals from EU and Asian Indian ethnic groups, no causal link between T2D and 25(OH)D was found [27]. Our current findings again failed to establish a causal relationship between genetically instrumented increase in 25(OH)D to reduce susceptibility to T2D and other cardiovascular diseases and traits after including the genome-wide polygenic scores and an expanded study cohort of more than 450,000 individuals from UKBB. These results support the prior published studies from other groups, where MR analyses have shown no causal association between vitamin D levels and the risk of T2D [60], including MR analysis of large consortia studies (European Prospective Investigation into Cancer and Nutrition [EPIC]–InterAct, EPIC–Norfolk, EPIC–CVD, Ely, and the SUNLIGHT consortium) [61]. Similarly, a study by La Barrera et al. [2023] found that increased 25(OH)D levels did not affect youth-onset T2D risk [62]. Even when restricting the PGS analysis to variants in established vitamin D pathway genes and lipid metabolism genes, which were strongly associated with 25(OH)D levels, we observed no association with T2D and cardiometabolic traits.

On the contrary, our results demonstrate a strong causal association between genetically enhanced T2D susceptibility due to PRS for significantly reducing 25(OH)D levels across multiple ethnic groups. A genetically instrumented per SD increase in T2D PRS would reduce 25(OH)D levels to 8.9 nmol/L with 95%CI from 8.5 nmol/L to 9.3 nmol/L ($p = 3.6 \times 10^{-28}$). These results also support epidemiological evidence, including studies from our group, which have consistently shown that vitamin D deficiency is more prevalent in individuals with T2D than in people without T2D in multiple populations [63,64]. Prospective studies have shown that vitamin D deficiency accelerates the development, progression, and severity of T2D [57]. In patients with T2D, circulating 25(OH)D levels are often reduced due to sequestration of vitamin D in adipose tissue, metabolic dysfunction, and diabetes-related complications [65]; however, the exact molecular mechanism remains unexplored.

Animal studies in diabetic rat models have demonstrated reduced renal expression of key vitamin D–regulating genes, including *Lrp2* (megalin), *Cubn* (cubilin), and *Dab2*, resulting in impaired vitamin D metabolism. As shown in **Supplementary Figure 2**, downregulation of the *Lrp2*–*Cubn*–*Dab2* complex disrupts renal reabsorption of vitamin D bound to D-binding protein, leading to its increased elimination through the urinary tract [66]. Furthermore, gene expression studies have reported downregulation of the vitamin D receptor (*VDR*) in diabetic individuals, which may also contribute to reduced circulating 25(OH)D levels in T2D [67]. Additionally, epigenetic mechanisms may further exacerbate vitamin D deficiency in metabolic disease. Hypermethylation of *VDR* and vitamin D metabolizing genes, such as *CYP27B1*, *CYP2R1* has been shown to reduce gene expression of these proteins, leading to decreased synthesis of active vitamin D, leading to impaired calcium homeostasis, and metabolic dysfunction [68–70]. Similarly, reduced 25(OH)D levels have been reported in obese Saudi women due to hypermethylation of *CYP2R1* and *CYP27B1* [71]. Collectively, these findings underscore the need for further investigation into the biological and molecular mechanisms underlying reduced vitamin D levels in T2D.

This study has several notable strengths. First, it investigates the impact of genome-wide vitamin D PGS on T2D and cardiometabolic disease risk across multi-ethnic populations. To our knowledge, no studies have yet explored this potentially causal connection using a genome-wide bidirectional MR approach. The genome-wide risk score information was from summary statistics obtained from large consortium studies to reduce the genetic score inflation, and sensitivity analysis was performed to check the reliability of causal inference. The inclusion of diverse ethnic groups improved the generalizability of our findings and reinforced the evidence of no association between vitamin D and protection against T2D. Similarly, this study has limitations, including a lack of diversity in the sample; over 80% of participants are from the EU. Also, the lack of genome-wide data and vitamin D measures in other diverse non-EU cohorts might have contributed to creating a weak genetic

instrument. There was observable phenotypic and genotypic heterogeneity between SA populations from UKBB and AIDHS/SDS. The SA cohort from UKBB is a random collection of SAs living in the UK originating from all over the world, and the AIDHS/SDS included one homogenous population of Punjabis and predominantly Sikhs from North India. Despite limitations, our polygenic score models demonstrated robustness for predicting T2D and were significantly effective for predicting 25(OH)D status across all ethnic cohorts. Our MR results have revealed important insights into the causal link between cumulative genetic risk for T2D and vitamin D levels.

In summary, our findings provide no evidence for an association between the genetically increased vitamin D levels or exogenous supplementation for protecting against T2D and cardiovascular risk. In contrast, our new findings using genome-wide scores demonstrated that people with genetically enhanced T2D risk are more prone to vitamin D insufficiency, which can substantiate T2D-related complications across ethnic groups. These findings may partly explain why individuals with T2D are 2 to 4 times more likely to develop fatal CAD, heart failure, and AIS than those without T2D. And that improved vitamin D status can be a modifying factor for endocrine and cardiovascular health. Given the rising prevalence of T2D, these results are timely and important and highlight the need for developing a deeper understanding of the biological mechanisms linking diabetes to vitamin D deficiency and consequent cardiovascular complications affecting aging and health span.

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Abbreviations

AIS: Acute Ischemic Stroke

AIDHS/SDS: Asian Indian Diabetic Heart Study/Sikh Diabetes Study

AF: Africans

BG: Blood glucose

BMI: Body mass index

BP: Blood pressure

CABG: Coronary artery bypass graft

CAD: Coronary artery disease

CVD : Cardiovascular disease

DBP: Diastolic blood pressure

EU: European

GSA: Global Screening Arrays

GWAS: Genome-wide association studies

HDL-C: High-density lipoprotein cholesterol
HWE: Hardy-Weinberg equilibrium
LD: Linkage disequilibrium
LDL-C: Low-density lipoprotein cholesterol
MAF: Minor allele frequency
MR: Mendelian randomization
PC: Principal components
PGS: Polygenic score
PRS: Polygenic risk score
SA: South Asians
SBP: Systolic blood pressure
T2D: Type 2 diabetes
TC: Total cholesterol
TG: Triglycerides
UKBB: UK Biobank
Vitamin D: 25(OH)D
WHR: Waist-to-hip ratio

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