Using Mendelian randomisation to identify opportunities for type 2 diabetes prevention by repurposing medications used for lipid management

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Summary

Background Maintaining a healthy lifestyle to reduce type 2 diabetes (T2D) risk is challenging and additional strategies for T2D prevention are needed. We evaluated several lipid control medications as potential therapeutic options for T2D prevention using tissue-specific predicted gene expression summary statistics in a two-sample Mendelian randomisation (MR) design.

Methods Large-scale European genome-wide summary statistics for lipids and T2D were leveraged in our multi-stage analysis to estimate changes in either lipid levels or T2D risk driven by tissue-specific predicted gene expression. We incorporated tissue-specific predicted gene expression summary statistics to proxy therapeutic effects of three lipid control medications [i.e., statins, icosapent ethyl (IPE), and proprotein convertase subtilisin/kexin type-9 inhibitors (PCSK-9i)] on T2D susceptibility using two-sample Mendelian randomisation (MR).

Findings IPE, as proxied via increased FADS1 expression, was predicted to lower triglycerides and was associated with a 53% reduced risk of T2D. Statins and PCSK-9i, as proxied by reduced HMGCR and PCSK9 expression, respectively, were predicted to lower LDL-C levels but were not associated with T2D susceptibility.

Interpretation Triglyceride lowering via IPE may reduce the risk of developing T2D in populations of European ancestry. However, experimental validation using animal models is needed to substantiate our results and to motivate randomized control trials (RCTs) for IPE as putative treatment for T2D prevention.

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**Research in context**

**Evidence before this study**

Previous studies have employed the Mendelian randomisation (MR) method to evaluate the putative causal association between an exposure and outcome. Many studies have also incorporated expression quantitative trait loci (eQTL) data in the genetic instruments used in the MR analysis. However, studies combining the MR approach with gene expression data to proxy current medications are limited. One study examined angiotensin-converting enzyme (ACE) expression in lung tissue as a proxy for anti-hypertensive medication via ACE-inhibitors on risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

**Added value of this study**

Our study estimated tissue-specific gene expression summary statistics using S-PrediXcan which leveraged existing lipid genome-wide association studies (GWAS) and eQTL information from the Genotype-Tissue Expression (GTEx) project. To identify potential repurposing opportunities for type 2 diabetes (T2D) prevention via these lipid control medications, we utilized these gene expression summary statistics as instruments in a MR analysis. The estimated gene expression summary statistics were used to proxy three lipid control therapies: LDL-C lowering via statins and proprotein convertase subtilisin/kexin type-9 inhibitors (PCSK-9i), and triglyceride lowering via icosa-pent ethyl (IPE).

**Implication of all the available evidence**

Using our *in silico* approach, we observed a potential opportunity for T2D primary prevention with triglyceride lowering IPE. Along with experimental validation, we hope our approach may serve as an initial step for identifying potential repurposing opportunities for existing therapies.

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**Background**

The burden of type 2 diabetes (T2D) is high with nearly 1.5 million new cases diagnosed in the United States (US) in 2018. Cardiovascular disease (CVD) complications, such as heart disease, stroke, and high blood pressure, may worsen after a T2D diagnosis. Current T2D management strategies are centered upon glycemic control. However, many individuals either do not achieve adequate blood glucose control, do not benefit from glucose reduction, or experience undesirable side effects from glucose-lowering medications. The complexity of T2D treatment and high prevalence of comorbidities emphasizes the importance of prevention. Lifestyle changes including improved diet and increased physical activity have been shown to reduce T2D risk. However, maintaining a healthy lifestyle is challenging, necessitating additional T2D prevention strategies.

Statins are commonly prescribed to lower low-density lipoprotein cholesterol (LDL-C) to reduce CVD risk. However, individuals on statins may have residual CVD risk or, in some populations, have increased risk for T2D. Hypertriglyceridemia is a possible culprit for the increased risks observed among statin users. Thus, triglyceride lowering strategies using long-chain omega-3 polysaturated fatty acid eicosapentaenoic acid (EPA) have been evaluated for CVD prevention. In a randomized control trial (RCT) conducted among a high-risk population, compared with patients prescribed a mineral oil placebo, patients prescribed icosapent ethyl (IPE), a purified version of EPA, had lower triglycerides and reduced CVD risk. Moreover, EPA metabolites have been shown to prevent hyperglycemia and hyperinsulinemia in humans, and in mice IPE is protective against high-fat diet-induced glucose intolerance, insulin resistance, and β-cell dysfunction. We therefore hypothesized that IPE could perhaps be used for T2D prevention. While researchers have rightfully focused on identifying agents for CVD prevention, discovering new putative therapies for T2D prevention could help lower T2D burden and ensuing complications, including CVD.

Mendelian randomisation (MR) utilizes single nucleotide polymorphisms (SNPs) as instrumental variables, or proxies, for exposures of interest. This approach allows for potential causal inference if the instrumental variable satisfies a set of strict MR assumptions. In our analysis, we expand upon the traditional two-sample MR approach by incorporating genetically predicted tissue-specific gene expression as the instrumental variable (Figure 1). Gene expression summary statistics for specific genes coding proteins targeted by lipid medications were used as instrumental variables in our MR
Figure 1. Directed acyclic graphs representing Mendelian randomisation (MR) approach undertaken in this analysis to evaluate the potential effect of lipid therapies on type 2 diabetes (T2D). Panel a represents the total effect of drug on T2D risk via changes in the primary indication or trait (i.e., lipids) via genetically predicted gene expression (GPGE). Panel b represents the effect of drug on T2D risk adjusted (indicated using a box) for the potential pleiotropic influence of other traits on T2D risk using multivariable MR (MVMR). Panel c represents the effect of drug on T2D risk via changes in glucose/insulin traits adjusted for the drug’s primary therapeutic effect. Panel d represents the effect of statins on T2D risk via lowering LDL-C. Panel e represents the effect of PCSK-9 inhibitors (PCSK-9i) on T2D risk via lowering LDL-C. Panel f represents the effect of icosapent ethyl (IPE) on T2D risk via lowering triglycerides.
The T2D-wide association study (GWAS) were leveraged and the component of the largest trans-ethnic T2D genome-canalonic T2D markers.

We examined the following: (1) the total effect of proxied medication on T2D risk accounting for the potential pleiotropic effects of other lipids on T2D risk; (2) the effect of proxied medication on T2D risk using two-sample MR; and (3) the effect of proxied medication on T2D risk via predicted changes in lipid levels (mg/dL) per standard deviation increase in lipid traits representing the therapeutic action of statins and icosapent ethyl (IPE) on lowering LDL-C and triglycerides, respectively.

Table 1: Random-effects summary of genetically predicted gene expression (GPGE) on changes in lipid traits representing the therapeutic action of statins and IPE on lowering LDL-C and triglycerides, respectively.

| Lipid control medication | Gene expression to proxy medication effect | Lipid trait (primary indication listed first) | GTEx tissues (eQTLs used in S-PrediXcan) | Random-effects meta-analysis of GPGE changes in lipid trait across tissues |
|--------------------------|------------------------------------------|---------------------------------------------|-----------------------------------------|--------------------------------------------------|
| Statins                  | Decreased HMGCR expression                | LDL-C                                       | 5 (126)                                 | -0.14 0.07 0.10                                    |
| PCSK-9i                  | Decreased PCSK9 expression                | Total cholesterol                           | 4 (116)                                 | -0.15 0.08 0.15                                   |
| IPE                      | Increased FADS1 expression                | LDL-C                                       | 6 (54)                                  | -0.31 0.17 0.13                                   |
|                          |                                          | HDL-C                                       | 2 (4)                                   | 0.10 0.05 0.31                                    |
|                          |                                          | Triglycerides                               | 2 (13)                                  | -0.10 0.09 0.47                                   |
|                          |                                          | Total cholesterol                           | 7 (56)                                  | -0.28 0.12 0.06                                   |
|                          |                                          | Triglycerides                               | 23 (307)                                | -0.11 0.01 9.8 × 10⁻¹⁰                              |

| Lipid control medication | Gene expression to proxy medication effect | Lipid trait (primary indication listed first) | GTEx tissues (eQTLs used in S-PrediXcan) | Random-effects meta-analysis of GPGE changes in lipid trait across tissues |
|--------------------------|------------------------------------------|---------------------------------------------|-----------------------------------------|--------------------------------------------------|
| Statins                  | Decreased HMGCR expression                | LDL-C                                       | 23 (306)                                | 0.12 0.01 6.4 × 10⁻¹⁰                              |
| PCSK-9i                  | Decreased PCSK9 expression                | HDL-C                                       | 23 (309)                                | 0.09 0.01 9.5 × 10⁻¹⁰                              |
| IPE                      | Increased FADS1 expression                | Total cholesterol                           | 23 (307)                                | 0.12 0.01 4.2 × 10⁻¹⁰                              |

Table 1: Random-effects summary of genetically predicted gene expression (GPGE) on changes in lipid traits representing the therapeutic action of statins and IPE on lowering LDL-C and triglycerides, respectively.

A Number of statistically significant tissues (P < 0.05) from each lipid-specific GPGE model, and the number of eQTLs used to estimate GPGE in S-PrediXcan. No statistically significant tissues were observed for HMGCR GPGE for HDL-C and triglycerides.

B Random-effects meta-analysis quantitatively summarizing the statistically significant tissue-specific GPGE per lipid trait. Summarized GPGE represent predicted changes in lipid levels (mg/dL) per standard deviation increase in FADS1 gene expression.

We sought to identify drug repurposing opportunities for T2D prevention by combining causal inference MR methodology with tissue-specific predicted gene expression to proxy therapeutic effects in a multi-stage in silico design. We examined the following: (1) the total effect of proxied medication on T2D risk using two-sample MR; (2) the effect of proxied medication on T2D risk accounting for the potential pleiotropic effects of other lipids on T2D risk using multivariable MR (MVMR); and (3) the effect of proxied medication on T2D risk via predicted changes in canonical T2D markers.

Methods

Summary statistics

We utilized summary statistics from independent GWAS for lipids and T2D. For lipids, summary statistics from the Global Lipids Genetics Consortium (GLGC) were used for LDL-C, high density lipoprotein cholesterol (HDL-C), triglycerides, and total cholesterol. For T2D, summary statistics from the European component of the largest trans-ethnic T2D genome-wide association study (GWAS) were leveraged and details of which are published elsewhere. The T2D GWAS included non-overlapping European participants from DIAMANTE, Million Veteran Program (MVP), Penn Medicine Biobank, Malmo Diet and Cancer Study, MedStar, and PennCath. Overall, 148,726 T2D cases and 965,732 controls were included in the European T2D GWAS utilized in our study. DIAMANTE and MVP contributed over 98% of participants included in the T2D GWAS, among whom 59% were male, with mean age of 55.7 years (SD=11.8), mean BMI of 27.2 kg/m² (SD=4.7), 37% were hypertensive, and 30% had dyslipidemia. Summary statistics from a Finnish population (FinnGen) including 29,166 T2D cases and 185,185 controls were used for replication.

Genetically predicted gene expression (GPGE)

Using S-PrediXcan, GPGE effects on changes in lipid traits and T2D risk were estimated using summary statistics from the GLGC and MVP T2D GWAS. Details regarding S-PrediXcan are published elsewhere. Briefly, using an elastic net framework, independent effects of expression quantitative trait loci (eQTL) were used to predict changes in a particular trait driven by gene expression in 48 different tissues. S-PrediXcan combines weights from gene expression prediction models from the Gene-Tissue Expression Project (GTEx v7), covariances from a reference set (i.e., 1,000 Genomes), and SNP-specific effect estimates and standard errors from GWAS summary statistics. This information is used to predict tissue-
Figure 2. Genetically predicted gene expression (GPGE) driven changes in circulating lipids for decreased HMGCR, decreased PCSK9, and increased FADS1 expression in 48 GTEx tissues. Depicted above are tissues with GPGE resulting in either increased lipid levels (green rectangles), decreased lipid levels (red rectangles), or no effect (grey rectangles). Statistically significant ($P < 0.05$) GPGE effects are bolded with a black border. Detailed GPGE summary statistics are provided in Supplementary Tables 1-3.
specific changes in a particular trait per standard deviation (SD) increase in gene expression. Using random-effects meta-analysis, GPGE effects were summarized across tissues for proxied statin, PCSK-9i, and IPE medications (Table 1). Tissue-specific GPGE for HMGCR, PCSK9, and FADS1 gene expression with changes in lipid traits are displayed for all GTEx tissues (excluding placental tissue) in Figure 2. Detailed GPGE summary statistics for proxied lipid control medications, European T2D GWAS, and FinnGen used in this analysis are provided in Supplementary Tables 1–3. These GPGE summary statistics were used as instrumental variables in our MR analysis.

Selection of proxy genes
Genes coding proteins known to be either directly targeted by lipid medications or involved in lipid metabolism were selected as proxies for the lipid control therapies examined in our analysis. Statins lower LDL-C by inhibiting the rate-limiting 3-Hydroxy-3-Methylglutaryl-CoA reductase (HMGCR). The LDL-C lowering effect of reduced HMGCR expression was also observed in our S-PrediXcan result (GPGE = –0.14 mg/dL, P = 0.10; Table 1). Thus, reduced HMGCR expression was chosen to proxy the LDL-C lowering effects of statins. We also proxied LDL-C lowering via PCSK-9i. These LDL-C lowering medications are known to influence downstream levels of these proteins, and reduced PCSK9 gene expression was selected to proxy PCSK9i. We also observed that one SD decrease in PCSK9 expression was predicted to decrease LDL-C levels (GPGE = –0.31 mg/dL, P = 0.13; Table 1).

FADS1, one of the genes coding for Δ5- and Δ6-desaturation enzymes, has been demonstrated in FADS1-knockdown mice, and triglyceride lowering has been observed in subjects supplemented with purified EPA. To identify the best possible gene to proxy IPE, we estimated GPGE effects for circulating EPA for five genes (i.e., FADS1, FADS2, ELOVL2, PTGS2, and PTGS3) involved in polysaturated fatty acid metabolism. Compared to other genes involved in the pathway, we observed that increased FADS1 expression resulted in the largest increase in circulating EPA (0.20 percent EPA increase; P = 5.2 × 10⁻⁶⁰; Supplementary Table 4). Also, increased FADS1 expression was predicted to lower triglycerides by 0.11 mg/dL (P = 9.8 × 10⁻¹⁰; Table 1), which further supported our decision to proxy the triglyceride-lowering IPE via increased FADS1 expression.

Mendelian randomisation (MR)
Tissue-specific GPGE effects for HMGCR and FADS1 gene expression that met our threshold for statistical significance (P < 0.05) were utilized as the MR instrument. To perform MR, S-PrediXcan-derived tissue-specific GPGE summary statistics were estimated for T2D in the European T2D GWAS and FinnGen. S-PrediXcan summary statistics were harmonized to proxy the pharmacologic action of each lipid control medication. Reduced HMGCR and PCSK9 expression were used to proxy statins and PCSK-9i, respectively. Whereas increased FADS1 expression was used to proxy IPE. The T2D GPGE used were harmonized according to the medication proxied (i.e., for statins and PCSK-9i, all T2D GPGE represented predicted T2D risk per SD decrease in either HMGCR or PCSK9 gene expression; for IPE, all T2D GPGE represented predicted T2D risk per SD increase in FADS1 gene expression). A fixed-effects inverse-variance weighted (IVW) MR analysis was performed using ‘TwoSampleMR’ in R to estimate the total effect of lipid lowering medications on T2D risk (proxied by HMGCR, PCSK9, and FADS1 driven lipid changes; Figure 1d–f). Briefly, for each gene and t number of GTex tissues, GPGE summary statistics for lipids and T2D were combined to calculate the IVW MR association as follows:

\[ \hat{\beta}_{MR-IVW} = \frac{\sum_{i=1}^{1} \hat{\beta}_{lipid} \beta_{T2D} \sigma_{T2D}^{-2}}{\sum_{i=1}^{1} \hat{\beta}_{lipid}^2 \sigma_{T2D}^{-2}} \times se(\hat{\beta}_{MR-IVW}) = \frac{1}{\sqrt{\sum_{i=1}^{1} \hat{\beta}_{lipid}^2 \sigma_{T2D}^{-2}}} \]

where \( \hat{\beta}_{lipid} \) represents GPGE per lipid trait; and \( \beta_{T2D} \) and \( \sigma_{T2D} \) represent T2D GPGE and standard error, respectively. Corresponding odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated using \( \hat{\beta}_{MR-IVW} \) and \( se(\hat{\beta}_{MR-IVW}) \).

We conducted several analyses to test the robustness of our MR results including assessing instrument strength and validity, examining bias due to population stratification, and the potential mediatary effects of FADS1 expression on T2D susceptibility via changes in circulating EPA. Details regarding these sensitivity analyses are provided in the Supplementary Materials. Of note, MR Egger was conducted to evaluate bias due to directional pleiotropy at the tissue level, with statistically significant regression intercepts (P < 0.05) as an indication of directional pleiotropy (suggesting the presence of unknown indirect pathways towards T2D). Furthermore, given lipids are highly correlated with one another, a multivariable MR (MVMR) was conducted to estimate the adjusted MR effects for lipids. Physical activity has also been shown to affect lipid levels, thus we additionally adjusted for total physical activity using S-PrediXcan derived tissue-specific GPGE representing changes in total physical activity using GWAS summary
Mendelian randomisation (MR)
The MR analyses estimating the total effect of proxied medications on T2D risk are presented in Table 2. In the European T2D GWAS, statins were associated with 63% increased T2D risk via HMGCR-predicted LDL-C lowering (IVW MR= 1.63, 95% CI= 1.44, 1.85; P= 7.2 × 10⁻¹⁰). After accounting for other lipids in the MVMR analysis, the increased risk associated with HMGCR-predicted LDL-C lowering was attenuated and less precise (MVMR= 1.36, 95% CI= 0.82, 2.26; P= 0.36; physical activity adjusted MVMR= 0.85, 95% CI= 0.43, 1.68, P= 0.72). For PCSK-9i, LDL-C lowering per SD decrease in PCSK9 expression indicated a modest T2D risk reduction (IVW MR= 0.92, 95% CI= 0.85, 0.99; P=0.04). However, after adjustment for other lipids and physical activity, no association between proxied PCSK-9i medication and T2D was observed. IPE proxied via FADS1-predicted triglyceride lowering was associated with an increase in T2D risk (IVW MR= 1.44, 95% CI= 1.29, 1.61; P= 1.9 × 10⁻¹⁰). However, after accounting for putative pleiotropic effects of FADS1 on HDL-C and LDL-C in the MVMR, FADS1-predicted triglyceride lowering was associated with an approximately 54% reduced T2D risk (MVMR= 0.46, 95% CI= 0.24, 0.87; P= 2.7 × 10⁻⁴⁵), which was also consistent in the physical activity adjusted MVMR analysis (MVMR=0.47, 95% CI=0.25, 0.89; P=3.1 × 10⁻⁴⁵). Although tissue-specific pleiotropic effects of varying magnitudes on T2D may still exist, there was no evidence of directional pleiotropy assessed via MR Egger regression (all Egger intercepts P > 0.05). Furthermore, MVMR results were robust across different MR sensitivity analyses (Supplementary Table 5). Replication in FinnGen resulted in similar magnitude of effects, however, the confidence intervals were less precise.

In Table 3, MVMR results are presented for FADS1-predicted changes in canonical T2D markers (i.e., fasting glucose, fasting insulin, HOMA-B, and HOMA-IR; Figure 1c). Proxied IPE resulted in increases in fasting glucose (mmol/L), decreases in fasting insulin (log-transformed mmol/L) and HOMA-B, and had no effect on HOMA-IR (summarized using random-effects meta-analysis across 23 different GTEx tissues; Supplementary Table 6). After adjustment for FADS1-predicted changes in LDL-C and triglycerides, proxied IPE resulted in reduced T2D risk in both the European and FinnGen T2D GWAS. However, these MVMR estimates were imprecise and were not statistically significant.

We also examined the effect of FADS1-predicted increases in circulating EPA on T2D risk (Supplementary Table 7). FADS1-predicted increase in circulating EPA was associated with a 23% T2D risk reduction (IVW MR= 0.77, 95% CI= 0.74, 0.81; P= 4.3 × 10⁻¹⁸), which was consistently observed in several MR sensitivity analyses. Finally, our MR analysis using natural hair colour as the negative control outcome indicated that our results are unlikely to be a consequence of population stratification bias (Supplementary Table 8).
Lipid control medication | Gene expression to proxy medication effect \(^a\) | Inverse-variance weighted (IVW) MR \(^b\) | Multivariable MR (MVMR) \(^d\) | MVMR adjusted for physical activity \(^e\)
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 |                 | OR | 95% CI | \(P\) | OR | 95% CI | \(P\) | OR | 95% CI | \(P\) |
| **European T2D GWAS** \(^f^\) |                 |     |         |     |     |         |     |     |         |     |
| Statins         | Decreased HMGCR expression | 1.63 | 1.44, 1.85 | \(7.2 \times 10^{-15}\) | 0.64 | 1.36 | 0.82, 2.26 | 0.36 | 0.85 | 0.43, 1.68 | 0.72 |
| PCSK-9i         | Decreased PCSK9 expression | 0.92 | 0.85, 0.99 | 0.04 | 0.35 | 0.82 | 0.60, 1.12 | 0.30 | 1.26 | 0.59, 2.69 | 0.61 |
| IPE             | Increased FADS1 expression | 1.44 | 1.29, 1.61 | \(1.9 \times 10^{-10}\) | 0.54 | 0.46 | 0.24, 0.87 | \(2.7 \times 10^{-02}\) | 0.47 | 0.25, 0.89 | \(3.1 \times 10^{-02}\) |
| **FinnGen T2D GWAS** \(^g^\) |                 |     |         |     |     |         |     |     |         |     |
| Statins         | Decreased HMGCR expression | 1.44 | 1.16, 1.80 | \(1.0 \times 10^{-03}\) | 0.68 | 2.86 | 0.97, 7.60 | 0.18 | 2.97 | 0.73, 12.06 | 0.37 |
| PCSK-9i         | Decreased PCSK9 expression | 0.87 | 0.73, 1.04 | 0.12 | 0.52 | 0.78 | 0.37, 1.61 | 0.55 | 2.41 | 0.55, 10.51 | 0.36 |
| IPE             | Increased FADS1 expression | 2.27 | 2.04, 2.53 | \(1.5 \times 10^{-50}\) | 0.35 | 0.44 | 0.11, 1.71 | 0.25 | 0.44 | 0.11, 1.72 | 0.25 |

**Table 2:** Mendelian randomisation (MR) estimates for the association between one standard deviation change in genetically predicted gene expression (GPGE) for proxied lipid control medications and type 2 diabetes (T2D).

- \(^a\) Decreased HMGCR and PCSK9 expression was predicted to lower LDL-C, and increased FADS1 expression was predicted to lower triglycerides (Table 1).
- \(^b\) Inverse-variance weighted (IVW) Mendelian randomisation (MR) analysis for T2D estimating odds ratios (ORs) and 95% confidence intervals (CIs) per GPGE changes in lipid trait as proxied by standard deviation changes in gene expression.
- \(^c\) Statistically significant Egger intercepts (\(P < 0.05\)) indicate potential for directional pleiotropy of the GPGE instruments used, suggesting potential alternative pathways from predicted gene expression to T2D.
- \(^d\) Multivariable MR (MVMR) analysis for T2D estimating adjusted odds ratios (ORs) and 95% confidence intervals (CIs) per GPGE changes in LDL-C or triglycerides (as proxied by reduced HMGCR and increased FADS1 expression, respectively). GPGE predicted changes in lipids are mutually adjusted for one another (e.g., LDL-C adjusted for HDL-C and triglycerides, triglycerides adjusted for LDL-C and HDL-C).
- \(^e\) Multivariable MR (MVMR) analysis additionally adjusted for total physical activity GPGE.
- \(^f\) Includes 148,726 T2D cases and 965,732 controls of European ancestry including non-overlapping participants from DIAMANTE, Million Veteran Program (MVP), Penn Medicine Biobank, Malmo Diet and Cancer Study, MedStar, and PennCath.
- \(^g\) Includes 29,166 T2D cases and 183,185 controls from a Finland T2D GWAS.
RCTs. However, after additional adjustment for physical expression corroborate the evidence from existing observed for statins proxied via reduced MVMR results indicating an increased T2D risk by approximately 30%.25 However, in a meta-analysis, LDL-C lowering effect has been shown to reduce CVD risk of new-onset diabetes.26 The biologic mechanism of 17 RCTs, statin therapy was reported to increase to reduced insulin sensitivity. 27 Our lipid-adjusted by which statins may increase T2D risk may be related depends upon the statin type and dosage,24 and this canonical T2D markers. IPE were not explained by

gested that the T2D risk reductions observed for proxied across adjustment sets. Furthermore, our analysis sug-
tions were observed for IPE in both the European and
FinnGen T2D GWAS populations. In the IVW MR anal-
ysis, statins proxied via reduced HMGCR expression, which was adjusted for the putative pleiotropic effects of FADS1 gene expression on LDL-C, HDL-C, and physical activity on T2D risk. Similar risk reduc-
tions were observed for IPE in both the European and FinnGen T2D GWAS populations. In the IVW MR anal-

Discussion
We examined the potential for repurposing lipid control medications for primary T2D prevention by combining publicly available GWAS and eQTL data to estimate genetically predicted gene expression which were then incorporated as instruments in an MR analysis examining T2D susceptibility. Triglyceride lowering via IPE was proxied by increased FADS1 expression and was associated with a 53% reduced T2D risk in the MVMR analysis, which was adjusted for the putative pleiotropic effects of FADS1 gene expression on LDL-C, HDL-C, and physical activity on T2D risk. Similar risk reductions were observed for IPE in both the European and FinnGen T2D GWAS populations. In the IVW MR analysis, statins proxied via reduced HMGCR expression, which was suggested to increase T2D risk; whereas PCSK-9i, proxied via reduced PCSK9 expression, were suggested to reduce T2D risk. However, the MVMR results for statins and PCSK-9i were imprecise and inconsistent across adjustment sets. Furthermore, our analysis suggested that the T2D risk reductions observed for proxied IPE were not explained by FADS1-predicted changes in
canonical T2D markers.

Statins reduce LDL-C levels to an extent which depends upon the statin type and dosage,3,4 and this LDL-C lowering effect has been shown to reduce CVD risk by approximately 30%.15 However, in a meta-analysis of 17 RCTs, statin therapy was reported to increase risk of new-onset diabetes.26 The biologic mechanism by which statins may increase T2D risk may be related to reduced insulin sensitivity.27 Our lipid-adjusted MVMR results indicating an increased T2D risk observed for statins proxied via reduced HMGCR expression corroborate the evidence from existing RCTs. However, after additional adjustment for physical activity in the MVMR, statins were no longer associated with increased T2D risk. Similarly, in a meta-analysis for various PCSK9 monoclonal antibodies no association was observed with T2D risk.2,8

Among patients using statins in the Reduction of Cardiovascular Events with Icosapent Ethyl – Intervention Trial (REDUCE-IT), IPE lowered triglycerides and reduced the hazard of first cardiovascular event by 30% compared to placebo.29 One possible benefit of IPE as observed in our analysis was the striking 53% T2D risk reduction. In REDUCE-IT, among patients without T2D at baseline, no effect was observed for IPE compared to placebo for new onset T2D (HR= 1.04; 95% CI= 0.73, 1.47). However, estimating the effect of IPE on primary prevention of T2D in REDUCE-IT would have been difficult given 60% of the participants had diabetes at baseline and T2D was evaluated as a tertiary endpoint. The Japan EPA Lipid Intervention Study (JELIS) RCT also observed benefits of EPA ethyl ester compared to placebo for prevention of coronary events (HR= 0.81; 95% CI= 0.69, 0.95).30 However, when examining increased blood sugar as an adverse event, no association for the EPA ethyl ester intervention group compared to placebo was observed (risk difference = 0.12%; 95% CI= −0.05% to 0.29%). It is possible that the reduced T2D risk associated with IPE is only observed in the context of a high-fat diet, such as was observed in mice.9 Despite the null associations reported for IPE in primary T2D prevention, a substantial CVD risk reduction (RR= 0.77, 95% CI= 0.66, 0.88; P= 3.0 × 10−4) was observed among REDUCE-IT participants with baseline T2D who were treated with IPE compared to placebo — indicating the potential interaction (perhaps synergistic effect) between statins and IPE on CVD prevention.31

| Gene expression to proxy IPE medication | GPGE changes on glucose/insulin traits | Multivariable MR (MVMR) |
|----------------------------------------|--------------------------------------|-------------------------|
|                                        |                                      |                         |
| European T2D GWAS                      |                                      |                         |
| Increased FADS1 expression             | Increased fasting glucose            | 0.66 (0.34, 1.30)       |
|                                       | Decreased fasting insulin            | 0.73 (0.26, 2.04)       |
|                                       | Decreased HOMA-B                     | 0.65 (0.20, 2.09)       |
|                                       | No change in HOMA-IR                 | 0.53 (0.18, 1.61)       |
| FinnGen T2D GWAS                       |                                      |                         |
| Increased FADS1 expression             | Increased fasting glucose            | 0.57 (0.13, 2.49)       |
|                                       | Decreased fasting insulin            | 0.74 (0.06, 8.54)       |
|                                       | Decreased HOMA-B                     | 0.25 (0.02, 2.97)       |
|                                       | No change in HOMA-IR                 | 0.16 (0.02, 1.63)       |

Table 3: Type 2 diabetes (T2D) risk per standard deviation increase in genetically predicted FADS1 expression (a proxy for the therapeutic effect of icosapent ethyl, IPE) on changes in glucose and insulin traits estimated via multivariable Mendelian randomisation (MVMR).

a Summarized from GPGE effects presented in Supplementary Table 6.

b Multivariable MR (MVMR) odds ratios (ORs) and 95% confidence intervals (95% CIs) estimate the potentially causal effect of one standard deviation increase in FADS1 gene expression (proxy for IPE) on changes in glucose and insulin traits on T2D risk, while adjusting for the effects of FADS1 gene expression on changes in triglycerides and LDL-C.
The biologic rationale for T2D risk reductions observed in our analysis of IPE is unknown. One putative mechanism is reduced hyperglycemia through the EPA-derived resolvin E1 metabolite. However, as indicated in our MVMR results, IPE may act independently of predicted changes in glucose and/or insulin to reduce T2D risk. One possible hypothesis relates to the preservation of membrane fluidity achieved via integration of EPA into the phospholipid bilayer. Increased erythrocyte membrane fluidity has been shown to facilitate glucose transport, and improve insulin signaling and control. Similarly, FADS1 protein has been shown to increase integration of polyunsaturated fatty acids in hepatic cell membranes in an omega-3 rich environment. It is plausible that IPE acts via similar biologic mechanisms to reduce T2D risk by enhancing erythrocyte membrane fluidity. Our sensitivity analysis examining the independent effect of FADS1-predicted increases in circulating EPA on reducing T2D risk may provide additional evidence in support of this hypothesis. Experimental studies in mice also point to other potential mechanisms for the beneficial effects of IPE including G-protein coupled receptor binding to improve insulin sensitivity, and protecting changes to the gut microbiome due to a high-fat diet.

Previous studies examined lipid-associated SNPs with T2D risk. Two studies incorporated targeted approaches by examining LDL-C lowering variants in HMGCR in relation to T2D risk. Lotta et al. reported a 40% increase in T2D risk (OR= 1.39, 95% CI= 1.12, 1.73; P= 0.003) associated with genetically predicted decreases in LDL-C incorporating three variants in HMGCR. Using a similar approach, Swerdlow et al. reported a 2-6% increase in T2D risk per LDL-C lowering allele for two SNPs located in HMGCR (rs72138484 and rs12916). The results are similar to ours when considering the direction of the reported T2D associations. Others examined the association between statin response and T2D susceptibility in a bi-directional MR in which no effect was observed for statin response and T2D risk and the effect of statin-induced methylation on T2D susceptibility. A previous MR study utilizing four LDL-C lowering PCSK9 variants were associated with 30% increased T2D risk, which is consistent with the increased T2D risks suggested in our PCSK9 physical activity adjusted MVMR result. However, these prior studies examined the association between SNPs in specific genomic regions with T2D susceptibility, rather than examining tissue-specific gene expression which makes direct comparison to our results difficult.

Causal inference in MR assumes: (1) the instrument (i.e., GPGE) is associated with the trait (i.e., lipids); (2) the instrument only affects the outcome (i.e., T2D susceptibility) via the trait; and (3) the instrument is not associated with any confounders of the trait-outcome association. We incorporated variants from a large lipid GWAS, and the largest T2D GWAS to date conducted among Europeans, to derive GPGE tissue-specific summary statistics via S-PrediXcan. All GPGE included in each lipid-specific instrument were associated with lipids as evidenced by their high prediction performance [i.e., FADS1 tissue-specific R² range from 2-42%; HMGCR tissue-specific R² range from 1-20%; PCSK9 tissue-specific R² range from 2-36%], and all were deemed valid instruments (I² > 0;9; Supplementary Table 9).

Although several sensitivity analyses were undertaken to test the robustness of MR results and we adjusted for total physical activity in the MVMR analysis, we cannot rule out the potential for bias due to unknown pleiotropy of SNPs included in S-PrediXcan GPGE models and are unable to assess potential off-target effects of predicted gene expression. Compared to the IVW MR, the magnitude of the IPE result was opposite after adjustment for correlated lipids and physical activity in the MVMR analysis. We attempted to reconcile these different results by conducting a sensitivity analysis examining the effects according to different GPGE adjustment sets (Supplementary Table 10). While our sensitivity analysis showed that holding predicted LDL-C constant resulted in a consistent T2D risk reduction for IPE, we still believe our results should be interpreted with caution. The possibility for bias due to unknown pleiotropic effects of FADS1 predicted gene expression on potential confounders of the other lipids and T2D remains (i.e., bias due to potential unmeasured confounding of the mediator and outcome). Nevertheless, we believe our IPE result is interesting given the IPE results (with LDL-C adjustment) could potentially reflect the effect of an intervention in which IPE is administered to patients currently taking statins.

Our negative control MR analysis indicated that population stratification bias was not a major concern in our study. Given that our analysis utilized two different European GWAS (i.e., European T2D GWAS with replication in the FinnGen T2D GWAS), our results are generalizable only to individuals of European ancestry. The efficacy of IPE therapy in other populations may differ due to potential T2D heterogeneity across populations. Additional research should be conducted to elucidate the putative effects of IPE on primary T2D prevention in diverse populations. Finally, GPGE estimates were not identified in tissues fundamental to lipid metabolism (e.g., liver), which is likely due to the limited number of high integrity samples available for various tissues in GTEx. We considered multiple lipid control medications in our analysis. However, due to limited availability of GPGE summary statistics, we were only able to identify adequate proxies for three current medications (i.e., statins, PCSK9-gi, and IPE). However, the three lipid control medications analysed in this manuscript are typically used as first-line treatment options for dyslipidemia, are effective strategies for reducing CVD risk, have limited side effects, and are generally...
lower in cost.15,38 Thus, our analysis focused on the most promising candidates for drug repurposing given these medications are commonly prescribed and well-tolerated in the patient population. Furthermore, future analyses should consider proxying drug metabolism rates to further understand the effectiveness of repurposing medications and how drug metabolism might influence any beneficial effects observed.

Overall, using gene expression to proxy current therapies, we identified a potential repurposing opportunity for T2D prevention for the hypertriglyceridemia medication, IPE. Regrettably, comparing our IPE results to current RCTs is difficult given that existing trials have not examined T2D as a primary endpoint. Utilizing gene-based approaches along with causal inference methods for known therapeutic gene targets may help illuminate avenues for possible drug repurposing. Combining our approach with experimental biologic validation, may help to motivate future RCT initiation to examine existing FDA-approved therapies with new primary endpoints.

Contributors
NKK conducted the analysis with input from all authors regarding the interpretation of the data. JMK curated the data and conducted a portion of the analysis. NKK wrote the original draft and revised the manuscript with input from all authors. NKK, JMK, and TLE were responsible for the study concept, design, and have verified the underlying data. All authors (NKK; JMK; VMW; KML; MMS; SLC; KRH; DRM; PDR; JAL; MV; and TLE) read and approved the final version of this manuscript.

Declaration of interests
All authors have nothing to disclose.

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Data sharing statement
Our analysis utilized only summary statistics from published GWAS. All GPGE summary statistics used in our analysis are provided in the Supplementary Materials.

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104038.

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