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

Nikhil K. Khankari,[a,b]* Jacob M. Keaton,[c,d] Venexia M. Walker,[e,f,g] Kyung Min Lee,[h] Megan M. Shuey,[b] Shoa L. Clarke,[i] Kent R. Heberer,[j,k] Donald R. Miller,[l,m] Peter D. Reaven,[n,o] Julie A. Lynch,[h,p] Marijana Vujkovic,[q,r] and Todd L. Edwards [b,d,s]**

[a]Division of Genetic Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
[b]Vanderbilt Genetics Institute, Vanderbilt University Medical Center, 2525 West End Ave, Suite 700, Nashville, TN 37203, USA
[c]Medical Genomics and Metabolic Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA
[d]Division of Epidemiology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
[e]Medical Research Council, Integrative Epidemiology Unit, University of Bristol, Bristol, UK
[f]Bristol Medical School: Population Health Sciences, University of Bristol, Bristol, UK
[g]Department of Surgery, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA
[h]VA Informatics and Computing Infrastructure, VA Salt Lake City Health Care System, Salt Lake City, UT, USA
[i]Departments of Medicine and Pediatrics, Stanford University School of Medicine, Stanford, CA, USA
[j]VA Palo Alto Health Care System, Palo Alto, CA, USA
[k]Departments of Medicine and Endocrinology, Stanford University School of Medicine, Stanford, CA, USA
[l]Center for Healthcare Organization and Implementation Research, Bedford VA Healthcare System, Bedford, MA, USA
[m]Center for Population Health, Department of Biomedical and Nutritional Sciences, University of Massachusetts, Lowell, MA, USA
[n]Phoenix VA Health Care Center, Phoenix, AZ, USA
[o]College of Medicine, University of Arizona, Phoenix, AZ, USA
[p]College of Nursing and Health Sciences, University of Massachusetts, Lowell, MA, USA
[q]Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA
[r]Corporal Michael J. Crescenz VA Medical Center, Philadelphia, PA, USA
[s]Nashville VA Medical Center, Nashville, TN, USA

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.

eBioMedicine 2022;80: 104038
Published online xxx
https://doi.org/10.1016/j.ebiom.2022.104038

*Corresponding author at: Dr. Nikhil K. Khankari, Division of Genetic Medicine, Vanderbilt University Medical Center, 2525 West End Ave, Suite 700, Nashville, TN 37203, USA.
**Corresponding author at: Dr. Todd L. Edwards, Division of Epidemiology, Vanderbilt University Medical Center, 2525 West End Ave, Suite 600, Nashville, TN 37203, USA.
E-mail addresses: nikhil.khankari@vumc.org (N.K. Khankari), todd.l.edwards@vumc.org (T.L. Edwards).

Funding Only summary statistics were used in this analysis. Funding information is detailed under Acknowledgments.

Keywords: Mendelian randomisation; Lipids; Statins; Icosapent ethyl; Gene expression; Type 2 diabetes; Drug repurposing

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 icosapent 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.

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.[1] Cardiovascular disease (CVD) complications, such as heart disease, stroke, and high blood pressure, may worsen after a T2D diagnosis.[1] 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.[2] 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.[3] 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.[4] Hypertriglyceridemia is a possible culprit for the increased risks observed among statin users.[5] Thus, triglyceride lowering strategies using long-chain omega-3 polyunsaturated fatty acid eicosapentaenoic acid (EPA) have been evaluated for CVD prevention.[6] 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.[7] Moreover, EPA metabolites have been shown to prevent hyperglycemia and hyperinsulinemia in humans,[8] and in mice IPE is protective against high-fat diet-induced glucose intolerance, insulin resistance, and β-cell dysfunction.[9] 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.[10] 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

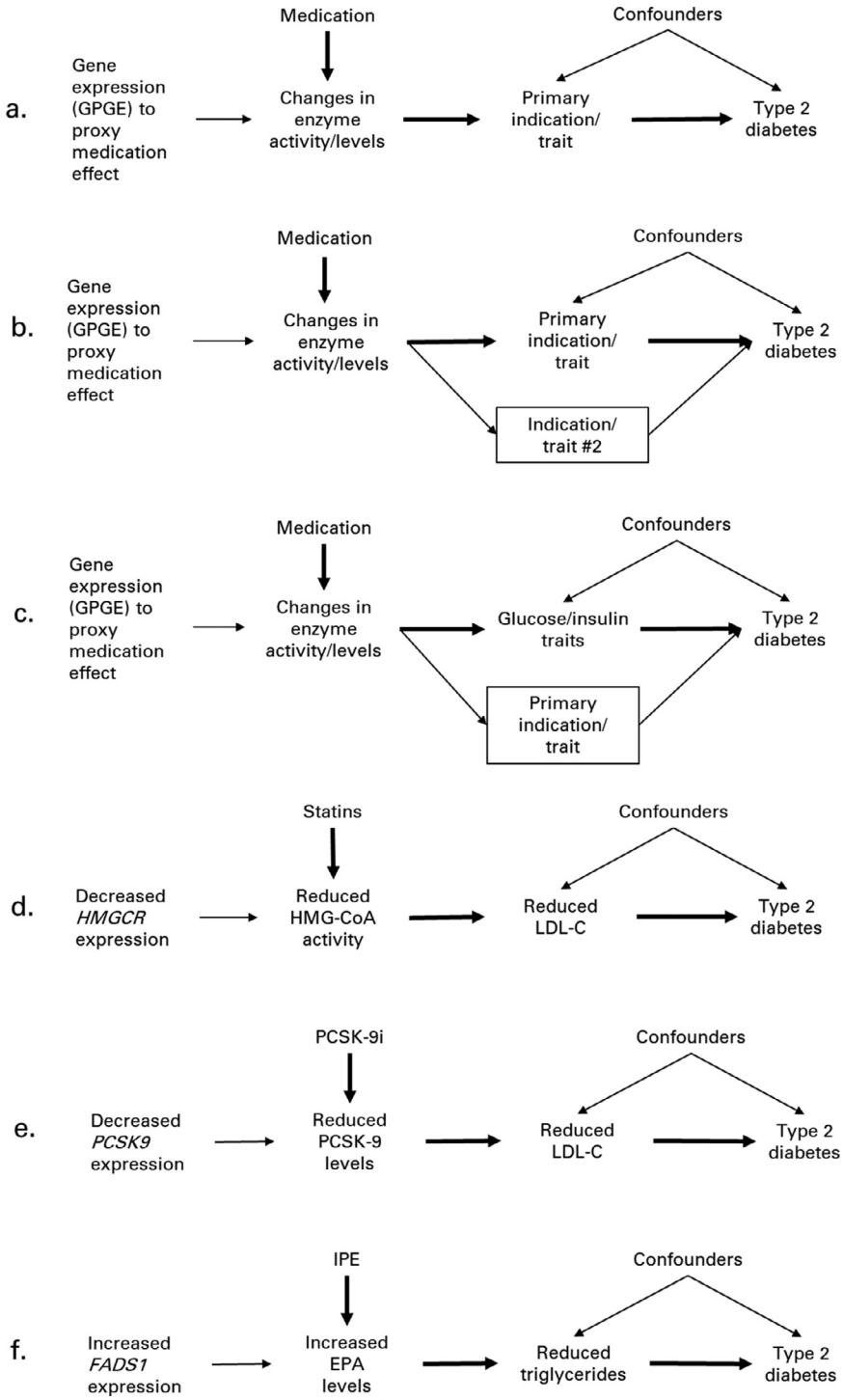

Figure 1. Directed acyclic graphs representing Mendelian randomisation (MR) approach undertaken in this analysis to evaluate the potential effect of lipid therapies on type 2 diabetes (T2D). **Panel a** represents the total effect of drug on T2D risk via changes in the primary indication or trait (i.e., 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.

Lipid control medication	Gene expression to proxy medication effect	Lipid trait (primary indication listed first)	GTEx tissues (eQTLs used in S-PrediXcan)[a]	Random-effects meta-analysis of GPGE changes in lipid trait across tissues[b]		
				mg/dL change	Std Err	*P*
Statins	Decreased *HMGCR* expression	LDL-C	5 (126)	-0·14	0·07	0·10
		Total cholesterol	4 (116)	-0·15	0·08	0·15
PCSK-9i	Decreased *PCSK9* 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
IPE	Increased *FADS1* expression	Triglycerides	23 (307)	-0·11	0·01	$9{\cdot}8 \times 10^{-10}$
		LDL-C	23 (306)	0·12	0·01	$6{\cdot}4 \times 10^{-10}$
		HDL-C	23 (309)	0·09	0·01	$9{\cdot}5 \times 10^{-10}$
		Total cholesterol	23 (307)	0·12	0·01	$4{\cdot}2 \times 10^{-10}$

***Table 1*: Random-effects summary of genetically predicted gene expression (GPGE) on changes in lipid traits representing the therapeutic action of statins and icosapent ethyl (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 decrease in *HMGCR* and *PCSK9* gene expression. Summarized GPGE represent predicted changes in lipid levels (mg/dL) per standard deviation increase in *FADS1* gene expression.

analysis. The following lipid control medications were considered: statins, IPE, proprotein convertase subtilisin/kexin type-9 inhibitors (PCSK-9i), Neimann-Pick C1-like 1 inhibitors (NPC1L1i), and fibrates.

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.[11] 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.[12] 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/m^2 (SD=3·9), 37% were hypertensive, and 30% had dyslipidemia. Summary statistics from a Finnish population (FinnGen) including 29,166 T2D cases and 183,185 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.[13] 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., 1000 Genomes), and SNP-specific effect estimates and standard errors from GWAS summary statistics. This information is used to predict tissue-

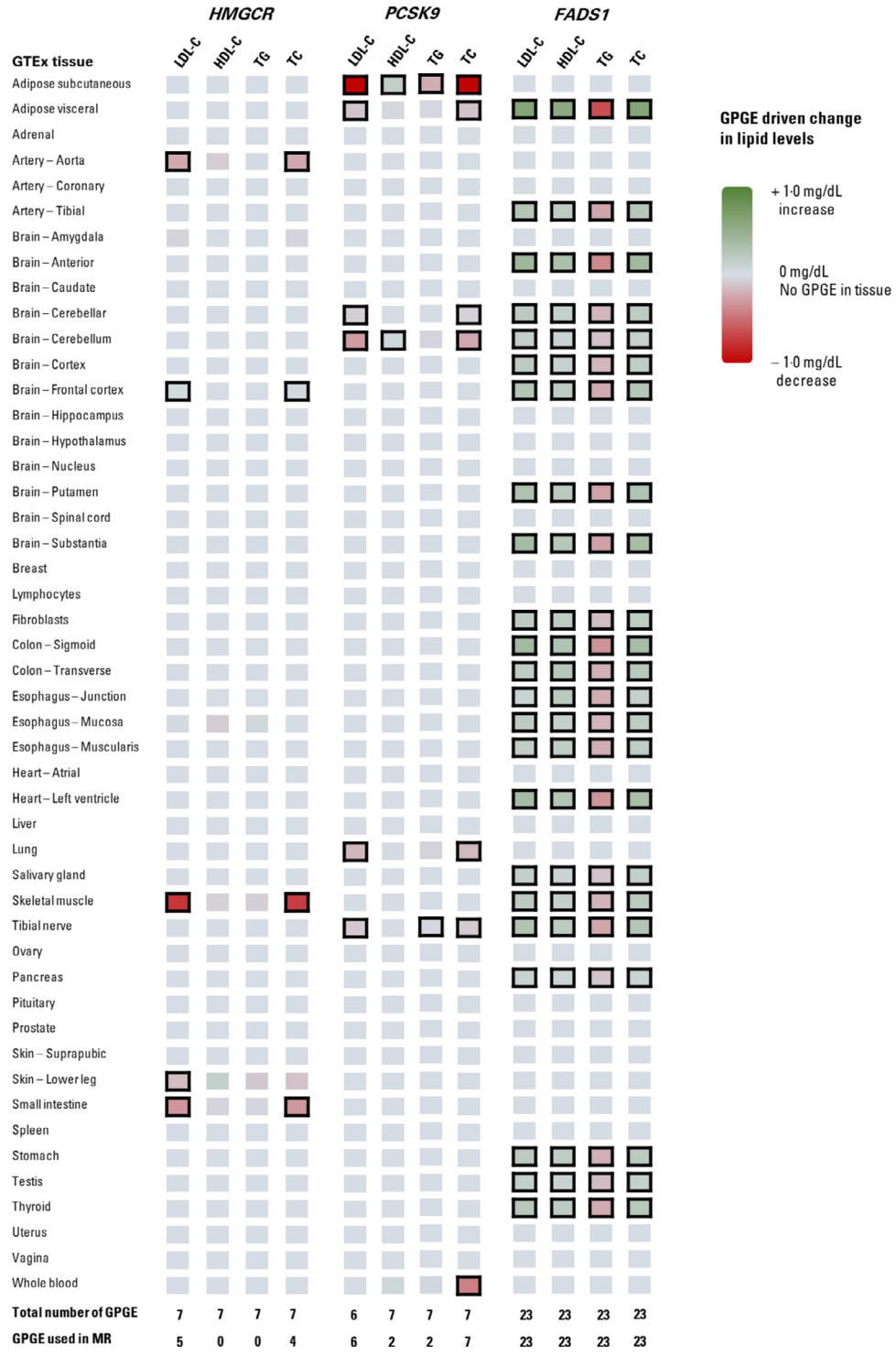

Figure 2. Genetically predicted gene expression (GPGE) driven changes in circulating lipids for decreased *HMGCR*, decreased *PCSK9*, and increased *FADS1* expression in 48 GTEx tissues. Depicted above are tissues with GPGE resulting in either increased lipid levels (green rectangles), decreased lipid levels (red rectangles), or no effect (grey rectangles). Statistically significant ($P < 0{\cdot}05$) 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*).[14] 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.[15] 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**)**.

The effect of FADS1 on increasing EPA levels has been demonstrated in *FADS1*-knockdown mice,[16] and triglyceride lowering has been observed in subjects supplemented with purified EPA.[17] To identify the best possible gene to proxy IPE, we estimated GPGE effects for circulating EPA for five genes (i.e., *FADS1, FADS2, ELOVL2, PTGS1*, and *PTGS2*) involved in polyunsaturated 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; $P = 5{\cdot}2 \times 10^{-09}$; **Supplementary Table 4**. Also, increased *FADS1* expression was predicted to lower triglycerides by 0·11 mg/dL ($P = 9{\cdot}8 \times 10^{-10}$; Table 1), which further supported our decision to proxy the triglyceride-lowering IPE via increased *FADS1* expression.

Triglyceride lowering achieved by fibrates [peroxisome proliferator-activated receptor α (PPARα) agonists] and LDL-C lowering via NPC1L1i were also considered. However, MR analyses were not conducted for fibrates and NPC1L1i due to the limited number of tissue-specific GPGE summary statistics estimated using S-PrediXcan.

Mendelian randomisation (MR)

Tissue-specific GPGE effects for *HMGCR* and *FADS1* gene expression that met our threshold for statistical significance ($P < 0{\cdot}05$) 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'[18] 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:

$$\hat{\beta}_{\mathrm{MR-IVW}} = \frac{\sum_{i=1}^{t} \beta_{\mathrm{lipid}} \beta_{\mathrm{T2D}} \sigma_{\beta_{\mathrm{T2D}}}{}^{-2}}{\sum_{i=1}^{t} \beta_{\mathrm{lipid}}{}^{2} \sigma_{\beta_{\mathrm{T2D}}}{}^{-2}}, \mathrm{se}\left(\hat{\beta}_{\mathrm{MR-IVW}}\right) = \sqrt{\frac{1}{\sum_{i=1}^{t} \beta_{\mathrm{lipid}}{}^{2} \sigma_{\beta_{\mathrm{T2D}}}{}^{-2}}},$$

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

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 mediatory 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,[19] with statistically significant regression intercepts ($P < 0{\cdot}05$) 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.[20,21] Physical activity has also been shown to affect lipid levels,[22] thus we additionally adjusted for total physical activity using S-PrediXcan derived tissue-specific GPGE representing changes in total physical activity using GWAS summary

statistics.[23] For IPE only (since the MVMR estimate for statins and PCSK-9i with T2D susceptibility were imprecise), we estimated the effect of increased *FADS1* expression on T2D susceptibility potentially driven by changes in glucose and/or insulin traits (Figure 1c; Table 3). MVMR estimates for the effect of glucose and/or insulin traits on T2D susceptibility were adjusted for *FADS1* predicted changes in triglycerides (the primary indication for IPE) and for *FADS1* predicted changes in LDL-C (which was the only other *FADS1* predicted lipid associated with T2D susceptibility in the MVMR analysis; MVMR OR=0·40, 95% CI=0·32, 0·48, P=5·7 × 10^{-09}).

Role of the funding source

The funding sources listed at the end of the manuscript did not have a role in the study design, analysis, interpretation, or writing of the manuscript. The decision to submit the manuscript for publication was made solely by the authors listed.

Ethics

Our analysis utilized only summary statistics and ethical approval was not required for this study.

Findings

Genetically predicted gene expression (GPGE)

S-PrediXcan identified seven GTEx tissues for which reduced *HMGCR* expression (to proxy statins) resulted in LDL-C changes, six tissues for reduced *PCSK9* expression (to proxy PCSK-9i) resulted in LDL-C changes, and 23 tissues were identified for triglyceride changes resulting from increased *FADS1* expression (to proxy IPE; Figure 2). One SD reduction in *HMGCR* expression was predicted to lower LDL-C by nearly 0·14 mg/dL (summarized using random-effects meta-analysis over five tissues) and lower total cholesterol by approximately 0·15 mg/dL (summarized over four tissues). One SD decrease in *PCSK9* expression was predicted to lower LDL-C by 0·31 mg/dL (summarized over six tissues) and decrease total cholesterol by 0·28 mg/dL. One SD decrease in *PCSK9* expression in two tissues were predicted to affect changes in HDL-C (0·10 mg/dL increase) and triglycerides (0·10 mg/dL decrease). One SD increase in *FADS1* expression (summarized over 23 tissues) was predicted to increase LDL-C by approximately 0·12 mg/dL, increase HDL-C by 0·09 mg/dL, and increase total cholesterol by 0·12 mg/dL. For triglycerides, one SD increase in *FADS1* expression was predicted to lower levels by 0·11 mg/dL (Table 1).

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^{-15}). After accounting for other lipids in the MVMR analysis, the increased T2D 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^{-02}), which was also consistent in the physical activity adjusted MVMR analysis (MVMR=0·47, 95% CI=0·25, 0·89; P=3·1 × 10^{-02}). 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, MR 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^{-28}), 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*	Egger *P* [c]	OR	95% CI	*P*	OR	95% CI	*P*
European T2D GWAS [f]											
Statins	Decreased *HMGCR* expression	1·63	1·44, 1·85	$7{\cdot}2 \times 10^{-15}$	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	$1{\cdot}9 \times 10^{-10}$	0·54	0·46	0·24, 0·87	$2{\cdot}7 \times 10^{-02}$	0·47	0·25, 0·89	$3{\cdot}1 \times 10^{-02}$
FinnGen T2D GWAS [g]											
Statins	Decreased *HMGCR* expression	1·44	1·16, 1·80	$1{\cdot}0 \times 10^{-03}$	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	$1{\cdot}5 \times 10^{-50}$	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.

Gene expression to proxy IPE medication	GPGE changes on glucose/insulin traits [a]	Multivariable MR (MVMR)[b]		
		T2D OR	95% CI	*P*
European T2D GWAS				
Increased *FADS1* expression	Increased fasting glucose	0·66	0·34, 1·30	0·25
	Decreased fasting insulin	0·73	0·26, 2·04	0·56
	Decreased HOMA-B	0·65	0·20, 2·09	0·47
	No change in HOMA-IR	0·53	0·18, 1·61	0·28
FinnGen T2D GWAS				
Increased *FADS1* expression	Increased fasting glucose	0·57	0·13, 2·49	0·46
	Decreased fasting insulin	0·74	0·06, 8·54	0·81
	Decreased HOMA-B	0·25	0·02, 2·97	0·29
	No change in HOMA-IR	0·16	0·02, 1·63	0·14

***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.

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, 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,[24] and this LDL-C lowering effect has been shown to reduce CVD risk by approximately 30%.[25] 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.[28]

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; $P = 3{\cdot}0 \times 10^{-4}$) 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]

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.[8] 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.[6] Increased erythrocyte membrane fluidity has been shown to facilitate glucose transport, and improve insulin signaling and control.[32] Similarly, FADS1 protein has been shown to increase integration of polyunsaturated fatty acids in hepatic cell membranes in an omega-3 rich environment.[16] 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.[9]

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.[33,34] 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* (rs17238484 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[35] and the effect of statin-induced methylation on T2D susceptibility.[36] A previous MR study utilizing four LDL-C lowering *PCSK9* variants were associated with 30% increased T2D risk[37], which is consistent with the increased T2D risks suggested in our PCSK9i 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.[10] 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 R^2 range from 2-42%; *HMGCR* tissue-specific R^2 range from 1-20%; *PCSK9* tissue-specific R^2 range from 2-36%], and all were deemed valid instruments ($I^2 > 0{\cdot}9$; **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, PCSK-9i, 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.

Acknowledgements

We would like to acknowledge the Million Veteran Program (MVP) participants. The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health (NIH), and by National Cancer Institute (NCI), National Human Genome Research Institute (NHGRI), National Heart, Lung, and Blood Institute (NHLBI), National Institute on Drug Abuse (NIDA), National Institute of Mental Health (NIMH), and National Institute of Neurological Disorders and Stroke (NINDS). The GTEx data used for this were obtained from dbGaP Accession number phs000424v7p2. GWAS summary statistics were for lipid traits were downloaded from the Global Lipids Genetics Consortium (http://csg.sph.umich.edu//abecasis/public/lipids2013). GWAS summary statistics for the negative control trait (natural hair color) were downloaded from the UK Biobank (dataset: ukb-d-1747_5; https://gwas.mrcieu.ac.uk/datasets/). We want to acknowledge the participants and investigators of FinnGen study (https://finngen.gitbook.io/documentation/). NKK is supported by NIH R00 CA215360. VMW is supported by the MRC Integrative Epidemiology Unit at the University of Bristol (MC UU 00011/1 and MC UU 00011/4).

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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