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ORIGINAL RESEARCH

Exploring Endoplasmic Reticulum Stress in Gestational Diabetes Mellitus: MultiOmics Insights Through Mendelian Randomization

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Objective: Recent studies suggest a correlation between endoplasmic reticulum (ER) stress and gestational diabetes mellitus (GDM). Nevertheless, the potential causal role of ER stress-related genes remains largely unexplored. This study aims to generate hypotheses regarding these connections through an integrative analysis of multiomics data.

Methods: We utilized an exploratory list of genes related to ER stress and integrated quantitative trait loci (QTL) for gene expression (eQTLs), DNA methylation (mQTLs), and protein levels (pQTLs). Genome-wide association studies (GWAS) summary statistics for GDM were obtained from the publicly accessible FinnGen database, with replication attempted using data from the GWAS Catalog. The summary-data-based Mendelian Randomization (SMR) method was applied to explore the genetic ties between these genes and GDM, followed by colocalization analysis to pinpoint overlapping causal genetic variants. Placental endothelial transcriptome data (GSE103552) were used for validation.

Results: The SMR and colocalization identified potential causal links for 27 mQTLs, 8 eQTLs, and 4 pQTLs with GDM risk. Integration of evidence across mQTL and eQTL levels suggested potential causal roles for the *NUP133, VHL, TAPBP*, and *GPX1* genes in GDM. Notably, *NUP133* shows suggestive colocalization evidence at the eQTL level. Analysis relating methylation to expression suggested hypermethylation at the CpG site cg17439967 may upregulate *NUP133*, potentially associating with reduced GDM risk. Transcriptomic validation in placental endothelial cells further showed differential expression of these four genes between GDM and controls.

Conclusion: Our findings provide suggestive genetic evidence linking specific ER stress-related genes, particularly *NUP133*, with GDM risk, highlighting potential pathways that warrant further investigation.

Keywords: diabetes mellitus, gestational, endoplasmic reticulum stress, multiomics, Mendelian randomization analysis, quantitative trait loci, genetic linkage

Introduction

Gestational diabetes mellitus (GDM) is typically characterized by hyperglycemia occurring at any point during pregnancy, but falling below the diagnostic criteria for diabetes mellitus, regardless of whether it appears before or after pregnancy.[1] GDM affects between 2 to 38% of pregnancies worldwide, which imposes a significant health burden on affected individuals and is linked to an increased incidence of negative maternal and fetal outcomes.[2,3] Key risk factors for GDM include maternal obesity, certain ethnic backgrounds, polycystic ovary syndrome, prediabetes, a history of GDM, familial Type 2 diabetes, prior fetal loss, and advancing maternal age.[4] Despite research into mechanisms such as pancreatic β-cell dysfunction and chronic insulin resistance, the precise etiology of GDM remains incompletely understood,[5] highlighting the need to investigate additional biological mechanisms that may contribute to disease development.

Disrupted endoplasmic reticulum (ER) homeostasis has been linked to reproductive tissue disorders and complications during pregnancy, including preterm labor. The ER is essential for protein folding, modification, and trafficking,[6,7]

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but various stressors—such as the accumulation of misfolded proteins, calcium imbalances, and glucose scarcity—can induce ER stress.[8] If unresolved, this stress triggers cell death through pro-apoptotic pathways.[9] In GDM, ER stress has been linked to inflammation and β-cell dysfunction, both critical for maintaining glucose homeostasis.[10,11] The ER stress response can also upregulate specific genes and proteins, including C/EBP homologous protein (CHOP) and the pro-apoptotic factor BAX, which are implicated in GDM pathogenesis.[12,13] Additionally, recent research has suggested that ghrelin, a hormone involved in appetite regulation, may modulate ER stress in GDM, offering a potential therapeutic target for intervention.[14] Moreover, supplementation with trace elements like zinc, selenium, and chromium has shown promise in alleviating ER stress and improving insulin resistance in GDM models.[15] These observations indicate that ER stress may represent an important but incompletely defined mechanism in GDM, warranting further investigation.

Mendelian randomization (MR) serves as an epidemiological instrument, leveraging genetic variations to explore causal links between risk factors and diseases.[16] Conventional MR analyses typically rely on single-omics data, which may not adequately capture the complex pathways involved in multifactorial diseases.[17] In contrast, the summary-data-based Mendelian randomization (SMR) method, when applied to multiomics data, offers a potential avenue to integrate information from various fields, including genomics, transcriptomics, epigenomics, and proteomics. This comprehensive integration enhances the accuracy and reliability of potential associations.[18]

Despite increasing evidence implicating ER stress in GDM, it remains unclear whether specific ER stress–related genes play a causal role in disease risk. To address this gap, we conducted SMR analysis to explore potential causal links between ER stress-related molecular traits (methylation, expression, protein abundance) and GDM risk. The purpose of this research is to provide preliminary evidence that could inform future research into the mechanisms of GDM.

Material and Methods

Study Design

In our research, we identified instrumental variables (IVs) associated with a list of candidate ER stress-related genes at three distinct molecular levels: DNA methylation, gene expression, and protein levels, primarily using data derived from blood or plasma. SMR analyses were conducted at each level to explore the potential links to GDM. The term "randomization" in this study refers to Mendelian randomization, which leverages the random segregation of alleles during gamete formation. The primary dataset for the discovery phase originated from the FinnGen R10 cohort, while replication was attempted using GWAS data from the GWAS Catalog (https://www.ebi.ac.uk/gwas/). It is crucial to emphasize that there was a distinct separation between the cohorts for exposure and outcome, ensuring no overlap in the primary analysis. To reinforce the inferences regarding causality, a colocalization analysis was also conducted to assess whether QTL and GDM signals likely share the same causal variant. Figure 1 provides a comprehensive outline of the study's design, delineating the approach for genetic variant selection and analysis, adhering to the principles outlined in the STROBE-MR guidelines.[19] The summary statistics for the MR analysis were sourced from studies previously reported in the literature, each with the appropriate ethical approval. The present study was confirmed by the Medical Ethics Committee of the Second Hospital of Jilin University to be exempt from additional ethical review (approval date: August 14, 2025; Serial Number: 2025013).

Data Sources

We obtained the GWAS summary statistics for GDM from the FinnGen R10 cohort (https://storage.googleapis.com/finngen-public-data-r10/summary_stats/finngen_R10_GEST_DIABETES.gz), encompassing 14,718 cases and 215,592 controls. The large sample size of this cohort provides substantial statistical power for the discovery phase of our SMR analysis. For the validation of our results, we employed GWAS data from two distinct GDM cohorts: one designated as Gestational diabetes (GCST90043951) with 864 cases and 6,977 controls of European ancestry,[20] and the other as Gestational diabetes mellitus (GCST90297786) comprising 3317 cases and 19,565 controls of East Asian ancestry.[21]

We obtained blood eQTL data from the eQTLGen Consortium, which included gene expression profiles for 31,684 individuals.[22] The mQTL data were derived from a meta-analysis of two European cohorts: the Brisbane Systems Genetics Study with 614 participants and the Lothian Birth Cohorts with 1,366 participants.[23] Furthermore, pQTL data

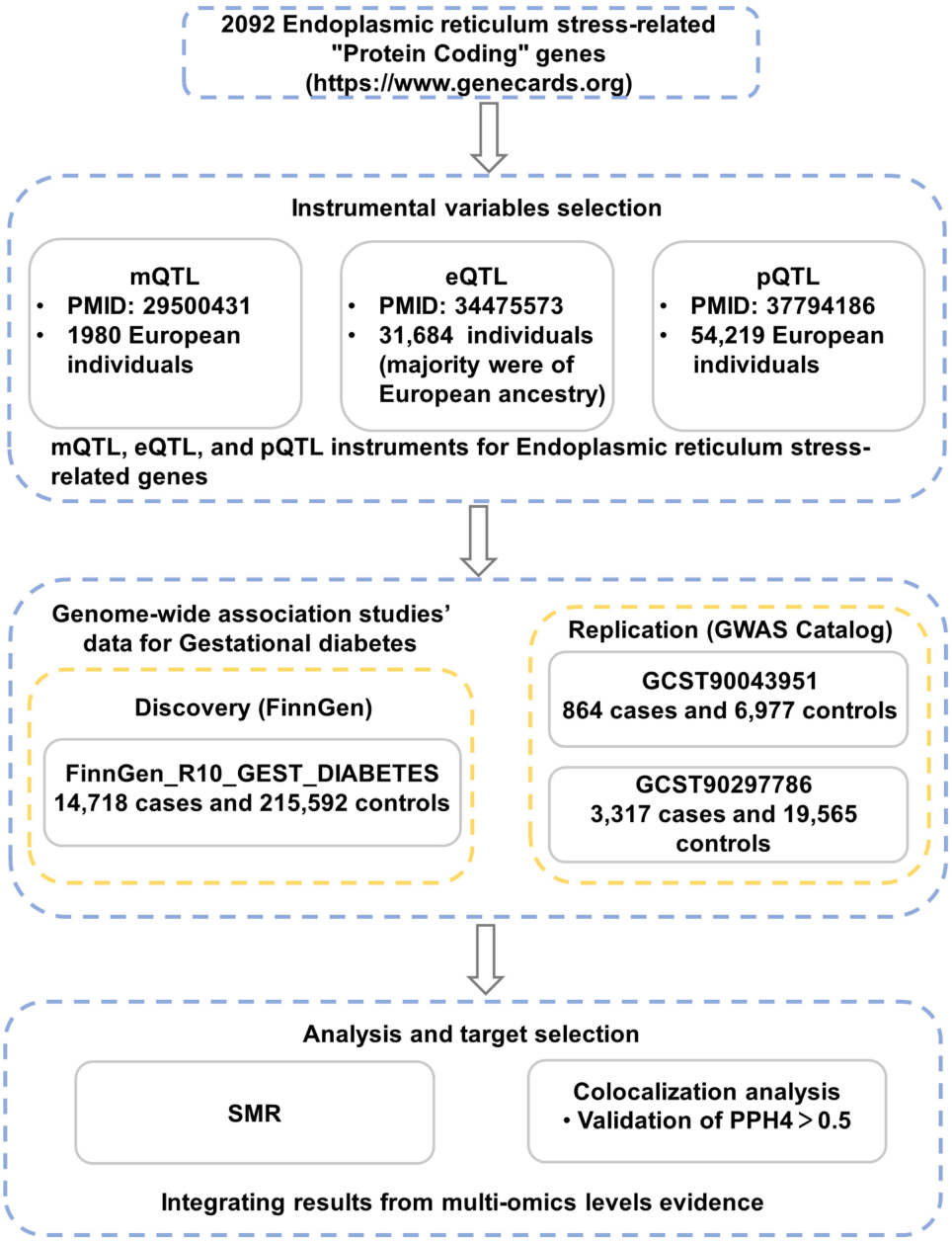

Figure 1 Flowchart of the analyses performed.

were sourced from a study by Sun BB et al, involving 54,219 participants from the UK Biobank.[24] All the summary statistics employed in the MR analysis were sourced from studies that have already been made public (Supplementary Table S1). Participant inclusion and exclusion criteria were determined by the original GWAS and QTL consortia as described in their respective publications.

In the GeneCards database (https://www.genecards.org), a search was conducted using the keyword "endoplasmic reticulum stress". The results were sorted by relevance in descending order, and the top 75% of the protein-coding genes associated with ER stress were downloaded, totaling 2,092 genes.

For additional validation, we analyzed transcriptomic data from the Gene Expression Omnibus (GEO, GSE103552), which profiled primary feto-placental arterial and venous endothelial cells isolated from 8 healthy and 14 GDM pregnancies, comprising 37 RNA expression samples (16 from controls and 21 from GDM cases). In the original study, GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Groups

(IADPSG) criteria based on a 75 g oral glucose tolerance test, and controls were women with uncomplicated singleton pregnancies and normal glucose tolerance.[25]

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Functional Enrichment Analysis

Functional enrichment analyses were performed using the R package clusterProfiler (v4.8.1). The candidate ER stress–related genes obtained from GeneCards were subjected to GO enrichment in biological process, molecular function, and cellular component categories. KEGG pathway analysis was conducted to identify enriched signaling pathways. Pathways classified under "Human Diseases" were excluded to reduce annotation bias. Statistical significance was assessed using the Benjamini–Hochberg procedure to control the false discovery rate (FDR), and pathways with adjusted p-values < 0.05 were considered significant.

Summary-Data-Based MR Analysis

Utilizing SMR analysis to investigate the association between methylation, expression levels, and protein abundance of ER stress genes with GDM. When exposures and outcomes are derived from two large, independent cohorts, SMR analysis, based on top-ranked cis-quantitative trait loci (QTLs), offers greater statistical power compared to conventional MR approaches.[26] The identification of top cis-QTLs is based on a genomic region extending ±1000 kb around the gene of interest, with a rigorous P-value threshold set at 5.0×10^{-8}.[27,28] SNPs showing allele frequency variance exceeding 0.2 across various datasets, such as linkage disequilibrium references, QTL databases, and outcome databases, are methodically excluded. The maximum allowable ratio of SNPs with allele frequency discrepancies is maintained at the pre-established limit of 0.05. Due to the absence of allele frequency data in the GCST90297786 cohort dataset, we applied filters of –maf 0.01 and –diff-freq 1 based on SNPs mentioned in prior literature.[29]

The multi-SNP SMR approach implemented in the SMR software was used, which extends the standard SMR by jointly modeling multiple SNPs within the cis-QTL region (±1 Mb). For each locus, all SNPs passing quality control and allele frequency filters in both the QTL and GWAS datasets were included. The number of SNPs per locus varied according to local variant density and data availability. To control for multiple testing, FDRs were estimated using the Benjamini–Hochberg procedure. In the SMR framework, the Heterogeneity in Dependent Instruments (HEIDI) test serves as the sensitivity analysis. It evaluates whether observed associations are consistent with a single shared causal variant underlying both the QTL and GDM signals. A non-significant HEIDI result (P-HEIDI > 0.05) suggests that heterogeneity due to linkage disequilibrium with distinct causal variants is unlikely, thereby strengthening causal inference.[26] Results satisfying the thresholds of P-SMR < 0.05, P-SMR-multi < 0.05, and P-HEIDI > 0.05 underwent subsequent colocalization analysis and multiomics integration.

Integration of Multiomics Evidence

To prioritize candidate genes supported by multiple molecular layers, we applied a structured, rule-based integration strategy. SMR analyses were first conducted independently for mQTLs, eQTLs, and pQTLs with GDM as the outcome. Genes that showed significant SMR associations (P-SMR < 0.05) and passed the HEIDI test (P-HEIDI > 0.05) at more than one QTL level were prioritized. For genes supported by both mQTL and eQTL data, an additional directional SMR analysis was performed using mQTLs as the exposure and eQTLs as the outcome to assess potential regulatory relationships.

Colocalization Analysis

The colocalization analysis was employed to evaluate whether the identified associations between ER stress-related cis-QTLs, which include eQTLs, mQTLs, and pQTLs, with gestational diabetes mellitus (GDM) are likely mediated by the same underlying causal genetic variant, as opposed to linkage disequilibrium between distinct variants. This analysis is predicated on the premise that colocalization of GWAS signals with QTLs strengthens the evidence that the genomic loci implicated in GWAS are influencing the phenotype by modulating gene-related biological mechanisms. The colocalization analysis delineates

five unique posterior probabilities aligned with five distinct hypotheses: 1) no genetic association of the SNP with either trait in the region (H0); 2) genetic association with the first trait only (H1); 3) genetic association with the second trait only (H2); 4) separate genetic associations of the SNP with both traits through different causal variants (H3); 5) shared genetic association of the SNP with both traits via a common causal variant (H4). The analysis is performed on SNPs within a 1000 kb range flanking the top cis-QTL colocalization region windows.[30,31] A threshold of PPH4 > 0.5 is applied to infer colocalization.[32,33]

All statistical analyses were conducted utilizing R software, version 4.3.0. For the generation of Manhattan plots, the "ggplot2" R package was implemented, while forest plots were produced using the "forest plot" package. The code for SMR Locus Plot and SMR Effect Plot was sourced from the research conducted by Zhu et al.[26]

Results

ER Stress-Related Gene Methylation and GDM

Before performing SMR analyses, we first evaluated whether the GeneCards-derived gene set indeed captured ER stress biology. GO analysis showed significant enrichment for processes related to ER stress, unfolded protein response, apoptosis, and protein quality control. KEGG analysis identified stress-responsive signaling pathways, including PI3K-Akt, TNF, MAPK, and HIF-1 (Supplementary Figure S1). These results support the biological relevance of the selected gene set. The significant enrichments are summarized in Supplementary Tables S2 and S3.

In the discovery phase, after applying the predefined criteria (P-SMR < 0.05, P-HEIDI > 0.05), we identified 354 CpG sites associated with 184 genes that were potentially linked to GDM (Supplementary Table S4). Colocalization analysis then provided evidence for shared causal variants at 27 of these sites, corresponding to 17 genes, all of which exceeded the stringent threshold of PPH4 ≥ 0.8 (Figure 2A). The colocalization results for cg03098644 in *EPHB4* are presented in Figure 2B as an example.

Replication attempts for CpG sites correlated with GDM within the GCST90043951 cohort yielded nominal significance (P < 0.05) and consistent direction of effect for CpG sites related to genes *ATXN7L3, FOXO1, HKDC1*, and *PIK3R2*, with confirmation results listed in Supplementary Table S5. Furthermore, the GCST90297786 cohort replication attempt showed nominal significance and consistent direction for sites related to *ANKFY1, GALNT2, HKDC1, IRF1, MEF2A*, and *NOTCH1*, with validation outcomes elaborated in Supplementary Table S6.

ER Stress-Related Gene Expression and GDM

In the discovery phase, we identified 41 ER stress–related genes associated with GDM risk based on SMR analysis (P-SMR < 0.05, P-HEIDI > 0.05) (Supplementary Table S7). Among these, the expression of 24 genes showed positive associations with GDM risk, whereas 17 showed inverse associations. Colocalization analysis provided suggestive evidence (PPH4 > 0.5) for shared causal variants for 8 genes. However, none of these exceeded the more stringent threshold of PPH4 ≥ 0.8 (Figure 3A). The colocalization results for *NUP133* (PPH4 = 0.787) are presented in Figure 3B.

The eQTL SMR results were not validated in the GCST90043951 cohort. In the GCST90297786 cohort, a total of one gene (*ANKFY1*) was validated, and the gene expression level's relationship with the risk of GDM was consistent with the results from the discovery cohort (Supplementary Table S8).

ER Stress-Related Protein Abundance and GDM

In the discovery phase, SMR analysis (P-SMR < 0.05, P-HEIDI > 0.05) identified 10 ER stress–related proteins associated with GDM risk (Supplementary Table S9). Colocalization analysis provided suggestive evidence (PPH4 > 0.5) for shared causal variants for 4 proteins, with SPTLC1 additionally exceeding the stringent threshold of PPH4 ≥ 0.8 (Figure 4A). The colocalization findings for SPTLC1 are shown in Figure 4B. The results obtained from the pQTL SMR analysis were not replicated in either the GCST90043951 or the GCST90297786 cohorts.

Integrating Evidence from Multiomics Levels

To identify genes with potential causal links to GDM supported by evidence across multiple molecular layers derived from blood/plasma, we integrated the SMR analysis results from mQTLs, eQTLs, and pQTLs based on gene names. We

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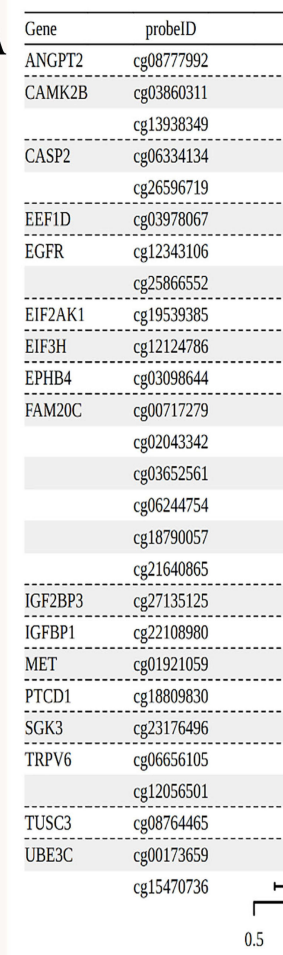
Gene	probeID	OR (95%CI)	p_SMR	Multi_pvalue	PPH4
ANGPT2	cg08777992	0.856(0.743 - 0.987)	0.032	0.032	0.895 *
CAMK2B	cg03860311	0.914(0.85 - 0.983)	0.015	0.015	0.872 *
	cg13938349	0.913(0.848 - 0.982)	0.014	0.014	0.872 *
CASP2	cg06334134	0.94(0.888 - 0.995)	0.034	0.034	0.872 *
	cg26596719	0.965(0.934 - 0.997)	0.032	0.005	0.872 *
EEF1D	cg03978067	0.977(0.958 - 0.996)	0.02	0.04	0.895 *
EGFR	cg12343106	0.847(0.742 - 0.967)	0.014	0.014	0.872 *
	cg25866552	0.848(0.744 - 0.967)	0.014	0.014	0.872 *
EIF2AK1	cg19539385	0.918(0.863 - 0.978)	0.008	0.006	0.872 *
EIF3H	cg12124786	1.167(1.057 - 1.289)	0.002	0.002	0.895 *
EPHB4	cg03098644	0.91(0.855 - 0.969)	0.003	0.016	0.872 *
FAM20C	cg00717279	1.074(1.004 - 1.148)	0.037	0.037	0.872 *
	cg02043342	1.119(1.004 - 1.248)	0.043	0.043	0.872 *
	cg03652561	1.128(1.003 - 1.268)	0.044	0.044	0.872 *
	cg06244754	1.118(1.01 - 1.237)	0.032	0.032	0.872 *
	cg18790057	1.058(1.004 - 1.115)	0.036	0.03	0.872 *
	cg21640865	1.098(1.024 - 1.177)	0.008	0.032	0.872 *
IGF2BP3	cg27135125	1.099(1.003 - 1.205)	0.044	0.044	0.872 *
IGFBP1	cg22108980	0.885(0.799 - 0.981)	0.02	0.02	0.872 *
MET	cg01921059	1.033(1.013 - 1.055)	0.002	0.006	0.872 *
PTCD1	cg18809830	1.073(1.015 - 1.133)	0.012	0.023	0.872 *
SGK3	cg23176496	0.86(0.746 - 0.991)	0.038	0.038	0.895 *
TRPV6	cg06656105	0.886(0.797 - 0.984)	0.024	0.024	0.872 *
	cg12056501	0.883(0.793 - 0.984)	0.024	0.024	0.872 *
TUSC3	cg08764465	0.833(0.708 - 0.98)	0.027	0.027	0.895 *
UBE3C	cg00173659	0.942(0.889 - 0.998)	0.043	0.037	0.872 *
	cg15470736	0.768(0.627 - 0.941)	0.011	0.011	0.872 *



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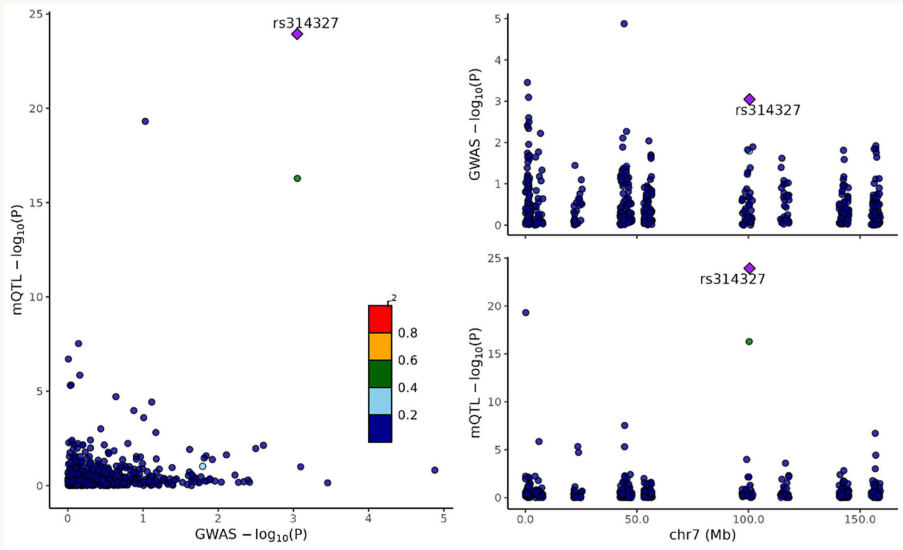

Figure 2 Associations of endoplasmic reticulum (ER) stress–related CpG methylation with gestational diabetes mellitus (GDM) in summary-data-based Mendelian randomization (SMR) analysis. (**A**) Forest plot of significant associations between genetically predicted CpG methylation and GDM risk in the FinnGen_R10_GEST_DIABETES cohort. Asterisks (*) indicate loci with posterior probability for hypothesis 4 (PPH4) ≥ 0.5, indicating suggestive evidence of colocalization. (**B**) Colocalization plots for cg03098644 in *EPHB4*, showing the alignment between methylation quantitative trait loci (mQTL) and GDM genome-wide association study (GWAS) signals.

Abbreviations: OR, Odds Ratio; CI, Confidence Interval.

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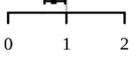
Gene	OR (95%CI)	p_SMR	Multi_pvalue	PPH4
ANKFY1	1.455(1.125 - 1.882)	0.004	0.004	0.556 *
ATG13	1.704(1.191 - 2.439)	0.004	0.01	0.218
BBC3	1.927(1.132 - 3.281)	0.016	0.016	0.406
CASP3	1.08(1.005 - 1.162)	0.037	0.005	0.173
CCPG1	0.792(0.676 - 0.929)	0.004	0.024	0.446
CDKAL1	0.889(0.8 - 0.988)	0.029	1.8e-04	0
CLU	0.642(0.426 - 0.969)	0.035	0.035	0.21
CYCS	1.279(1.078 - 1.517)	0.005	0.005	0.725 *
DNAJC3	0.633(0.474 - 0.847)	0.002	0.001	0.339
EEF1A2	1.062(1.002 - 1.125)	0.041	0.032	0.186
EPHB4	1.064(1.021 - 1.11)	0.004	1.4e-04	0.573 *
FCF1	1.377(1.048 - 1.81)	0.022	0.031	0.384
FOSL2	0.61(0.391 - 0.954)	0.03	0.03	0
FTL	1.378(1.058 - 1.795)	0.017	0.017	0.174
GNAI1	1.336(1.008 - 1.772)	0.044	0.019	0.264
GPX1	1.321(1.054 - 1.656)	0.016	0.028	0.21
HRK	0.918(0.867 - 0.972)	0.004	9.4e-05	0.646 *
IER3	1.083(1.003 - 1.169)	0.041	0.024	0.031
IKBIP	0.902(0.823 - 0.989)	0.028	2.3e-04	0.131
IL32	1.064(1.009 - 1.121)	0.021	0.05	0.111
KAT5	1.681(1.058 - 2.672)	0.028	0.028	0.141
MAN2C1	0.965(0.934 - 0.997)	0.033	0.004	0.112
MICA	1.044(1.007 - 1.082)	0.021	5.7e-07	0.496
MRPL19	1.089(1.004 - 1.181)	0.04	0.045	0.177
NGFR	0.637(0.422 - 0.963)	0.032	0.032	0.176
NUP133	0.834(0.747 - 0.932)	0.001	6.2e-04	0.787 *
PPP2CA	0.648(0.438 - 0.96)	0.03	0.03	0.167
PSAT1	1.086(1.02 - 1.156)	0.009	0.01	0.368
RNF168	0.792(0.668 - 0.938)	0.007	0.016	0.471
RYR1	1.172(1.031 - 1.332)	0.015	0.008	0.348
SAMD8	1.757(1.095 - 2.821)	0.02	0.02	0.532 *
SIAH1	0.765(0.587 - 0.996)	0.047	0.024	0.165
TAPBP	1.14(1.018 - 1.276)	0.023	0.005	0
TOMM70	0.861(0.748 - 0.992)	0.038	0.023	
TUBB4A	0.956(0.922 - 0.991)	0.014	0.005	0.407
TUT1	1.522(1.021 - 2.267)	0.039	0.005	0.107
UBTF	1.413(1.117 - 1.787)	0.004	0.039	0.687 *
ULK1	1.382(1.092 - 1.749)	0.007	0.005	0.508 *
VHL	1.272(1.019 - 1.589)	0.034	0.016	0.263
YWHAH	0.926(0.867 - 0.99)	0.023	0.003	0.249
ZC3H12A	0.783(0.624 - 0.982)	0.034	0.004	0.221



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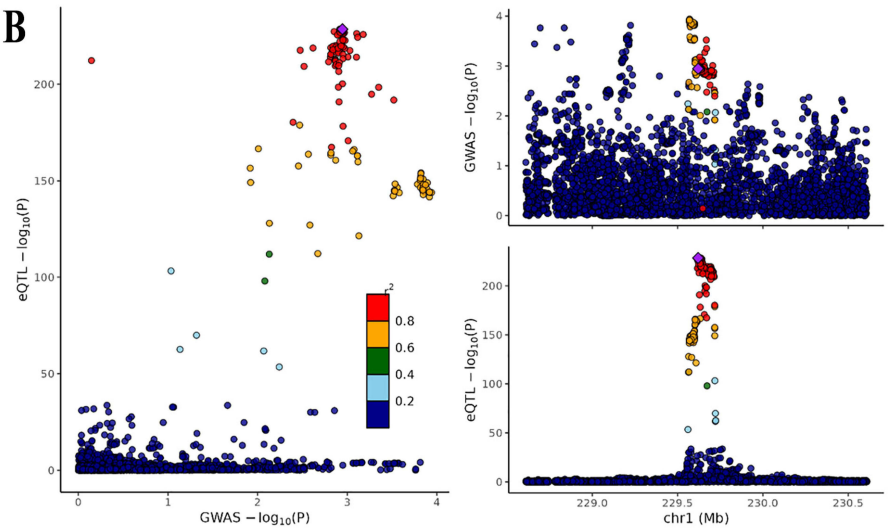

Figure 3 Associations of ER stress–related gene expression with GDM in SMR analysis. (**A**) Forest plot showing genes with significant associations between genetically predicted expression and GDM in the FinnGen_R10_GEST_DIABETES cohort. Asterisks (*) indicate loci with PPH4 ≥ 0.5, indicating suggestive evidence of colocalization. (**B**) Colocalization plots for *NUP133*, showing the overlap between expression quantitative trait loci (eQTL) and GDM GWAS signals.
Abbreviations: OR, Odds Ratio; CI, Confidence Interval.

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A

probeID	OR (95%CI)	p_SMR	Multi_pvalue	PPH4
AGER	0.763(0.634 - 0.918)	0.004	4.3e-06	0
CASP1	1.786(1.103 - 2.89)	0.018	0.018	0.451
CLU	0.715(0.547 - 0.934)	0.014	0.006	0.346
EPHB4	0.812(0.692 - 0.951)	0.01	0.001	0.407
HYOU1	1.25(1.08 - 1.446)	0.003	0.034	0.734 *
MDH1	1.669(1.166 - 2.389)	0.005	0.028	0.167
MICA	1.027(1.003 - 1.051)	0.025	4.5e-06	0
PARP1	0.784(0.655 - 0.937)	0.008	0.009	0.581 *
SPTLC1	0.719(0.586 - 0.883)	0.002	0.019	0.938 *
TNFRSF1A	1.904(1.222 - 2.967)	0.004	0.004	0.745 *

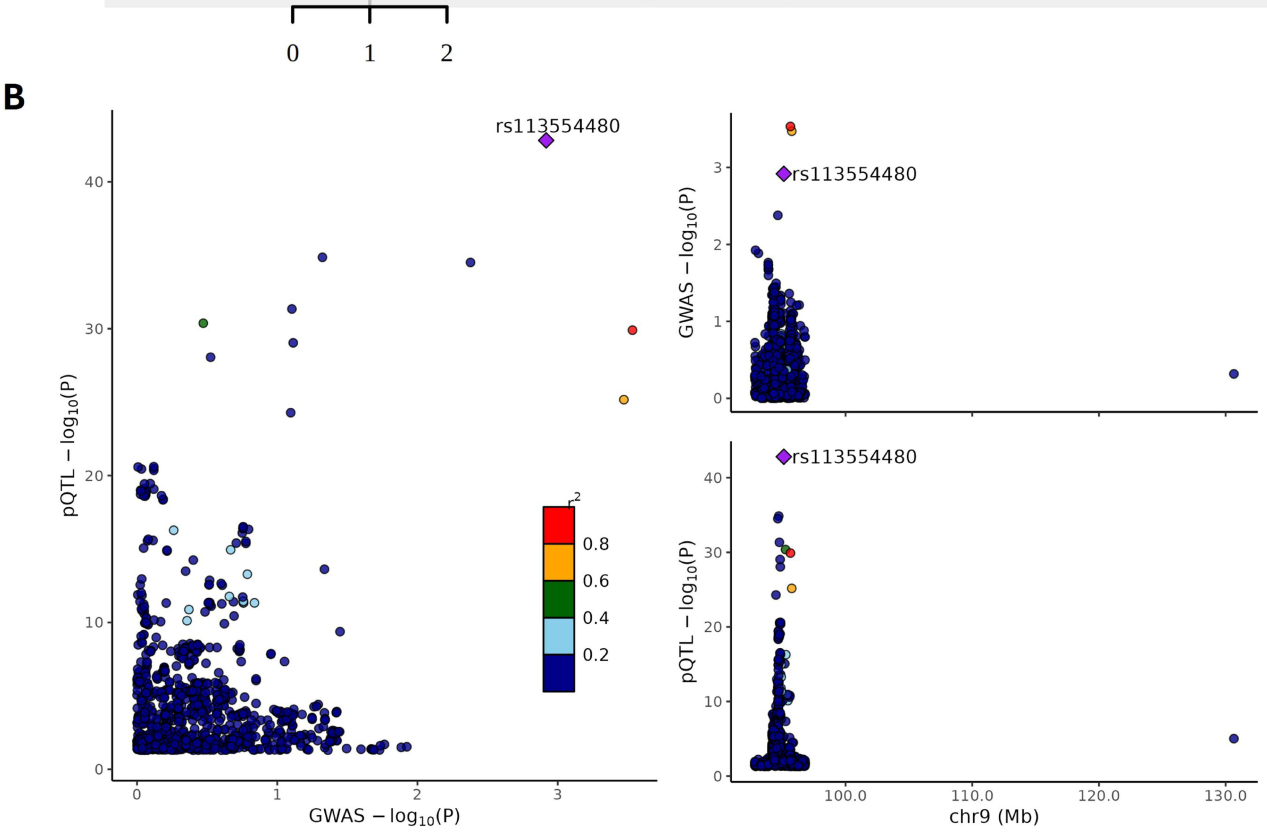

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Figure 4 Associations of ER stress–related protein abundance with GDM in SMR analysis. (**A**) Forest plot showing proteins with significant associations between genetically predicted abundance and GDM in the FinnGen_R10_GEST_DIABETES cohort. Asterisks (*) denote loci with PPH4 ≥ 0.5, indicating suggestive evidence of colocalization. (**B**) Colocalization plots for SPTLC1, showing overlap between protein quantitative trait loci (pQTL) and GDM GWAS signals.
Abbreviations: OR, Odds Ratio; CI, Confidence Interval.

identified 13 genes (*YWHAH, TAPS2, IL32, EPHB4, MICA, UBTF, RNF168, ANKFY1, VHL, NUP133, RYR1, GPX1, FOSL2*) that showed significant SMR associations (P-SMR < 0.05, FDR < 0.05, P-HEIDI > 0.05) with GDM across at least two molecular levels (mQTL, eQTL, or pQTL) (Figure 5A, Table 1 and Supplementary Table S10). Further investigating potential regulatory links using mQTL->eQTL SMR analysis for genes associated with GDM at both methylation and expression levels revealed suggestive evidence for methylation influencing expression for *NUP133, VHL, TAPBP*, and *GPX1* (Figure 5B). There was no intersection between the pQTL findings and the genes highlighted by the mQTL-eQTL integration.

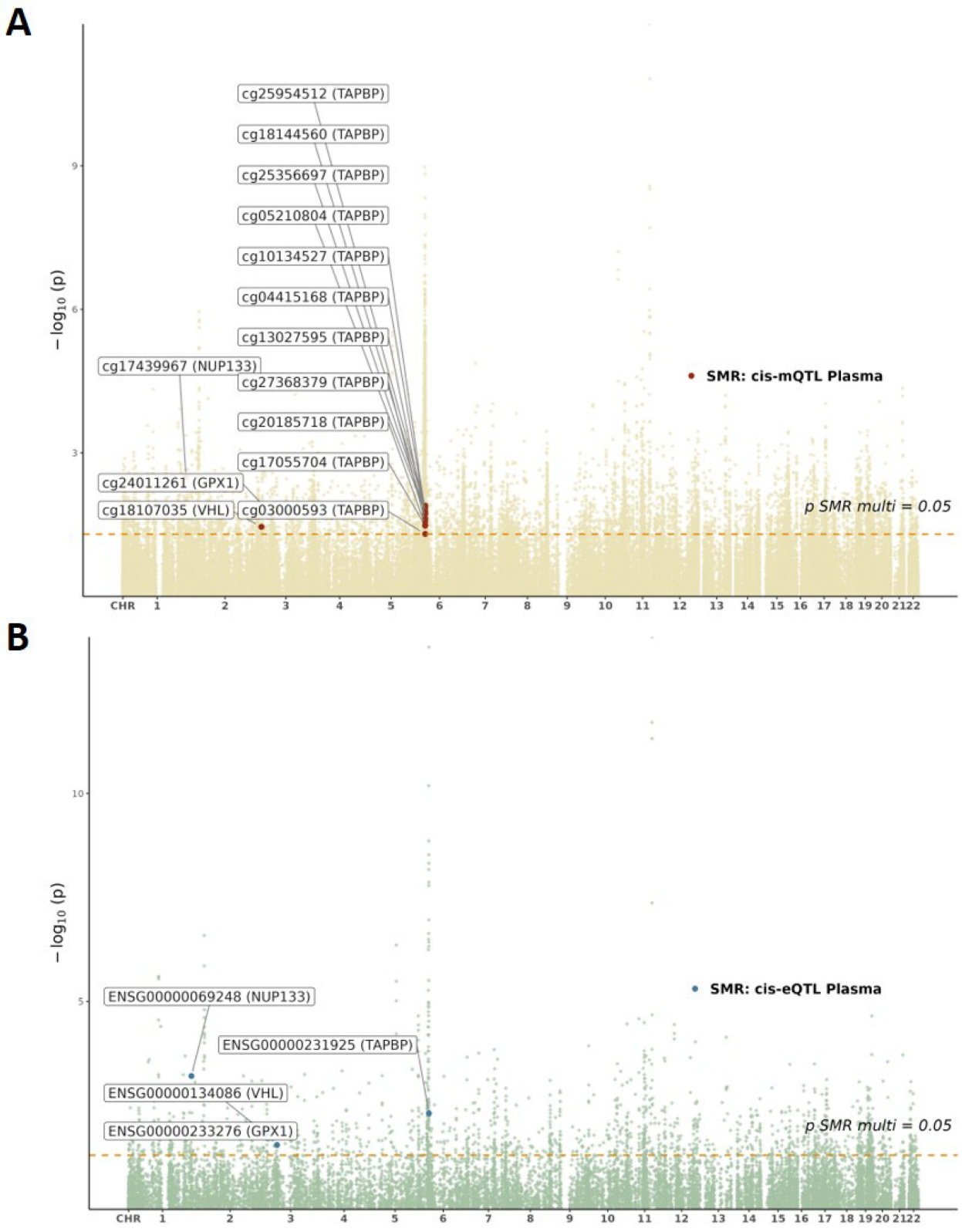

Figure 5 Manhattan plot of associations between ER stress-related molecular features and GDM (FinnGen_R10_GEST_DIABETES). (**A**) Associations of ER stress–related gene methylation with GDM. (**B**) Associations of ER stress–related gene expression with GDM.

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Table 1 Tier of Genetically Predicted Methylation and Expression of Endoplasmic Reticulum Stress-Related Gene with GDM in MR Analysis

Expo_probe	Outco_Gene	p_SMR	Multi_pvalue_SMR	FDR	OR_SMR	95% CI_SMR
cg24011261	GPX1	1.94E-09	1.94E-09	3.01E-08	1.568	1.354–1.816
cg17439967	NUP133	2.23E-12	2.23E-12	5.03E-11	2.993	2.204–4.065
cg03000593	TAPBP	1.91E-25	4.66E-24	1.21E-23	1.69	1.531–1.865
cg04415168	TAPBP	1.20E-17	1.20E-17	4.46E-16	1.98	1.693–2.315
cg05210804	TAPBP	3.51E-41	2.48E-31	4.72E-39	1.415	1.345–1.489
cg18144560	TAPBP	5.42E-33	4.29E-28	5.09E-31	1.547	1.44–1.662
cg10134527	TAPBP	5.28E-38	5.77E-32	6.22E-36	1.462	1.38–1.549
cg20185718	TAPBP	9.67E-08	9.67E-08	1.16E-06	3.282	2.12–5.079
cg13027595	TAPBP	2.82E-22	5.11E-21	1.47E-20	1.79	1.591–2.013
cg25356697	TAPBP	1.72E-22	1.39E-20	9.06E-21	1.779	1.585–1.997
cg17055704	TAPBP	3.91E-34	2.62E-28	3.88E-32	1.506	1.41–1.608
cg25954512	TAPBP	1.88E-27	9.49E-25	1.33E-25	1.637	1.497–1.789
cg27368379	TAPBP	1.05E-11	1.05E-11	2.18E-10	2.495	1.917–3.247
cg18107035	VHL	3.27E-31	3.27E-31	2.81E-29	1.198	1.162–1.235

Supplementary Figure S2A presents the genetic consistency between GDM and eQTL in the vicinity of *NUP133* (ENSG00000069248) using SMR Locus Plot. Supplementary Figure S2B displays the SMR Effect Plot between the eQTL of *NUP133* (ENSG00000069248) and the GWAS for GDM. Supplementary Figure S3A presents the genetic consistency between GDM and mQTL near cg17439967 using a SMR locus plot. Supplementary Figure S3B illustrates the SMR Effect Plot between the mQTL of cg17439967 and the GWAS for GDM. Given that the potential causal association of the *NUP133* gene with GDM was supported by suggestive colocalization evidence at the eQTL level, and our mQTL->eQTL analysis suggested a link between methylation at cg17439967 and NUP133 expression, it is hypothesized that a higher methylation level at cg17439967 may upregulate the expression of the *NUP133* gene, thereby reducing the risk of GDM.

Placental Validation of Prioritized Genes

Considering that all QTL data were derived from blood or plasma, we examined placental endothelial cell transcriptomic data (GSE103552) for validation. *GPX1, TAPBP*, and *VHL* were significantly upregulated, and *NUP133* was down-regulated in GDM compared with controls, consistent with the directions indicated by the SMR analysis (Figure 6).

Discussion

In this exploratory multiomics Mendelian randomization study, we identified four ER stress–related genes—*NUP133, VHL, TAPBP*, and *GPX1*—with suggestive causal links to GDM. *NUP133* was most prominent, supported by colocalization at the eQTL level and by methylation–expression integration. Placental transcriptomic data showed consistent differential expression of these genes in GDM, providing tissue-level support.

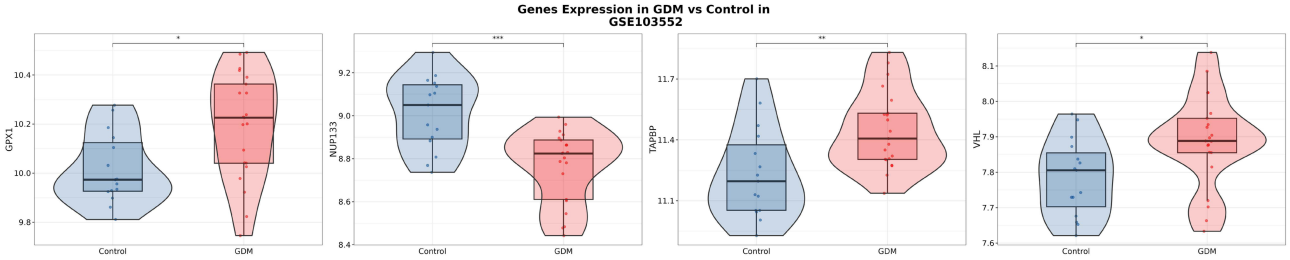

Figure 6 Expression of SMR-prioritized genes in placental endothelial cells (GSE103552). Violin plots showing differential expression of *GPX1, NUP133, TAPBP*, and *VHL* between GDM (pink) and control (blue) groups. *P < 0.05, **P < 0.01, ***P < 0.001.

NUP133 emerged as the most consistent candidate in our multiomics analyses, with converging evidence from methylation and expression data suggesting a protective role in GDM. *NUP133*, a core component of the nuclear pore complex (NPC), is essential for NPC assembly and nucleocytoplasmic transport.[34] Prior studies also highlight its involvement in ER dynamics during mitosis, linking it indirectly to ER-associated processes.[35] Our SMR study suggests that hypermethylation at cg17439967 may upregulate *NUP133* expression, which was associated with reduced GDM risk. This aligns with evidence that *NUP133* regulates mitochondrial and oxidative stress responses through nuclear respiratory factor 1 (NRF1),[36] consistent with the well-established role of oxidative stress in insulin resistance and β-cell impairment.[37] Together, these findings suggest that *NUP133* may exert a protective effect against GDM by modulating stress-response pathways, which warrants functional investigation in pancreatic islets and placental cells.

Our analysis identified *VHL* as a potential risk-associated gene for GDM. *VHL* encodes a tumor suppressor protein known for regulating hypoxia-inducible factors through ubiquitination and proteasomal degradation.[38] Beyond its role in cancer biology,[39] *VHL* has been linked to metabolic stress, as *VHL*-deficient cells show ER stress and activation of the unfolded protein response.[40] Loss of *VHL* impairs endocrine pancreas development by reducing neurogenin-3–positive cells and β-cell maturation.[41] These functions provide biological plausibility for an involvement of *VHL* in glucose regulation. In our study, genetically predicted higher *VHL* expression conferred increased risk of GDM. While this directionality differs from prior reports focusing on *VHL* deficiency, it suggests that both insufficient and excessive *VHL* activity may disrupt β-cell homeostasis through distinct mechanisms. Interestingly, *VHL* mutations underlie Von Hippel–Lindau disease, a rare hereditary tumor syndrome. A published case described a woman with a *VHL* mutation who developed GDM along with other manifestations of VHL disease, providing clinical support for a potential link between *VHL* dysregulation and glucose intolerance in pregnancy.[42] Further functional studies are warranted to clarify the precise role of *VHL* in GDM pathophysiology.

TAPBP also demonstrated a potential causal link to GDM in our analysis. *TAPBP* encodes tapasin, an ER-resident chaperone that stabilizes MHC class I complexes and facilitates antigen presentation,[43] which contributes to maternal–fetal immune tolerance.[44] Epigenetic studies further support its involvement in metabolic and developmental processes. In the Newborn Epigenetics Study (NEST) cohort, methylation of *TAPBP* was associated with offspring weight and blood pressure in a sex-dependent manner, supporting its metabolic relevance.[45] Additionally, night-shift work was associated with altered placental methylation at *TAPBP*, suggesting sensitivity of this gene to environmental stressors relevant to pregnancy.[46] Taken together, these findings suggest that *TAPBP* dysregulation may disrupt immune homeostasis at the maternal–fetal interface, thereby linking ER stress pathways to GDM susceptibility. Future functional studies in placental tissues are needed to clarify its mechanistic role.

GPX1 encodes a ubiquitously expressed antioxidant enzyme that detoxifies hydrogen peroxide and limits oxidative damage.[47] Our analysis showed that genetically predicted higher expression of *GPX1* was associated with increased GDM risk. This result contrasts with prior studies reporting reduced *GPX1* expression in maternal obesity and GDM,[48] in yolk sacs affected by maternal diabetes,[48,49] and in embryos with congenital anomalies from diabetic rat models,[50] which have generally suggested a protective role. One explanation is that *GPX1* regulation may differ across tissues, and blood-based QTLs reflect systemic or compensatory responses that do not mirror placental or pancreatic expression. Alternatively, increased *GPX1* expression in blood may represent an adaptive upregulation to oxidative stress rather than a causal protective mechanism. Reduced expression of M3 muscarinic acetylcholine receptors (M3 mAChRs) in GDM placentas has recently been linked to vascular dysfunction and placental insufficiency,[51] and disruption of *GPX1*-dependent antioxidant systems has been shown to aggravate cholinergic receptor downregulation, including M3 mAChRs,[52] under methamphetamine-induced oxidative stress. These findings suggest that *GPX1* may influence cholinergic signaling pathways relevant to placental function. Thus, *GPX1* appears to play a complex, context-dependent role in GDM pathophysiology, reinforcing the need for functional validation in the placenta and other metabolically active tissues.

At the integrative level, our analysis revealed limited overlap between eQTL and pQTL findings. This likely reflects the complexity of gene regulation, as post-transcriptional and post-translational mechanisms can decouple mRNA abundance from protein levels. Smaller sample sizes of current pQTL resources compared with large eQTL consortia may also have reduced statistical power. These patterns emphasize the value of multiomics integration and highlight the need for larger proteomic datasets to clarify how ER stress–related variation translates into protein-level effects relevant to GDM.

Strengths and Limitations

This study systematically evaluated ER stress–related genes in GDM using a multiomics SMR framework and colocalization. Large GWAS datasets from European and East Asian populations increased power and generalizability. Evidence was cross-checked across methylation, expression, and protein QTLs, and further supported by placental endothelial cell transcriptome validation. However, several limitations should be acknowledged. Firstly, all QTL datasets were derived from blood or plasma, which may not capture regulatory processes in metabolically active tissues such as the pancreas, liver, adipose tissue, and placenta that are involved in GDM pathophysiology. Second, the initial gene set was obtained from GeneCards based on relevance ranking. Although enrichment analyses confirmed links to ER stress–related pathways, this approach does not provide full biological validation. Third, our analyses were restricted to cis-QTLs, which reduces pleiotropy but prevents the detection of trans-effects. Fourth, incorporating DNA methylation data into SMR may have reduced power because only associations passing stringent SMR and HEIDI criteria were retained. Fifth, replication was limited by the relatively small sample sizes of available cohorts, particularly GCST90043951, which weakened statistical confirmation. Sixth, no formal sample size or power calculations were performed, as this study relied on secondary analyses of publicly available GWAS and QTL datasets with fixed sample sizes. Although the large scale of these resources provides substantial power, the absence of de novo power estimation remains a limitation, particularly for small effect sizes. Finally, we did not perform conditional analyses (eg, COJO) to exclude potential secondary association signals. Although the HEIDI test mitigates this risk to some extent, complex loci with multiple causal variants cannot be fully resolved.

Conclusion

This study systematically evaluated ER stress–related genes in GDM using a multiomics SMR and colocalization framework, supported by placental transcriptomic validation. We identified *NUP133, VHL, TAPBP*, and *GPX1* as candidate genes with suggestive causal roles, suggesting potential links between ER stress, immune regulation, oxidative balance, and GDM pathogenesis. These findings provide novel genetic insights into GDM and generate testable hypotheses for future functional studies in disease-relevant tissues. Further work integrating larger QTL resources, conditional analyses, and experimental validation will be essential to refine these causal inferences and clarify the underlying biological mechanisms.

Abbreviations

GDM, Gestational diabetes mellitus; QTL, Quantitative trait loci; GWAS, Genome-wide association studies; SMR, Summary-data-based Mendelian Randomization; ER, Endoplasmic reticulum; MR, Mendelian randomization; IVs, Instrumental variables; Nrf1, Nuclear respiratory factor; VHL, Von Hippel-Lindau; GPX1, Glutathione peroxidase 1.

Data Sharing Statement

All data generated or analysed during this study are included in this published article.

Ethics Approval and Informed Consent

This study used only publicly available summary-level data from previously published GWAS with existing ethical approval. According to Article 32 of the Measures for the Ethical Review of Life Science and Medical Research Involving Human Beings (2023, China), such analyses are exempt from additional ethical review. The Medical Ethics Committee of the Second Hospital of Jilin University confirmed this exemption (approval date: August 14, 2025; Serial Number: 2025013).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors confirm that there is no conflict of interest related to the manuscript.

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