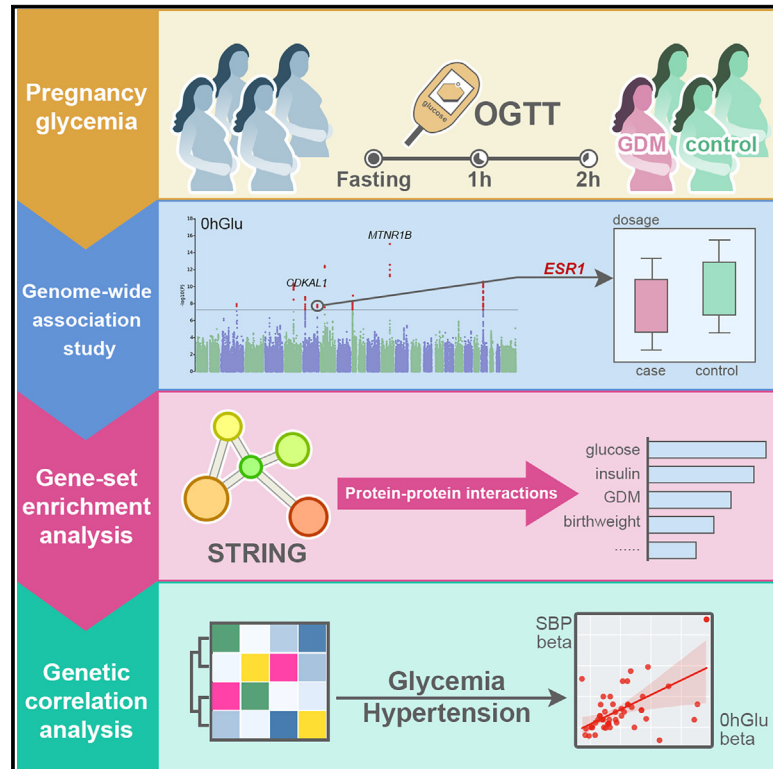


Novel insights into the genetic architecture of pregnancy glycemic traits from 14,744 Chinese maternities

Graphical abstract



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In brief

Zhu et al. analyzed 14,744 Chinese pregnant women, identifying 25 genetic associations with glycemic traits via GWAS. They discovered a novel link between fasting glucose and the *ESR1* gene and validated this by an independent study. Further analyses provided a genetic insight into the relationship between pregnancy glycemic traits and hypertension.

Highlights

- Glycemic traits GWAS involving 14,744 Chinese pregnant women identify 25 genetic loci
- Newly discovered *ESR1* association provides insights into maternal glucose levels
- Highlighted a potential causal relationship between hypertension and glycemic traits



Article

Novel insights into the genetic architecture of pregnancy glyceemic traits from 14,744 Chinese maternities

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SUMMARY

Glyceemic traits are critical indicators of maternal and fetal health during pregnancy. We performed genetic analysis for five glyceemic traits in 14,744 Chinese pregnant women. Our genome-wide association study identified 25 locus-trait associations, including established links between gestational diabetes mellitus (GDM) and the genes *CDKAL1* and *MTNR1B*. Notably, we discovered a novel association between fasting glucose during pregnancy and the *ESR1* gene (estrogen receptor), which was validated by an independent study in pregnant women. The *ESR1*-GDM link was recently reported by the FinnGen project. Our work enhances the findings in East Asian populations and highlights the need for independent studies. Further analyses, including genetic correlation, Mendelian randomization, and transcriptome-wide association studies, provided genetic insights into the relationship between pregnancy glyceemic traits and hypertension. Overall, our findings advance the understanding of genetic architecture of pregnancy glyceemic traits, especially in East Asian populations.

INTRODUCTION

Pregnancy glyceemic traits (e.g., fasting glucose) play a critical role in assessing and managing maternal health during pregnancy and serve as indicators of glucose metabolism and gestational diabetes mellitus (GDM).¹ Understanding the genetic basis of pregnancy glyceemic traits may provide insights into the underlying genetic factors influencing maternal glucose regulation during pregnancy. To date, numerous genome-wide association studies (GWASs) have identified candidate genetic variants associated with glyceemic traits in normal populations.^{2–4} How-

ever, there has been limited research on glyceemic traits during pregnancy, with only a few studies exploring this area, especially in the Chinese population.^{5–7}

Clinical studies have focused on potential risk factors and adverse outcomes associated with abnormal glucose levels during pregnancy. In general, maternal age, obesity, polycystic ovary syndrome, and previous history of hyperglycemia increase a woman's susceptibility to developing GDM.^{8–10} Elevated maternal blood glucose levels lead to increased fetal growth, causing macrosomia, infants born large for their gestational age, and a higher likelihood of cesarean section.^{11–13} Moreover,



hyperglycemia during pregnancy is often associated with other complications during and after pregnancy, notably hypertension.^{11,14} However, the genetic architecture underlying the relationship between pregnancy glycemic traits and hypertension has not been fully explored.

Non-invasive prenatal testing (NIPT) has emerged as an effective approach to reduce the birth rate of children with fetal autosomal trisomies.¹⁵ NIPT involves sequencing cell-free DNA fragments, including fetal cell-free DNA, obtained from maternal peripheral plasma utilizing next-generation sequencing technology. Compared to the traditional genetic research pipeline of “sampling, sequencing, and analyzing,” using NIPT data significantly reduces both sequencing time and research costs, as genotype data are generated alongside clinical prenatal tests. Additionally, with the growing popularity of NIPT technology worldwide, it has generated an immense amount of genotype data, nearly ten times that of whole-genome sequencing samples.¹⁶ Previously, we demonstrated the validity of using large-scale NIPT sequencing data for population genetics and GWASs.^{17,18}

In this study, we investigate the genetic basis of five pregnancy glycemic traits using NIPT sequence data, which include three glucose levels based on the oral glucose tolerance test (OGTT), the area under the curve of these three measurements, and GDM. In total, we identified 25 locus-trait associations, including several well-known examples such as the association of GDM with the genes *CDKAL1* and *MTNR1B*, and fasting glucose levels with the gene *PCSK1*. A significant new association was discovered between fasting glucose levels during pregnancy and the *ESR1* gene (estrogen receptor). This finding was subsequently confirmed by an independent study in pregnant women. Notably, the *ESR1*-GDM association was recently reported by the FinnGen project.¹⁹ Our work not only enriches the findings specific to East Asian populations but also underscores the necessity for independent studies. Additional analyses, including genetic correlation, Mendelian randomization (MR), transcriptome-wide association study (TWAS), and drug target enrichment analysis (DTEA), have provided deeper genetic insights into the relationship between glycemic traits during pregnancy and hypertension. These findings offer insights into the genetic landscape of pregnancy glycemic traits, particularly within East Asian populations.

RESULTS

Participants and data types

In this study, we used two data types: whole-genome sequence (WGS) data from the NIPT test and pregnancy glycemic traits from the OGTT test. For participants with NIPT WGS data, we included those with a sequencing depth greater than 0.05 and a mapping rate above 90%, resulting in a cohort of 38,668 individuals. All were used in genotype imputation to ensure the highest possible accuracy. Additionally, there were 21,813 subjects who underwent the OGTT and had glycemic measurements. We employed all 21,813 subjects for subsequent regression analysis involving other clinical phenotypes. For the GWAS analysis, we used participants who possessed both NIPT sequence data and glycemic data, totaling 14,889 individuals (Figure S1A).

For the 38,668 individuals with NIPT sequence data, we assessed gestational age at the time of the NIPT test and fetal fraction in maternal plasma (Figure S1B), as provided in the NIPT reports. Specifically, gestational age information was available for 28,037 participants, with an average of 16.16 weeks (113.13 days). Additionally, fetal fraction data were obtained for 15,837 participants, with an average of 9.76%, aligning with our literature review (STAR Methods). For the 21,813 subjects with pregnancy glycemic traits, we provided distributions of maternal information and birth outcomes (Figures S1C–S1D). Maternal characteristics included age (29.58 ± 4.03 years), BMI (21.39 ± 3.08 kg/m²), first-time pregnancy status, and hypertension. Birth outcomes included gestational age (38.74 ± 1.58 weeks), birth weight (3286.43 ± 473.71 g), delivery type, and newborn gender.

Pregnancy glycemic traits

Applying the criteria of 0hGlu ≥ 5.1 mmol/L, 1hGlu ≥ 10.0 mmol/L, and 2hGlu ≥ 8.5 mmol/L, 2,502 out of 21,813 pregnant women were diagnosed with GDM, resulting in a disease rate of 11.47% (Figure S2A). This rate is slightly lower than the 14.8% reported in a 2019 meta-analysis conducted in mainland China.²⁰ After integrating NIPT sequence data, we retained 14,744 subjects, among which 1,619 were GDM cases, and 13,125 were controls. This subset was used for the GWAS analysis. For the 0hGlu, 1hGlu, and 2hGlu measurements, the available sample sizes for the GWAS were 14,889, 14,790, and 14,760, respectively. We provided the distributions of the three glucose levels among the GDM case and control groups (Figure S2A).

Additionally, we computed the area under the curve (AUC) for the three OGTT glucose levels, which served as a metric for glucose tolerance during pregnancy and was denoted as GDM_{AUC} (Figure S2B). The sample size for the GWAS of GDM_{AUC} was 14,726.

Regression analysis between GDM and multi-phenotypes

Using a significance threshold of $5.15E-4$, univariate regression analysis identified 26 risk factors for GDM and 29 for GDM_{AUC} (Tables 1 and S1 and Figure S2C). Among the maternal information, age was most significant (for GDM: odds ratio [OR] = 1.112 and p value = $4.19E-98$, for GDM_{AUC}: beta = 0.135 and p value = $5.20E-283$), followed by BMI, multiparity, and hypertension. Among the 93 early-stage laboratory tests, the most significant features were blood glucose (GLU), prealbumin (PA), γ -glutamyl transferase (GGT), urine glucose (GLU_U), and uric acid, being correlated with GDM in previous studies.^{21–29} In the multivariate regression analysis, to ensure an effective sample size of 3,000 for each variable, we tested 20 out of 26 factors for GDM and 24 out of 29 for GDM_{AUC}, identifying five and nine significant risk factors, respectively (Tables S2 and S3). Notably, age was the only significant maternal factor for GDM multivariately (p value = $2.78E-10$).

Women with GDM tend to have shorter gestational lengths (OR = 0.662, p value = $8.84E-35$), undergo cesarean sections at higher rates (OR = 1.481, p value = $2.76E-19$), and deliver a low-birth-weight (OR = 1.517, p value = $3.26E-06$) or macrosomic infant

Table 1. Regression results for GDM/GDM_{AUC} and risk factors and outcomes

Category	Traits	Mean ± std./n (%)		Regression results of GDM				Regression results of GDM _{AUC}				
		All samples	In GDM control	In GDM case	n	OR	Std.	p value	n	Beta	Std.	p value
Risk factors	age (years)	29.58 ± 4.03	29.37 ± 3.94	31.20 ± 4.32	21,808	1.112	0.005	4.19E-98	21,786	0.135	0.004	5.20E-283
	BMI (kg/m ²)	21.39 ± 3.08	21.22 ± 2.98	22.67 ± 3.45	19,161	1.143	0.007	3.42E-87	19,140	0.159	0.005	3.04E-199
	first-time pregnancy, n (%)	7,769 (55.73%)	7,176 (57.09%)	593 (43.25%)	13,941	0.573	0.057	2.98E-22	13,931	-0.539	0.038	3.36E-46
Outcomes	hypertension, n (%)	308 (1.41%)	233 (1.21%)	75 (3.00%)	21,813	2.530	0.134	5.12E-12	21,791	1.143	0.129	1.06E-18
	gestational weeks (weeks)	38.74 ± 1.58	38.78 ± 1.55	38.37 ± 1.74	21,808	0.662	0.033	8.84E-35	21,786	-0.077	0.005	1.88E-60
	birth weight (g)	3,286.43 ± 473.71	3,287.08 ± 464.74	3,281.42 ± 537.96	21,672	0.003	10.096	5.75E-01	21,650	4.475	1.424	1.68E-03
	low birthweight, n (%)	979 (4.52%)	821 (4.28%)	158 (6.35%)	21,672	1.517	0.090	3.26E-06	21,650	0.069	0.013	1.48E-07
	giant baby, n (%)	1,054 (4.86%)	889 (4.63%)	165 (6.63%)	21,672	1.462	0.088	1.43E-05	21,650	0.100	0.012	1.37E-16
newborn gender, n (%)	c-section, n (%)	11,789 (54.10%)	10,225 (53.01%)	1,564 (62.56%)	21,790	1.481	0.044	2.76E-19	21,768	0.096	0.006	4.11E-52
	newborn gender, n (%)	10,418 (48.07%)	9,218 (48.05%)	1,200 (48.25%)	21,673	1.008	0.043	8.47E-01	21,651	0.005	0.006	3.89E-01

Notes: in the columns of "All sample," "In GDM control" and "In GDM case," for quantitative traits, we provide the mean and standard deviation of each variable; for dichotomous traits, we provide the number of positive samples for the corresponding trait in each group and the percentage of positive samples to the total sample size in that group.

(OR = 1.462, *p* value = 1.43E-05) (Table 1). The gender of newborn is not a significant birth outcome with GDM (OR = 1.008, *p* value = 0.847). At a 3.33E-03 (= 0.05/15) threshold, four diagnoses were significantly associated with GDM and seven with GDM_{AUC}, including pregnancy with uterine scar and intrauterine distress (Table S4). Additional diagnoses significantly associated with GDM_{AUC} were eclampsia (*t* = 5.272, *p* value = 1.37E-07), pregnancy with vomiting (*t* = -3.924, *p* value = 8.75E-05), and gestational hypertension (*t* = 3.795, *p* value = 1.48E-04) (Figure S2D). Numerous studies have reported positive associations between preeclampsia/eclampsia and GDM.^{14,30,31}

Genotype imputation performance

Note that, from December 2018, the sequencing depth of the NIPT product was increased. We excluded 1,719 samples with unknown test dates, resulting in 10,795 pregnant women with an average depth of 0.1049× before December 2018 and 26,154 participants with an average depth of 0.1644× afterward, with an overall average depth of 0.1525× (Figure S3A).

After STITCH imputation (STAR Methods), we obtained a total of 8,134,302 SNPs. Filtering out SNPs with a Hardy-Weinberg equilibrium (HWE) *p* value < 1E-6 and minor allele frequency (MAF) < 0.05 resulted in 2,818,480 high-quality SNPs. We assessed imputation accuracy of these high-quality SNPs using Pearson's correlation between the imputed genotype dosage and the original high-depth genotype of 30 Chinese samples from the 1000 Genomes Project (1KGP). On average, the imputation accuracy *R* was 87.61%, and *R*² was 79.34% (Figure S3B). More imputation evaluation results, including additional datasets and results at the specific SNP level, are described in our companion work.³² Additionally, we computed the Pearson's correlation (*R*²) for the frequency of non-reference alleles of these high-quality SNPs in our dataset and the East Asian samples from the 1KGP (Figure S3C). The *R*² of 0.97 indicates a high consistency of our imputed variants with those in the 1KGP.

We utilized BaseVar for population variation detection and conducted principal component analysis (PCA) on the identified variants to reveal population structure (STAR Methods). Additionally, we acquired population structure information derived from the PCA plot of 585 samples representing 30 administrative divisions in China. These divisions were further categorized into seven main geographical regions spanning from north to south China. These samples were independently recruited in a study approved by the institutional review board at BGI (BGI-IRB 21163) and authorized by the Human Genetic Resources Administration of China ([2022] CJ0197). We projected the principal components plot of the main data onto the reference data and presented the plot in Figure S3D. Notably, the main samples exhibited a significant overlap with samples from the Huazhong area, which corresponds to central China and aligns with the location of Wuhan.

GWAS analysis of glycemic traits

We performed GWAS analysis for three OGTT traits, GDM, and GDM_{AUC} (Figures 1 and 2 and Table S5). The full GWAS summary statistics for these traits are available on our MANE (Maternal

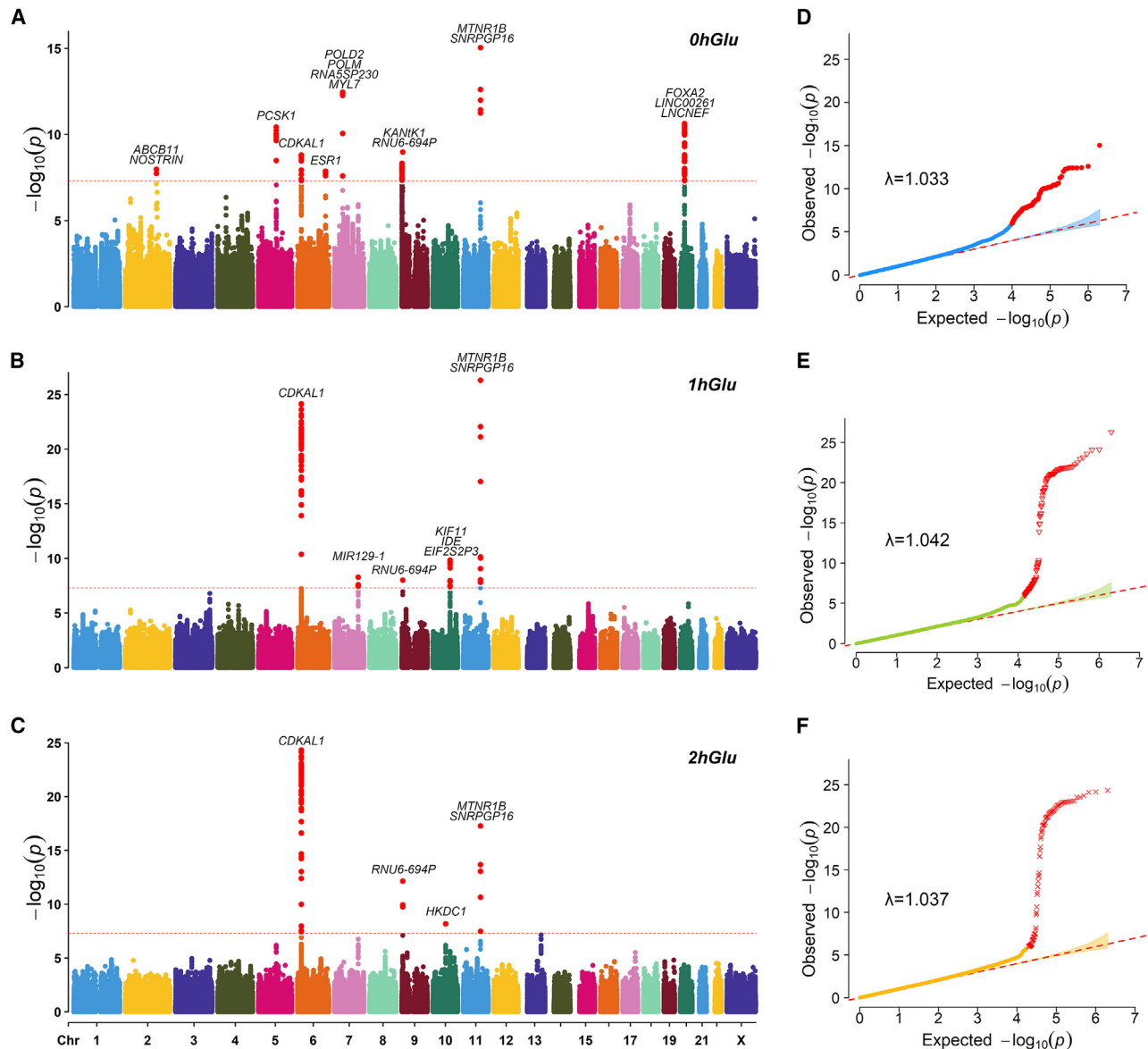


Figure 1. The GWAS results of three OGTT measurements

(A–C) Manhattan plots of three OGTT measurements; SNPs that pass the genome-wide threshold ($5E-8$) were colored in red. (D–F) QQ-plots of three OGTT measurements with genomic inflation factor λ .

and Neonatal) PheWeb website.³³ In total, we identified 25 trait-locus associations, with many previously associated either with pregnancy glycaemic traits (e.g., *CDKAL1*, *MTNR1B*, and *PCSK1*)⁶ or with glucose levels in the general population (e.g., *POLD2*).^{34–36}

Two genes, *CDKAL1* and *MTNR1B*, were consistently associated with all five traits, as previously reported to be associated with GDM.^{5,7,19,37,38} *CDKAL1* has been associated with proinsulin conversion defect and glucose-stimulated insulin response defect.^{39,40} *MTNR1B* (melatonin receptor 1B) encodes one of the two high-affinity forms of a receptor for the hormone melatonin, playing a role in glucose homeostasis.⁴¹

For GDM status, the lead SNPs mapped on the two genes were rs35261542 (*CDKAL1*, p value = $3.88E-12$) and rs3781637 (*MTNR1B*, p value = $3.29E-09$), respectively. In Table S6, we provided details on the two SNPs and known GDM-associated SNPs. Notably, rs35261542 was significant in the mixed population study,³⁷ while rs3781637 has not previously been reported in association with GDM. Additionally, allele frequency of rs3781637 significantly differed between East Asian and European populations.

The heritabilities (standard error) for 0hGlu, 1hGlu, 2hGlu, GDM, and GDM_{AUC} were 0.1308 (0.0372), 0.1395 (0.04), 0.0611 (0.04), 0.0817 (0.0977), and 0.1429 (0.0421), respectively.

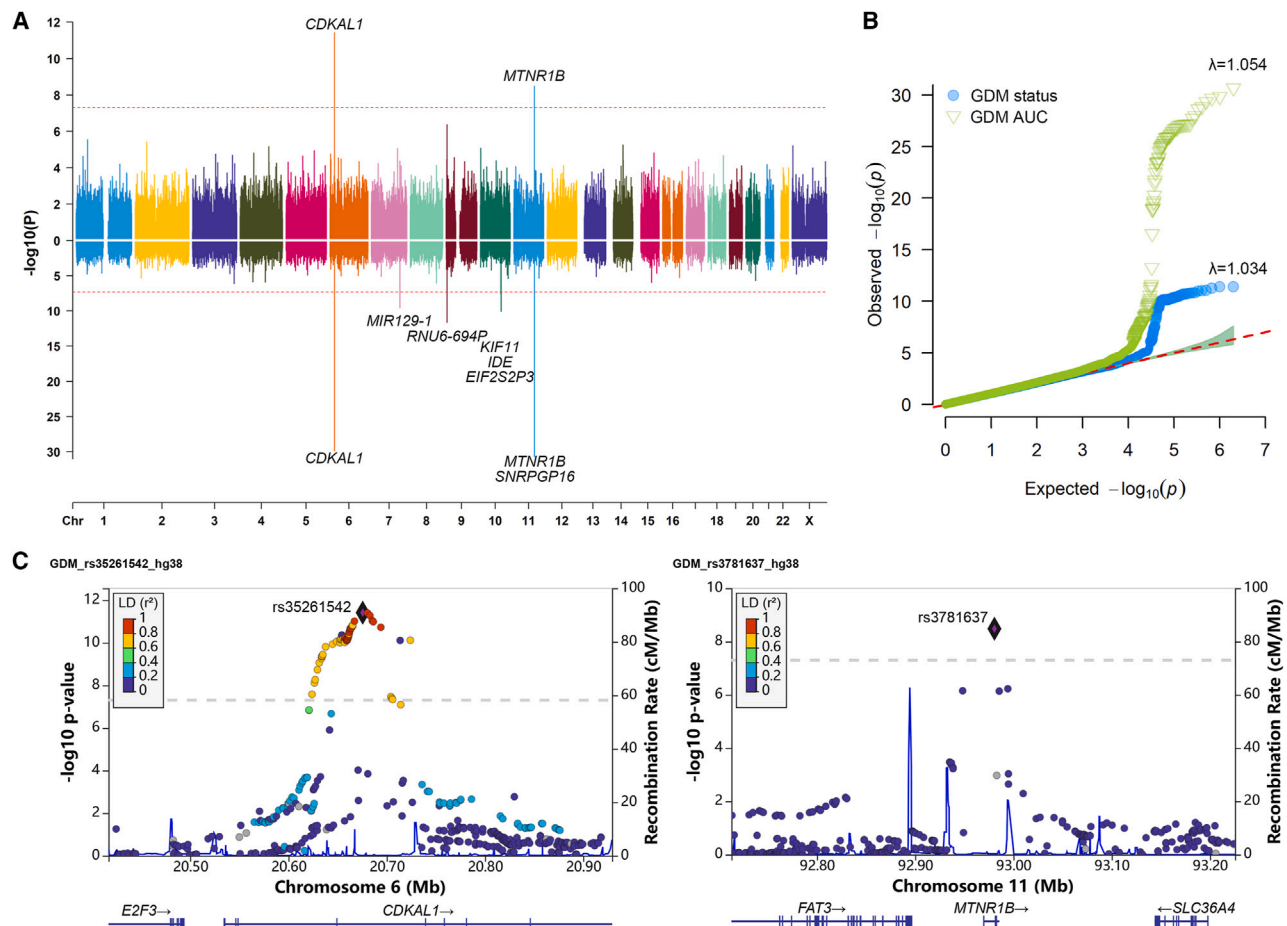


Figure 2. The GWAS results of GDM status and GDM_{AUC}

(A) Mirrored Manhattan plot indicates the GWAS results of GDM status and GDM_{AUC} , located on top and bottom, respectively; the red dashed line represents the genome-wide significance threshold ($5E-8$).
 (B) QQ plot for GDM status and GDM_{AUC} with genomic inflation factor λ .
 (C) Regional plots of two loci *CDKAL1* and *MTNR1B* for testing GDM.

Validation study

Here, we selected a recently published study involving 22,882 Chinese pregnant women from Shenzhen Bao'an Women's and Children's Hospital,⁷ referred to as the Shenzhen cohort. This cohort underwent the same OGTT protocol as our study participants. We downloaded their GWAS results of fasting glucose, 1-h and 2-h glucose levels after consuming 75 g of sugar, and GDM status from the GWAS catalog.

In detail, 20 out of the 25 trait-locus associations were subject to replication, as 5 GDM_{AUC} -associated loci were removed because the Shenzhen cohort did not test for GDM_{AUC} . For each of the 20 trait-locus associations, we examined a flanking window of 500 kb in the Shenzhen cohort. An association was considered replicated if at least one SNP within this window had a p value less than $1E-4$ and a linkage disequilibrium (LD) r^2 greater than 0.1. Among the 20 associations, 19 were successfully replicated, with the sole exception being 1hGLU-*MIR129-1* (Figure 3). The gene *MIR129-1* has previously been reported to be associated with type 2 diabetes in

East Asian populations.^{42,43} The replication study underscores the robustness of our GWAS findings on the pregnancy glycaemic traits.

A novel gene *ESR1*

We identified *ESR1* as a candidate gene associated with fasting glucose (0hGlu), with lead SNP rs3020430 (p value = $1.36E-08$). Recently, the FinnGen project, with 12,332 GDM cases and 131,109 parous female controls, identified *ESR1* as a novel gene associated with GDM through GWAS, with rs537224022 as the lead SNP.¹⁹ The MAF of this SNP is 0.006 in European populations and undetectable (0.00) in East Asian populations, which explains its absence in our dataset.

The gene *ESR1* encodes an estrogen receptor and ligand-activated transcription factor, which regulates estrogen-inducible genes that play a role in growth, metabolism, sexual development, gestation, and other reproductive system.⁴⁴ To validate our finding of the effects of *ESR1* on fasting glucose,

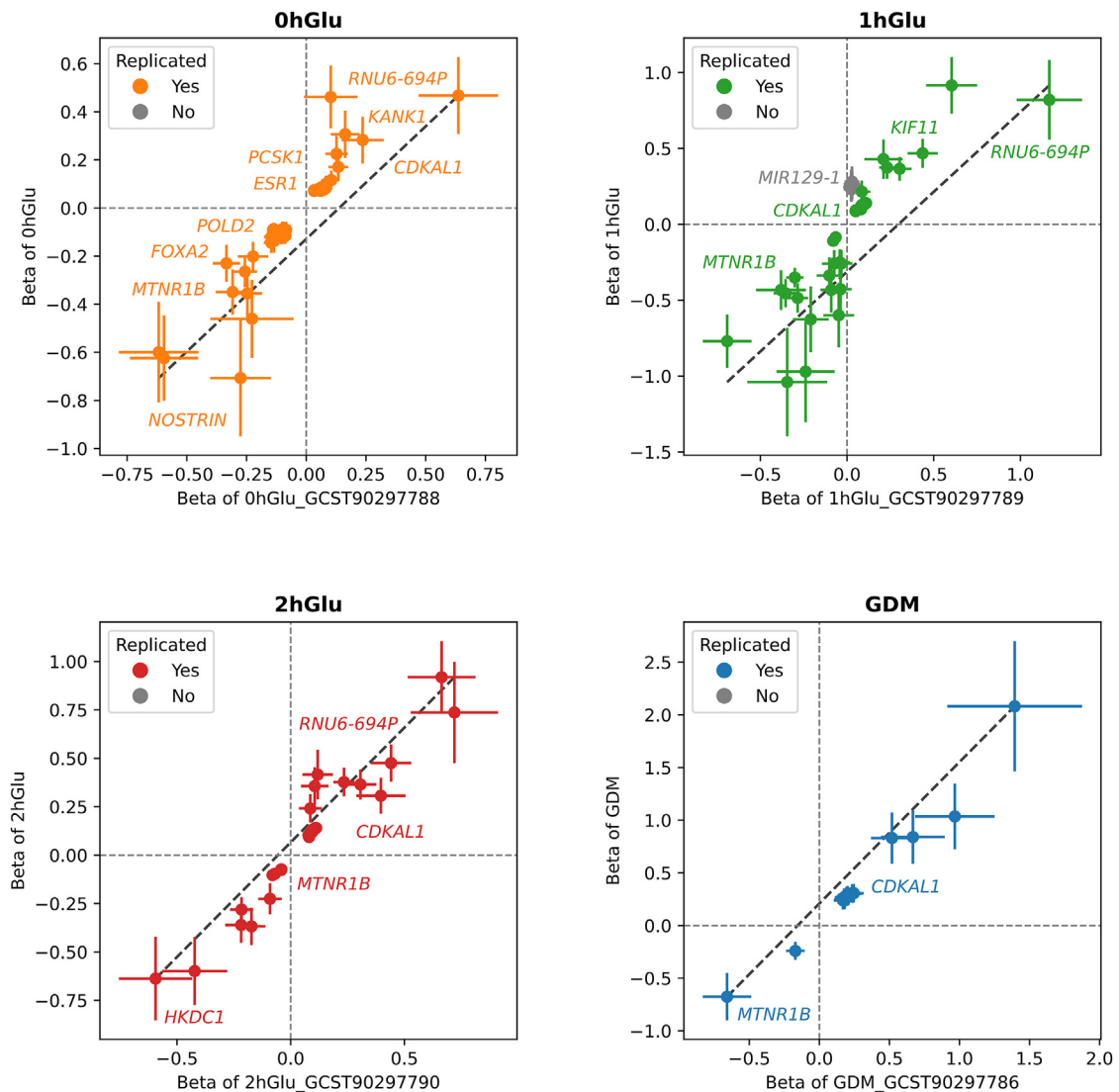


Figure 3. The comparison between the GWAS results in this study and the replication study

The forest plots between this study and the replication study, for 0hGlu, 1hGlu, 2hGlu, and GDM, respectively; the position of the dots represents the beta values of the two studies, and the lines represent the range of one standard deviation above and below; the successfully replicated variants in both studies are marked in color, or otherwise are marked in gray.

we performed a GWAS-GWAS colocalization analysis⁴⁵ with the Shenzhen cohort⁷ using the *coloc::coloc.abf*⁴⁶ in R. In the Shenzhen cohort's GWAS summary of fasting glucose, the lead SNP in the *ESR1* gene is rs9322351, with a p value of $6.91E-06$ (in LD with rs3020430, $r^2 = 0.4008$, in the 1KGP EAS samples, GRCh38). The posterior probability of H4 (indicating one common causal variant) was 0.84, surpassing the threshold of 0.75, signifying a shared causal signal. We employed the R function *locuscompare*⁴⁷ to generate regional plots (Figure S4). Additional literature has reported associations between *ESR1* and pregnancy, such as its link to susceptibility to recurrent pregnancy loss in a Tunisian study,⁴⁸ as well as estrogen dysregulation contributing to preeclampsia occurrence.^{49,50}

Upon analyzing all 1,536 SNPs within the *ESR1* gene from our dataset, we conducted annotation analysis to glean functional insights for each mutation site (Figure 4A). Notably, 96.2% of the SNP sites are situated within intron regions, lacking direct involvement in protein encoding. Upon further investigation beyond our dataset to encompass all SNPs in *ESR1*, we observed a region within the *ESR1* association peak that falls within exon 5 (Figure 4B). This region harbors three pathogenic/likely pathogenic variants (rs397509428, rs1131692059, and rs1057519827) according to the ClinVar database,⁵¹ with linked diseases encompassing estrogen resistance syndrome and breast neoplasms.^{52,53} We hypothesize that these SNPs may exert influence on glycemic levels during pregnancy by modulating estrogen resistance and interacting with insulin levels.^{54–56}

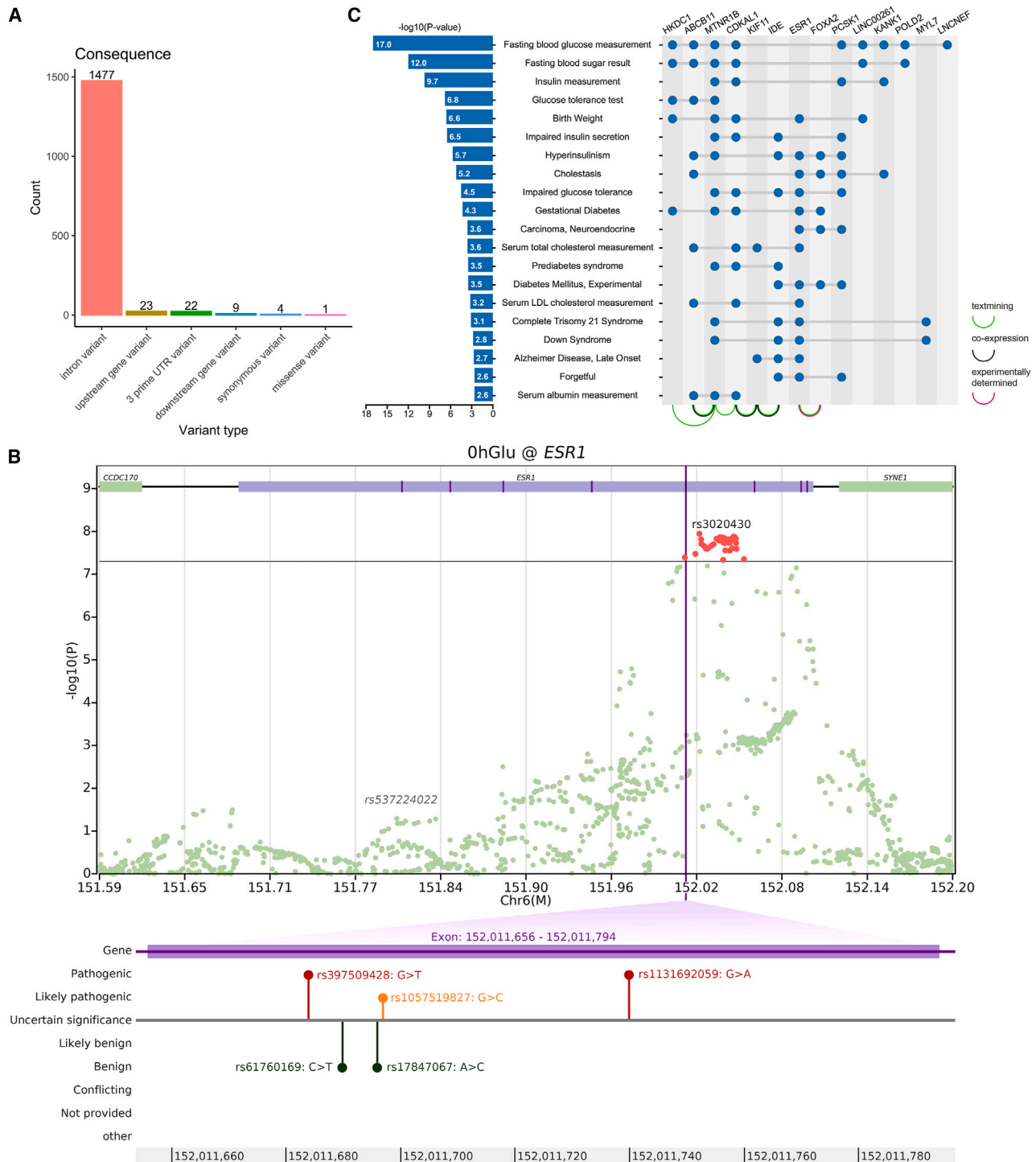


Figure 4. The GWAS result and annotation information on *ESR1* and the results of gene set enrichment analysis

(A) The bar plot showing the variant count of each category for functional annotation on *ESR1*.

(B) The regional plot for OhGlu on *ESR1*, with the fifth exon and potential pathogenic variants indicated below.

(C) The results of the gene set enrichment analysis for all genes correlated to glycemic traits, the $-\log_{10} p$ values are displayed on the left side using a bar plot, the genes corresponding to each pathway are marked with blue dots on the right side, and the interactions between each gene are indicated with curves below.

Gene set enrichment analysis

A total of 21 mapped genes associated with pregnancy glycemic traits were identified, including 14 protein-coding genes, 4 pseudogenes, and 3 non-coding RNAs (Table S5). Since PPI (protein-protein interactions) analysis excludes pseudogenes and non-coding RNAs, the 14 protein-coding genes were included in the STRING PPI analysis (Figure 4C). The majority of PPIs were derived from text mining, with a minority being co-expressed and one interaction experimentally determined. Among the gene pairs potentially influencing pregnancy glycemic levels through co-expression are *MTNR1B* and *ABCB11*, *CDKAL1* and *KIF11*, as well as *KIF11* and *IDE*. Notably, an experimentally determined interaction was observed between *ESR1* and *FOXA2*. Studies using a *Foxa2* knockout mouse model have demonstrated that *Foxa2* regulates the estrogen responsiveness and influences *ESR1* transcriptional activity in the uterus.⁵⁷

At a significance threshold of p value <0.01 , requiring a minimum gene count of 3, and an enrichment factor >1.5 , the gene set enrichment analysis identified enrichment in 20 functional pathways (Figure 4C). The top five pathways included fasting blood glucose measurement, fasting blood sugar result, insulin measurement, glucose tolerance test, and birth weight. Notably, genes such as *HKDC1*, *MTNR1B*, *CDKAL1*, *ESR1*, and *LINC00261* were found in the birth weight pathway. Additionally, the pathway associated with gestational diabetes featured genes including *HKDC1*, *MTNR1B*, *CDKAL1*, *ESR1*, and *FOXA2*. Both the birth weight and the gestational diabetes pathway involved gene *ESR1*, suggesting its essential role in maternal and fetal conditions during pregnancy.

Genetic correlation analysis

Building upon our earlier discovery that *ESR1* is a gene associated with pregnancy glycemic traits and considering the prominence of hormone signaling revealed in the pathway-based analysis, we delved deeper into the association between *ESR1* and pregnancy glycemic traits (Figure 5A). First, we evaluated the genetic correlation between established *ESR1*-associated phenotypes and pregnancy glycemic traits, including 0hGlu, GDM, and GDM_{AUC} . The *ESR1*-associated phenotypes were acquired by searching “*ESR1*” in the GWAS catalog,⁵⁸ and we downloaded the available full summary statistics. In total, GWAS summaries for 15 phenotypes were downloaded, encompassing traits such as height (GCST90018959), systolic blood pressure (GCST90018972), and pulse pressure (GCST90018970), among others (Table S7).

At a significance threshold of $3.33E-3$ ($= 0.05/15$), we observed two significant associations for 0hGlu, including systolic blood pressure (SBP, $r_g = 0.2436 \pm 0.0694$, p value $= 4.00E-04$) and diastolic blood pressure (DBP, $r_g = 0.2586 \pm 0.0803$, p value $= 1.30E-03$). Notably, for GDM_{AUC} , the two most associated phenotypes were also SBP ($r_g = 0.185 \pm 0.070$, p value $= 7.80E-03$) and DBP ($r_g = 0.190 \pm 0.070$, p value $= 6.30E-03$) (Table S8). While the phenotypic correlation between GDM and hypertension has been confirmed,^{59,60} only limited studies have explored this correlation from a genetic perspective.

We also conducted a literature review to explore the potential influence of hypertension during pregnancy on blood glucose

levels, summarizing our findings into three pathways. Firstly, it may worsen insulin resistance, impeding insulin's capacity to transport glucose into cells effectively.⁶¹ Secondly, hypertension may impact maternal vascular function and hemodynamics, thereby impairing the blood supply to the placenta and uterus, disrupting insulin metabolism.⁶² Additionally, hypertension may lead to increased inflammation and oxidative stress, negatively affecting insulin sensitivity and secretion.⁶³ These factors collectively may result in elevated glycemic levels during pregnancy, increasing the risk of gestational diabetes.

Mendelian randomization analysis

In this section, we investigated the causal effects of the associated GWAS phenotypes on pregnancy glycemic traits with variants associated with *ESR1*. Specifically, for each exposure phenotype, we identified all independently significant (ind.sig) variants ($r^2 < 0.2$, p value $< 5E-8$) within 50 kb on both sides of *ESR1* (chr6:151606672–152179619, GRCh38). This identified eight phenotypes with more than three ind.sig SNPs. Inference of phenotypes with fewer than three SNPs may lack power; therefore, we excluded the corresponding phenotypes. At the significant threshold of $6.25E-3$ ($= 0.05/8$), we observed three, zero, and one causal relationships for 0hGlu, GDM, and GDM_{AUC} , respectively (Figure 5B and Table S9). For 0hGlu, the most significant causality was observed in pulse pressure (p value $= 2.14E-04$), followed by serum phosphate density (p value $= 3.40E-03$) and appendicular lean mass (p value $= 3.91E-03$). For GDM_{AUC} , the top two potentially causal phenotypes were height (p value $= 4.51E-03$) and pulse pressure (p value $= 9.41E-03$). The MR analysis highlighted the relationship between blood pressure and pregnancy glycemic traits in a causal manner, supposed by observations that women with chronic hypertension are more prone to developing GDM.⁶⁴

To ensure the validity of our MR analysis, we examined the pleiotropic effects of instrumental variables and also performed a reverse MR analysis. We combined all instrumental variables across the exposure phenotypes and checked their GWAS p values relative to glycemic traits. We found no genome-wide significant signals with the smallest p value for rs488133- GDM_{AUC} (p value $= 0.050$). For the reverse MR analysis, we employed 0hGlu as the exposure variable and pulse pressure as the outcome variable and conducted a two-sample MR analysis using the R function *TwoSampleMR*.⁶⁵ The p value from the inverse variance weighted method was 0.250 (>0.05), signifying the absence of reverse causality.

Transcriptome-wide association study

TWASs can identify significant associations underlying genetically regulated genes and traits of interest.^{66,67} We thus performed a TWAS analysis to investigate whether *ESR1* affects GDM by modulating its expressions (Figure S5 and Table S10). To predict the genetically regulated gene expression (GReX), we multiplied the independent significant *cis*-eQTL (expression quantitative trait loci) effects of *ESR1* by the corresponding genotype dosages in our dataset.

At the significance threshold of $1.0E-03$ ($= 0.05/49$), we identified 17, 0, and 2 tissues with potentially causal GReX on 0hGlu,

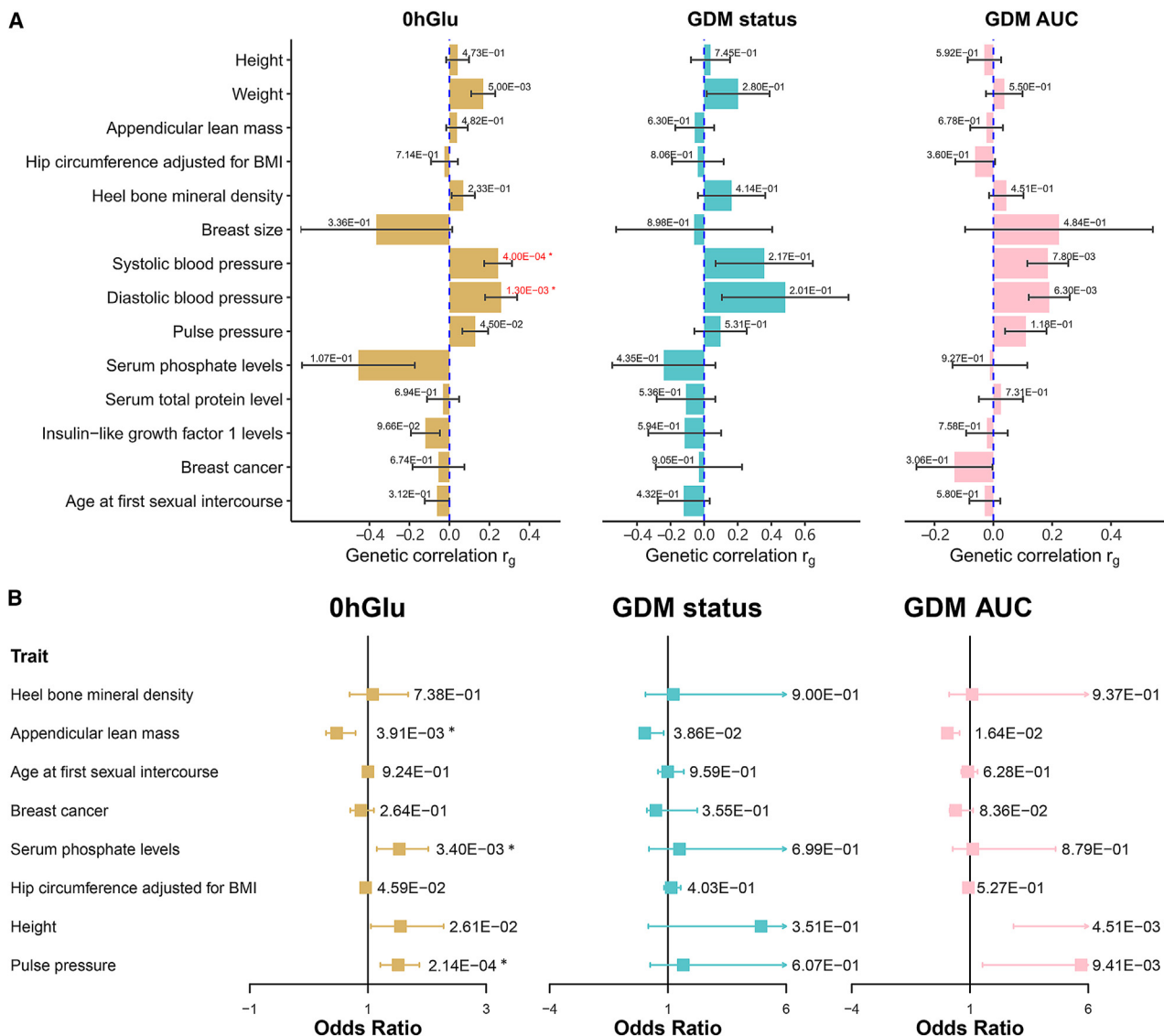


Figure 5. Results of genetic correlation and Mendelian randomization between glycemic traits and *ESR1*-associated phenotypes from GWAS catalog

(A) The bar plots indicate genetic correlation results. The value marked on each bar indicates the p value of the genetic correlation test.

(B) The forest plots indicate MR results. The value marked on each bar indicates the p value of the MR test, and the asterisks (*) means that the p value has reached the Bonferroni corrected threshold $6.25E-3$ ($= 0.05/8$).

GDM, and GDM_{AUC} , respectively. The top three tissues for 0hGlu were brain basal ganglia (p value = $1.69E-07$), artery tibial (p value = $3.61E-07$), and cell-transformed fibroblasts (p value = $5.00E-07$). For GDM_{AUC} , the two significant tissues were artery tibial (p value = $1.81E-04$) and cell-transformed fibroblasts (p value = $6.92E-04$). Here, we wish to underscore the hypothesis that *ESR1* plays a crucial role in influencing pregnancy glycemic traits through the regulation of gene expressions in the tibial artery. This is expected given the well-established association between diabetes and arterial disease.^{68–70} Our study offers a potential pathway through which these two diseases may be interconnected.

Significant overlap of GWAS loci and drug targets

The DTEA translates GWAS results into potential applications. Analysis of 19,365 genes and 10,722 drug gene sets, followed by filtering (STAR Methods), retained 1,072 drug gene sets from 66 classifications. At a significance threshold of $7.58E-04$ and $AUC > 0.5$, we noted significant overlaps of 3, 0, and 6 Anatomical Therapeutic Chemical classifications for 0hGlu, GDM, and GDM_{AUC} , respectively. Notably, for GDM_{AUC} , significant associations were found with hypertension (C07A, p value = $1.21E-07$; C08C, p value = $7.01E-04$) and hypotension (C01C, p value = $5.93E-05$) (Figure S6). This observation aligns with the genetic correlation and MR

analysis results, indicating a putative causal relationship between blood pressure and pregnancy glycemic traits. The potential utility of drug targets for hypertension in addressing hyperglycemia necessitates comprehensive evaluation through rigorous experimental and clinical trials.

DISCUSSION

Glycemic traits during pregnancy play a crucial role in maternal and fetal health. Abnormal glycemic traits during pregnancy may indicate a risk of developing gestational diabetes, which can have adverse outcomes for both the mother and the baby. Understanding the genetic architecture of pregnancy glycemic traits is essential for facilitating the effective management of associated complications. In our study, we uncovered an associated signal, *ESR1*, which encodes an estrogen receptor known to play a significant role in metabolism and gestation. Our investigation revealed potential mechanisms through which *ESR1* was linked to pregnancy glycemic traits. One pathway involved its impact on blood pressure, while another pathway involved the regulation of gene expressions in the tibial artery. Further investigation is required to elucidate the underlying biological mechanisms involved in these associations.

In recent years, the rapid advancement of high-throughput multi-omics technologies has facilitated the acquisition of transcriptomics, proteomics, and metabolomics data from study participants. Numerous placental multi-omics studies have been conducted to identify biomarkers derived from diverse omics datasets and subsequently construct predictive models to distinguish women at high risk of pregnancy hyperglycemia.^{71–76} While these analyses enable the identification of associated biomarkers from each omics data, the explanations of the underlying biological mechanisms and the genetic factors involved are still obscure. The integration of genomic data with other multi-omics data is essential for improving our understanding of disease etiology comprehensively. A TWAS offers an approach to integrate genomic and transcriptomic data, revealing disease-associated genes through the regulation of expressions.⁶⁶ Additionally, proteome-wide association study represents a novel method for detecting gene-phenotype associations mediated by alterations in protein function.⁷⁷ Metabolome-wide association study has unveiled associations between metabolic phenotypes and disease risk, taking into account metabolic quantitative trait loci.⁷⁸ We believe that the integrative analysis of genomic and other multi-omics data holds significant promise for providing a comprehensive exploration of the disease etiology of pregnancy hyperglycemia.

Limitations of the study

While our study offered valuable insights into the genetic landscape of pregnancy glycemic traits, it is essential to acknowledge its limitations. First, despite the recruitment of nearly 40,000 participants, the valid sample size for the GWAS was limited to 14,000 due to the absence of laboratory measurements in 65% of the participants. Conducting a GWAS analysis requires larger sample sizes to achieve sufficient statistical power. Additionally, it's important to acknowledge that our sample collection was confined to Wuhan, central China, potentially

limiting the generalizability of our findings to the entire Chinese population. To address this, we are currently working on recruiting additional samples with both genotype and laboratory data from various regions across China. Simultaneously, we are planning a large-scale meta-analysis within the East Asian population. Second, while our analytical approaches have provided insights into how *ESR1* may influence glycemic traits during pregnancy, biological experiments remain the gold standard for validation. These experiments typically include mouse and cell models. However, our literature review revealed that knocking out the estrogen receptor (*ESR1*) can result in infertility in female mice,^{79,80} which poses significant challenges for designing models to study glycemic traits in pregnant mice. We are actively pursuing ongoing efforts for future biological replication. Additionally, most of the GWAS hits are non-coding intronic variants, which complicates the direct inference of their functional consequences on glycemic traits. This adds another layer of complexity to experimental replication, as it is challenging to pinpoint the precise regulatory mechanisms affected by these non-coding variants in *ESR1*. Third, we acknowledge that the *ESR1*-associated phenotypes retrieved from the GWAS catalog predominantly originated from European populations. Despite our efforts to harmonize data on allele type and frequency, minimizing disparities in variants spectrum between European and East Asian populations, there may persist some bias in our results. Furthermore, we downloaded some *ESR1* associations from East Asian populations; however, they either did not provide full summary statistics^{81–84} or had a limited number of signals that were subsequently excluded during harmonization.^{85,86} We strive for undertaking robust replication studies involving larger East Asian populations to enhance the reliability of our findings. Fourth, we note that studying environmental factors alongside genetic analysis in GDM research is crucial. Environmental influences such as diet, lifestyle, and pollutants play a significant role in GDM development. Understanding these factors enhances our ability to predict, prevent, and manage GDM effectively, improving maternal and fetal health outcomes. Unfortunately, we did not include a questionnaire to gather data on environmental factors in this study, but we plan to collect these variables in future investigations.

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact Xin Jin (jinxin@genomics.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The data that support the findings of this study have been deposited into CNGB Sequence Archive (CNSA)⁸⁷ of China National GeneBank DataBase (CNGBdb)⁸⁸ with accession number CNP0003674 and can also be downloaded directly from <https://db.cngb.org/MANE.PheWeb/>. A summary of analysis software and tools were provided in [key resources table](#). All original code has been deposited at Zenodo and is publicly available through <https://doi.org/10.5281/zenodo.11614736>. Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

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AUTHOR CONTRIBUTIONS

A.Z. and X.J. conceived the study, designed the research program, and managed the project. H.X., M.Y., J. Zhou, Y. Zhou, J.L., Y. Zhong, Z.C., H.M., X.C., L.H., and R.Z. collected the data. L.L., X.L., and P.L. preprocessed the data and finished the quality control. H.Z., H.X., L.L., M.Y., Y.L., X.Z., X.Q., and J. Zeng performed the statistical analysis and results visualization. H.Z., H.X., L.L., M.Y., and Y.L. wrote the manuscript. All authors participated in revising the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
GWAS summary statistics	This paper; China National GeneBank DataBase	CNSA: CNP0003674
Human reference genome NCBI build 38, GRCh38	Genome Reference Consortium	http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/
1000 Genomes Project reference panel EAS	The International Genome Sample Resource	https://www.internationalgenome.org/data
Summary statistics from GWAS Catalog (See Table S7)	Sollis E. et al., 2023 ⁵⁸	https://www.ebi.ac.uk/gwas/
The reference transcriptome data form GTEx	Gamazon E.R. et al., 2015 ⁶⁶	https://www.gtexportal.org/home/
The effect sizes for all 49 tissues	Barbeira A.N. et al., 2021 ⁸⁹	Zenodo: 5709385
Phase 3 downloaded from the MAGMA website	de Leeuw C.A. et al., 2015 ⁹⁰	https://ctg.cncr.nl/software/magma
The drug-gene-set built from DGIdb v5	Freshour S.L. et al., 2021 ⁹¹	https://www.dgldb.org/
Anatomical Therapeutic Chemical (ATC) classification of the drug data	The WHO Collaborating Center for Drug Statistics Methodology	https://www.whocc.no/
DisGeNET	Piñero J. et al., 2017 ⁹²	https://www.disgenet.org
Software and algorithms		
Original Code for analyses	This paper	Zenodo: 11614736
BWA-MEM 0.7.16a-r1181	Li H. and Durbin R., 2009 ⁹³	https://bio-bwa.sourceforge.net/
SAMtools 1.3	Danecek P. et al., 2021 ⁹⁴	https://www.htslib.org/
BQSR of the GATK 4.0.4.0 toolset	McKenna A. et al., 2010 ⁹⁵	https://gatk.broadinstitute.org/hc/en-us/articles/360035890531-Base-Quality-Score-Recalibration-BQSR-
STITCH 1.6.6	Davies R.W. et al., 2016 ⁹⁶	https://github.com/rwdavies/STITCH
PLINK2	Chang C.C. et al., 2015 ⁹⁷	https://www.cog-genomics.org/plink/2.0/
LDSC 1.0.1	Bulik-Sullivan B.K. et al., 2015 ⁹⁸	https://github.com/bulik/ldsc
Metascape	Zhou Y. et al., 2019 ⁹⁹	https://metascape.org/
STRING	Szklarczyk D. et al., 2023 ¹⁰⁰	https://string-db.org/cgi/
R software version 4.0.2	R project	https://www.r-project.org/
BaseVar	Liu S. et al., 2018 ¹⁷	https://pypi.org/project/basevar/
LiftOver	Hinrichs A.S. et al., 2006 ¹⁰¹	https://genome.ucsc.edu/cgi-bin/hgLiftOver
R package TwoSampleMR: clump_data	Hemani G. et al., 2018 ⁶⁵	https://mrcieu.github.io/TwoSampleMR/reference/clump_data.html
MAGMA v1.10	de Leeuw C.A. et al., 2015 ⁹⁰	https://ctg.cncr.nl/software/magma
R package coloc	Wallace C. et al., 2020 ⁴⁶	https://cran.r-project.org/web/packages/coloc/
R package locuscomparer	Liu B. et al., 2019 ⁴⁷	https://github.com/boxiangliu/locuscomparer
Other		
MANE (Maternal and Neonatal) PheWeb websiteThis paper	This paper	https://db.cngb.org/MANE.PheWeb/

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects

All pregnant women were recruited from the Wuhan Children's Hospital in Wuhan, Hubei Province, China, where they underwent routine prenatal tests—including blood tests, urinary tests, oral glucose tolerance tests, ultrasound screenings, and NIPT test—from 2017 to 2020. All the participants have signed the informed consent forms. This study was approved by the IRB at Wuhan

Children's Hospital (2021R062) and BGI Research (BGI-IRB 21088) and also authorized by the Human Genetic Resources Administration of China ([2021] CJ2002). We used NIPT sequencing data as the sole inclusion criterion and then performed filtering based on sequencing depth, mapping rate, and outlier values.

Phenotype

We collected four sets of phenotypic data: (1) maternal information from the NIPT questionnaire, (2) laboratory tests from prenatal exams, (3) neonatal information from childbirth outcomes, and (4) clinical diagnoses from doctor's notes. Maternal data included age, height, weight, systolic blood pressure (SBP), diastolic blood pressure (DBP), and obstetric history, collected pre-NIPT screening. BMI was calculated as weight (kg) divided by height (m²). Hypertension was defined as SBP/DBP over 140/90 mmHg. As a result, four maternal factors—age, BMI, obstetric history (first-time pregnancy status), and hypertension—were used for regression analysis.

The laboratory tests were primarily blood and urinary biochemical measurements. To detect early risk indicators of GDM, we focused on laboratory features in the first trimester. A total of 93 available phenotypes were measured and classified into 9 categories, including hematological ($n = 24$), urinalysis ($n = 24$), infection ($n = 14$), liver-related ($n = 8$), metabolism ($n = 6$), protein ($n = 5$), electrolyte ($n = 4$), hormone ($n = 4$), and kidney-related ($n = 4$). For certain phenotypes, some women had multiple records due to more than one examination or test. We defined an overall observation by taking average or ascertained incidence for quantitative and dichotomous traits, respectively.

The neonatal information included gestational week, birth weight, newborn gender, and delivery options. Low birth weight is defined as a birth weight under 2500g, while fetal macrosomia is diagnosed when a baby weighs over 4000g. The delivery options included natural birth and cesarean section. The clinical diagnosis was the doctor's descriptions of pregnancy status which spanned a wide spectrum of information, for example, pregnancy complications, how much amniotic fluid, what fetal positions for birth, etc.

Maternal glyceic traits

The oral glucose tolerance test (OGTT) was usually taken during the 24th to 28th week of pregnancy. Specifically, the pregnant women were asked to drink about 237mL liquid that contained 75g glucose. Before drinking the liquid, the pregnancy women would have blood drawn that for measuring the fasting glucose (0hGlu). After drinking the solution, blood was drawn two more times at 1-h (1hGlu) and 2-h (2hGlu) post consumption. By following the recommendations of international association of diabetes and pregnancy study groups,¹⁰² we defined a GDM case if at least one of the following factors were true: 0hGlu >5.1 mmol/L, 1hGlu >10.0 mmol/L, or 2hGlu >8.5 mmol/L. To consider the contributions of the three OGTT values simultaneously, we further computed the area under the curve (AUC) as $1hGlu + (0hGlu + 2hGlu)/2$.^{103,104} We used GDM_{AUC} as a complement to the GDM status and performed same statistical analysis.

METHOD DETAILS

Whole-genome sequencing

During gestational weeks 12–22, pregnant women who consented to the NIPT test had 5 mL of peripheral blood drawn. The blood samples were centrifuged to separate the plasma, which contained both maternal and fetal cell-free DNA (cfDNA). We then employed single-end whole genome sequencing using the BGISEQ-500 platform, with a read length of 35 base pairs (bp). On average, the sequencing depth ranged between 0.1× and 0.2×. Notably, at the time of NIPT testing, the fetal DNA fraction in maternal plasma is approximately 10%,^{105,106} with half of it being identical to the maternal DNA. For subsequent analyses, we did not distinguish between maternal and fetal DNA; instead, we treated all cfDNA as maternal.

Variants calling

The sequencing reads were stored in fastq (.fq) format. First, we used BWA-MEM algorithm⁹³ to align input reads to the human reference genome assembly GRCh38/hg38 and obtained the bam alignment files. Then, we used SAMtools⁹⁴ to sort sequences and remove duplicate sequences, thus to keep those sequences with highest mapping quality. The following step was to check the base quality in the aligned files and correct the quality value of the original bases to ensure the accuracy of base detection, which was achieved by Base Quality Score Recalibration (BQSR) of the GATK toolset.⁹⁵ Finally, to obtain the population-based variants, we used BaseVar that was designed to call variants especially for ultra-low-pass WGS data.¹⁰⁷ The detected variants were stored in VCF format.

Genotype imputation

Since the NIPT sequencing data was ultra-low depth and only about 10% of the genome were measured, resulting in a high rate of genotype missingness. To make the NIPT sequence data qualified for genetic analysis, we performed genotype imputation for the aligned bam files in STITCH,⁹⁶ which produced allele dosages of the imputed genotype. To evaluate the imputation performance, we used 30 randomly selected Chinese samples from the high-coverage 1000 genomes project (1KGP)¹⁰⁸ as the true set, down-sampled them to about 0.1×, and performed imputation for the down-sample data together with the original NIPT data. Then, we calculated the Pearson's correlation R^2 between the imputed dosages (test) and high coverage genotypes (truth) as the imputation accuracy.

QUANTIFICATION AND STATISTICAL ANALYSIS

Regression analysis

For the four types of phenotypes, we performed regression analysis to examine their relationship with GDM and GDM_{AUC} . In general, for potential risk factors (e.g., maternal age, early-stage laboratory tests), we fitted logistic and linear model by regressing GDM and GDM_{AUC} as dependent variable, respectively. For potential outcomes (e.g., birthweight, delivery type), we fitted either linear or logistic regression for quantitative or binary outcomes while treating GDM and GDM_{AUC} as predictive variable. For the clinical diagnosis that were all binary variables, it was uncertain whether they were potential risks or outcomes, we used chi-square and t testes for GDM and GDM_{AUC} , respectively.

For significant risk factors with p -value less than $5.15E-4$ ($=0.05/97$), we further performed multivariate logistic and linear regression analysis for GDM and GDM_{AUC} , respectively. It is important to acknowledge that not all phenotypes had measurements available for every participant, which could reduce the effective sample size in a multivariate model. To ensure robustness, we selected a subset of significant factors, ensuring that the sample size in the multivariate model remained at least 3,000.

Genome-wide association study

We executed GWAS analysis for the three glycemic traits (0hGlu, 1hGlu, and 2hGlu), GDM status and GDM_{AUC} . This was achieved in PLINK 2.0⁹⁷ with argument `-glm -maf 0.05 -hwe 1e-06 -geno 0.1` by testing SNPs with minor allele frequency (MAF) > 0.05, Hardy-Weinberg equilibrium (HWE) > $1E-6$, and genotype missing rate (geno) < 0.1. The logistic regression model was employed to assess GDM status, whereas linear regression models were utilized for evaluating OGTT traits and GDM_{AUC} . The covariates comprised maternal age, gestational week when taking OGTT screening, and the five principal components (PCs) of genotype variants. All covariates were standardized to have a mean of zero and a standard deviation of one by using the argument `-covar-variance-standardize` and the quantitative phenotype were normalized by `-pheno-quantile-normalize`. We set the genome-wide significance threshold as $5E-08$.

Heritability is a metric indicating the proportion of phenotypic variation in a trait that can be ascribed to genetic factors.¹⁰⁹ We used the LDSC (Linkage Disequilibrium Score Regression) method⁹⁸ to estimate the heritability of glycemic traits, GDM, and GDM_{AUC} . We note that, given the binary nature of GDM status, calculating heritability should be conducted using the liability scale rather than the observed scale. In particular, we specified the population prevalence of GDM as 0.1 and the sample prevalence as 0.113 by using arguments `-pop-prev` and `-samp-prev`, respectively.

Protein-protein interaction and gene set enrichment analysis

Protein-protein interaction (PPI) analysis aims to identify and characterize the network of interactions between two or more proteins, which is crucial for deciphering cellular processes and functions.¹¹⁰ Gene set enrichment analysis (GSEA) focuses on identifying biological pathways that are significantly enriched within a list of genes.¹¹¹ We conducted the PPI and GSEA analysis by combining all mapped genes associated with the five pregnancy glycemic traits. For PPI analysis, we utilized STRING to visualize the network.¹⁰⁰ This included known interactions (e.g., experimentally determined), predicted interactions (e.g., gene neighborhood), and other interactions (e.g., text-mining). GSEA analysis was performed using Metascape,⁹⁹ leveraging the DisGeNET database, which catalogs gene-disease associations.⁹²

Genetic correlation analysis

Genetic correlation refers to the correlation established at the genetic level between two phenotypes.¹¹² Understanding the genetic correlation between traits is helpful to understand the shared genetic etiology and reveal the genetic pleiotropy. We employed LDSC⁹⁸ to estimate the genetic correlations between 0hGlu, GDM, GDM_{AUC} , and potentially correlated traits. Specifically, we acquired full summary statistics for these traits from the GWAS catalog.⁵⁸ To ensure compatibility, we utilized LiftOver¹⁰¹ to convert the reference genome of the downloaded summary statistics to GRCh38. LD calculations were performed using the East Asian population data from the 1000 Genomes Project as a reference.

Mendelian randomization analysis

Mendelian randomization (MR) is a statistical technique to assess causal effects of a modifiable exposure on an outcome by using genetic variants as instrumental variables (IVs).¹¹³ In this study, we would like to explore the causal relationships between certain potentially correlated phenotypes and glycemic traits. For each examined exposure phenotype, we chose highly significant SNPs with p -value less than $5E-08$ and conducted LD clumping to ensure the retained SNPs were independent. Specifically, we used R function `TwoSampleMR: clump_data`⁶⁵ by setting clumping r^2 as 0.2 and other parameters in default values. The remaining SNPs were utilized to compute the polygenic score (PGS) for our participants, achieved by multiplying the exposure's genetic effects with individual-level genotype dosages. To assess the causality of the PGS on glycemic traits, we constructed appropriate regression models with the PGS as the predictor.

Transcriptome-wide association study

TWAS is a method that integrates gene expression data and genotype information to identify genes whose expression levels are associated with complex traits or diseases.^{66,67} It involves two datasets: reference transcriptome data and the main dataset. The reference data includes samples with genotype and gene expression information, used to study genetic impacts on gene expression. The main dataset consists of samples with genotype and complex traits. By integrating genetic effects from the reference data with the main dataset, we derived genetically regulated expression (GReX). Finally, we tested the association between GReX and the trait of interest.

In this study, we used the GTEx database (v8) as our reference transcriptome data to estimate GReX levels for our participants.⁶⁶ Specifically, we chose to use the cross-tissue gene expression imputation framework (CTIMP)¹¹⁴ and downloaded the effect sizes from Zenodo⁸⁹ for all 49 tissues. The *cis*-eQTL effects were obtained from all variants located within 500kb upstream and downstream of a target gene. These variants were clumped using an r^2 threshold of 0.2 to retain independent effects using R:*clump_data*.⁶⁵ GReX was calculated by multiplying eQTL effects with genotype dosages. Logistic regression was then used to analyze GDM status, and linear regression was applied to 0hGlu and GDM_{AUC}, with a significance threshold adjusted to 0.001 using Bonferroni correction.

Drug target enrichment analysis

In this section, we conducted drug target enrichment analysis (DTEA) to determine if a group of candidate genes was significantly enriched for known drug targets. This analysis can provide insights into drug repurposing opportunities, uncover novel therapeutic targets, and aid in understanding the molecular mechanisms underlying diseases.¹¹⁵ Using MAGMA v1.10,⁹⁰ we performed drug-gene set analysis on glycemia-associated genes. The drug-gene set was curated from the Drug-Gene Interaction Database (DGIdb) version 5 interactions file.⁹¹ Gene-level analyses were performed using the default competitive model.¹¹⁶

The follow-up analysis involved drug classification. For this, we needed data on the Anatomical Therapeutic Chemical (ATC) classification of drugs.^{117,118} Using previously described enrichment curves, we annotated the drug-gene set analysis results according to ATC classifications, excluding those with fewer than 10 valid drug-gene sets.^{116,119} The sets were ranked by association ($-\log_{10}(p)$) and divided into groups based on ATC classification. We then calculated the area under the curve (AUC) and p -value using the Wilcoxon Mann-Whitney test, with a Bonferroni-corrected significance threshold set at $p < 7.58E-04$ for 66 drug classifications.

Cell Genomics, Volume 4

Supplemental information

**Novel insights into the genetic architecture
of pregnancy glycaemic traits
from 14,744 Chinese maternities**

Huanhuan Zhu, Han Xiao, Linxuan Li, Meng Yang, Ying Lin, Jieqiong Zhou, Xinyi Zhang, Yan Zhou, Xianmei Lan, Jiuying Liu, Jingyu Zeng, Lin Wang, Yuanyuan Zhong, Xiaobo Qian, Zhongqiang Cao, Panhong Liu, Hong Mei, Mingzhi Cai, Xiaonan Cai, Zhuangyuan Tang, Liqin Hu, Rui Zhou, Xun Xu, Huanming Yang, Jian Wang, Xin Jin, and Aifen Zhou

Figure S1. The basic information of study participants, related to Table 1

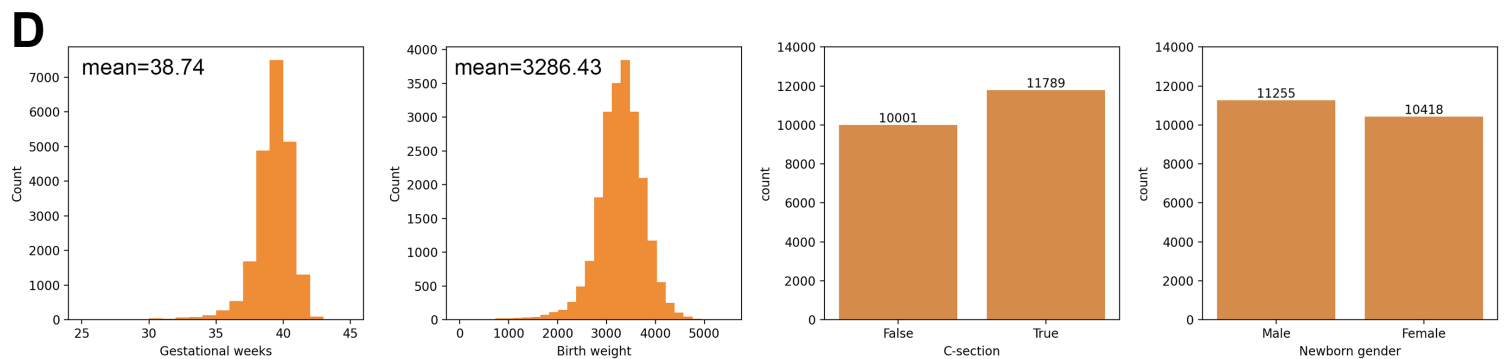
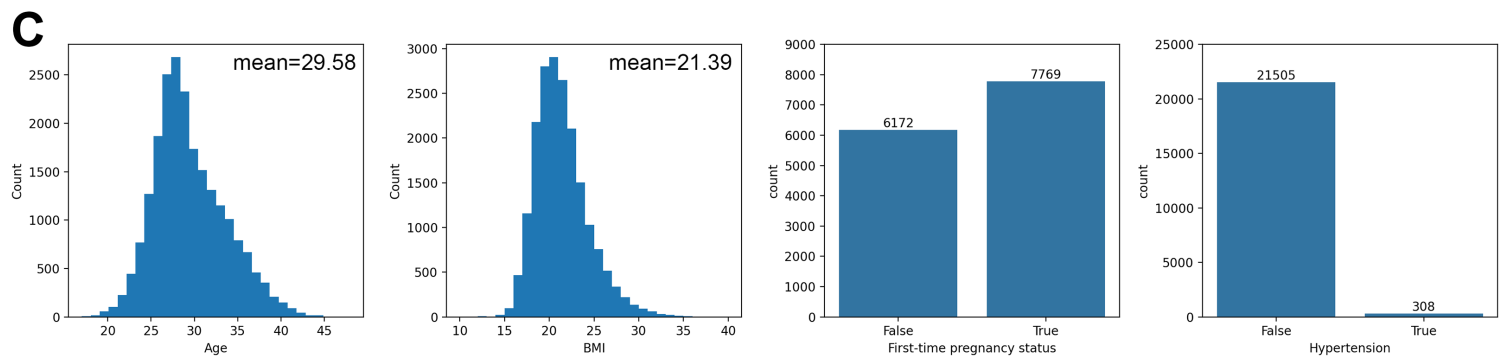
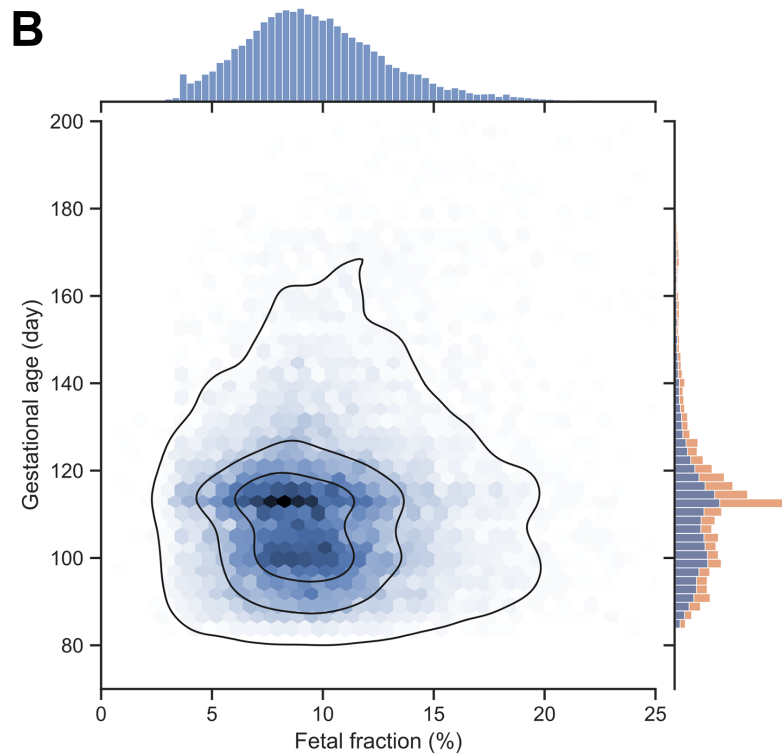
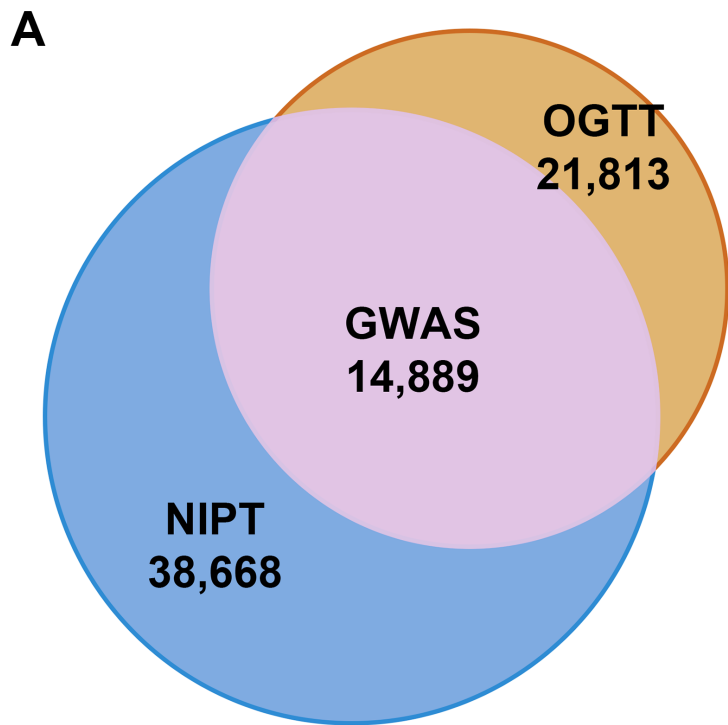


Figure S2. Statistics on glyceimic traits and regression analysis with laboratory features, related to Table 1

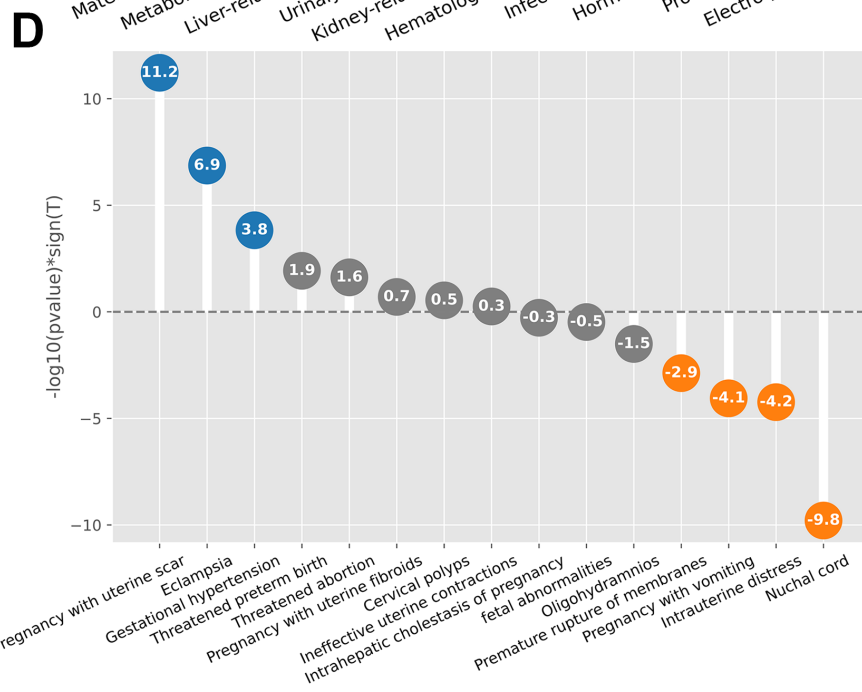
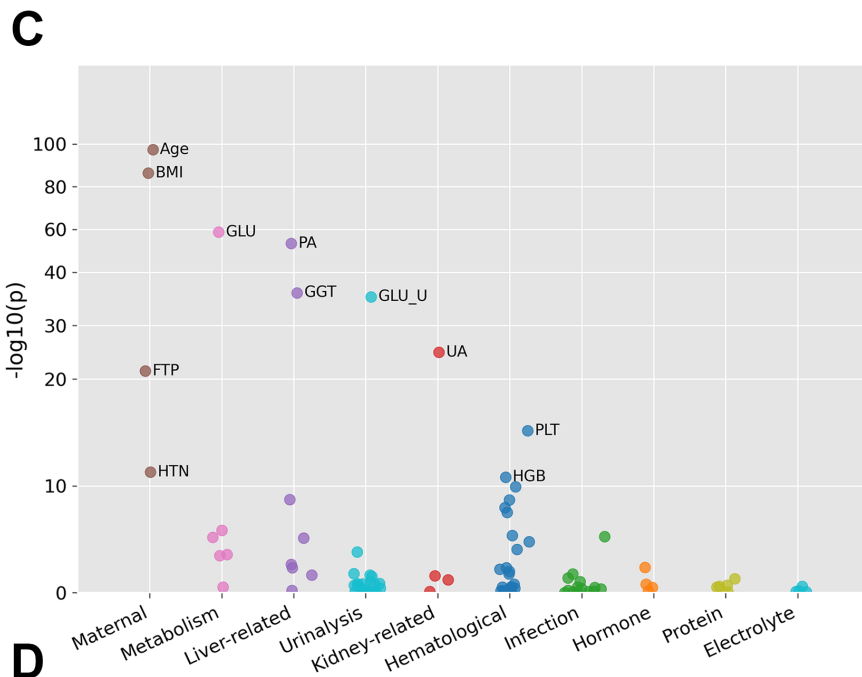
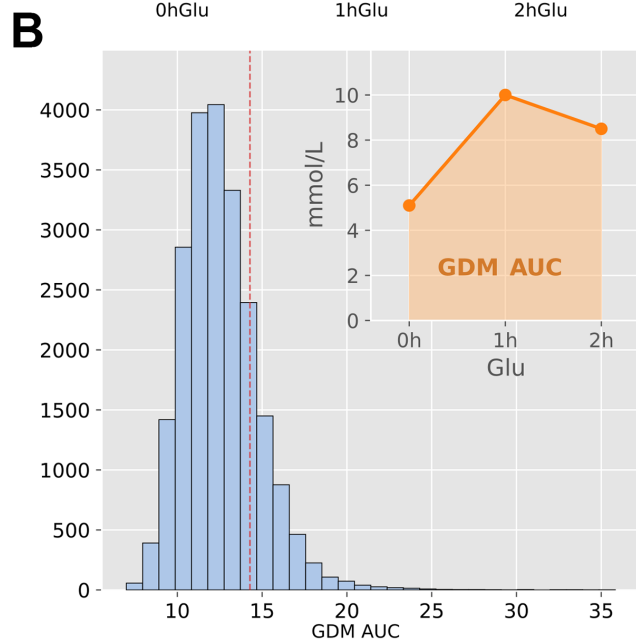
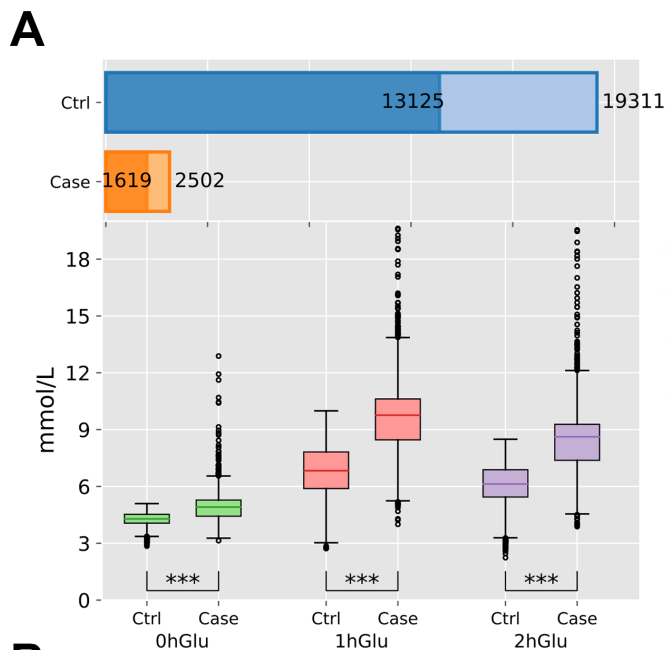


Figure S3. The assessment of sequencing and genotype imputation, related to STAR Methods

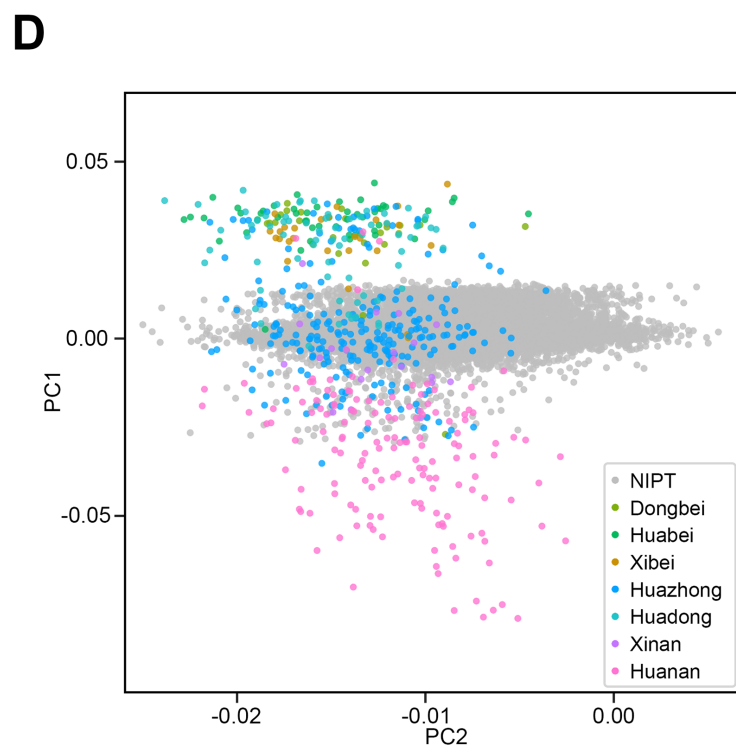
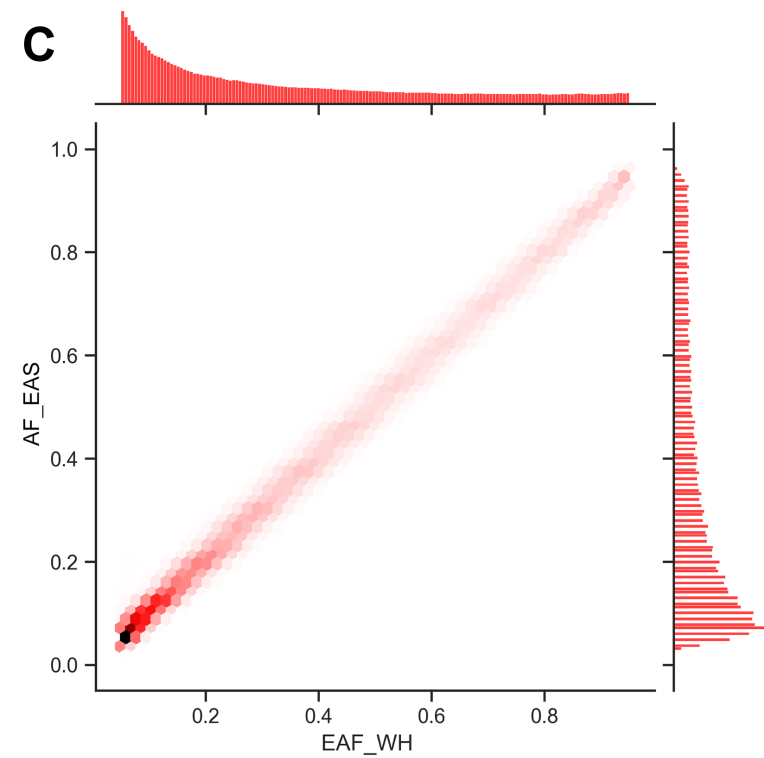
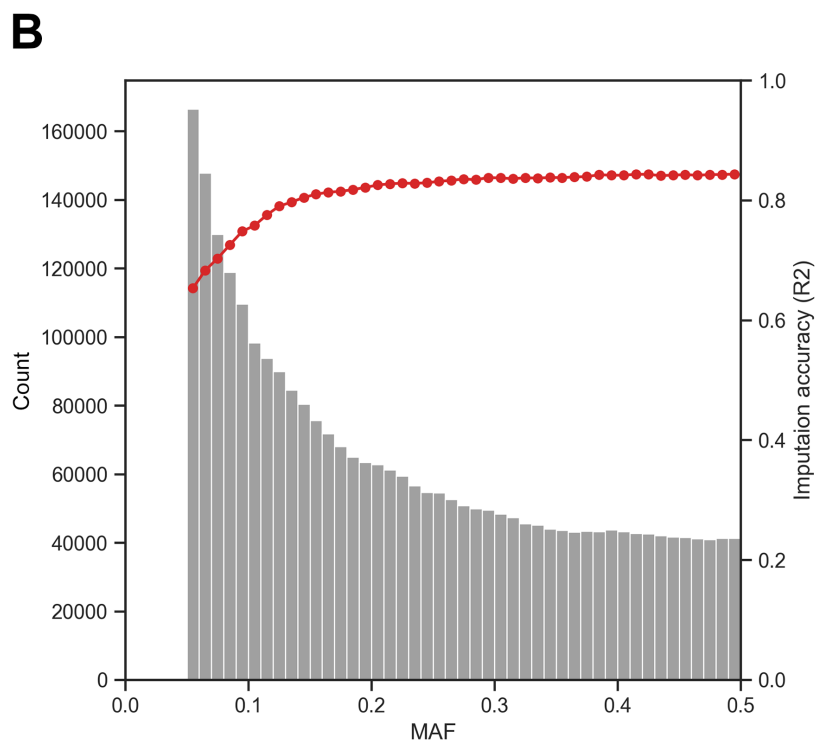
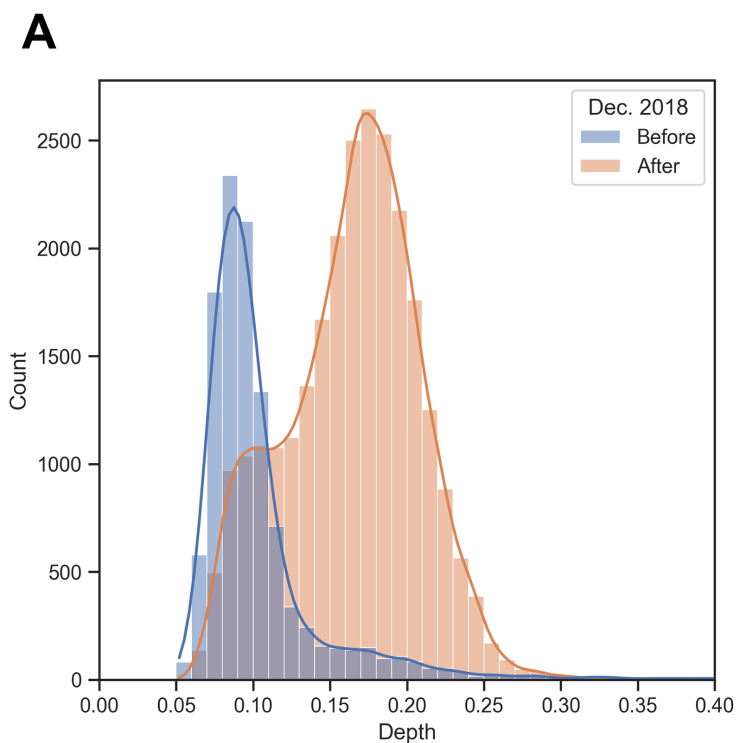


Figure S4. The results of colocalization analysis between discovery and replication studies in the region of gene *ESR1*, related to Figure 1

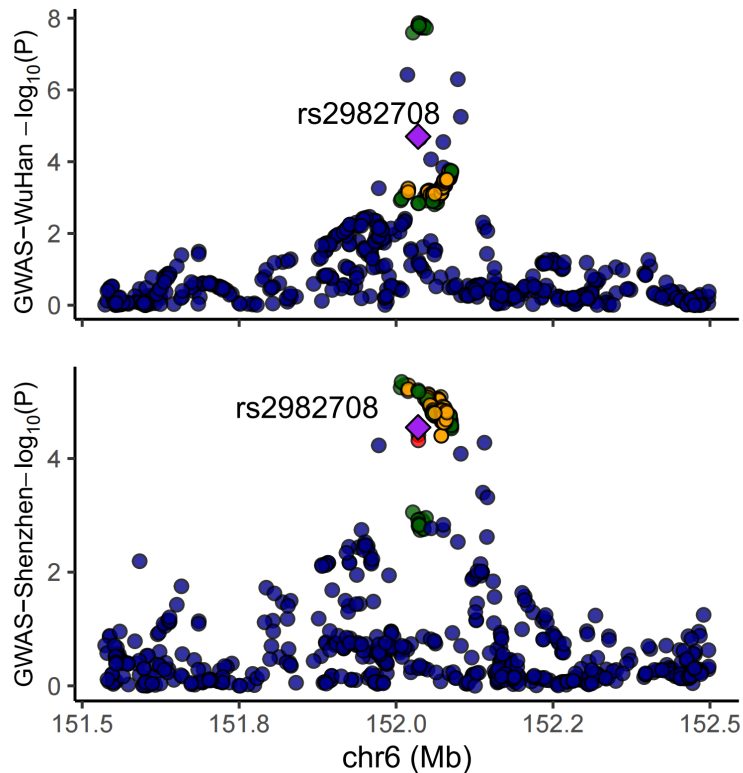
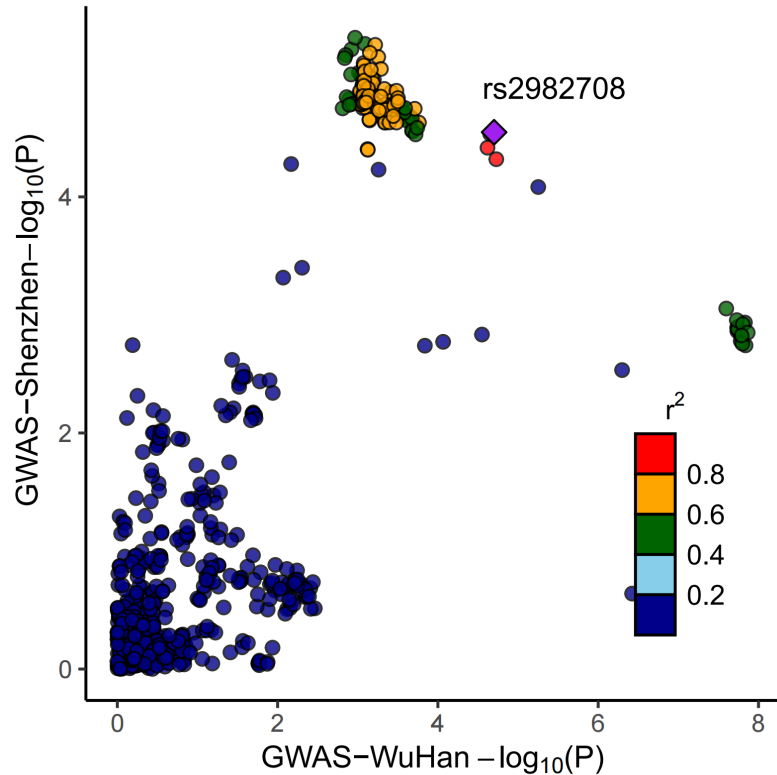


Figure S5. The TWAS results between GTEx data and glycemic traits, related to STAR Methods

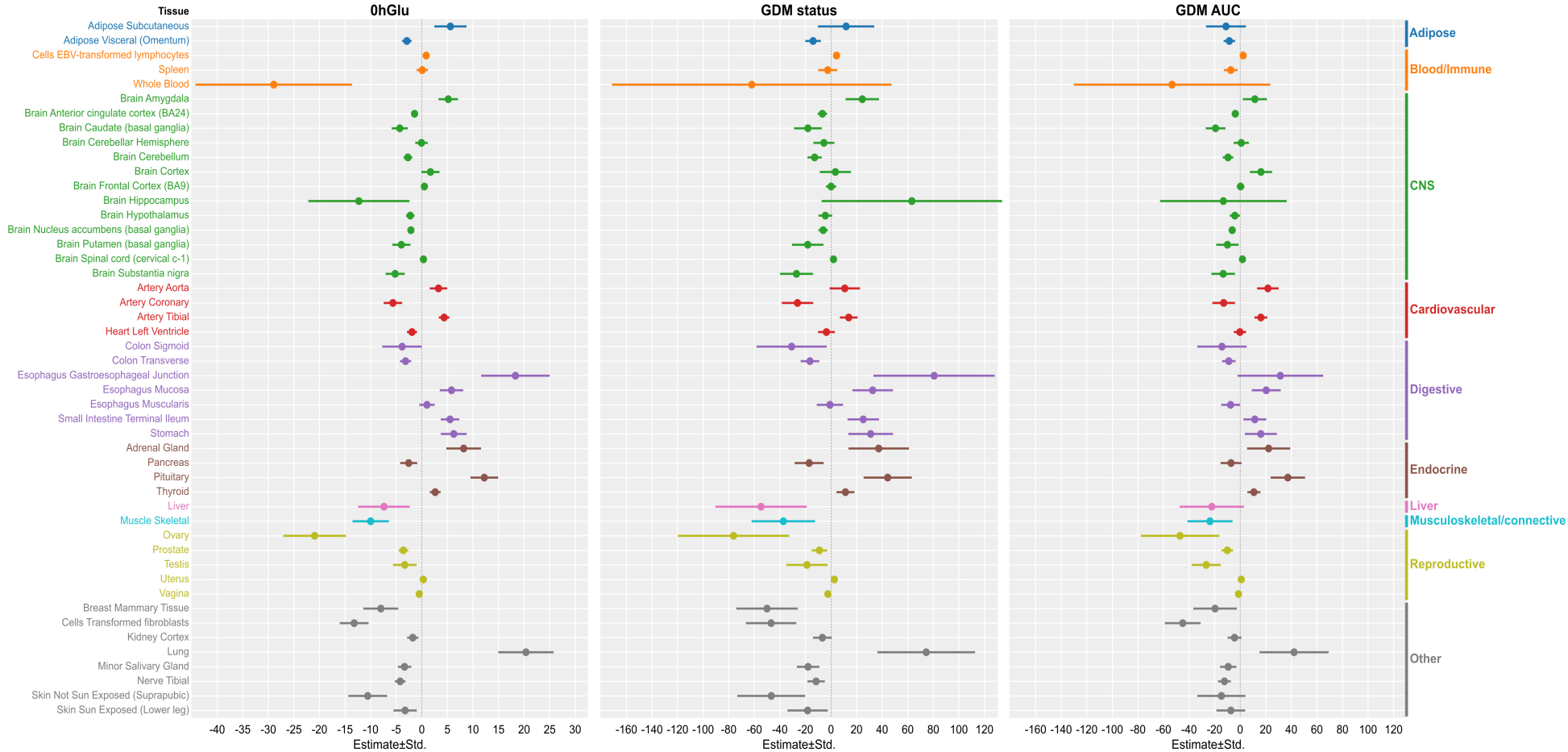
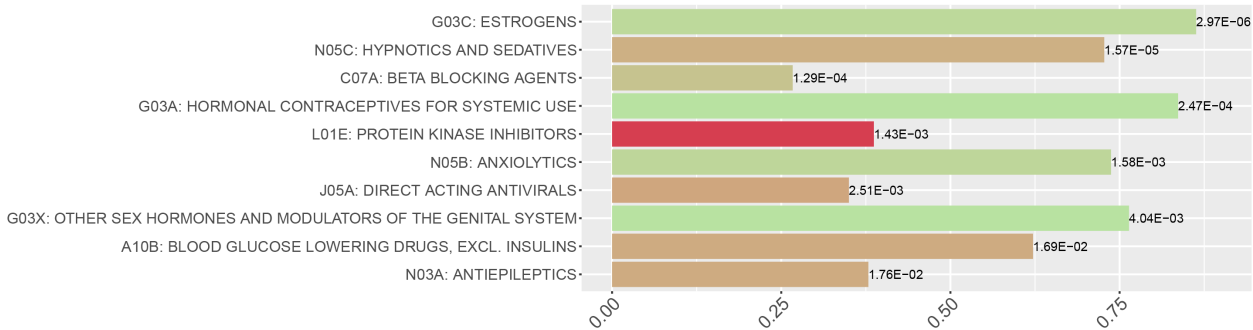


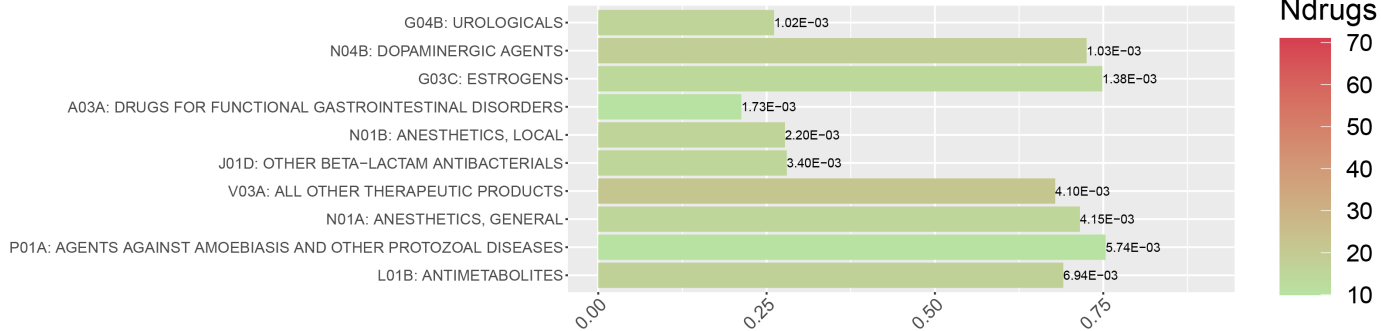
Figure S6. The top ten drug ATC classifications from DTEA analysis, related to STAR Methods

Anatomical therapeutic chemical classification

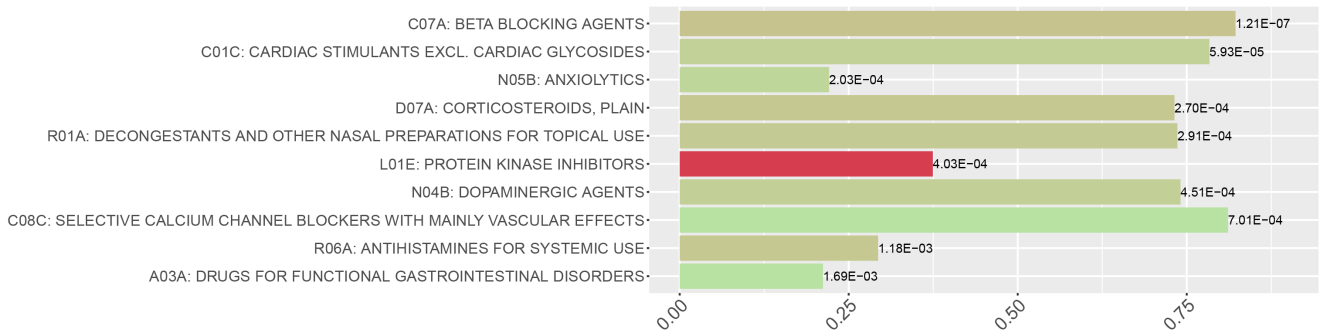
0hGlu



GDM status



GDM AUC



Area under the curve

Table S1. The univariate regression analysis of GDM status and GDM AUC on early-stage laboratory traits, related to Table 1

Trait information					GDM status univariate regression			GDM AUC univariate regression		
Category	Trait	Abbreviation	n	mean±std case(%)	beta	std.	pvalue	beta	std.	pvalue
Metabolism	Blood glucose	GLU	11557	4.51±0.48	1.101	0.068	1.75E-59	1.219	0.048	3.29E-140
Liver-related	Prealbumin	PA	17417	224.17±36.74	0.011	0.001	3.26E-54	0.011	0.001	1.83E-114
Liver-related	γ -glutamyl transferase	GGT	17417	14.30±10.11	0.027	0.002	7.36E-37	0.033	0.002	1.74E-67
Urinalysis	Urine glucose	GLU U	18619	2187(11.75%)	0.825	0.066	4.29E-36	0.957	0.056	3.77E-65
Kidney-related	Uric acid	UA	17807	212.06±48.87	0.005	0.001	9.21E-26	0.005	0.000	2.45E-44
Hematological	Platelet count	PLT	18790	237.63±55.22	0.004	0.000	6.86E-16	0.004	0.000	2.09E-30
Hematological	Hemoglobin	HGB	18790	125.17±10.25	0.017	0.003	1.55E-11	0.015	0.002	6.46E-18
Hematological	White blood cell count	WBC	18790	9.17±2.13	0.076	0.012	1.21E-10	0.077	0.008	1.91E-19
Liver-related	Alkaline phosphatase	ALP	17417	44.24±10.41	0.015	0.002	1.94E-09	0.018	0.002	5.60E-24
Hematological	Plateletcrit	PCT	15752	0.23±0.06	2.866	0.478	2.02E-09	2.698	0.341	2.63E-15
Hematological	Red blood cell count	RBC	18790	4.26±0.38	0.380	0.067	1.12E-08	0.367	0.047	9.55E-15
Hematological	Hematocrit	HCT	18790	37.67±2.98	0.048	0.009	2.97E-08	0.047	0.006	1.47E-14
Metabolism	Oral glucose tolerance test-fasting	OGTT0h	102	5.02±1.07	4.227	0.877	1.43E-06	2.925	0.270	3.04E-18
Hematological	platelet larger cell ratio	P-LCR	15646	28.46±7.90	-0.017	0.004	4.30E-06	-0.014	0.003	3.71E-08
Infection	Herpes simplex virus type 2 specific IgG antibody	HSV-2 IgG	6591	474(7.19%)	0.640	0.141	5.48E-06	0.410	0.121	6.86E-04
Metabolism	Hemoglobin A1c	HbA1C	230	5.20±0.56	2.527	0.560	6.38E-06	3.758	0.328	7.14E-23
Liver-related	Alanine aminotransferase	ALT	17419	16.92±16.50	0.006	0.001	7.99E-06	0.010	0.001	5.51E-18
Hematological	Neutrophil count	NEU	12698	6.64±1.81	0.073	0.017	1.75E-05	0.079	0.012	8.34E-11
Hematological	Lymphocyte count	LYM	12698	1.81±0.50	0.245	0.063	9.11E-05	0.192	0.044	1.36E-05
Urinalysis	Urine PH level	pH	18619	6.54±0.71	-0.143	0.038	1.53E-04	-0.082	0.026	1.71E-03
Metabolism	Oral glucose tolerance test-1 hour	OGTT1h	68	9.75±2.81	0.734	0.201	2.68E-04	1.496	0.056	2.87E-37
Metabolism	Oral glucose tolerance test-2 hour	OGTT2h	68	8.16±2.65	0.727	0.204	3.54E-04	1.499	0.087	4.95E-26
Liver-related	Total bilirubin	TBIL	17417	8.43±3.47	-0.025	0.008	2.24E-03	-0.027	0.005	8.34E-07
Hormone	Free triiodothyronine	FT3	17500	3.39±0.77	0.086	0.030	4.32E-03	0.082	0.024	5.72E-04
Liver-related	Direct bilirubin	DBIL	17417	3.06±1.20	-0.068	0.024	4.49E-03	-0.090	0.016	8.04E-09
Hematological	Monocyte count	MONO	12698	0.51±0.18	0.471	0.167	4.66E-03	0.360	0.118	2.29E-03
Hematological	Platelet size deviation width	PDW	18634	13.03±2.44	-0.030	0.011	6.56E-03	-0.025	0.007	6.60E-04
Hematological	Mean platelet volume	MPV	18634	9.95±1.45	-0.046	0.018	1.00E-02	-0.049	0.013	9.50E-05
Urinalysis	Ketones in urine	KET	18619	6057(32.53%)	0.131	0.054	1.62E-02	0.102	0.039	9.13E-03
Hematological	Mean corpuscular hemoglobin concentration	MCHC	18790	332.57±15.96	0.004	0.002	1.75E-02	0.002	0.001	5.56E-02
Infection	C-reactive protein	CRP	4134	5.17±6.45	0.017	0.007	1.80E-02	0.014	0.007	3.31E-02
Urinalysis	Crystals in Urine	X'TAL U	18453	7.97±117.71	0.001	0.000	2.21E-02	0.000	0.000	9.79E-02
Liver-related	Aspartate aminotransferase	AST	17417	17.70±8.81	0.006	0.003	2.22E-02	0.010	0.002	1.08E-06
Kidney-related	Serum creatinine	Cr	17807	43.45±6.23	-0.010	0.004	2.63E-02	-0.015	0.003	3.17E-07
Urinalysis	Small round epithelial cells in urine	SRC U	18453	3.48±4.76	0.013	0.006	3.18E-02	0.009	0.005	5.27E-02
Infection	Hepatitis B envelope antibody	HBeAb	17851	1.41±0.76	-0.079	0.039	4.33E-02	-0.063	0.024	9.01E-03
Protein	Ferritin	Ferr	938	82.26±66.15	0.003	0.002	5.09E-02	0.001	0.001	3.06E-01
Kidney-related	Cystatin C	CysC	17624	0.67±0.16	0.325	0.173	6.07E-02	0.287	0.119	1.62E-02
Infection	Hepatitis B core antibody	HBcAb	17851	1.46±0.86	-0.051	0.030	9.61E-02	-0.073	0.022	7.44E-04
Urinalysis	Urine specific gravity	SG	18619	1.02±0.01	5.653	3.430	9.93E-02	1.187	2.405	6.22E-01
Urinalysis	Leukocytes esterase in urine	LEU	18619	9282(49.85%)	-0.076	0.052	1.42E-01	-0.089	0.037	1.56E-02
Urinalysis	Casts in urine	CAST U	18452	3.17±4.20	-0.010	0.007	1.44E-01	-0.014	0.005	1.79E-03
Urinalysis	Urine clarity	Clarity	18608	8491(45.63%)	-0.076	0.052	1.46E-01	-0.086	0.037	1.97E-02
Urinalysis	Urine conductivity	Cond	18452	18.07±8.34	-0.004	0.003	1.52E-01	-0.003	0.002	2.08E-01
Hormone	Free thyroxine	FT4	17501	2.81±5.19	-0.007	0.005	1.62E-01	-0.005	0.004	1.98E-01
Hematological	Mean corpuscular volume	MCV	18790	88.61±5.43	-0.006	0.005	1.69E-01	-0.004	0.003	1.93E-01

Urinalysis	Protein in urine	PRO	18602	11542(62.05%)	0.072	0.054	1.86E-01	0.047	0.038	2.11E-01
Protein	Albumin/globulin ratio	AGR	17417	1.68±0.24	-0.147	0.115	1.99E-01	-0.110	0.080	1.69E-01
Urinalysis	Unlysed red blood cell percentage in urine	Unlysed RBC(%)	18453	68.27±22.97	-0.001	0.001	2.03E-01	0.000	0.001	8.09E-01
Electrolyte	Sodium	Na	150	137.15±2.33	0.209	0.183	2.54E-01	-0.013	0.076	8.69E-01
Protein	Globulin	GLB	17417	26.73±3.16	0.010	0.008	2.56E-01	0.008	0.006	1.78E-01
Hematological	Neutrophil percentage	NEU%	12698	72.67±5.89	0.006	0.006	2.84E-01	0.009	0.004	2.23E-02
Protein	Total protein	TP	17417	70.89±3.83	0.007	0.007	2.97E-01	0.005	0.005	3.48E-01
Metabolism	Vitamin D	Vit D	1401	19.60±6.93	-0.012	0.012	2.98E-01	0.000	0.009	9.74E-01
Hematological	Red cell distribution width-coefficient of variation	RDW-CV	18782	13.31±1.37	-0.020	0.020	3.07E-01	-0.016	0.013	2.37E-01
Infection	Cytomegalovirus IgG antibody	CMV IgG	6928	6445(93.03%)	0.176	0.174	3.11E-01	0.133	0.118	2.57E-01
Infection	Hepatitis B envelope antigen	HBeAg	17851	10.88±140.44	0.000	0.000	3.18E-01	0.000	0.000	3.44E-01
Hormone	β-human chorionic gonadotropin	β-hCG	8250	36380.08±48609.71	0.000	0.000	3.24E-01	0.000	0.000	2.72E-01
Urinalysis	Bacteria count in urine	BACT U	18606	1037.48±2397.54	0.000	0.000	3.74E-01	0.000	0.000	2.32E-02
Urinalysis	Bilirubin in urine	BIL	18619	129(0.69%)	0.242	0.279	3.85E-01	-0.372	0.215	8.39E-02
Hematological	Lymphocyte percentage	LYM%	12698	20.41±5.07	-0.005	0.006	3.96E-01	-0.009	0.004	4.06E-02
Hematological	Eosinophil percentage	EOS%	12698	1.05±1.01	-0.028	0.033	3.98E-01	-0.013	0.022	5.50E-01
Infection	Rubella IgM antibody	Rub IgM	8675	72(0.83%)	-0.381	0.467	4.15E-01	-0.160	0.278	5.66E-01
Infection	Rubella IgG antibody	Rub IgG	7250	5765(79.52%)	-0.074	0.099	4.58E-01	-0.066	0.071	3.54E-01
Hematological	Monocyte percentage	MONO%	12698	5.67±1.77	-0.012	0.018	4.95E-01	-0.021	0.012	9.51E-02
Urinalysis	White blood cell count in urine	WBC U	18607	72.35±270.76	0.000	0.000	5.04E-01	0.000	0.000	5.69E-01
Infection	Herpes simplex virus type 2 specific IgM antibody	HSV-2 IgM	6592	18(0.27%)	0.371	0.770	6.30E-01	-0.539	0.627	3.90E-01
Liver-related	Total bile acid	TBA	230	2.36±2.82	-0.069	0.150	6.46E-01	0.037	0.078	6.39E-01
Electrolyte	Calcium	Ca	150	2.36±0.10	1.504	3.602	6.76E-01	-2.491	1.669	1.38E-01
Hematological	Mean corpuscular hemoglobin	MCH	18790	29.45±1.94	0.005	0.013	6.87E-01	0.001	0.009	9.20E-01
Hematological	Basophil count	BASO	12698	0.02±0.04	0.237	0.596	6.91E-01	1.095	0.544	4.43E-02
Hematological	Eosinophil count	EOS	12698	0.10±0.10	0.110	0.322	7.31E-01	0.170	0.225	4.48E-01
Infection	Hepatitis B surface antibody	HBsAb	17851	335.96±390.31	0.000	0.000	7.42E-01	0.000	0.000	6.38E-01
Hormone	Thyroid-stimulating hormone	TSH	17627	1.52±2.16	-0.004	0.014	7.50E-01	-0.005	0.008	5.80E-01
Urinalysis	Red blood cell count in urine	RBC U	18607	28.61±176.91	0.000	0.000	7.88E-01	0.000	0.000	3.11E-01
Urinalysis	Epithelial cell count in urine	EC U	18452	36.36±44.17	0.000	0.001	7.90E-01	0.000	0.000	4.56E-01
Kidney-related	Blood urea nitrogen	BUN	17807	2.77±0.74	0.009	0.036	8.00E-01	0.007	0.025	7.85E-01
Infection	Toxoplasma gondii IgG antibody	TOX IgG	6974	553(7.93%)	-0.043	0.171	8.01E-01	-0.071	0.121	5.55E-01
Electrolyte	Chloride	CL	150	100.65±3.02	0.031	0.126	8.07E-01	0.093	0.057	1.07E-01
Infection	Hepatitis C virus antibody	Anti-HCV	18244	0.19±3.01	0.002	0.009	8.28E-01	0.004	0.006	5.64E-01
Urinalysis	Nitrites in urine	NIT	18619	176(0.95%)	0.053	0.267	8.43E-01	-0.079	0.192	6.81E-01
Urinalysis	Fibers in urine	FIBERS	18608	2.03±8.55	0.001	0.003	8.55E-01	0.000	0.002	9.90E-01
Urinalysis	Yeast-like cell in urine	YLC	18608	3.39±24.87	0.000	0.001	8.57E-01	0.000	0.001	9.85E-01
Protein	Albumin	ALB	17417	44.17±2.52	0.002	0.011	8.74E-01	-0.002	0.007	7.92E-01
Electrolyte	Potassium	K	150	3.92±0.40	0.128	0.821	8.76E-01	0.276	0.403	4.96E-01
Urinalysis	Urinary tract infection	UTI	18453	13304(72.10%)	0.009	0.058	8.78E-01	0.019	0.041	6.46E-01
Urinalysis	Lysed red blood cell percentage in urine	Lysed RBC(%)	18453	5.18±13.95	0.000	0.002	8.80E-01	0.000	0.001	7.52E-01
Infection	Toxoplasma gondii IgM antibody	TOX IgM	6975	19(0.27%)	-0.128	1.055	9.03E-01	0.213	0.718	7.66E-01
Hematological	Basophil percentage	BASO%	12698	0.20±0.24	0.014	0.114	9.04E-01	0.153	0.085	7.25E-02
Hematological	Red cell distribution width-standard deviation	RDW-SD	15799	41.60±3.24	-0.001	0.009	9.26E-01	0.000	0.006	9.64E-01
Urinalysis	Urobilinogen	UBG	18619	1376(7.39%)	-0.006	0.100	9.51E-01	-0.161	0.070	2.22E-02
Urinalysis	Blood in urine	BLD	18619	4787(25.71%)	-0.003	0.059	9.54E-01	0.052	0.042	2.14E-01
Infection	Cytomegalovirus IgM antibody	CMV IgM	6929	36(0.52%)	-0.013	1.062	9.90E-01	0.806	0.757	2.87E-01

Table S2. The multiple regression results of GDM status with potential risk factors, related to Table 1

Model:	Logit			
Method:	Maximum Likelihood Estimation			
Df Model:	20			
No. Observations:	3472			
Pseudo R-squared:	0.132			
Log-Likelihood:	-872.266			
LL-Null:	-970.913			
Prob (F-statistic):	3.86E-31			
variables	coef	stderr	z	P> z
Intercept	-12.524	1.597	-7.841	4.46E-15
Age	0.122	0.019	6.311	2.78E-10
GLU U	0.907	0.176	5.156	2.52E-07
GGT	0.031	0.006	4.721	2.35E-06
UA	0.006	0.001	4.075	4.61E-05
PA	0.007	0.002	3.555	3.78E-04
ALT	-0.016	0.006	-2.701	6.90E-03
BMI	0.032	0.02	1.556	1.20E-01
HTN	0.533	0.406	1.312	1.89E-01
ALP	0.009	0.007	1.294	1.96E-01
FTP	-0.166	0.146	-1.135	2.56E-01
HGB	0.016	0.017	0.941	3.47E-01
NEU	0.18	0.229	0.788	4.31E-01
WBC	-0.148	0.219	-0.674	5.01E-01
RBC	-0.195	0.32	-0.61	5.42E-01
PLT	0.004	0.008	0.53	5.96E-01
LYM	0.153	0.297	0.514	6.07E-01
pH	0.046	0.095	0.478	6.33E-01
P-LCR	0.008	0.023	0.34	7.34E-01
PCT	-2.755	8.146	-0.338	7.35E-01
HCT	0.007	0.06	0.12	9.05E-01

Notes: HTN, FTP, Age and BMI are abbreviations for hypertension, first-time pregnancy, maternal age and body mass index, respectively. The full names of other abbreviations are provided in [Table S1](#).

Table S3. The multiple regression analysis of GDM AUC with potential risk factors, related to Table 1

Model:	Ordinary Least Square			
Method:	Least Squares			
Df Model:	24			
No. Observations:	3472			
R-squared:	0.132			
F-statistic:	21.783			
Prob (F-statistic):	4.50E-88			
variables	coef	stderr	z	P> z
Intercept	4.202	2.287	1.837	6.62E-02
Age	0.123	0.011	11.186	1.47E-28
UA	0.005	0.001	6.193	6.61E-10
PA	0.006	0.001	5.559	2.92E-08
GLU U	0.625	0.114	5.491	4.28E-08
Cr	-0.032	0.006	-5.416	6.52E-08
GGT	0.023	0.005	5.035	5.02E-07
DBIL	-0.387	0.095	-4.074	4.73E-05
BMI	0.046	0.012	3.887	1.04E-04
TBIL	0.115	0.033	3.477	5.14E-04
AST	-0.029	0.009	-3.036	2.41E-03
ALT	0.014	0.005	2.727	6.43E-03
FTP	-0.096	0.075	-1.277	2.02E-01
HGB	0.008	0.009	0.902	3.67E-01
PLT	0.004	0.005	0.872	3.83E-01
HTN	0.247	0.316	0.780	4.35E-01
PCT	-3.295	4.788	-0.688	4.91E-01
MPV	0.186	0.294	0.633	5.27E-01
ALP	-0.002	0.004	-0.615	5.39E-01
NEU	0.053	0.131	0.403	6.87E-01
P-LCR	-0.013	0.035	-0.375	7.07E-01
WBC	-0.033	0.125	-0.266	7.90E-01
LYM	-0.015	0.165	-0.089	9.29E-01
HCT	0.002	0.031	0.059	9.53E-01
RBC	0.007	0.159	0.047	9.63E-01

Notes: HTN, FTP, Age and BMI are abbreviations for hypertension, first-time pregnancy, maternal age and body mass index, respectively. The full names of other abbreviations are provided in [Table S1](#).

Table S4. Correlation tests between GDM traits and clinical diagnosis, related to Table 1

Traits info	n(%)			GDM status		GDM AUC	
	Name	All samples	In GDM control	In GDM case	OR	Pvalue	T
Pregnancy with uterine scar, n(%)	1844(11.34%)	1583(10.98%)	261(14.22%)	1.344	4.50E-05	6.883	6.08E-12
Premature rupture of membranes, n(%)	2469(15.19%)	2266(15.72%)	203(11.06%)	0.667	1.95E-07	-3.211	1.33E-03
Intrauterine distress, n(%)	1419(8.73%)	1306(9.06%)	113(6.15%)	0.658	4.02E-05	-4.023	5.77E-05
Threatened preterm birth, n(%)	404(2.49%)	348(2.41%)	56(3.05%)	1.272	1.16E-01	2.516	1.19E-02
Threatened abortion, n(%)	319(1.96%)	278(1.93%)	41(2.23%)	1.162	4.25E-01	2.256	2.41E-02
Eclampsia, n(%)	220(1.35%)	182(1.26%)	38(2.07%)	1.653	6.67E-03	5.272	1.37E-07
Gestational hypertension, n(%)	224(1.38%)	198(1.37%)	26(1.42%)	1.032	9.66E-01	3.795	1.48E-04
Ineffective uterine contractions, n(%)	287(1.77%)	266(1.84%)	21(1.14%)	0.616	4.00E-02	0.583	5.60E-01
Oligohydramnios, n(%)	371(2.28%)	346(2.40%)	25(1.36%)	0.561	6.49E-03	-2.156	3.11E-02
Nuchal cord, n(%)	773(4.76%)	758(5.26%)	15(0.82%)	0.148	6.22E-17	-6.399	1.61E-10
Intrahepatic cholestasis of pregnancy, n(%)	112(0.69%)	98(0.68%)	14(0.76%)	1.123	7.99E-01	-0.631	5.28E-01
Pregnancy with vomiting, n(%)	132(0.81%)	120(0.83%)	12(0.65%)	0.784	5.06E-01	-3.924	8.75E-05
Pregnancy with uterine fibroids, n(%)	96(0.59%)	83(0.58%)	13(0.71%)	1.232	5.92E-01	1.282	2.00E-01
fetal abnormalities, n(%)	122(0.75%)	110(0.76%)	12(0.65%)	0.856	7.13E-01	-0.969	3.32E-01
Cervical polyps, n(%)	68(0.42%)	56(0.39%)	12(0.65%)	1.687	1.43E-01	1.061	2.89E-01

Table S5. Identified associated loci from GWAS, related to Figures 1-2

Trait	Locus INFO						Top SNP INFO						
	name	chrom	start	end	width	snp_count	locus_gene	pos	SNP	gene	pvalue	beta/log(OR)	se
GDM		6	20623948	20724019	100072	54	CDKAL1	20675561	rs35261542	CDKAL1	3.88E-12	0.306	0.044
GDM		11	92980604	92980604	1	1	MTNR1B	92980604	rs3781637	MTNR1B	3.29E-09	-0.676	0.114
GDM_AUC		6	20614483	20829886	215404	76	CDKAL1	20680447	rs9348441	CDKAL1	1.40E-30	0.155	0.013
GDM_AUC		7	128203335	128217273	13939	13	MIR129-1	128212012	rs11981584	<MIR129-1	2.74E-10	0.284	0.045
GDM_AUC		9	4291435	4307369	15935	3	RNU6-694P	4307369	rs79321323	RNU6-694P>	2.41E-12	0.262	0.037
GDM_AUC		10	92492827	92664301	171475	12	IDE, KIF11, EIF2S2P3	92627577	rs1573051	KIF11	8.97E-11	0.090	0.014
GDM_AUC		11	92934966	92994860	59895	11	SNRPGP16, MTNR1B	92980604	rs3781637	MTNR1B	2.09E-31	-0.378	0.032
0hGlu		2	168797218	168956919	159702	3	NOSTRIN, ABCB11	168797218	rs12612019	NOSTRIN>	1.05E-08	-0.707	0.123
0hGlu		5	96379021	96428986	49966	9	PCSK1	96383972	rs10213823	PCSK1>	3.72E-11	-0.091	0.014
0hGlu		6	20664850	20713683	48834	11	CDKAL1	20680447	rs9348441	CDKAL1	1.57E-09	0.083	0.014
0hGlu		6	152026723	152047623	20901	15	ESR1	152036096	rs3020430	ESR1	1.36E-08	0.074	0.013
0hGlu		7	44078957	44137627	58671	8	POLM, POLD2, RNA5SP230, MYL7	44124463	rs3217944	<POLD2	3.57E-13	-0.116	0.016
0hGlu		9	627420	679916	52497	23	KANK1	655864	rs75885458	KANK1	4.78E-09	0.171	0.029
0hGlu		9	4291435	4291435	1	1	RNU6-694P	4291435	rs57132962	RNU6-694P>	1.07E-09	0.306	0.050
0hGlu		11	92948256	92994860	46605	5	SNRPGP16, MTNR1B	92980604	rs3781637	MTNR1B	9.10E-16	-0.264	0.033
0hGlu		20	22540023	22625059	85037	48	LINC00261, FOXA2, LNCNEF	22580744	rs6048206	FOXA2>	2.34E-11	-0.122	0.018
1hGlu		6	20620906	20724019	103114	58	CDKAL1	20675561	rs35261542	CDKAL1	7.41E-25	0.139	0.014
1hGlu		7	128205603	128212012	6410	7	MIR129-1	128212012	rs11981584	<MIR129-1	5.58E-09	0.264	0.045
1hGlu		9	4307369	4307369	1	1	RNU6-694P	4307369	rs79321323	RNU6-694P>	9.89E-09	0.215	0.037
1hGlu		10	92492827	92664301	171475	12	IDE, KIF11, EIF2S2P3	92627577	rs1573051	KIF11	1.50E-10	0.090	0.014
1hGlu		11	92936503	92994860	58358	10	SNRPGP16, MTNR1B	92980604	rs3781637	MTNR1B	5.09E-27	-0.351	0.033
2hGlu		6	20620906	20829886	208981	63	CDKAL1	20680447	rs9348441	CDKAL1	4.61E-25	0.140	0.014
2hGlu		9	4291435	4307369	15935	3	RNU6-694P	4291435	rs57132962	RNU6-694P>	7.22E-13	0.357	0.050
2hGlu		10	69215297	69215297	1	1	HKDC1	69215297	rs58433451	HKDC1>	6.64E-09	-0.638	0.110
2hGlu		11	92948256	92994245	45990	5	SNRPGP16, MTNR1B	92980604	rs3781637	MTNR1B	5.31E-18	-0.281	0.032

Table S6. The summary of GDM associated SNP, related to Figure 2

SNP	rs35261542	rs3781637	rs7754840	rs9348441	rs10830962	rs10830963
Chr	6	11	6	6	11	11
Pos	20675561	92980604	20661019	20680447	92965261	92975544
Gene	<i>CDKALI</i>	<i>MTNR1B</i>	<i>CDKALI</i>	<i>CDKALI</i>	<i>MTNR1B</i>	<i>MTNR1B</i>
A1	A	C	C	A	G	G
A1 freq	0.3729	0.1409	0.3875	0.3606	0.4322	0.4207
Freq EAS	0.3859	0.1349	0.4008	0.3869	0.4296	0.4226
Freq EUR	0.2793	0.0089	0.3181	0.2783	0.4125	0.2883
pv in GDM status	3.88E-12	3.29E-09	4.90E-11	4.00E-12	NA	NA
pv in GDM_{AUC}	1.97E-30	2.09E-31	3.22E-27	1.40E-30	NA	NA
pv in GCST90239750	2.36E-14	2.97E-01	1.51E-11	1.57E-14	3.01E-37	4.30E-54
HWE	1.33E-02	8.31E-02	1.59E-03	4.24E-01	0.00E+00	0.00E+00
Note					removed due to HWE	removed due to HWE

Notes : A1 freq indicates the allele frequency of A1 allele, and HWE indicates hardy-Weinberg equilibrium p-value for each SNP. The Freq EAS and Freq EUR are allele frequencies in EAS and EUR population, respectively, obtained from 1000 genomes project phase 3.

Table S7. Detailed information of studies downloaded from the GWAS catalog, related to Figures 5-6

Index	GWAS catalog study ID	Trait name	Journal	Title	Population	Publication date	URL
1	GCST90018959	Height	Nat Genet	A cross-population atlas of genetic associations for 220 human phenotypes. ¹	360,388 European ancestry individuals, 165,056 East Asian ancestry individuals	2021/9/30	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90018001-GCST90019000/GCST90018959
2	GCST90018949	Weight	Nat Genet	A cross-population atlas of genetic associations for 220 human phenotypes. ¹	360,116 European ancestry individuals, 165,419 East Asian ancestry individuals	2021/9/30	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90018001-GCST90019000/GCST90018949
3	GCST90000025	Appendicular lean mass	Commun Biol	The genetic architecture of appendicular lean mass characterized by association analysis in the UK Biobank study. ²	450243 European	2020/10/23	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90000001-GCST90001000/GCST90000025
4	GCST90020028	Hip circumference adjusted for BMI	Sci Rep	GWAS of allometric body-shape indices in UK Biobank identifies loci suggesting associations with morphogenesis, organogenesis, adrenal cell renewal and cancer. ³	219872 European	2021/5/21	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90020001-GCST90021000/GCST90020028
5	GCST006979	Heel bone mineral density	Nat Genet	An atlas of genetic influences on osteoporosis in humans and mice. ⁴	426824 European	2018/12/31	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST006001-GCST007000/GCST006979
6	GCST006655	Breast size	Sci Rep	Japanese GWAS identifies variants for bust-size, dysmenorrhea, and menstrual fever that are eQTLs for relevant protein-coding or long non-coding RNAs. ⁵	5,609 Japanese ancestry female individuals	2018/5/31	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST006001-GCST007000/GCST006655
7	GCST90018972	Systolic blood pressure	Nat Genet	A cross-population atlas of genetic associations for 220 human phenotypes. ¹	340,159 European ancestry individuals, 145,505 East Asian ancestry individuals	2021/9/30	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90018001-GCST90019000/GCST90018972
8	GCST90018952	Diastolic blood pressure	Nat Genet	A cross-population atlas of genetic associations for 220 human phenotypes. ¹	340,162 European ancestry individuals, 145,515 East Asian ancestry individuals	2021/9/30	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90018001-GCST90019000/GCST90018952
9	GCST90018970	Pulse pressure	Nat Genet	A cross-population atlas of genetic associations for 220 human phenotypes. ¹	360,863 European ancestry individuals, 145,445 East Asian ancestry individuals	2021/9/30	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90018001-GCST90019000/GCST90018970
10	GCST90019516	Serum phosphate levels	Nat Genet	Genetics of 35 blood and urine biomarkers in the UK Biobank. ⁶	312888 European, 5568 African unspecified, 6685 South Asian	2021/1/18	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90019001-GCST90020000/GCST90019516
11	GCST90018976	Serum total protein level	Nat Genet	A cross-population atlas of genetic associations for 220 human phenotypes. ¹	314,921 European ancestry individuals, 133,321 East Asian ancestry individuals	2021/9/30	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90018001-GCST90019000/GCST90018976
12	GCST90019511	Insulin-like growth factor 1 levels	Nat Genet	Genetics of 35 blood and urine biomarkers in the UK Biobank. ⁶	340,567 European ancestry individuals, 5,974 African ancestry individuals, 7,283 South Asian ancestry individuals	2021/1/18	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90019001-GCST90020000/GCST90019511
13	GCST90018799	Breast cancer	Nat Genet	A cross-population atlas of genetic associations for 220 human phenotypes. ¹	17,389 European ancestry cases, 240,341 European ancestry controls, 6,325 East Asian ancestry cases, 73,225 East Asian ancestry controls	2021/9/30	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90018001-GCST90019000/GCST90018799
14	GCST90000047	Age at first sexual intercourse	Nat Hum Behav	Identification of 371 genetic variants for age at first sex and birth linked to externalising behaviour. ⁷	397338 European	2021/7/1	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90000001-GCST90001000/GCST90000047
15	GCST90011804	Breast cancer	Nat Commun	Pan-cancer study detects genetic risk variants and shared genetic basis in two large cohorts. ⁸	428231 European	2020/9/4	http://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90011001-GCST90012000/GCST90011804

Table S8. Genetic correlation between glycemc traits and ESR1-associated traits, related to Figure 5

Traits info		0hGlu				GDM status				GDM AUC			
GWAS catalog study ID	Traits name	Rg	Stderr.	Zscore	Pvalue	Rg	Stderr.	Zscore	Pvalue	Rg	Stderr.	Zscore	Pvalue
GCST90018952	Diastolic blood pressure	0.259	0.080	3.219	1.30E-03	0.483	0.377	1.280	2.01E-01	0.190	0.070	2.734	6.30E-03
GCST90018972	Systolic blood pressure	0.244	0.069	3.512	4.00E-04	0.358	0.289	1.236	2.17E-01	0.185	0.070	2.662	7.80E-03
GCST90018949	Weight	0.169	0.060	2.810	5.00E-03	0.202	0.187	1.082	2.80E-01	0.037	0.062	0.598	5.50E-01
GCST006979	Heel bone mineral density	0.069	0.058	1.194	2.33E-01	0.164	0.200	0.817	4.14E-01	0.044	0.058	0.754	4.51E-01
GCST90000047	Age at first sexual intercourse	-0.062	0.061	-1.012	3.12E-01	-0.121	0.154	-0.786	4.32E-01	-0.029	0.052	-0.553	5.80E-01
GCST90019516	Serum phosphate levels	-0.455	0.282	-1.613	1.07E-01	-0.240	0.307	-0.781	4.35E-01	-0.012	0.127	-0.091	9.27E-01
GCST90018970	Pulse pressure	0.129	0.064	2.005	4.50E-02	0.098	0.156	0.626	5.31E-01	0.110	0.070	1.565	1.18E-01
GCST90018976	Serum total protein level	-0.032	0.080	-0.393	6.94E-01	-0.108	0.174	-0.619	5.36E-01	0.026	0.075	0.344	7.31E-01
GCST90019511	Insulin-like growth factor 1 levels	-0.120	0.072	-1.662	9.66E-02	-0.116	0.217	-0.533	5.94E-01	-0.022	0.071	-0.308	7.58E-01
GCST90000025	Appendicular lean mass	0.038	0.054	0.704	4.82E-01	-0.056	0.116	-0.482	6.30E-01	-0.023	0.056	-0.415	6.78E-01
GCST90018959	Height	0.041	0.057	0.717	4.73E-01	0.038	0.116	0.325	7.45E-01	-0.030	0.057	-0.536	5.92E-01
GCST90020028	Hip circumference adjusted for BMI	-0.025	0.067	-0.367	7.14E-01	-0.038	0.154	-0.245	8.06E-01	-0.062	0.068	-0.916	3.60E-01
GCST006655	Breast size	-0.365	0.380	-0.963	3.36E-01	-0.059	0.465	-0.128	8.98E-01	0.223	0.319	0.699	4.84E-01
GCST90018799	Breast cancer	-0.055	0.130	-0.421	6.74E-01	-0.031	0.257	-0.120	9.05E-01	-0.132	0.129	-1.023	3.06E-01

Table S9. Mendelian randomization of ESRI-associated traits on glycemic traits, related to Figure 6

Exposure info		Outcome: 0hGlu						Outcome: GDM status					Outcome: GDM AUC						
GWAS catalog study ID	Traits name	Estimate	Stderr.	Zvalue	OR	Pvalue	(95% CI)	Estimate	Stderr.	Zvalue	OR	Pvalue	(95% CI)	Estimate	Stderr.	Zvalue	OR	Pvalue	(95% CI)
GCST006979	Heel bone mineral density	0.076	0.227	0.334	1.079	7.38E-01	(0.69, 1.68)	0.206	1.640	0.125	1.228	9.00E-01	(0.05, 30.60)	0.090	1.142	0.079	1.094	9.37E-01	(0.12, 10.26)
GCST90000025	Appendicular lean mass	-0.747	0.259	-2.886	0.474	3.91E-03	(0.29, 0.79)	-3.878	1.875	-2.069	0.021	3.86E-02	(0.00, 0.82)	-3.123	1.302	-2.400	0.044	1.64E-02	(0.00, 0.56)
GCST90000047	Age at first sexual intercourse	-0.004	0.037	-0.095	0.996	9.24E-01	(0.93, 1.07)	-0.014	0.270	-0.051	0.986	9.59E-01	(0.58, 1.67)	-0.091	0.188	-0.485	0.913	6.28E-01	(0.63, 1.32)
GCST90011804	Breast cancer	-0.131	0.117	-1.118	0.877	2.64E-01	(0.70, 1.10)	-0.719	0.777	-0.925	0.487	3.55E-01	(0.11, 2.24)	-0.935	0.540	-1.730	0.393	8.36E-02	(0.14, 1.13)
GCST90019516	Serum phosphate levels	0.422	0.144	2.930	1.525	3.40E-03	(1.15, 2.02)	0.402	1.038	0.387	1.495	6.99E-01	(0.20, 11.44)	0.111	0.724	0.153	1.117	8.79E-01	(0.27, 4.62)
GCST90020028	Hip circumference adjusted for BMI	-0.043	0.022	-1.997	0.958	4.59E-02	(0.92, 1.00)	0.130	0.155	0.835	1.138	4.03E-01	(0.84, 1.54)	-0.069	0.108	-0.632	0.934	5.27E-01	(0.75, 1.15)
GCST90018959	Height	0.437	0.196	2.224	1.548	2.61E-02	(1.05, 2.28)	1.598	1.714	0.933	4.943	3.51E-01	(0.17, 142.15)	3.366	1.185	2.840	28.963	4.51E-03	(2.84, 295.49)
GCST90018970	Pulse pressure	0.409	0.111	3.703	1.506	2.14E-04	(1.21, 1.87)	0.499	0.970	0.515	1.647	6.07E-01	(0.25, 11.03)	1.740	0.670	2.597	5.700	9.41E-03	(1.53, 21.20)

Table S10. Transcriptome-wide association study for glycemic traits, related to STAR Methods

Tissue info		0hGlu			GDM status			GDM AUC			
Category	Tissue	Beta	Stderr.	Pvalue	Beta	Stderr.	Pvalue	Beta	Stderr.	Pvalue	
Adipose	Adipose Subcutaneous	5.594	2.950	5.80E-02	11.784	21.243	5.79E-01	-11.120	14.835	4.54E-01	
	Adipose Visceral (Omentum)	-2.922	0.739	7.77E-05	-14.039	5.319	8.31E-03	-8.498	3.720	2.24E-02	
Blood/Immune	Cells EBV-transformed lymphocytes	0.851	0.252	7.48E-04	4.321	1.816	1.73E-02	2.335	1.270	6.59E-02	
	Spleen	0.089	0.918	9.23E-01	-2.579	6.655	6.98E-01	-7.382	4.619	1.10E-01	
	Whole Blood	-28.945	15.127	5.57E-02	-61.981	108.560	5.68E-01	-53.266	76.046	4.84E-01	
CNS	Brain Amygdala	5.180	1.720	2.60E-03	24.459	12.324	4.72E-02	11.483	8.646	1.84E-01	
	Brain Anterior cingulate cortex (BA24)	-1.414	0.412	5.95E-04	-6.762	2.958	2.23E-02	-3.831	2.071	6.43E-02	
	Brain Caudate (basal ganglia)	-4.321	1.380	1.74E-03	-18.077	10.047	7.20E-02	-19.259	6.942	5.54E-03	
	Brain Cerebellar Hemisphere	-0.065	1.032	9.50E-01	-5.569	7.425	4.53E-01	0.849	5.186	8.70E-01	
	Brain Cerebellum	-2.715	0.666	4.57E-05	-12.835	4.799	7.48E-03	-9.493	3.350	4.61E-03	
	Brain Cortex	1.688	1.579	2.85E-01	3.383	11.420	7.67E-01	16.232	7.942	4.10E-02	
	Brain Frontal Cortex (BA9)	0.496	0.449	2.69E-01	0.035	3.241	9.91E-01	0.302	2.257	8.94E-01	
	Brain Hippocampus	-12.308	9.696	2.04E-01	63.231	69.789	3.65E-01	-13.170	48.767	7.87E-01	
	Brain Hypothalamus	-2.257	0.640	4.22E-04	-4.449	4.601	3.34E-01	-4.158	3.220	1.97E-01	
	Brain Nucleus accumbens (basal ganglia)	-2.137	0.408	1.69E-07	-6.178	2.919	3.43E-02	-6.236	2.056	2.43E-03	
	Brain Putamen (basal ganglia)	-3.997	1.587	1.18E-02	-18.198	11.534	1.15E-01	-9.970	7.985	2.12E-01	
	Brain Spinal cord (cervical c-1)	0.318	0.218	1.45E-01	1.960	1.576	2.14E-01	1.764	1.099	1.09E-01	
	Brain Substantia nigra	-5.209	1.684	1.99E-03	-27.008	12.140	2.61E-02	-13.290	8.475	1.17E-01	
	Cardiovascular	Artery Aorta	3.261	1.522	3.22E-02	10.779	11.085	3.31E-01	21.671	7.660	4.67E-03
		Artery Coronary	-5.648	1.605	4.34E-04	-26.222	11.520	2.28E-02	-12.912	8.073	1.10E-01
Artery Tibial		4.367	0.858	3.61E-07	13.848	6.157	2.45E-02	16.170	4.318	1.81E-04	
Heart Atrial Appendage		1.106	1.711	5.18E-01	-6.927	12.338	5.75E-01	7.991	8.596	3.53E-01	
Heart Left Ventricle		-1.919	0.803	1.68E-02	-3.601	5.781	5.33E-01	-0.204	4.041	9.60E-01	
Colon Sigmoid		-3.848	3.696	2.98E-01	-30.791	26.708	2.49E-01	-14.250	18.585	4.43E-01	
Digestive	Colon Transverse	-3.170	0.908	4.86E-04	-16.483	6.535	1.17E-02	-8.894	4.570	5.17E-02	
	Esophagus Gastroesophageal Junction	18.303	6.506	4.91E-03	80.647	46.713	8.43E-02	31.393	32.730	3.37E-01	
	Esophagus Mucosa	5.802	2.101	5.75E-03	32.588	15.131	3.13E-02	20.353	10.566	5.41E-02	
	Esophagus Muscularis	1.008	1.311	4.42E-01	-0.880	9.480	9.26E-01	-7.531	6.598	2.54E-01	
	Small Intestine Terminal Ileum	5.524	1.615	6.26E-04	25.208	11.580	2.95E-02	11.449	8.124	1.59E-01	
	Stomach	6.243	2.339	7.61E-03	31.010	16.714	6.35E-02	16.135	11.754	1.70E-01	
	Adrenal Gland	8.190	3.202	1.05E-02	37.301	22.863	1.03E-01	22.234	16.093	1.67E-01	
Endocrine	Pancreas	-2.558	1.479	8.38E-02	-17.109	10.611	1.07E-01	-7.161	7.434	3.35E-01	
	Pituitary	12.214	2.518	1.24E-06	44.345	18.084	1.42E-02	37.282	12.682	3.29E-03	
	Thyroid	2.616	0.865	2.50E-03	11.291	6.244	7.06E-02	10.724	4.353	1.38E-02	
	Liver	-7.394	4.853	1.28E-01	-54.706	34.872	1.17E-01	-22.213	24.392	3.62E-01	
Musculoskeletal/connective	Muscle Skeletal	-10.001	3.362	2.94E-03	-37.286	24.057	1.21E-01	-23.627	16.902	1.62E-01	
Reproductive	Ovary	-20.964	5.951	4.28E-04	-76.249	42.746	7.45E-02	-47.004	29.927	1.16E-01	
	Prostate	-3.601	0.740	1.15E-06	-9.072	5.291	8.64E-02	-10.074	3.723	6.82E-03	
	Testis	-3.334	2.109	1.14E-01	-18.805	15.407	2.22E-01	-26.571	10.610	1.23E-02	
	Uterus	0.290	0.219	1.84E-01	2.592	1.575	9.99E-02	0.858	1.099	4.35E-01	
	Vagina	-0.498	0.150	9.18E-04	-2.448	1.081	2.35E-02	-1.328	0.755	7.88E-02	
	Breast Mammary Tissue	-8.010	3.230	1.32E-02	-50.069	23.264	3.14E-02	-19.646	16.245	2.27E-01	

Other

Cells Transformed fibroblasts	-13.224	2.630	5.00E-07	-46.871	18.969	1.35E-02	-44.936	13.242	6.92E-04
Kidney Cortex	-1.762	0.924	5.65E-02	-6.758	6.599	3.06E-01	-4.428	4.644	3.40E-01
Lung	20.361	5.216	9.53E-05	74.485	37.386	4.63E-02	42.162	26.222	1.08E-01
Minor Salivary Gland	-3.379	1.122	2.61E-03	-17.953	8.067	2.60E-02	-9.387	5.643	9.62E-02
Nerve Tibial	-4.228	0.844	5.58E-07	-11.668	6.036	5.32E-02	-12.328	4.247	3.70E-03
Skin Not Sun Exposed (Suprapubic)	-10.573	3.592	3.25E-03	-46.750	25.791	6.99E-02	-14.686	18.055	4.16E-01
Skin Sun Exposed (Lower leg)	-3.269	2.084	1.17E-01	-18.319	14.996	2.22E-01	-7.293	10.477	4.86E-01

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