Cardiovascular Diabetology

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Metabolic pathways mediating insulin resistance and gestational diabetes mellitus discovered by high-dimensional systematic Mendelian randomization

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Abstract

Background Gestational diabetes mellitus (GDM), characterized by insulin resistance (IR) and β-cell dysfunction, is one of the most common complications of pregnancy with unmet needs of prevention methods.

Objective To investigate the causal role of insulin resistance and metabolic pathways in the pathogenesis of GDM with our proposed high-dimensional systematic Mendelian randomization (hdsMR) framework.

Methods Cases with GDM and controls with normal glucose tolerance were recruited at the University of Hong Kong–Shenzhen Hospital from 2015 to 2018. A total of 566 participants (aged > 18 years), including 274 with GDM, were enrolled after excluding subjects with major chronic diseases or long-term use of medications affecting glycolipid metabolism. Clinical characteristics and serum samples were collected during the GDM screening stage, and the genome and metabolome were tested. A novel hdsMR framework was proposed to estimate the causal role of IR index (Homeostasis Model Assessment of Insulin Resistance, HOMA-IR) and metabolic pathways in the pathogenesis of GDM.

Results Our hdsMR method confirmed that HOMA-IR was causal to GDM (odds ratio, 1.17; 95% confidence interval, 1.04–1.32) and revealed that two metabolic pathways (glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway) mediated 14.6% and 8.4%, respectively, between HOMA-IR and GDM. In an independent validation cohort comprising 255 pre-diabetic individuals, we showed that both pathways could be intervened

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through diet ($P < 0.05$). Furthermore, glyoxylate and dicarboxylate metabolism pathway was significantly associated with adverse pregnancy outcomes in GDM.

Conclusions These results indicated that targeting specific metabolic pathways through dietary intervention is worth exploring as a possible GDM prevention approach, and hdsMR is more efficient in finding causal mediating metabolic pathways than traditional MR methods.

Keywords Gestational diabetes mellitus, Mendelian randomization, Insulin resistance, Adverse pregnancy outcome, Metabolic pathway

Research insights

What is currently known about this topic?

- Gestational diabetes mellitus (GDM), characterized by metabolic disorders, including increased insulin resistance (IR) and β-cell defects, is one of the most common complications of pregnancy with unmet needs for prevention methods. However, the metabolic mechanism between IR and the pathogenesis of GDM remains unclear.

What is the key research question?

- What is the relationship among metabolome, IR, and GDM?

What is new?

- This study proposed a novel high-dimensional systematic Mendelian randomization framework. We identified the causal mediation effect of two metabolic pathways (glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway) from HOMA-IR to GDM and their impact on adverse pregnancy outcomes in GDM subjects.

How might this study influence clinical practice?

- These results indicated that targeting specific metabolic pathways through dietary modifications could be explored as a possible GDM prevention approach.

Background

Gestational diabetes mellitus (GDM) affects 14.2% of pregnant women globally and is associated with a variety of adverse pregnancy outcomes (APOs), including preeclampsia, macrosomia, preterm labor, stillbirth, and neonatal hyperinsulinemia [1–3]. Risk factors and underlying mechanisms of GDM have been studied extensively [4–7]. Nevertheless, there is still a scarcity of effective prevention methods to reduce the occurrence of GDM. GDM characterized by metabolic disorders include increased insulin resistance (IR) and β-cell defects [2]. As shown in previous studies, high IR increases the risk of GDM [8], whose resulting metabolic changes may be a potential target for GDM intervention. However, the metabolic mechanism between IR and the pathogenesis of GDM remains unclear.

Given that GDM is a metabolic disease, there have been many studies on the relationship between maternal metabolites and GDM [7, 9–12]. In addition to this, there is also evidence that modifications of metabolites involved in IR during pregnancy contribute to GDM development [13, 14], which provides a novel source of prevention and treatment targets for GDM. Recent research has focused on evaluating the causal relationship between serum metabolites and GDM by Mendelian randomization (MR), but few metabolites were found [15–17], which indicates the limited effects of single metabolites. This also illustrates that traditional MR approaches face challenges in handling high-dimensional metabolomic data and identifying pathway-level mediators. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, collaborative clusters with the defined biological function, have a more stable and sufficient influence on elucidating disease pathogenesis than individual molecules [18, 19]. This provides methodological direction to better quantify the role of metabolic pathways in mediating disease causation.

The aim of this study was to propose a high-dimensional systematic MR (hdsMR) framework that integrates MR and pathway quantification to assess causal relationships among IR, metabolic pathways and GDM. Building on these findings, we further examined whether these causal metabolic pathways influence APOs in women with GDM. Finally, using an independent external dataset of pre-diabetic individuals, we validated the potential for dietary interventions to modulate these metabolic pathways in glycemic management.

Methods

Study participants and ethical approval

The present study was conducted at the University of Hong Kong–Shenzhen Hospital from 2015 to 2018. A total of 566 pregnant women who met the following criteria were recruited: (a) Pregnant women aged 18 years or older; (b) performed oral glucose tolerance test (OGTT);

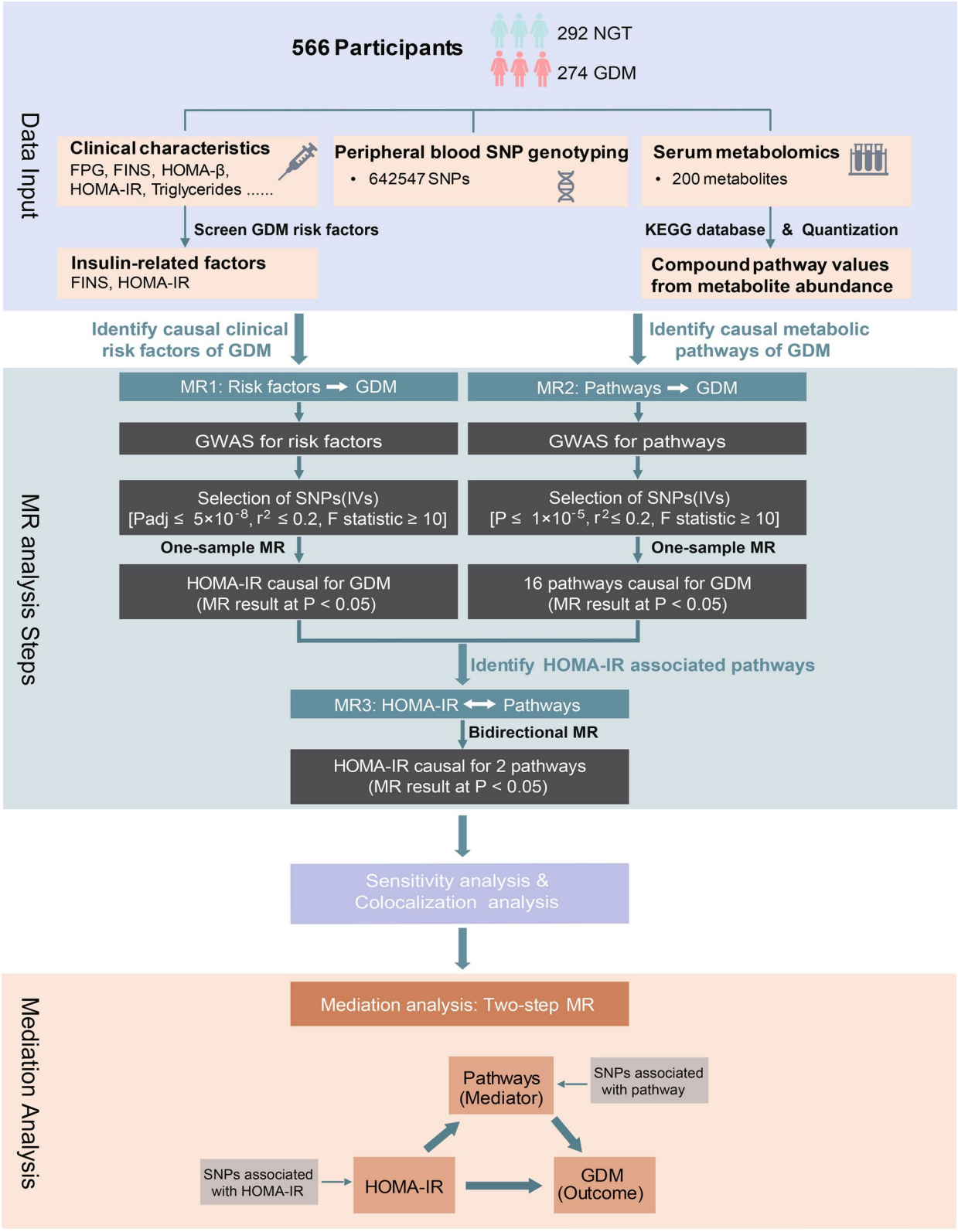

Fig. 1 (See legend on next page.)

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Fig. 1 Overview of hdsMR framework. Insulin-related factors (FINS and HOMA-IR) were screened as GDM risk factors by GLM analysis. Metabolic pathways were quantified by PC1 scores obtained by PCA for the metabolites based on the KEGG database. MR analysis was carried out for the following three parts: (1) MR1 was used to identify causal risk factors of GDM and HOMA-IR; (2) MR2 was used to identify causal metabolic pathways of GDM, and 16 pathways were likely causal for GDM; (3) MR3 was bidirectional MR analysis to identify HOMA-IR–associated pathways, and HOMA-IR was observed to have potential causal impacts on two pathways. Finally, mediation analysis based on two-step MR was performed to estimate the effects of HOMA-IR on GDM via pathways.

and (c) singleton pregnancy. Women with preexisting diabetes mellitus or chronic diseases (including cardiovascular, cerebrovascular, hepatic, renal, or autoimmune disorders), and those receiving long-term medications that affect glycolipid metabolism (e.g., glucocorticoids) were excluded.

The study was approved by the Institutional Review Board of the University of Hong Kong–Shenzhen Hospital ([2017]13) and conducted in accordance with the principles of the Declaration of Helsinki as revised in 2013. All of the participants signed written informed consent prior to enrolment. The workflow of the study is illustrated in Fig. 1.

Diagnosis of GDM

The standards recommended by the International Association of the Diabetes and Pregnancy Study Group (IADPSG) were used to diagnose GDM based on 2-h 75-g OGTT [20]. The pregnant women took 75 g of glucose between 24 and 28 weeks of gestation (OGTT week), and their venous plasma glucose level was measured at fasting and at 1 and 2 h after glucose administration. GDM was diagnosed if fasting plasma glucose (FPG) was ≥ 5.1 mmol/L, 1-h plasma glucose (1 h-PG) was ≥ 10.0 mmol/L, or 2-h plasma glucose (2 h-PG) was ≥ 8.5 mmol/L. All values for the OGTT lower than the thresholds were considered normal.

Data collection

Clinical characteristics

Serum samples and clinical characteristics, including age, height, pre-pregnancy weight, pregnancy weight gain, gestational week, FPG, 1 h-PG, 2 h-PG, hemoglobin A1c (HbA1c), total cholesterol (TC), triglycerides (TG), low-density lipoprotein–cholesterol (LDL-C), high-density lipoprotein–cholesterol (HDL-C), fasting insulin (FINS), and total bile acid, were collected during the GDM screening stage. Details of the measurement of these biochemical indicators have been described in previous research [21]. HOMA-β and HOMA-IR were computed from FPG and FINS, as shown in Eqs. (1) and (2) [22].The aera under curve of glucose (G_{AUC}) from the 75-g OGTT was calculated as shown in Eq. (3) [23]. Triglyceride-glucose (TyG) index was calculated as Eq. (4) [24].

$$HOMA-\beta = 20 \times FINS(\mathrm{mU/L})/[FPG(\mathrm{mmol/L}) - 3.5] \quad (1)$$

$$HOMA-IR = FINS(\mathrm{mU/L}) \times FPG(\mathrm{mmol/L})/22.5 \quad (2)$$

$$\begin{aligned} G_{AUC} = & \frac{1}{2} \times [FPG\,(\mathrm{mmol/L})] + 1\,\mathrm{h} - PG\ (\mathrm{mmol/L}) \times 1\,\mathrm{h} \\ & + \frac{1}{2} \times [1\,\mathrm{h} - PG\,(\mathrm{mmol/L}) + 2\,\mathrm{h} - PG\ (\mathrm{mmol/L})] \times 1\,\mathrm{h} \end{aligned} \quad (3)$$

$$\mathrm{TyG} = \ln(TG(\mathrm{mg/dL}) \times FPG(\mathrm{mg/dL})/2) \quad (4)$$

The definition of APOs was based on Simmons et al. and included any of the following, such as birth before 37 weeks of gestation, birth weight of 4500 g or greater, birth trauma, neonatal respiratory distress, phototherapy, stillbirth or neonatal death, or shoulder dystocia [25]. Among the GDM population, 64 individuals had APOs.

Serum metabolomics

Targeted metabolomic analysis was performed using Metabo-Profile (Shanghai, China). Detailed serum sample preparation, chemical materials for targeted metabolomics, and mass spectrometry (MS) acquisition and chromatographic conditions have been described previously by Luo et al [21]. Raw data files generated by ultra-high performance liquid chromatography (UPLC)-MS/MS were processed with Masslynx software (v4.1, Waters, Milford, MA, USA). A total of 200 metabolites were detected. Limit of detection was applied to fill in missing values of quantitative metabolomic data.

Single-nucleotide polymorphisms (SNP) genotyping and quality control

Genotyping was performed using Infinium Asian Screening Array-24 v1.0 BeadChip (Illumina, Inc., San Diego, CA, United States). The genotype data underwent strict quality control, and PLINK files were generated for subsequent analysis via PLINK (Version 1.9) [26]. SNPs with minor allele frequency < 0.01 and those not in Hardy–Weinberg equilibrium (HWE) were removed. Specifically, SNPs with HWE $P < 1 \times 10^{-6}$ in controls and $P < 1 \times 10^{-10}$ across all samples were excluded. After filtering, genotype imputation was done with 1000 Genomes Project data. To reduce linkage disequilibrium (LD), SNP pairs with $r^2 > 0.2$ were removed. Principal component analysis (PCA) was then applied to adjust for population substructure, retaining 566 samples and 479,053 SNPs for further analysis.

External metabolic validation data

We collected serum metabolite profiles from a randomized, controlled, single-blind dietary intervention study (NCT03222791) [27]. In that work, researchers conducted a 6-month dietary intervention in pre-diabetic individuals and 225 participants were randomly assigned to either a personalized postprandial glucose–targeting diet (PPT) (n = 113) or a Mediterranean diet (MED) (n = 112). The results demonstrated that diet intervention had a positive impact on glycemic control through changes in serum metabolites. The data can be accessed at https://static-content.springer.com/esm/art%3A10.1038%2Fs41467-023-41042-x/MediaObjects/41467_2023_41042_MOESM9_ESM.xlsx.

Metabolite landscape construction of the study population

MetaboAnalyst 6.0 (https://www.metaboanalyst.ca) [28] was used to analyze the metabolites in the NGT and GDM groups by univariate and multivariate analyses online. The differentially expressed metabolites (DEMs) were detected through a volcano plot, which combined the results from fold change (FC) analysis (FC > 1) and *t* tests (FDR < 0.05) into a single graph based on both biological significance and statistical significance. PCA was utilized for unsupervised clustering of metabolites among all samples, and one GDM subject that deviated from other samples was removed. Metabolite set enrichment analysis (MSEA) was performed to directly investigate the biological functions of the DEMs in the KEGG database with $P < 0.05$ regarded as statistically significant.

Framework of hdsMR

Metabolic pathways quantization

The latest KEGG pathways and metabolites contained in the pathways were downloaded by the KEGGREST package in R (https://doi.org/10.18129/B9.bioc.KEGGREST). Of the 200 metabolites detected, 92 were mapped to 205 pathways in the KEGG database. A hypergeometric test was performed to calculate the P values corrected by Bonferroni for those matched pathways, which was completed by the magrittr package in R (https://doi.org/10.32614/CRAN.package.magrittr). FDR < 0.05 was taken as the significant enrichment, and 52 of the 92 mapped pathways met this condition. Then, we performed PCA on the metabolites that each significant pathway contains and extracted the PC1 score as the quantitative characterization of the pathway. PCA was conducted by prcomp function in R (https://www.r-project.org/).

Genetic variants associated with insulin-related factors and metabolic pathways

To test for the association between genetic variants and GDM, a logistic regression model was used in PLINK 1.9 [26], including family history of diabetes, pre-gestational body mass index (BMI), changes in BMI, age, and principal component (PC) factors from population stratification as covariates. As for the associations of genetic variants with insulin-related factors (FINS, HOMA-IR) and metabolic pathways, a linear regression (additive model) was performed, adjusting for family history of diabetes, pre-gestational BMI, changes in BMI, age, and top 10 genotype-based PCs. The adjust parameter was used to obtain the P values of the multiple test correction for the abovementioned association analysis, providing Bonferroni-corrected P values along with FDR and other parameters.

Causal associations among insulin-related factors, metabolic pathways, and GDM

MR uses genetic variants to assess the causal relationships using observational data. A genetic variant can be considered an instrumental variable (IV) for a given exposure if it satisfies the following IV assumptions [29, 30]: (1) it is associated with the exposure; (2) it is not associated with the outcome due to confounding pathways; and (3) it does not affect the outcome, except potentially via the exposure. In this study, we performed MR using data from a single sample (known as one-sample MR), in which genetic variants, exposure, and outcome were measured in the same individuals [31]. All of the analyses were conducted using the MendelianRandomization (https://doi.org/10.32614/CRAN.package.MendelianRandomization) and TwoSampleMR (https://mrcieu.github.io/TwoSampleMR/) packages in R. MR analysis was carried out for three parts in our study as follows: (1) MR1 analysis step was to find out whether insulin-related factors were causal for GDM; (2) MR2 analysis step was to find metabolic pathways causal for GDM; and (3) MR3 analysis step was a bidirectional MR between insulin-related traits and metabolic pathways that were all associated with GDM.

SNPs selected for MR1 were based on a suggestive threshold of Bonferroni-corrected P values $< 5 \times 10^{-8}$ [32], and those for MR2 and MR3 were based on P values $< 1 \times 10^{-5}$ [33]. SNPs selected for IVs that are associated with GDM ($P < 1 \times 10^{-5}$) in PhenoScannerV2 (http://www.phenoscanner.medschl.cam.ac.uk/) were excluded when performing MR1 and MR2. F statistic was used to evaluate the effects of weak IVs [34], which was calculated with the following formula: $F = \frac{N-k-1}{k} \times \frac{R^2}{1-R^2}$, where N is the sample size in GWAS analysis, k is the number of IVs, and R^2 is the extent to which the IVs explain the exposure. R^2 was obtained from the get_r_from_pn function of the TwoSampleMR package. The SNPs with an F statistic greater than 10 were considered strong IVs and remained for the analysis. To avoid LD, the IVs were clumped using the criterion $r^2 < 0.2$ with a clumping

window of 50 kb for independence. The inverse-variance weighting (IVW) method, either in a fixed-effect framework (IVs ≤ 3) or in a multiplicative random-effect meta-analysis framework (IVs > 3) [35], was used to generate an overall estimate of the causal effect in each MR analysis. For each single SNP that remained after clumping, the Wald ratio was used, which is the most basic method. Other methods such as MR Egger, Weighted median, Simple mode, and Weighted mode were also completed in each MR analysis. The suggested threshold of $P < 0.05$ was used as a significance level for MR results.

Sensitivity analysis

Several sensitivity analyses were conducted to assess the robustness of results to potential violations of the MR assumptions [36]. First, heterogeneity tests, which could use MR Egger and IVW, were estimated by the Cochran Q test. When the P value of Cochran Q-test results was below 0.05, the heterogeneity of the MR results was indicated. Second, the intercept term in MR Egger regression was used as an indication of whether directional horizontal pleiotropy was driving the results of the MR analysis [37]. Furthermore, leave-one-out analysis was performed to identify whether a single SNP was driving the association. In the leave-one-out analysis, the MR was performed again, but leaving out each SNP one by one. If the result changed greatly after the elimination of an SNP, it indicated that there was an SNP with a great influence on the result.

Table 1 Clinical characteristics of the study participants with or without GDM

	NGT (n = 292)	GDM (n = 274)	*P*
Age (years)	29 (28, 31)	29 (28, 30)	0.168
Gestational age (week)	27 (26, 28)	27 (26, 28)	0.641
Pre-gestational BMI (kg/m^2)	20.65 ± 2.53	20.97 ± 2.68	0.148
Changes in BMI (kg/m^2)	5.43 ± 1.33	4.72 ± 1.53	< 0.001
FPG (mmol/L)	4.39 ± 0.28	4.67 ± 0.47	< 0.001
1 h-PG (mmol/L)	7.27 ± 1.40	9.70 ± 1.40	< 0.001
2 h-PG (mmol/L)	6.33 ± 0.97	8.56 ± 1.36	< 0.001
HbA1c (%)	5.15 ± 0.30	5.26 ± 0.30	< 0.001
Total cholesterol (mmol/L)	6.40 ± 1.18	5.92 ± 1.13	< 0.001
Triglycerides (mmol/L)	2.49 ± 1.26	2.60 ± 1.28	0.309
LDL-C (mmol/L)	3.14 ± 0.85	3.31 ± 0.94	0.025
HDL-C (mmol/L)	2.01 ± 0.37	1.96 ± 0.41	0.121
G_{AUC} (mmol/L·h)	12.63 ± 1.74	16.31 ± 1.70	< 0.001
Fasting insulin (mU/L)	6.05 (4.15, 9.22)	8.69 (5.83, 12.20)	< 0.001
HOMA-β	144.65 (88.68, 215.74)	155.01 (109.43, 248.92)	0.013
HOMA-IR	1.20 (0.79, 1.79)	1.84 (1.12, 2.63)	< 0.001
Total bile acid (μmol/L)	2.27 (1.67, 2.96)	2.22 (1.53, 3.07)	0.802
Family history of diabetes, n (%)	22 (7.5)	68 (24.6)	< 0.001
TyG	9.0 ± 0.5	9.1 ± 0.4	0.014

BMI body mass index; *FPG* fasting plasma glucose; *1 h-PG* one hour postprandial glucose; *2 h-PG* two hours postprandial glucose; *HbA1c* hemoglobin A1c; *LDL-C* low-density lipoprotein cholesterol; *HDL-C* high-density lipoprotein cholesterol; G_{AUC} area under the curve of glucose from the 75-g OGTT; *HOMA-β* homeostasis model assessment index of β-cell secretion; *HOMA-IR* homeostasis model assessment of insulin resistance; *TyG* triglyceride-glucose

Mediation analysis

For insulin-related factors that causally associate with both metabolic pathways and GDM, a mediation analysis based on two-step MR [38] was used to quantify the effects of the risk factors on GDM via pathways. Total effect of exposure on outcome included both direct and indirect effects through mediators. A univariate MR model was carried out to estimate the effect of the exposure on the mediator. To estimate the indirect effect, results from two-step MR were used. The Product method was chosen to estimate the beta of the indirect effect, and the Delta method was used to estimate the standard error (SE) and confidence interval (CI).

Statistical analysis

Clinical characteristics between the normal glucose tolerance (NGT) and GDM groups or between the normal pregnancy outcome (NPO) and APO groups were compared using the *t* test or Wilcoxon test for continuous variables and the chi-square test for categorical variables with R version 4.3.2. Spearman correlation analysis was used to assess relationships between continuous variables. A generalized linear model (GLM) was used to investigate the relationship between HOMA-IR and APOs. In the validation study, PC1 scores of metabolic pathways were derived using the hdsMR method, and changes in PC1 before and after dietary intervention were statistically evaluated via a paired T-test. Two-sided *P* values lower than 0.05 were considered significant. Results were visualized using the ggplot2 package in R (https://doi.org/10.32614/CRAN.package.ggplot2).

Results

Clinical characteristics and metabolite landscape of the study population

A total of 566 (aged > 18 years) participants with GDM or NGT were recruited in line with the diagnostic criteria of the IADPSG at the University of Hong Kong–Shenzhen Hospital from 2015 to 2018 (Fig. 1). The clinical characteristics of the 566 study participants from the two groups are summarized in Table 1. Compared with the NGT group, the GDM group had higher levels of HbA1c, FINS, LDL-C, FPG, 1 h-PG, 2 h-PG, G_{AUC}, HOMA-β, HOMA-IR, TyG and family history of diabetes, and lower levels of TC and changes in BMI during pregnancy ($P < 0.05$).

Classical metabolome analysis showed that serum metabolism in GDM patients was significantly different

from that in pregnant women with NGT (Fig. 2). The detected metabolites were grouped into 13 chemical classes (Fig. 2A). Namely, 37 DEMs and corresponding metabolic pathways were obtained (Fig. 2C, D; Tables S1 and S2). Weighted correlation network analysis (WGCNA) detected one module (MEbrown) that positively correlated ($P < 0.05$) with GDM and many clinical traits, such as 2 h-PG, FINS, HOMA-β, and HOMA-IR (Fig. 2E; Tables S3 and S4).

Identification of the causal role of HOMA-IR and metabolic pathways in GDM

GLM analysis revealed that FINS (odds ratio [OR] 1.20; 95% CI 1.14–1.26; $P < 0.001$) and HOMA-IR (OR 2.55; 95% CI 2.04–3.25; $P < 0.001$) were both significantly associated with GDM (Fig. S1). Thus, we used the hdsMR framework (Fig. 1) for causal analysis among these insulin-related factors (i.e., FINS and HOMA-IR), metabolic pathways, and GDM (Fig. 3A). We identified 23 SNPs associated with FINS and HOMA-IR ($P_{adj} < 5 \times 10^{-8}$; Table S5). HOMA-IR was confirmed as a causal GDM risk factor in the MR1 analysis step by the IVW test (OR 1.17; 95% CI 1.04–1.32; $P = 0.007$; Fig. 3B). In the MR2 analysis step, 16 metabolic pathways characterized with PC1 scores were causal for GDM ($P < 0.05$) by the IVW test (Table S6; Fig. 3C). In the MR3 analysis step, bidirectional MR analysis was performed on HOMA-IR and 16 GDM-associated pathways (Fig. 3D). We found that HOMA-IR had a potential causal risk effect on glyoxylate and dicarboxylate metabolism pathway (IVW: OR 1.08; 95% CI 1.08–1.15; $P = 0.006$) and a causal protective effect on lysine degradation pathway (IVW: OR 0.95; 95% CI 0.91–0.98; $P = 0.006$). By contrast, the causal effect of these two metabolic pathways on HOMA-IR was not confirmed (Fig. 3D).

The robustness of the abovementioned MR analyses was confirmed by the results of sensitivity analyses. The IVs had no horizontal pleiotropy in the association of HOMA-IR and any of the two key metabolic pathways, as measured by MR-Egger intercept $P > 0.05$ (Table S7). There was no evidence of heterogeneity in each MR analysis step, according to Cochran's Q-test $P > 0.05$ by both the MR Egger and IVW methods (Table S9). Furthermore, the results of leave-one-out analysis showed that no single SNP was driving the association (Fig. S2). However, there was no evidence for colocalization between the exposure and outcome at any of the MR analysis steps based on a Bayesian algorithm (PP.H4.abf < 60%; Table S7), which was possibly due to violation of its single-causal-variable hypothesis.

Besides, the HOMA-IR value was increased in the GDM group (Fig. 4D), and correlations between the two metabolic pathways and GDM/HOMA-IR were consistent with the MR estimation. PC1 score of glyoxylate and dicarboxylate metabolism pathway was higher in GDM and that of lysine degradation pathway was lower (Fig. 4D). HOMA-IR showed a risk trend with glyoxylate and dicarboxylate metabolism pathway and a protective trend with the lysine degradation pathway (Fig. 4E).

Mediation effect of the key metabolic pathways between HOMA-IR and GDM

Next, we conducted a two-step MR analysis to infer the causal mediating effect of HOMA-IR on GDM through two key metabolic pathways (Fig. 4A). Glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway significantly mediated the association between HOMA-IR and GDM, explaining 14.6% (indirect effect = 1.02; 95% CI 1.01–1.10; Fig. 4B) and 8.4% (indirect effect = 1.013; 95% CI 1.00–1.03; Fig. 4C) of the total effect, respectively.

We also performed conventional MR analysis on the metabolite level (Fig. S3A; Table S8) to investigate the causal relationships among HOMA-IR, metabolites (mediators), and GDM (outcomes). Although eight metabolites were causally related to GDM and one was related to HOMA-IR, none of them mediated HOMA-IR to GDM (Fig. S3C, D).

External validation of the key metabolic pathways on GDM development and prevention

To explore the interactions between metabolic pathways as well as their relationship with GDM, we constructed a regularized partial correlation network using the metabolites involved in glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway (Fig. S4). This network showed that dense pathway crosstalk occurred between the two pathways, indicating that they may regulate each other. According to the STRING database [39], the *GLYCTK* gene involved in the glyoxylate and dicarboxylate metabolism pathway was linked to the well-known GDM risk gene *HKDC1* [40] (Fig. S5A). Genes *EZH2* and *PRDM16*, involved in the lysine degradation pathway, were linked to the known GDM-related gene *PPARG* [41] (Fig. S5B).

We also validated the effects of these two pathways on diabetes prevention with dietary intervention using an external independent dataset from pre-diabetic individuals. PC1 scores of the two key pathways significantly changed after the dietary intervention ($P < 0.05$), namely the PC1 score of glyoxylate and dicarboxylate metabolism pathway decreased, while that of the lysine degradation pathway increased (Fig. 4F).

Association between glyoxylate and dicarboxylate metabolism pathway and APOs in GDM

We further evaluated the impact of the two pathways on pregnancy outcomes in GDM patients and found

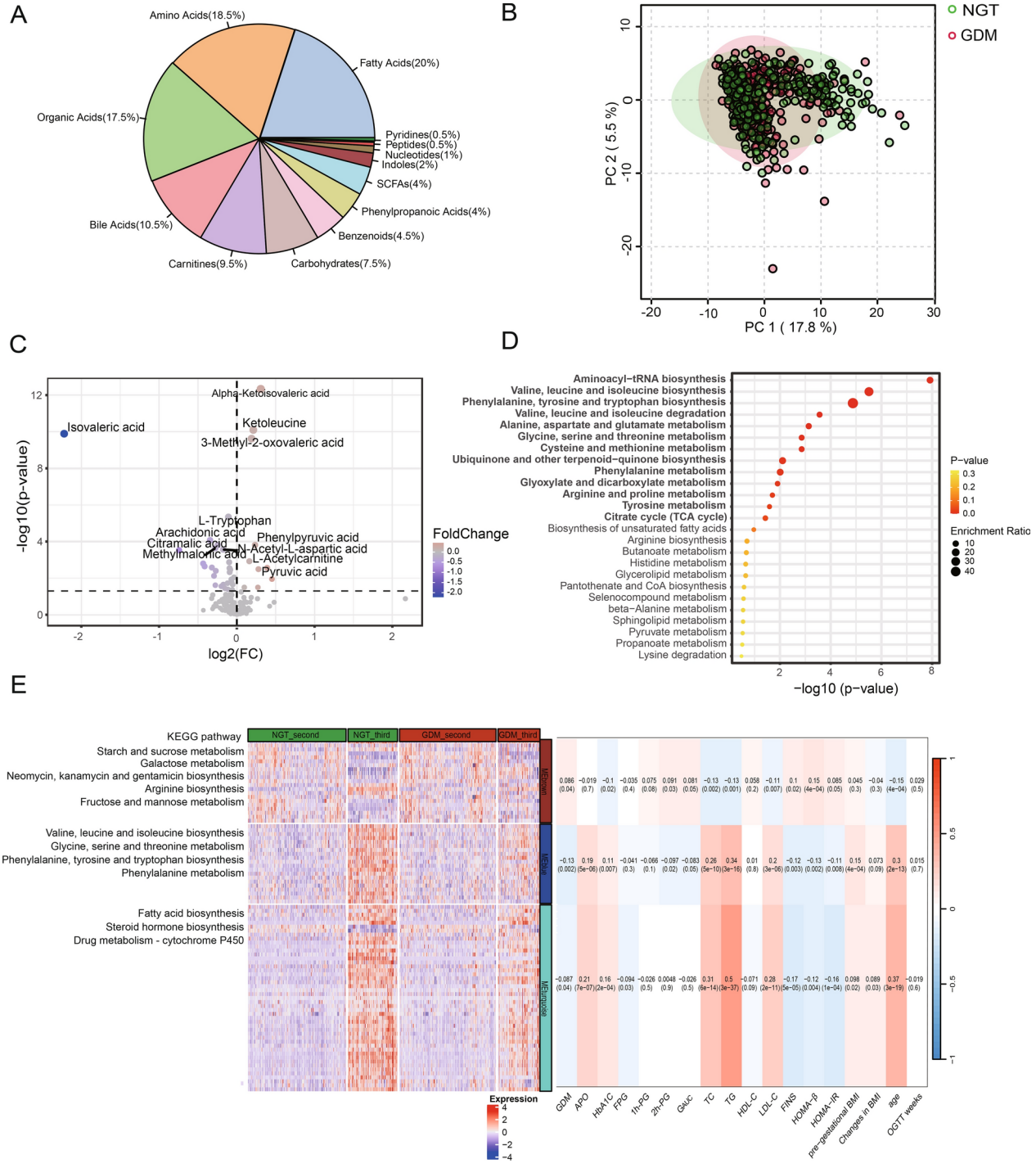
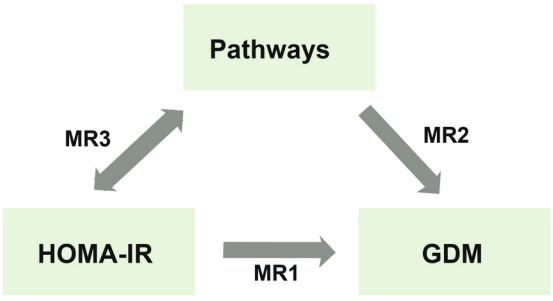


Fig. 2 Metabolite profile characteristics between the NGT and GDM groups. **A** The statistics of metabolite compositions detected in serum samples from the GDM and NGT groups. **B** Sample distributions by PCA based on the metabolite profiles from all participants. **C** Differentially expressed metabolites (DEMs) between the GDM and NGT groups shown in a volcano plot. **D** Functional enrichments of DEMs and recognition of GDM-associated biological pathways. **E** Metabolite modules newly identified by WGCNA and their association with clinical indicators, especially their relationships with GDM outcome. NGT, normal glucose tolerance; GDM, gestational diabetes mellitus.

that the glyoxylate and dicarboxylate metabolism pathway was not only associated with GDM but also contributed to APOs (Fig. 5). The APO subjects had higher TC, TG, FINS, and HOMA-IR compared with the NPO subjects in the GDM group (Table 2; Fig. 5A). HOMA-IR also showed a risk effect on APOs in GLM analysis after adjusting for TG, TC, age, changes in BMI, and pre-gestational BMI (Fig. 5B). Through evaluating the association

A



B **MR1 HOMA-IR → GDM**

Exposure	Outcome	nsnp	Method	OR(95% CI)	Pvalue
HOMA−IR	GDM	6	MR Egger	1.38 (0.48 to 3.94)	0.578
			Weighted median	1.15 (1.01 to 1.32)	**0.042**
			Inverse variance weighted	1.17 (1.04 to 1.32)	**0.007**
			Simple mode	1.13 (0.91 to 1.39)	0.318
			Weighted mode	1.12 (0.93 to 1.36)	0.288

0 2

C **MR2 Pathways → GDM**

Exposure	Outcome	nsnp	Method	OR(95% CI)	Pvalue
Glyoxylate and dicarboxylate metabolism	GDM	4	MR Egger	1.29 (0.84 to 2.00)	0.366
			Weighted median	1.41 (1.12 to 1.77)	**0.003**
			Inverse variance weighted	1.42 (1.17 to 1.73)	**0.001**
			Simple mode	1.52 (1.10 to 2.09)	0.085
			Weighted mode	1.32 (0.94 to 1.86)	0.206
Lysine degradation	GDM	12	MR Egger	0.91 (0.58 to 1.43)	0.692
			Weighted median	0.78 (0.61 to 0.99)	**0.041**
			Inverse variance weighted	0.76 (0.63 to 0.92)	**0.005**
			Simple mode	0.87 (0.58 to 1.31)	0.521
			Weighted mode	0.88 (0.59 to 1.31)	0.538

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D **MR3 HOMA-IR ↔ Pathways**

Exposure	Outcome	nsnp	Method	OR(95% CI)	Pvalue
HOMA−IR	Glyoxylate and dicarboxylate metabolism	23	MR Egger	1.21 (0.86 to 1.71)	0.288
			Weighted median	1.10 (1.01 to 1.19)	**0.021**
			Inverse variance weighted	1.08 (1.02 to 1.15)	**0.006**
			Simple mode	1.18 (1.01 to 1.39)	**0.047**
			Weighted mode	1.18 (1.01 to 1.37)	**0.045**
HOMA−IR	Lysine degradation	23	MR Egger	1.01 (0.80 to 1.27)	0.923
			Weighted median	0.93 (0.88 to 0.98)	**0.003**
			Inverse variance weighted	0.95 (0.91 to 0.98)	**0.006**
			Simple mode	0.92 (0.83 to 1.02)	0.115
			Weighted mode	0.92 (0.83 to 1.01)	0.106
Glyoxylate and dicarboxylate metabolism	HOMA−IR	3	MR Egger	0.51 (0.28 to 0.93)	0.274
			Weighted median	1.24 (0.95 to 1.62)	0.121
			Inverse variance weighted	1.11 (0.78 to 1.57)	0.566
			Simple mode	1.30 (0.96 to 1.75)	0.228
			Weighted mode	1.30 (0.97 to 1.75)	0.224
Lysine degradation	HOMA−IR	12	MR Egger	1.14 (0.78 to 1.67)	0.516
			Weighted median	0.87 (0.70 to 1.08)	0.206
			Inverse variance weighted	0.88 (0.75 to 1.03)	0.113
			Simple mode	0.85 (0.60 to 1.20)	0.364
			Weighted mode	0.84 (0.62 to 1.13)	0.275

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Fig. 3 (See legend on next page.)

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Fig. 3 Key metabolic pathways with causal relationship for GDM outcome and HOMA-IR identified by one-sample MR. **A** The basic MR model used in the GDM study for inferring the causal relationship among HOMA-IR, key pathways, and GDM outcome, which includes three MR determinations. **B** Association between HOMA-IR and GDM determined by one-sample MR, which indicates the relation between biochemistry exposure and outcome. **C** Association between metabolic pathways and GDM determined by one-sample MR, which supports the relation between biological exposure and the same outcome. Here, 16 key metabolic pathways were selected. **D** Association between HOMA-IR and metabolic pathways determined by bidirectional MR, which identified the causal relation direction from HOMA-IR to two key metabolic pathways.

between pathway activation and pregnancy outcomes, glyoxylate and dicarboxylate metabolism pathway was significantly related to APOs (Fisher's exact test: OR 2.13; 95% CI 1.16–3.94; $P = 0.01$) (Table S9). The trend of the average activity of glyoxylate and dicarboxylate metabolism pathway in the APO group was also higher than that in the NPO group (Fig. 5C), consistent with its risk role in GDM development.

Besides, based on the STRING and KEGG databases, these two key pathways had cross talk with 17 biological pathways through their involved genes (Fig. 5D, top). According to previous studies (Table S10; Fig. 2D), 11 of the 17 pathways were related to GDM, but one pathway was related to APO [42], which only cross-talked with glyoxylate and dicarboxylate metabolism pathway (Fig. 5D, bottom; Methods).

Discussion

In this study, we elucidated the role of metabolic pathways in GDM development by providing a high-dimensional causal mediation analytic framework for investigating the relationship among metabolome, IR, and GDM. The proposed hdsMR framework applied the PC1 score of PCA to quantify metabolomic biological pathways, which overcame the high dimensionality and instability of the traditional metabolomic MR analysis. Under the hdsMR framework, we confirmed that HOMA-IR had a causal risk effect on GDM, indicating the essential role of IR in the pathogenesis of GDM, consistent with classical epidemiological studies [8, 43–47]. We found that the association between HOMA-IR and GDM could be mediated by glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway, the former being a risk factor for GDM and the latter being a protective factor for GDM. The two pathways were also enriched by DEMs through the KEGG pathway enrichment analysis (Fig. 2D). In addition, both key pathways were validated to be potential targets to prevent GDM through dietary intervention in an external independent dataset [27]. Finally, we showed glyoxylate and dicarboxylate metabolism pathway as a risk factor for APOs in GDM.

Traditional metabolomic MR studies have usually been based on metabolites to describe changes associated with disease states [15–17], but the single-molecule level is not systematic enough to gain an in-depth understanding of biological significance. Therefore, we focused on finding new ways to infer causality between metabolism and disease. Gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) have been applied for pathway quantification to reduce the dimensionality of multiple metabolites [48]. Our hdsMR framework utilized PCA [49] to quantify metabolic pathways to efficiently infer causality and mediating factors among metabolome, IR, and GDM, based on the existing MR mediation methods [38, 50]. Compared to traditional MR mediation methods that yielded no significant findings, our hdsMR framework identified two biologically plausible causal mediation pathways, demonstrating both superior statistical power and clearer mechanistic interpretations.

The consistency of results across multiple sensitivity analysis approaches (Cochran Q test, MR-Egger and Leave-one-out) supported the robustness of our findings. Especially leave-one-out tests confirmed that the MR results were not influenced by any single SNP. Based on rigorous screening processes and sensitivity tests, we ensured the precision and validity of the selected genetic variants. To validate the effectiveness of our hdsMR method, we conducted data simulation comparison with traditional MR analysis. Our simulation results (Fig. S6) demonstrated that the hdsMR method performed better than traditional MR analysis. Across different noise levels, our approach showed higher accuracy.

HOMA-IR is an index used to evaluate the level of IR in individuals [22]. In observational studies, high HOMA-IR before or at the early stage of pregnancy was associated with an increased risk of GDM [8, 43–46]. Consistent with this, we provided robust evidence that HOMA-IR was a causal risk factor for GDM ($P = 0.007$), indicating that HOMA-IR could be used for GDM risk screening in early pregnancy and even before pregnancy. Moreover, previous studies have indicated that strategies to improve IR may help reduce the risk of GDM [44].

We found that the causal relationship of HOMA-IR with GDM could be mediated by the glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway. Of the two mediating pathways, the glyoxylate and dicarboxylate metabolism pathway describes a variety of reactions involving glyoxylate or dicarboxylates in the KEGG database [18], which has previously been related to obesity [51], an important risk factor for GDM [52]. Glyoxylate and dicarboxylate metabolism was upregulated at the peri-implantation period of early pregnancy in mice [53]. The evidence indicates that this

pathway is physiologically upregulated during a normal pregnancy. In our study, we showed that glyoxylate and dicarboxylate metabolism was a direct causal risk for GDM. Our findings reveal that GDM is associated with an exaggerated activation of this pathway. Previous studies have shown that the activity of this pathway is increased in patients with type 2 diabetes (T2D) [51]. In animal studies, it was associated with a high glucose intake in prediabetes [54] and was related to IR in T2D rats [55]. Overall, the amplified pathway activity may reflect a response to IR.

In addition, we found that glyoxylate and dicarboxylate metabolism was a risk factor for APOs in GDM. This pathway has been reported to be involved in the development of fetal growth restriction [56], which leads to a higher risk of mortality and neonatal complications with long-term consequences [57]. This pathway was also altered in other APOs, such as neonates of preeclamptic pregnancies and preterm infants with bronchopulmonary dysplasia [58, 59]. Taken together, the inhibition of glyoxylate and dicarboxylate metabolism represents a promising intervention target to prevent GDM and APO, but further experimental studies are warranted to illustrate the mechanism.

The other pathway that mediates HOMA-IR to GDM development is the lysine degradation pathway, which occurs in the liver [60] and was shown to be a protective factor for GDM in our study. It was highly associated with metabolic syndrome of prediabetes according to metabolic pathway analysis in a population-based study [61]. Changes in the concentrations of metabolites for lysine degradation correlated with pre-diabetic state in an animal study [62]. L-lysine is the initial substrate of the lysine degradation pathway [18]. In T2D patients, L-lysine supplementation has been reported to reduce the protein glycation [63], which can be linked to long-term hyperglycemia [64]. L-lysine is abundant in legumes [65], so increasing their intake might lower the risk of GDM [66, 67]. The abovementioned studies suggest that the lysine degradation pathway highly correlates with abnormal glucose metabolism, which may become a potential dietary target for GDM prevention and therapy.

Furthermore, we showed that both key pathways could be altered after diet interventions according to an independent metabolome data assessment from pre-diabetes individuals [27]. Based on evidence suggesting shared pathogenic mechanisms and intervention between GDM and pre-diabetic individuals, we selected a metabolic dataset reflecting 6-month dietary intervention changes in pre-diabetic individuals to approximate whether the two metabolic pathways we identified could achieve glycemic management through dietary intervention. The original research based on this dataset showed that diets demonstrated a positive impact on both serum metabolites and glycemic control. Therefore, we analyzed the changes in metabolic pathways and found that the glyoxylate and dicarboxylate metabolism pathway was downregulated, while the lysine degradation pathway was upregulated after a PPT or MED intervention. Our findings suggest that early screening for HOMA-IR could identify pregnant women at high risk for GDM. For this high-risk population, dietary interventions (e.g., PPT diet or MED diet) or prebiotic supplements targeting both metabolic pathways [27, 66, 67] may be beneficial in reducing their risk of conversion to GDM. In addition, evidence from population studies and animal studies indicate that dietary interventions may mitigate the risk of GDM through the improvements in IR [68, 69]. The role of other lifestyles such as regular physical activity and adequate sleep duration on these metabolic pathways needs to be further investigated.

There are some limitations to our study. First, as a cross-sectional study, we only collected samples at the time of the OGTT, lacking samples from early pregnancy. Although MR methods were used to exclude confounding, future prospective studies of the association between early indices of IR and GDM are still needed. Second, we used one-sample MR with not a large sample size, which resulted in low statistical power; however, we performed various sensitivity analyses to ensure the reliability of our MR analysis, and the low power may cause false negative results that would not weaken our positive findings. Third, as this study was conducted in a single-center Chinese population, the generalizability of the results may be limited. Fourth, although our study advances the current understanding of metabolic pathways mediating GDM and its perinatal outcomes, the critical evaluation of long-term health outcomes (e.g., T2D, metabolic syndrome, and cardiovascular complications) in mothers and offspring necessitates future large-scale birth cohort studies with extended follow-up periods.

Collectively, in this study, we performed hdsMR analysis and discovered the mediating roles of metabolic biological pathways between IR and GDM. Our findings demonstrated that both mediating pathways, namely glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway, could be intervened by diet, which provides evidence for potential prevention methods of GDM. The proposed hdsMR framework overcomes the high dimensionality and instability of the traditional metabolomic MR analysis and is useful for investigating the underlying biological mechanism of diseases.

Conclusions

This study exhibited the utility of the hdsMR framework for estimating the causal role of HOMA-IR and metabolic pathways in the pathogenesis of GDM. We identified the causal mediation effect of two metabolic pathways

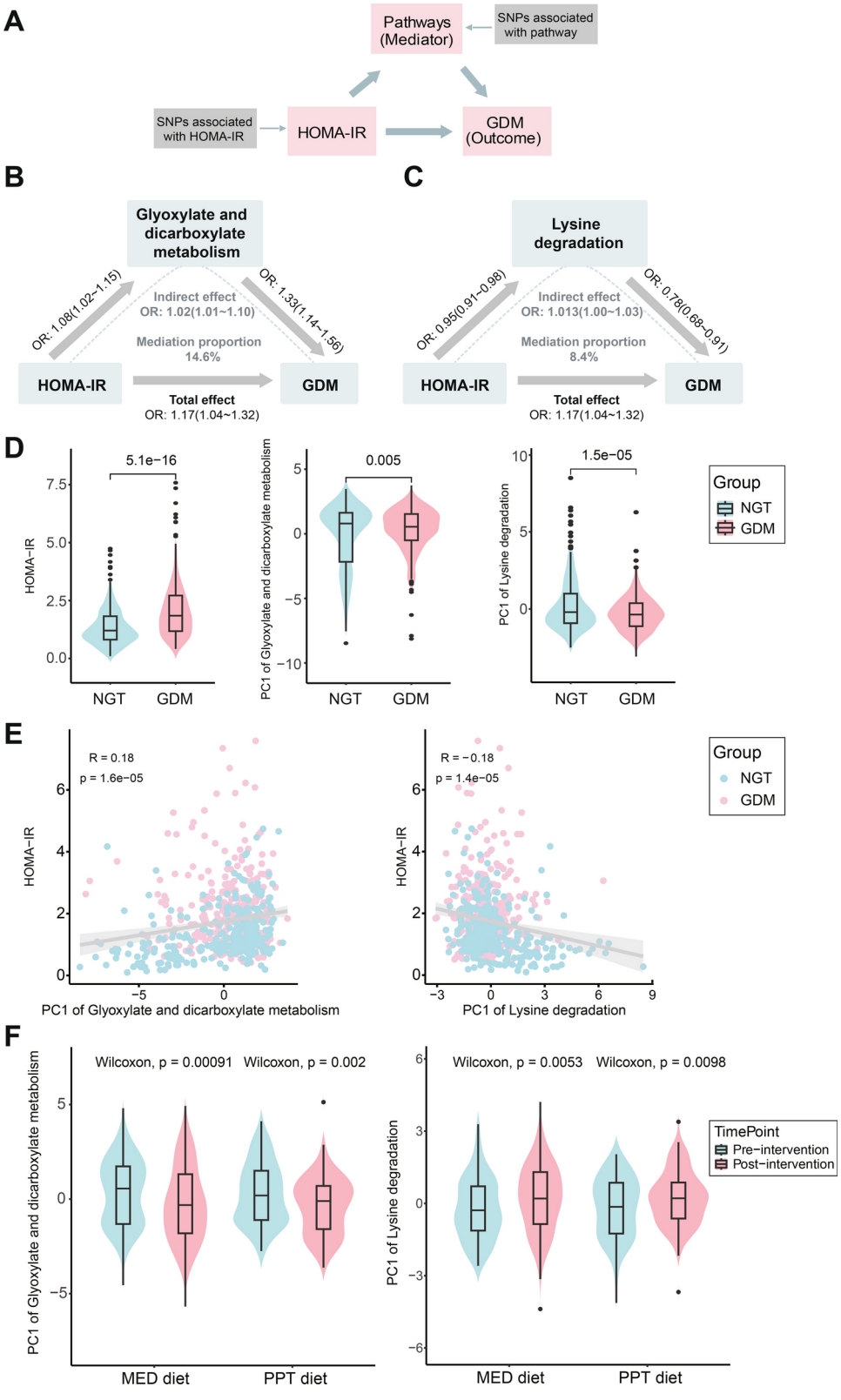

Fig. 4 (See legend on next page.)

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Fig. 4 Mediating role of the key metabolic pathways from HOMA-IR to GDM identified by two-step MR. **A** A two-step MR model for mediation analysis used in this study. **B** Mediation analysis of glyoxylate and dicarboxylate metabolism pathway, indicating its upregulation in GDM. **C** Mediation analysis of lysine degradation pathway, indicating its downregulation in GDM. **D** The consistent alteration between HOMA-IR and metabolic pathway for GDM. HOMA-IR was increased in GDM (left panel). The PC1 score of glyoxylate and dicarboxylate metabolism pathway was increased in GDM (middle panel). Meanwhile, the PC1 score of the lysine degradation pathway was decreased in GDM (right panel). **E** Significant association between PC1 scores of two key metabolic pathways and HOMA-IR. **F** The PC1 scores of glyoxylate and dicarboxylate metabolism pathway decreased after both MED and PPT diet intervention (left panel). Meanwhile, the PC1 scores of lysine degradation pathway increased after both MED and PPT diet intervention (right panel). MED diet, the standard of care Mediterranean diet; PPT diet, a personalized postprandial glucose–targeting diet.

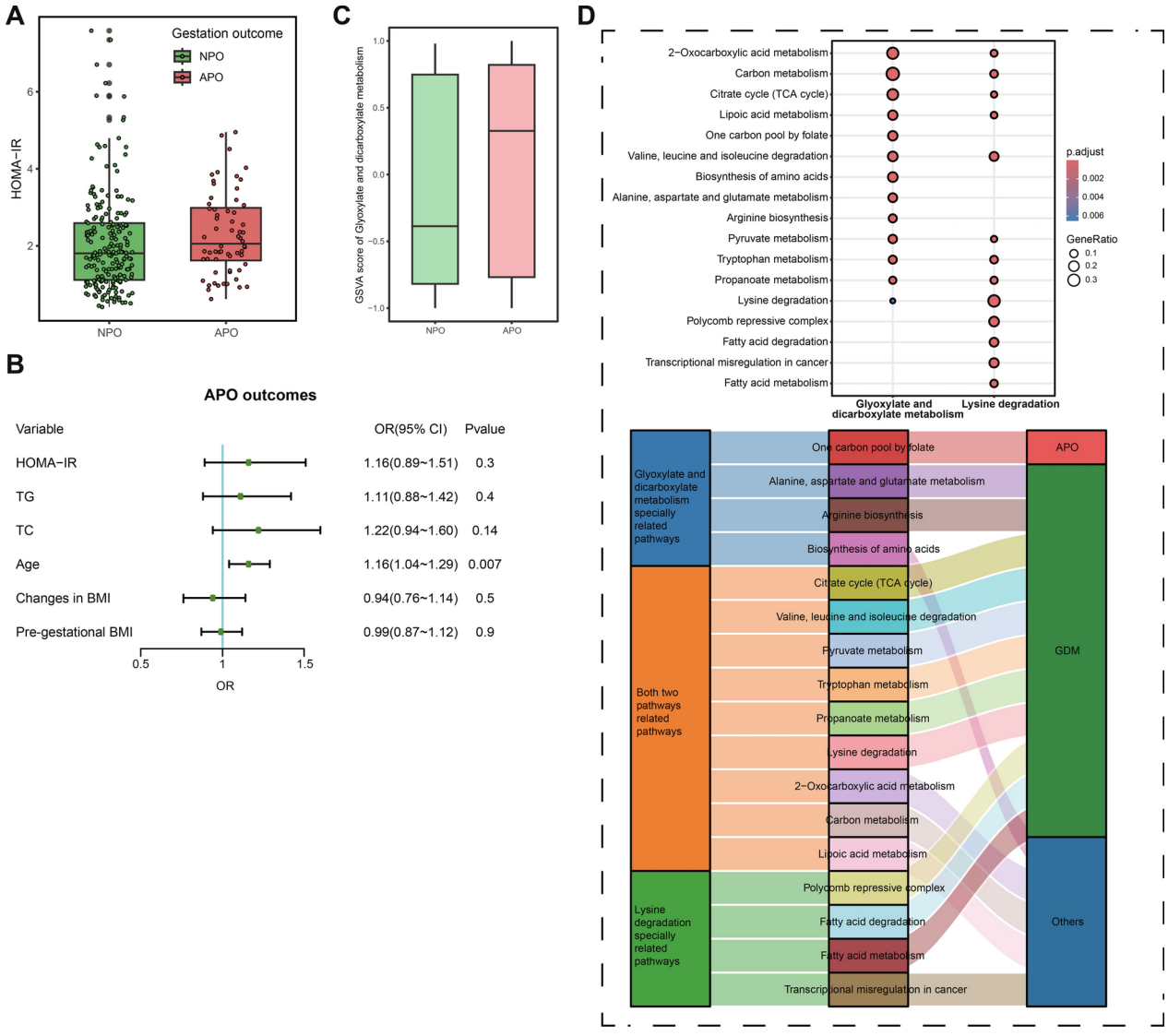

Fig. 5 Role of glyoxylate and dicarboxylate metabolism pathway in the association with HOMA-IR and APO of GDM. **A** HOMA-IR differences of GDM individuals between APO and NPO groups. NPO, normal pregnancy outcome; APO, adverse pregnancy outcome. **B** Risk model for APO outcome of GDM individuals. **C** Activity differences in glyoxylate and dicarboxylate metabolism pathway observed between the APO and NPO groups. **D** The enrichment of KEGG pathways related to the genes involved in glyoxylate and dicarboxylate metabolism and lysine degradation pathways (top panel), and the associations of those pathways with GDM and APO (bottom panel).

(glyoxylate and dicarboxylate metabolism pathway and lysine degradation pathway) from HOMA-IR to GDM and their impact on APOs in GDM subjects. We further highlighted that targeting specific metabolic pathways through dietary modifications could be explored as a possible GDM prevention approach, and hdsMR was more efficient in finding causal mediating metabolic pathways than traditional MR methods.

Table 2 Characteristics of the NPO and APO groups for GDM patients

	NPO (n = 209)	APO (n = 64)	*P*
Age (years)	30 (28, 31)	29 (28, 30)	0.001
Gestational age (week)	26 (25, 26)	26 (25, 27)	0.111
Pre-gestational BMI (kg/m^2)	20.90 ± 2.70	21.24 ± 2.59	0.375
Changes in BMI (kg/m^2)	4.73 ± 1.55	4.70 ± 1.50	0.921
FPG (mmol/L)	4.66 ± 0.46	4.71 ± 0.51	0.522
1 h-PG (mmol/L)	9.69 ± 1.38	9.71 ± 1.46	0.914
2 h-PG (mmol/L)	8.53 ± 1.35	8.66 ± 1.40	0.507
HbA1c (%)	5.24 ± 0.30	5.31 ± 0.30	0.155
Total cholesterol (mmol/L)	5.87 ± 1.14	6.16 ± 1.20	0.032
Triglycerides (mmol/L)	2.48 ± 1.04	2.97 ± 1.90	0.014
LDL-C (mmol/L)	3.28 ± 0.94	3.48 ± 0.92	0.071
HDL-C (mmol/L)	1.96 ± 0.40	1.90 ± 0.42	0.323
G_{AUC} (mmol/L·h)	16.28 ± 1.65	16.40 ± 1.88	0.621
Fasting insulin (mU/L)	8.62 (5.70, 12.15)	10.18 (7.58, 14.19)	0.024
HOMA-β	152.93 (110.13, 238.94)	190.34 (116.81, 268.75)	0.071
HOMA-IR	1.81 (1.11, 2.60)	2.06 (1.56, 3.03)	0.030
Total bile acid (μmol/L)	2.17 (1.55, 3.00)	2.53 (1.51, 3.54)	0.359
Family history of diabetes, n (%)	43 (20.6)	22 (34.4)	0.023

BMI body mass index; *FPG* fasting plasma glucose; *1 h-PG* one hour postprandial glucose; *2 h-PG* two hours postprandial glucose; *HbA1c* hemoglobin A1c; *LDL-C* low-density lipoprotein cholesterol; *HDL-C* high-density lipoprotein cholesterol; G_{AUC} area under the curve of glucose from the 75-g OGTT; *HOMA-β* homeostasis model assessment index of β-cell secretion; *HOMA-IR* homeostasis model assessment of insulin resistance

Abbreviations

APOs	Adverse pregnancy outcomes
BMI	Body mass index
CI	Confidence interval
DEMs	Differentially expressed metabolites
FC	Fold change
FINS	Fasting insulin
FPG	Fasting plasma glucose
G_{AUC}	The area under curve of glucose
GDM	Gestational diabetes mellitus
GLM	Generalized linear model
GSVA	Gene set variation analysis
HbA1c	Hemoglobin A1c
HDL-C	High-density lipoprotein cholesterol
hdsMR	High-dimensional systematic Mendelian randomization
HOMA-IR	Homeostasis model assessment of insulin resistance
HOMA-β	Homeostasis model assessment of β-cell function
HWE	Hardy–Weinberg equilibrium
IQR	Interquartile range
IR	Insulin resistance
IVs	Instrumental variables
IVW	Inverse variance weighting
KEGG	Kyoto Encyclopedia of Genes and Genomes
LD	Linkage disequilibrium
LDL-C	Low-density lipoprotein cholesterol
LOD	Limit of detection
MAF	Minor allele frequency
ME	Module eigengenes
MED	Mediterranean diet
MR	Mendelian randomization
MVMR	Multivariable Mendelian randomization
NPO	Normal pregnancy outcome
OGTT	Oral glucose tolerance test
PCA	Principal component analysis
PPT	Personalized postprandial glucose-targeting diet
SD	Standard deviation
SE	Standard error
SNP	Single-nucleotide polymorphisms
TC	Total cholesterol
TG	Triglycerides
1 h-PG	1-Hour plasma glucose
2 h-PG	2-Hour plasma glucose
TyG	Triglyceride-glucose

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-025-02746-0.

Supplementary Material 1.

Supplementary Material 2.

Acknowledgements
Not applicable.

Author contributions
All authors participated in the scientific discussion. XT.Y., WT.Z. and H.C. conceived the research. XT.Y., MJ.L. involved in study design, sample collection and experiment execution. W.C., JY.G., SN.W., DD.Y., XH.F., YT.H., L.S., R.Z., and J.Y. performed data analysis and discussed the results. W.C., XT.Y., and JY.G. wrote the manuscript. XT.Y., T.Z. and WT.Z. supervised the project. All authors commented on the manuscript.

Funding
This study was supported by the National Key R&D Program of China (2022YFA1004800, 2022YFF1202100), the National Natural Science Foundation of China (No.12371485), the Fundamental Research Funds for the Central Universities (YG2023QNB20), the Clinical Research Plan of SHDC (SHDC2022CRS044, SHDC2023CRS029) and the Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine Basic Scientific Research (No. ynms202118).

Availability of data and materials
External datasets used in this study were downloaded fromhttps://static-content.springer.com/esm/art%3A10.1038%2Fs41467-023-41042-x/MediaObjects/41467_2023_41042_MOESM9_ESM.xlsx. Data supporting the findings of this study can be reasonably require access to https://www.biosino.org/bmdc/, with ID SUB00040355. Code used to accomplish the main result is available at: https://gitee.com/chenwei_11/gestational-diabetes-mellitus.git.

Declarations

Ethics approval and consent to participate
The study was approved by the Institutional Review Board (IRB) of the University of Hong Kong-Shenzhen Hospital ([2017]13) and conducted according to the principles of the Declaration of Helsinki as revised in 2013. All participants signed written informed consent prior to enrolment.

Consent for publication
Not applicable.

Competing interests
All authors disclosed no competing interests.

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Received: 15 January 2025 / Accepted: 18 April 2025
Published online: 09 May 2025

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