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The causality between gut microbiota and ectopic pregnancy based on genome-wide association: A Mendelian randomization study

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Ning Zhang[1,2], Zhige Wang[2], Feixiang Yang[2,3], Tianrui Liu[4] and Dongmei Ji[1,4,5]*

Department of Obstetrics and Gynecology, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China[1]; First School of Clinical Medicine, Anhui Medical University, Hefei, Anhui 230032, China[2]; Department of Urology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui 230032, China[3]; Second School of Clinical Medicine, Anhui Medical University, Hefei, Anhui 230032, China[4]; NHC Key Laboratory of Study on Abnormal Gametes and Reproductive Tract (Anhui Medical University), No 81 Meishan Road, Hefei 230032, Anhui, China[5]; Key Laboratory of Population Health Across Life Cycle (Anhui Medical University), Ministry of Education of the People's Republic of China, No 81 Meishan Road, Hefei 230032, Anhui, China[6].

***For Correspondence:** Email: *jidongmei@ahmu.edu.cn*; Phone*:* +86-551-6590-8729.

Abstract

Ectopic pregnancy represents a prevalent gynecological emergency with incompletely characterized pathophysiology, creating substantial clinical challenges in timely diagnosis and effective management. The causal interplay between gut microbiota and ectopic pregnancy, particularly through plasma proteomics mediation, remains undefined. We derived Gut microbiota GWAS data (n=412) from the Dutch Microbiome Project meta-analysis and Ectopic pregnancy data from IEU OpenGWAS and FinnGen project. The results revealed nine kinds of gut microbiotas demonstrating causal associations with ectopic pregnancy risk. A total of 67 plasma proteins causally impact the risk of ectopic pregnancy. In addition, three kinds of gut microbiotas were mediated by 25 kinds of plasma proteins on ectopic pregnancy. Microbiota enriched in **L.rhamnose.degradation.I** affected ectopic pregnancy through 22 kinds of plasma proteins. This study establishes causal relationships between specific gut microbial pathways and ectopic pregnancy risk, mediated through distinct plasma protein signatures, providing directions for clinical interventions and future research.. *(Afr J Reprod Health 2026; 30 [8]: 66-75).*

Keywords: gut microbiota; ectopic pregnancy; Mendelian Randomization; GWAS

Résumé

La grossesse extra-utérine représente une urgence gynécologique fréquente dont la physiopathologie est incomplètement caractérisée, ce qui engendre des défis cliniques substantiels pour un diagnostic rapide et une prise en charge efficace. L'interaction causale entre le microbiote intestinal et la grossesse extra-utérine, en particulier par la médiation de la protéomique plasmatique, reste indéterminée. Nous avons obtenu les données d'étude d'association pangénomique (GWAS) sur le microbiote intestinal (n=412) à partir d'une méta-analyse du Projet Microbiome Néerlandais, et les données sur la grossesse extra-utérine des projets IEU OpenGWAS et FinnGen. Les résultats ont révélé que neuf types de microbiotes intestinaux présentent des associations causales avec le risque de grossesse extra-utérine. Un total de 67 protéines plasmatiques ont un impact causal sur le risque de grossesse extra-utérine. De plus, l'effet de trois types de microbiotes intestinaux sur la grossesse extra-utérine est médié par 25 types de protéines plasmatiques. Le microbiote enrichi dans la voie de dégradation du L-rhamnose affecte la grossesse extra-utérine par l'intermédiaire de 22 types de protéines plasmatiques. Cette étude établit des relations causales entre des voies spécifiques du microbiote intestinal et le risque de grossesse extra-utérine, médiées par des signatures distinctes de protéines plasmatiques, ouvrant ainsi des perspectives pour les interventions cliniques et les recherches futures.. *(Afr J Reprod Health 2026; 30 [8]: 66-75).*

Mots-clés: microbiote intestinal; grossesse extra-utérine; randomisation mendélienne; étude d'association pangénomique

Introduction

Ectopic pregnancy represents a prevalent obstetric emergency with a reported incidence rate of 1.3–2.0%.[1] As one of the leading causes of mortality during the first trimester, it contributes to 5%–10% of all pregnancy-associated maternal mortality.[2] Developing countries continue to experience disproportionately high mortality rates from ectopic pregnancies, compounded by substantial treatment

costs that create huge burdens for affected families and pose critical challenges to global maternal health.[3] In addition, the underlying pathogenesis of ectopic pregnancy remains incompletely understood, creating persistent obstacles for developing effective preventive strategies and optimized therapeutic interventions. Currently, ectopic pregnancy can be challenging to diagnose, as most women experience pain and vaginal bleeding during the first trimester of pregnancy; however, these nonspecific symptoms are very common in early pregnancy and frequently mimic other acute conditions including appendicitis, spontaneous abortion, or traumatic injuries, complicating differential diagnosis.[4] Existing research predominantly examines epidemiological associations and therapeutic approaches rather than elucidating mechanistic pathways. While studies have identified epidemiological associations between ectopic pregnancy and various risk factors including advanced maternal age and tobacco use, inherent limitations from confounding variables and reverse causation preclude definitive causal inference.[5,6]

The gut microbiome constitutes a complex ecosystem whose cellular abundance rivals that of human host cells. Existing evidence demonstrates that microbial β-glucuronidase enzymes critically modulate host systemic estrogen homeostasis via reactivating estrogen.[7,8] Reduced circulating estrogen has been implicated in the pathogenesis of obesity, metabolic dysregulation, cardiovascular disorders, and neurocognitive impairment, while elevated circulating estrogen potentially triggers endometriosis and malignancies.[9,10] The gut microbiota is associated with maternal and infant health. The gut microbiota alterations induced by pregnancy are related to pregnancy-related disorders and have a profound impact on the development of the neonatal immune system.[11,12] Previous studies mainly focused on microbe-hormone mediated mechanisms or the relationship between the pregnancy and gut microbiome; however, the causal gut microbiota-ectopic pregnancy relationship remains unexplored.

In this study, we performed bidirectional mendelian randomization analysis to assess putative causal associations between 412 gut microbiota and ectopic pregnancy risk utilizing GWAS-derived genetic instruments. In addition, we implemented proteome-wide mediation analysis (4,907 proteins) to identify potential mediating effects. Our analysis delineated candidate pathogenic microbial taxa and functional pathways, providing theoretical foundations for microbiome-uterine axis interactions and references for ectopic pregnancy prevention and clinical management..

Methods

The study was designed following the STROBE-MR guidelines.[13] **Figure 1** illustrates the flowchart of the study.

Data source

GWAS data of 412 gut microbiotas were derived from Esteban et al., including 207 taxa and 205 pathways representing microbial composition and function from 7,738 participants.[14] The GWAS summary statistics for ectopic pregnancies were obtained from the IEU Open GWAS Project (gwas.mrcieu.ac.uk/), using 1,514 cases and 250,492 controls. Proteomics data for 35,559 circulating proteins were obtained from the deCODE 2021 cohort.[15] The GWAS data for the validation analysis were extracted from the latest FinnGen Consortium R11 release data (https://r11.finngen.fi/) using 6,818 cases and 180,063 controls.

Genetic instrumental variable selection

Instrumental variables (IVs) significantly associated with exposure and outcomes were extracted via several quality control steps. A genome-wide significance threshold of $P < 1\times10^{-5}$ was used for IVs selection. First, SNPs exhibiting strong linkage disequilibrium (LD) were removed to avoid bias. A clustering process was conducted on European samples from the 1000 Genomes Project ($R^2<0.001$, 10,000 kb) to estimate the LD between SNPs.[16] Secondly, the PhenoScanner database (http://phenoscanner.medschl.cam.ac.uk) was used to exclude potential confounding SNPs with a threshold of $P<5\times10^{-8}$ to address potential confounders. Last, SNPs with F-statistics greater than 10 were considered IVs to maintain a strong

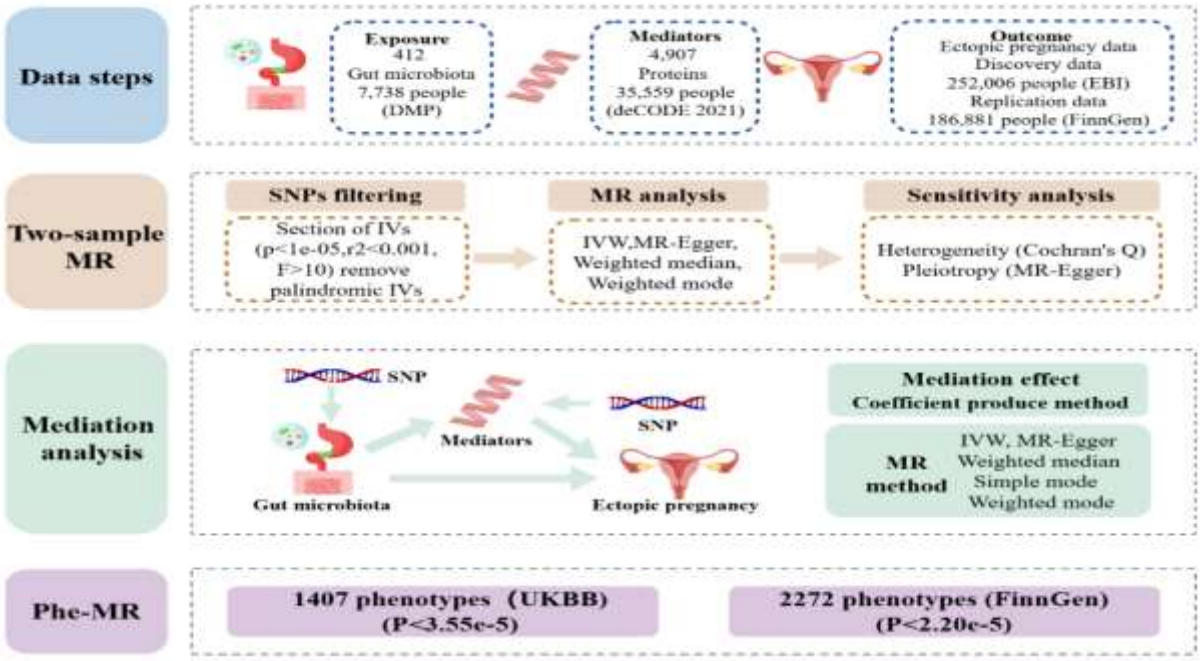

Figure 1: The flowchart of the study.

association with exposure and exclude interference from weak instrumental variables.[17] Palindromic SNPs with intermediate allele frequencies were excluded to enhance the accuracy of the results.[18]

Mendelian randomization analysis

Inverse variance-weighted (IVW), MR-Egger, Wald ratio, Weighted median, and Weighted mode were used for mendelian randomization analysis. Additionally, Cochran's Q test was used for evaluation of heterogeneity and MR-Egger regression was conducted to eliminate horizontal pleiotropy of the instrumental variables, with data of *P*-value below 0.05 excluded. Phenome-wide association study (PheWAS) was conducted using summary-level statistics from the UK Biobank (UKBB) cohort, which comprised 1,407 registered phenotypes. The analyses were conducted using the R package TwoSampleMR (version 0.5.7). Validation analysis using data from the FinnGen database was conducted following the same analytical procedures.[19]

Mediation analysis

Mediating Mendelian randomization analyses were performed on specific gut microbiota that exhibited positive results in the Mendelian randomization analysis, with sensitivity analyses revealing no evidence of horizontal pleiotropy or evidence of heterogeneity. A total of 4,907 proteins were incorporated as potential mediators and the product of coefficients method was used for each plasma protein to estimate the indirect effect. By determining the proportion of the indirect effect relative to the total effect, the portion mediated by each plasma protein in the total effect of gut microbiota on ectopic pregnancy can be estimated.[20]

Ethics approval and consent to participate

This study is based entirely on available public data, does not require an ethics approval.

Results

Causal associations between gut microbiota and ectopic pregnancy

After quality control, we identified 4014 unique SNPs associated with 407 gut microbiotas. Overall, we determined the causal effect of nine gut microbiotas on ectopic pregnancy (Table 1). According to IVW analysis, Microbiota enriched

Table 1: Causal relationships estimated for gut microbiotas and ectopic pregnancy.

Exposure	Method	N-SNP	*P* value	OR (95% CI)	Heterogeneity
GLUCOSE1PMETAB.PWY..glucose.and.glucose.1.phosphate.degradation	Inverse variance weighted	9	<0.01	1.40 (1.13 to 1.73)	0.95
GLUCOSE1PMETAB.PWY..glucose.and.glucose.1.phosphate.degradation	MR Egger	9	0.28	1.81 (0.68 to 4.80)	0.93
GLUCOSE1PMETAB.PWY..glucose.and.glucose.1.phosphate.degradation	Weighted median	9	0.04	1.35 (1.02 to 1.79)	
GLUCOSE1PMETAB.PWY..glucose.and.glucose.1.phosphate.degradation	Weighted mode	9	0.19	1.35 (0.90 to 2.01)	
POLYAMINSYN3.PWY..superpathway.of.polyamine.biosynthesis.I	Inverse variance weighted	11	0.03	0.83 (0.70 to 0.99)	0.97
POLYAMINSYN3.PWY..superpathway.of.polyamine.biosynthesis.I	MR Egger	11	0.65	0.86 (0.46 to 1.62)	0.94
POLYAMINSYN3.PWY..superpathway.of.polyamine.biosynthesis.I	Weighted median	11	0.08	0.81 (0.65 to 1.02)	
POLYAMINSYN3.PWY..superpathway.of.polyamine.biosynthesis.I	Weighted mode	11	0.23	0.80 (0.57 to 1.13)	
PWY.5973..cis.vaccenate.biosynthesis	Inverse variance weighted	15	0.02	0.78 (0.63 to 0.97)	0.75
PWY.5973..cis.vaccenate.biosynthesis	MR Egger	15	0.15	1.78 (0.85 to 3.74)	0.97
PWY.5973..cis.vaccenate.biosynthesis	Weighted median	15	0.12	0.80 (0.60 to 1.06)	
PWY.5973..cis.vaccenate.biosynthesis	Weighted mode	15	0.44	0.83 (0.53 to 1.31)	
PWY.7013..L.1.2.propanediol.degradation	Inverse variance weighted	11	0.04	0.89 (0.79 to 1.00)	0.65
PWY.7013..L.1.2.propanediol.degradation	MR Egger	11	0.76	1.09 (0.64 to 1.88)	0.62
PWY.7013..L.1.2.propanediol.degradation	Weighted median	11	0.28	0.92 (0.78 to 1.07)	
PWY.7013..L.1.2.propanediol.degradation	Weighted mode	11	0.97	0.99 (0.77 to 1.28)	
PWY.7446..sulfoglycolysis	Inverse variance weighted	10	0.04	1.14 (1.01 to 1.29)	0.89
PWY.7446..sulfoglycolysis	MR Egger	10	0.97	1.01 (0.58 to 1.78)	0.85
PWY.7446..sulfoglycolysis	Weighted median	10	0.21	1.10 (0.94 to 1.29)	
PWY.7446..sulfoglycolysis	Weighted mode	10	0.47	1.09 (0.87 to 1.37)	

RHAMCAT.PWY..L.rhamnose.degradation.I	Inverse variance weighted	13	0.03	0.76 (0.59 to 0.98)	0.28
RHAMCAT.PWY..L.rhamnose.degradation.I	MR Egger	13	0.45	1.96 (0.36 to 10.65)	0.3
RHAMCAT.PWY..L.rhamnose.degradation.I	Weighted median	13	0.42	0.87 (0.62 to 1.22)	
RHAMCAT.PWY..L.rhamnose.degradation.I	Weighted mode	13	0.84	1.06 (0.58 to 1.94)	
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria	Inverse variance weighted	6	0.04	0.71 (0.52 to 0.98)	0.93
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria	MR Egger	6	0.84	1.21 (0.21 to 7.00)	0.91
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria	Weighted median	6	0.07	0.69 (0.46 to 1.03)	
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria	Weighted mode	6	0.15	0.61 (0.35 to 1.07)	
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales.f_Desulfovibrionaceae	Inverse variance weighted	6	0.04	0.71 (0.52 to 0.98)	0.93
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales.f_Desulfovibrionaceae	MR Egger	6	0.84	1.21 (0.21 to 7.00)	0.91
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales.f_Desulfovibrionaceae	Weighted median	6	0.07	0.69 (0.46 to 1.03)	
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales.f_Desulfovibrionaceae	Weighted mode	6	0.13	0.61 (0.36 to 1.04)	
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales	Inverse variance weighted	6	0.04	0.71 (0.52 to 0.98)	0.93
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales	MR Egger	6	0.84	1.21 (0.21 to 7.00)	0.91
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales	Weighted median	6	0.07	0.69 (0.45 to 1.04)	
k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales	Weighted mode	6	0.13	0.61 (0.36 to 1.05)	

in glucose.and.glucose.1.phosphate.degradation (OR=1.40, 95%CI, 1.13-1.73, *P*=0.002), and sulfoglycolysis (OR=1.14, 95%CI, 1.01-1.29, *P*=0.038) were positively correlated with the risk of ectopic pregnancy. Microbiota enriched in superpathway.of.polyamine.biosynthesis.II (OR=0.83, 95%CI, 0.70-0.99, *P*=0.035), cis.vaccenate.biosynthesis (OR=0.78, 95%CI, 0.63-0.97,*P*=0.024), L.1.2.propanediol.degradation (OR=0.89, 95%CI, 0.79-1.00, *P*=0.044), L.rhamnose.degradation.I (OR=0.76, 95%CI, 0.59-0.98,P=0.033),k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria (OR=0.71, 95%CI, 0.52-0.98, *P*=0.038),k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales (OR=0.71, 95%CI,0.520.98,*P*=0.030),k_Bacteria.p_Proteobacteria.c_Deltaproteobacteria.o_Desulfovibrionales.f_Desulfovibrionaceae (OR=0.71, 95%CI, 0.52-0.98, *P*=0.039) were inversely related to ectopic pregnancy. No significant pleiotropy or heterogeneity was observed in the causal estimates.For further validation, we used data from the FinnGen database for confirmatory analysis. A total of 3926 SNPs were identified, and the results suggested two overlapped gut microbiotas causally associated with ectopic pregnancy: Microbiota enriched in L.1.2.propanediol.degradation (OR=0.94, 95%CI, 0.88-1.00, *P*=0.042) and L.rhamnose.degradation.I (OR=0.88, 95%CI, 0.78-1.00, *P*=0.045) (Figure 2). No significant pleiotropy or heterogeneity was observed in the causal estimates.

Causal associations between proteomics and ectopic pregnancy

A total of 5402 unique SNPs associated with 1227 plasma proteins were identified. Our analysis indicated the potential impact of 67 plasma proteins on the risk of ectopic pregnancy. No significant pleiotropy or heterogeneity was observed in the causal estimates.

PheWAS of gut microbiota associated ectopic pregnancy

To examine the potential relationship between gut microbiota related to ectopic pregnancy and various diseases, we performed PheWAS analyses. The results indicated no association between nine gut microbiota and 1406 phenotypes (*P*<3.55×10-5 (0.05/1407)) except for microbiota enriched in superpathway.of.polyamine.biosynthesis.II. For this kind of microbiota, it may be negatively related to cervicitis and endocervicitis (OR=0.60, 95%CI, 0.48-0.75, $P=1.20\times10^{-5}$). In addition, no association was observed using the data from FinnGen database (*P*<2.20×10-5(0.05/2272)).

Identification of mediators

We further identified that three of the nine gut microbiotas were mediated by 25 kinds of plasma proteins with ectopic pregnancy, and two of them were further validated via FinnGen database (L.1.2.propanediol.degradation & L.rhamnose.degradation.I). For these two kinds of microbiotas, we used the product of coefficients method as our main method to estimate the indirect effect (which is the effect of gut microbiota on ectopic pregnancy through the mediator). The results indicated that Microbiota enriched in L.1.2.propanediol.degradation acted on ectopic pregnancy via plasma protein FJX1 (β=-0.010, 95%CI, -0.023 to 0.0027). Microbiota enriched in sulfoglycolysis acted on ectopic pregnancy via LY9 (β=0.00471, 95%CI, -0.00151 to 0.01093) and CRELD1 (β=0.00396, 95%CI, -0.001 to 0.009). In addition, Microbiota enriched in L.rhamnose.degradation.I affected ectopic pregnancy through 22 kinds of plasma proteins, such as TOR4A (β=-0.237, 95%CI, -0.454 to -0.020), UXS1 (β=-0.155, 95%CI, -0.359 to 0.048), and NHEJ1 (β=-0.122, 95%CI, -0.259 to 0.015).

Discussion

To date, the role of gut microbiota in the pathogenesis of ectopic pregnancy is still not clearly defined. In this study, we identified that nine gut microbiotas may have the potential causal impact on the risk of ectopic pregnancy based on large GWAS summary datasets. Except for superpathway.of.polyamine.biosynthesis.II related to the risk of cervicitis and endocervicitis, all nine gut microbiotas have no association with 1406 kinds of diseases, indicating they might be considered potential targets for ectopic pregnancy interventions. In addition, we identified a potential network that three gut microbiotas causally

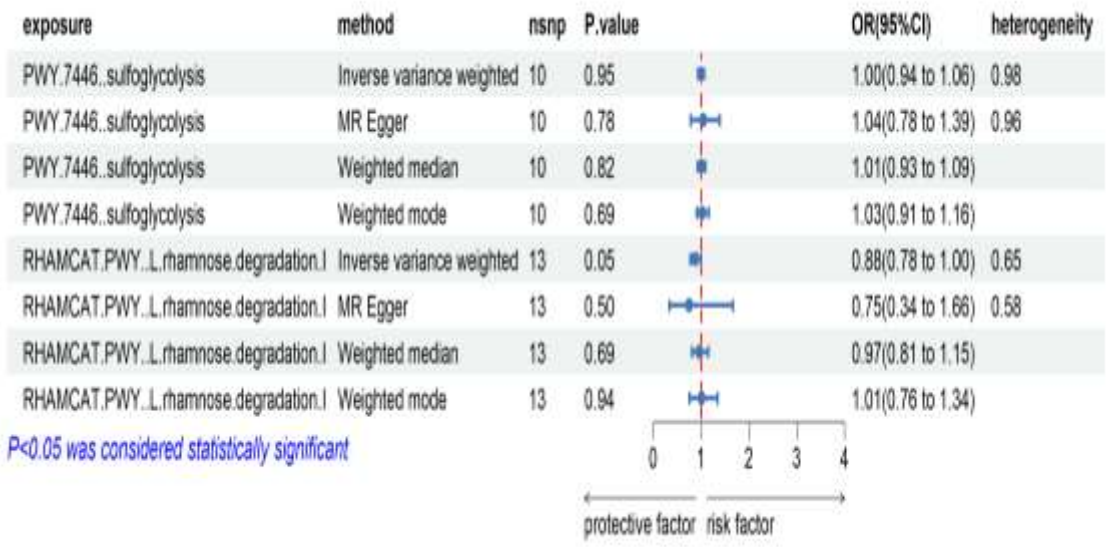

Figure 2. Forest plot of causal relationships estimated for gut microbiotas and ectopic pregnancy (FinnGen database).

impacted on ectopic pregnancy mediated by 25 kinds of plasma proteins, further revealing the possible mechanisms from the perspective of gut microbiota. To the best of our knowledge, this study represents the first comprehensive investigation systematically evaluating the causal relationship between gut microbiota and ectopic pregnancy from a genetic perspective.

Our study suggests that the gut microbiota may affect the occurrence of ectopic pregnancy through pathways such as L.1.2.propanediol.degradation, L.rhamnose.degradation.I, and sulfoglycolysis. The gut microbiota plays a crucial role in various physiological processes, including immune regulation, metabolism, and maintaining the integrity of the gut barrier.[21] Recent research has highlighted its potential impact on reproductive health, including pregnancy outcomes. The gut microbiota's influence on pregnancy is multifaceted, involving interactions with the immune system and metabolic pathways that can affect both maternal and fetal health.[22,23] For instance, alterations in gut microbiota have been associated with gestational diabetes mellitus (GDM), a condition that can complicate pregnancy and affect fetal development. Studies have shown that women with GDM exhibit distinct gut microbiota profiles compared to normoglycemic pregnant women, with differences persisting postpartum.[24,25] Moreover, the gut microbiota's role in immune modulation during pregnancy is significant. It has been observed that changes in gut microbiota composition can affect systemic inflammation and immune responses, which are critical for maintaining a healthy pregnancy.[26] For example, increased microbial translocation and systemic inflammation have been linked to depression during early pregnancy, indicating a possible connection between gut microbiota and mental health during pregnancy.[27]

Additionally, the gut microbiota's interaction with other microbial communities, such as the vaginal microbiota, can further influence pregnancy outcomes. Dysbiosis in the vaginal microbiota has been associated with adverse pregnancy outcomes, including preterm birth and recurrent pregnancy loss.[28, 29] This suggests that the gut microbiota, through its interaction with the vaginal microbiota, may play a role in maintaining reproductive health and preventing complications. Furthermore, maternal diet and environmental factors can shape the gut microbiota, influencing pregnancy outcomes. For instance, maternal exposure to certain environmental elements has

been shown to alter gut microbiota composition, which in turn can affect blood pressure during pregnancy.[30] This highlights the potential for dietary and environmental interventions to modulate gut microbiota and improve pregnancy outcomes.It is noteworthy that we identified FJX1 as a novel mediator linking gut microbial functions (L.1.2.propanediol.degradation and L.rhamnose.degradation.I pathways) to ectopic pregnancy risk. FJX1, or Four Joint Box-1 Protein, is a planar cell polarity protein and plays significant roles in multiple cancers.[31,32] Recently, FJX1 has been reported in conditions like endometriosis, where its overexpression has been noted in the endometrium of affected women. This suggests that FJX1 may influence reproductive outcomes through its regulatory effects on angiogenesis and inflammation, processes that are also critical in the establishment and maintenance of a healthy pregnancy.(32) Our findings demonstrate a potential association between FJX1 and gut microbiota with protective effects against ectopic pregnancy. Building upon established biological functions, we propose that its regulatory capacities in angiogenesis, inflammatory modulation, and cytoskeletal reorganization may mechanistically bridge this association, though specific mechanisms requiring further experimental validation..

Strengths and limitations

Our findings carry significant implications for public health policy and clinical practice. Firstly, the identification of specific gut microbial taxa and plasma protein signatures associated with ectopic pregnancy risk paves the way for developing non-invasive predictive biomarkers. Health policies could support research into integrating fecal microbiome profiling and targeted plasma proteomic panels into routine early-pregnancy screening protocols for high-risk populations. Secondly, the causal links identified, particularly the protective roles of certain microbial pathways, suggest potential for microbiome-targeted preventative strategies. Public health initiatives could promote further research into prebiotic, probiotic, or dietary interventions aimed at modulating these specific gut microbial communities to reduce ectopic pregnancy incidence at a population level. Finally, the discovery of FJX1 as a key mediating protein offers a new molecular target for therapeutic investigation. We recommend that funding agencies and health policymakers prioritize translational research that validates these biomarkers and explores targeted interventions, ultimately aiming to transform these mechanistic insights into tangible tools for risk stratification and prevention, thereby alleviating the global burden of this gynecological emergency. Our study has several limitations. First, while employing various methods to minimize pleiotropy, residual confounding remains inherent to the mendelian randomization study. Second, instrument strength limitations exist due to GWAS sample size constraints. Third, European ancestry restriction limits generalizability, and mendelian randomization captures lifelong exposure effects rather than time-specific impacts. Future studies with expanded, ethnically diverse cohorts are needed to validate these associations. Additional research is required to clarify the roles of gut microbiota in ectopic pregnancy pathogenesis

Conclusion

In conclusion, our study elucidates causal gut microbiota-ectopic pregnancy relationships and mechanistic pathways, establishing a framework for future investigations into microbial-driven reproductive pathophysiology. Furthermore, integrated fecal microbiome profiling and plasma proteome analysis might emerge as novel screening modalities for ectopic pregnancy risk stratification, facilitating timely clinical interventions for ectopic pregnancy and enhancing reproductive health.IVW: Inverse variance-weighted; PheWAS: Phenome-wide association study; UKBB: UK Biobank.

Availability of data and materials

The original data can be downloaded from the websites:https://gwas.mrcieu.ac.uk/,https://www.decode.com/summarydata/,and ttps://r11.finngen.fi/.

Conflict of interest

The authors have declared no conflicts of interest.

Author contributions

Ning Zhang and Zhige Wang contributed equally to this work and should be treated as co-first authors.

N.Z., and Z.W., conceptualization, data curation, methodology, visualization, formal analysis, investigation, validation, and writing – original draft; F.Y. and T.L., formal analysis, investigation, and validation; D.J., conceptualization, funding acquisition, resources, software, investigation, project administration, supervision, validation, and writing – review & editing. All authors have read and approved the final manuscript.

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