Twin Research and Human Genetics (2024), **27**, 80–84
doi:10.1017/thg.2024.16

CAMBRIDGE UNIVERSITY PRESS

Article

Genetic Causal Relationship Between Alanine Aminotransferase Levels and Risk of Gestational Diabetes Mellitus: Mendelian Randomization Analysis Based on Two Samples

Lihua Yin, Yifang Hu, Xiaoxia Hu, Xiaolei Huang, Yingyuan Chen and Yisheng Zhang
The Affiliated Lihuili Hospital of Ningbo University, Ningbo, Zhejiang 315040, P.R. China

Abstract

Gestational diabetes mellitus (GDM) is a frequent complication of pregnancy. The specific mechanisms underlying GDM have not yet been fully elucidated. Contemporary research indicates a potential association between liver enzyme irregularities and an increased risk of metabolic disorders, including diabetes. The alanine aminotransferase (ALT) level is recognized as a sensitive marker of liver injury. An increase in ALT levels is hypothesized to be linked to the pathogenesis of insulin resistance and diabetes. Nonetheless, the definitive causal link between ALT levels and GDM still needs to be determined. This investigation utilized two-sample Mendelian randomization (MR) to examine the genetic causation between alanine aminotransferase (ALT) and GDM. We acquired alanine aminotransferase (ALT)-related GWAS summary data from the UK Biobank, Million Veteran Program, Rotterdam Study, and Lifeline Study. Gestational diabetes data were obtained from the FinnGen Consortium. We employed various MR analysis techniques, including inverse-variance weighted (IVW), MR Egger, weighted median, simple, and weighted weighting. In addition to MR-Egger intercepts, Cochrane's Q test was also used to assess heterogeneity in the MR data, and the MR-PRESSO test was used to assess horizontal pleiotropy. To assess the association's sensitivity, a leave-one-out approach was employed. The IVW results confirmed the independent risk factor for GDM development, as indicated by the ALT level ($p = .011$). As shown by leave-one-out analysis, horizontal pleiotrophy did not significantly skew the causative link ($p > .05$). Our dual-sample MR analysis provides substantiated evidence of a genetic causal relationship between alanine aminotransferase (ALT) levels and gestational diabetes.

Keywords: Gestational diabetes mellitus; ferroptosis

(Received 30 January 2024; revise received 25 February 2024; accepted 28 February 2024; First Published online 18 April 2024)

Gestational diabetes mellitus (GDM) manifests as diabetes during pregnancy in women with previously normal glucose metabolism (McIntyre et al., 2019). As a significant public health concern, GDM incidence varies globally, estimated at approximately 14.0% worldwide (Wang et al., 2022) and 14.8% in China (Gao et al., 2019), as reported by the International Diabetes Federation (IDF). GDM jeopardizes maternal health and adversely impacts fetal development. It predisposes mothers to perinatal complications such as gestational hypertension and preeclampsia, along with adverse pregnancy outcomes such as macrosomia, cesarean delivery and preterm birth. Moreover, this condition heightens the risk of future type 2 diabetes and cardiovascular diseases for mothers. Additionally, fetuses face increased risks of neonatal complications, including hyperglycemia, hyperbilirubinemia, and respiratory distress syndrome, and in the long term, childhood obesity, metabolic syndrome, and cardiovascular diseases (Kondracki et al., 2022; Lee et al., 2018; Lenoir-Wijnkoop et al., 2015; McIntyre et al., 2019).

Corresponding author: Yisheng Zhang; Email: zhangysnbu@163.com
Cite this article: Yin L, Hu Y, Hu X, Huang X, Chen Y, Zhang Y. (2024) Genetic Causal Relationship Between Alanine Aminotransferase Levels and Risk of Gestational Diabetes Mellitus: Mendelian Randomization Analysis Based on Two Samples. *Twin Research and Human Genetics* **27**: 80–84, https://doi.org/10.1017/thg.2024.16

The alanine aminotransferase (ALT) level, a critical liver damage biomarker, is positively correlated with diabetes risk when it is persistently elevated, as demonstrated by prior research. Some articles also suggests that a substantial correlation between the two variables is lacking. Nonetheless, the epidemiological association between ALT and GDM has been subject to scrutiny (Hua et al., 2021). Traditional risk factor identification for GDM, primarily based on observational studies, is often limited by confounding factors. Mendelian randomization (MR) analysis, a novel methodological approach, overcomes these limitations by controlling for confounders, thereby elucidating the causal relationships between variables. This study employed two-sample Mendelian randomization, leveraging large-scale genomewide association study (GWAS) data and utilizing genetic markers as instrumental variables. This approach aims to delineate the causal relationship between ALT levels and GDM incidence, laying the groundwork for enhanced prediction and intervention strategies in GDM management.

Materials and Methods

Study Design and Data Sources

This study implemented two-sample MR to examine the causal link between ALT exposure and GDM outcomes. This method,

utilizing distinct and independent GWAS datasets, surpasses single-sample MR in efficacy and power. ALT levels were the exposure variable, while gestational diabetes status was the outcome of interest. Instrumental variables (IVs) for the analysis were single nucleotide polymorphisms (SNPs), chosen based on three fundamental two-sample MR assumptions: (1) a strong association of all selected IVs with ALT exposure ($p < 5\times10$^-8); (2) the independence of all selected IVs from confounders affecting ALT as well as GDM; (3) influence of all selected IVs on gestational diabetes exclusively via ALT, without alternative pathways.

There were 437,438 discovery samples and 315,572 replication samples in the ALT GWAS from the UK Biobank, Million Veteran Program, Rotterdam Study and Lifeline Study (Pazoki et al., 2021). All participants, of European descent, provided informed consent. The GWAS summary data for gestational diabetes were sourced from a Finnish database that included 6033 gestational diabetes patients and 110,330 controls, all of European descent.

Selecting Instrument Variables

MR analysis required strict adherence to three principles: relevance, independence and exclusion restriction. Consequently, all IVs selected for further analysis underwent stringent screening. SNPs strongly associated with ALT exposure ($p < 5 \times 10$^-8) were chosen. To ensure significance and mitigate weak IV bias, F values less than 10 were excluded. The F value was calculated as $F = R$^2 $\times$ (N-2)/(1-R^2), where R^2 is the variance in ALT explained by each IV. $R^2 = 2 \times EAF \times (1\text{-}EAF) \times \beta$^2, where beta indicates the allelic effect and EAF the effect allele frequency. To eliminate biases from high linkage disequilibrium among SNPs, a clumping process ($r^2 < .001$, physical distance = 10,000 kb) was used to ensure IV independence. Additionally, palindromic SNPs with intermediate allele frequencies were excluded to align effect alleles between the ALT and gestational diabetes datasets.

Statistical Analysis

The genetic association between ALT levels and gestational diabetes incidence was investigated using five methods: MR-Egger regression, the weighted median, the inverse-variance weighted (IVW) method, the simple mode, and the weighted mode. IVW, assuming the validity of all analyzed SNPs, was anticipated to provide the most accurate estimates and thus was the primary method in this study (Chen et al., 2024). The results were statistically significant when the p value of IVW was less than .05 and IVW and MR-Egger were in the same direction. Several tests, including the Cochrane Q test and funnel plot symmetry assessment, were used to validate the results. The MR-Egger intercept test and MR-PRESSO global test were used to detect multicollinearity, with MR-PRESSO also identifying and excluding outliers to provide adjusted estimates. A leave-one-out sensitivity analysis was used to assess the impact of individual SNPs on the overall association. Statistical analyses were conducted with R software (version 4.3.2) using the TwoSampleMR package; $p < .05$ indicated statistical significance.

Results

Selection of Instrumental Variables

Screening identified 252 SNPs strongly linked to ALT ($p < 5 \times 10$^-8; F value > 10) and independently related to ALT ($r^2 < .001$, physical distance ≤ 10,000 kb), initially serving as potential instrumental variables, with the lowest F value being 27.35. Postharmonization analysis of the ALT and gestational diabetes datasets was performed. For subsequent MR analysis, 238 SNPs were retained, including nine palindromic SNPs, namely, rs12609548, rs133015, rs13395911, rs1778793, rs4711750, rs4782568, rs7041363, rs7672435, and rs9788910. Consequently, 229 SNPs were finalized as instrumental variables.

Table 1. The MR results obtained by five methods.

Exposure	Outcome	Method	SNP (*n*)	*OR*	*OR* 95%CI	*p* value
ALT	GDM	MREgger	229	1.340	0.303, 5.917	.700
ALT	GDM	Weighted median	229	2.957	0.826, 10.581	.096
ALT	GDM	IVW	229	2.868	1.275, 6.451	.011
ALT	GDM	Simple mode	229	0.100	0.004, 2.322	.152
ALT	GDM	Weighted mode	229	1.848	0.364, 9.386	.459

Note: MR, Mendelian randomization; ALT, alanine aminotransferase; GDM, gestational diabetes mellitus; IVW, inverse-variance weighted.

Mendelian Randomization Analysis

Genetic links between ALT levels and gestational diabetes incidence were explored using the random-effects IVW method. A significant difference in the odds ratio (*OR*) was detected between people with gestational diabetes and those without gestational diabetes ($p = .011$, 95% CI = 2.868 [1.275-6.451]) (Table 1; Figure 1). The weighted median method corroborated a genetic causal relationship between ALT levels and gestational diabetes incidence (Figure 2). Table 1 details the five methodologies employed in our MR analysis, along with their respective outcomes.

The heterogeneity tests revealed significant variability in the impacts of genetic instrumental variables. The MR Egger method yielded a heterogeneity Q statistic of 302.6416 (degrees of freedom [*df*] = 227, $p = .00058$), highlighting notable heterogeneity among the genetic tools. The Q statistic of the IVW method was 304.5560 ($df = 228$, $p = .00052$), which further confirmed the heterogeneity. Additionally, funnel plots exhibited SNP symmetry (Figure 3).

Egger intercept and MR-PRESSO analyses indicated no pleiotropy ($p = .23205$), with no outliers identified in the MR-PRESSO during the analysis. The leave-one-out test confirmed that the MR analysis results were unaffected by individual SNPs, confirming the stability and robustness of the findings (Figure 4).

Discussion

This study leveraged large-scale GWAS data to examine the causal relationship between ALT levels and GDM incidence. We identified a notable association between SNPs affecting ALT levels and those affecting GDM prevalence, suggesting that prenatal interventions targeting liver disease affecting ALT can reduce the prevalence of GDM.

Increasing evidence supports a correlation between ALT levels and GDM risk. A study of 94 GDM patients reported by An et al. (2022) revealed a negative correlation between early pregnancy AST/ALT levels and GDM risk. Conversely, a prospective study involving 1128 patients indicated a positive association between

https://doi.org/10.1017/thg.2024.16 Published online by Cambridge University Press

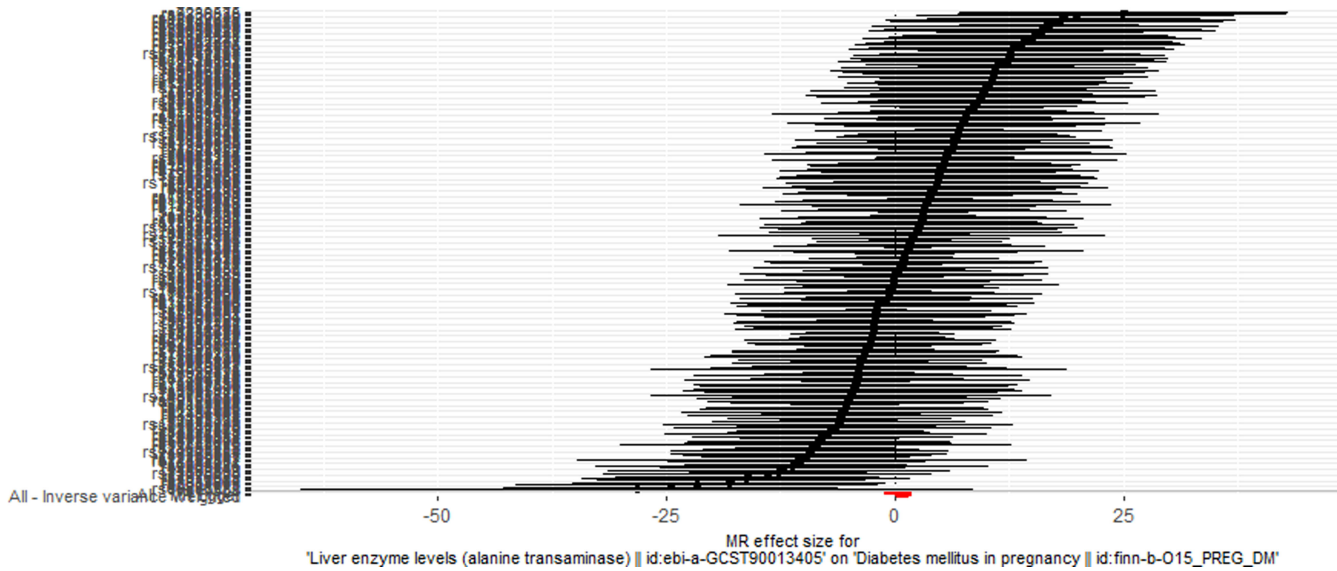

Figure 1. Forest plot of the effect of alanine aminotransferase (ALT) on gestational diabetes mellitus (GDM).

Figure 2. The scatter plot shows the causal effect of alanine aminotransferase (ALT) on gestational diabetes mellitus (GDM).

early pregnancy ALT/AST levels and GDM (Song et al., 2022), identifying them as independent risk factors. Research by Erdoğan et al. (2014) also suggested that the ALT concentration is a predictive marker for GDM. Nevertheless, some studies have reported no significant correlation between ALT levels and GDM risk (Kong et al., 2018; Zhao et al., 2020).

There is no evidence that ALT causes GDM based on observational studies. The cooccurrence of ALT with conditions such as intrahepatic cholestasis during pregnancy and elevated AST complicates its relationship with GDM. Genome-wide association studies are instrumental in dissecting complex diseases and identifying key genetic contributors beyond single-gene analyses. Our research, using extensive data, provides genetic insight into the causal relationship between ALT levels and GDM under both intricate and interrelated conditions.

The relationship between ALT and GDM is likely complex. Liver stress, metabolic imbalances, insulin resistance, and inflammation related to ALT have implications for GDM (Peracchi & Polverini, 2022). Insulin resistance, which is crucial in GDM development, may impair liver function and elevate ALT levels (Sakurai et al., 2021). GDM has been associated with metabolic disorders and chronic inflammation (Bakhshimoghaddam et al., 2023), potentially exacerbating liver stress and ALT levels (Huang et al., 2019). Moreover, the interplay between inflammation and insulin resistance could intensify GDM progression (Zheng et al., 2016). Fatty liver disease, a GDM risk factor, can increase ALT levels and

https://doi.org/10.1017/thg.2024.16 Published online by Cambridge University Press

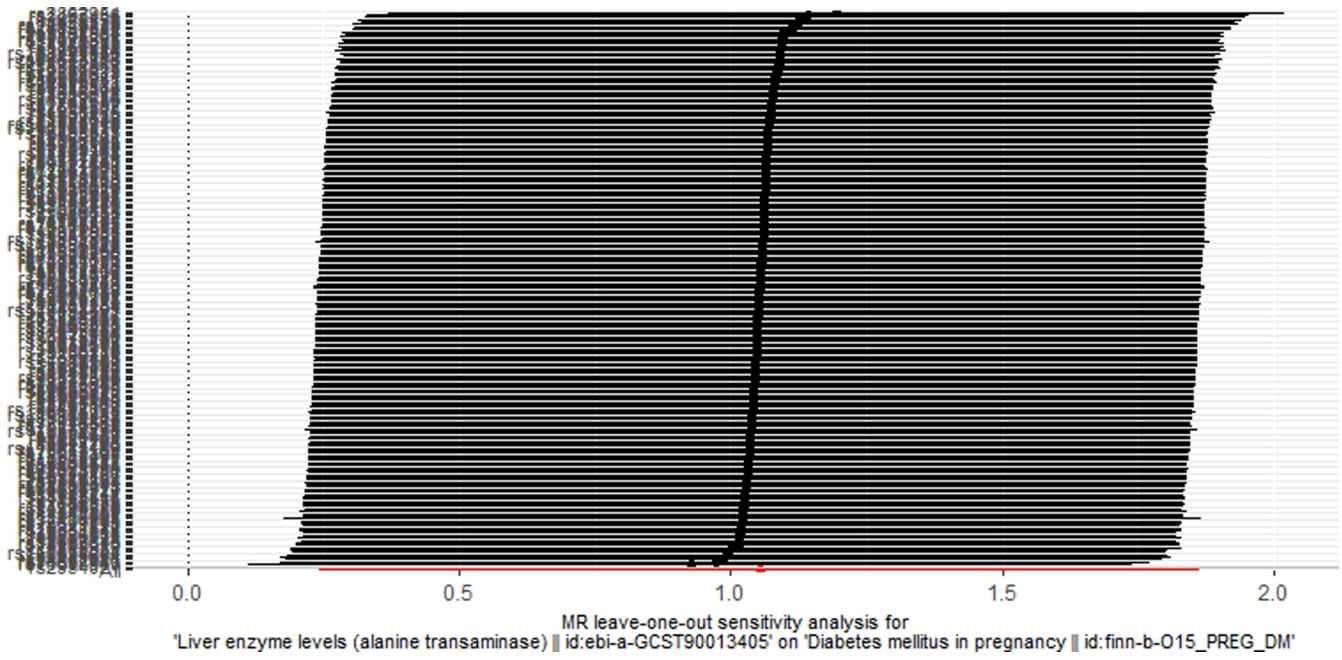

Figure 3. Mendelian randomization (MR) leave-one-out shows the sensitivity analysis of alanine aminotransferase (ALT) for gestational diabetes mellitus (GDM).

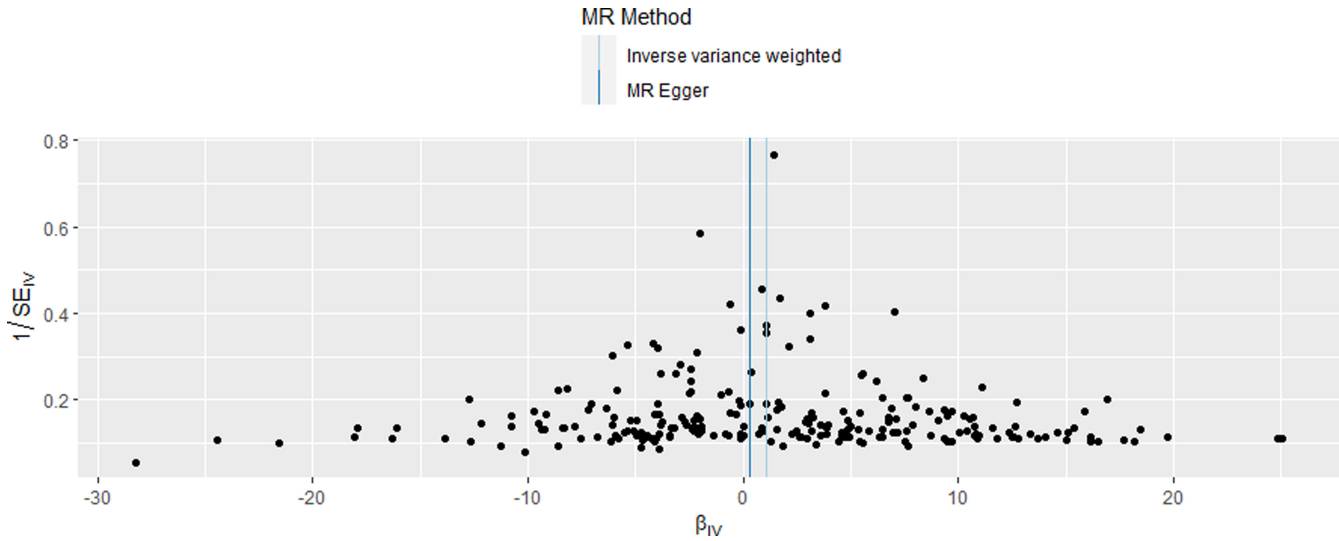

Figure 4. Funnel plot of the effect of alanine aminotransferase (ALT) on gestational diabetes mellitus (GDM).

contribute to GDM development (Ajmera et al., 2016; Chen et al., 2021). Hormonal changes during pregnancy may also impact insulin sensitivity and metabolism, affecting alanine aminotransferase (ALT) levels.

A strength of our study is that it is the first GWAS exploring the causal relationship between ALT and GDM. The two-sample MR method addresses observational study limitations such as reverse causation, confounding factors, and biases. Rigorous selection of instrumental variables ensured accurate results. Various tests for sensitivity, horizontal pleiotropy, and heterogeneity reinforced the stability and reliability of the ALT-GDM association.

However, there are limitations. The participants were exclusively of European descent, leaving the generalizability of our findings to other populations uncertain. Pleiotropy was adjusted using MR intercepts and MR-PRESSO global tests, and residual confounding factors could bias the results. Finally, reliance on genome-wide association meta-analyses limits stratified analyses by country, ethnicity, or age group, potentially restricting the applicability of the observed ALT effects to specific populations.

Conclusion

This study has established a link between ALT and GDM, enhancing our comprehension of their inherent connection and laying the groundwork for future targeted interventions. Further investigation is essential to ascertain the generalizability of these associations and their implications for clinical practice.

Author contribution. Lihua Yin: Conceptualization, methodology, software, visualization, writing — original draft, review and editing. Yifang Hu, Xiaoxia Hu: Software, Writing — Review and editing. Xiaolei Huang and Yingyuan

https://doi.org/10.1017/thg.2024.16 Published online by Cambridge University Press

Chen discussed and revised the manuscript. Yisheng Zhang: Project administration, funding acquisition. All the authors read and approved the final manuscript.

Financial support. This work was supported by the Science and Technology Project of Ningbo City (No. 2019C50091), the Medical and Health Science and Technology Project of Zhejiang Province (No. 2022KY297, No. 2021Y02), and the Key Technology Research and Development Project of Ningbo (No. 2023Z185).

Competing interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ajmera, V. H., Gunderson, E. P., VanWagner, L. B., Lewis, C. E., Carr, J. J., & Terrault, N. A. (2016). Gestational diabetes mellitus is strongly associated with non-alcoholic fatty liver disease. *The American Journal of Gastroenterology*, *111*, 658–664. https://doi.org/10.1038/ajg.2016.57

An, R., Ma, S., Zhang, N., Lin, H., Xiang, T., Chen, M., & Tan, H. (2022). AST-to-ALT ratio in the first trimester and the risk of gestational diabetes mellitus. *Frontiers in Endocrinology*, *13*, 1017448. https://doi.org/10.3389/fendo.2022.1017448

Bakhshimoghaddam, F., Razmi, H., Malihi, R., Mansoori, A., & Ahangarpour, A. (2023). The association between the dietary inflammatory index and gestational diabetes mellitus: A systematic review of observational studies. *Clinical Nutrition ESPEN*, *57*, 606–612. https://doi.org/10.1016/j.clnesp.2023.08.007

Chen, V. L., Hawa, F., Berinstein, J. A., Reddy, C. A., Kassab, I., Platt, K. D., Hsu, C.-Y., Steiner, C. A., Louissaint, J., Gunaratnam, N. T., & Sharma, P. (2021). Hepatic steatosis is associated with increased disease severity and liver injury in coronavirus disease-19. *Digestive Diseases and Sciences*, *66*, 3192–3198. https://doi.org/10.1007/s10620-020-06618-3

Chen, X., Zhang, S., Wu, X., Lei, Y., Lei, B., & Zhao, Z. (2024). Inflammatory cytokines and oral lichen planus: A Mendelian randomization study. *Frontiers in Immunology*, *15*, 1332317. https://doi.org/10.3389/fimmu.2024.1332317

Erdoğan, S., Ozdemir, O., Doğan, H. O., Sezer, S., Atalay, C. R., Meriç, F., Yilmaz, F. M., & Koca, Y. (2014). Liver enzymes, mean platelet volume, and red cell distribution width in gestational diabetes. *Turkish Journal of Medical Sciences*, *44*, 121–125. https://doi.org/10.3906/sag-1301-41

Gao, C., Sun, X., Lu, L., Liu, F., & Yuan, J. (2019). Prevalence of gestational diabetes mellitus in mainland China: A systematic review and meta-analysis. *Journal of Diabetes Investigation*, *10*, 154–162. https://doi.org/10.1111/jdi.12854

Hua, S., Qi, Q., Kizer, J. R., Williams-Nguyen, J., Strickler, H. D., Thyagarajan, B., Daviglus, M., Talavera, G. A., Schneiderman, N., Cotler, S. J., Cai, J., Kaplan, R., & Isasi, C. R. (2021). Association of liver enzymes with incident diabetes in US Hispanic/Latino adults. *Diabetic Medicine*, *38*, e14522. https://doi.org/10.1111/dme.14522

Huang, L.-L., Guo, D.-H., Xu, H.-Y., Tang, S.-T., Wang, X. X., Jin, Y.-P., & Wang, P. (2019). Association of liver enzymes levels with fasting plasma glucose levels in Southern China: A cross-sectional study. *BMJ Open*, *9*, e025524. https://doi.org/10.1136/bmjopen-2018-025524

Kondracki, A. J., Valente, M. J., Ibrahimou, B., & Bursac, Z. (2022). Risk of large for gestational age births at early, full and late term in relation to pre-pregnancy body mass index: Mediation by gestational diabetes status. *Paediatric and Perinatal Epidemiology*, *36*, 566–576. https://doi.org/10.1111/ppe.12809

Kong, M., Liu, C., Guo, Y., Gao, Q., Zhong, C., Zhou, X., Chen, R., Xiong, G., Yang, X., Hao, L., & Yang, N. (2018). Higher level of GGT during mid-pregnancy is associated with increased risk of gestational diabetes mellitus. *Clinical Endocrinology*, *88*, 700–705. https://doi.org/10.1111/cen.13558

Lee, K. W., Ching, S. M., Ramachandran, V., Yee, A., Hoo, F. K., Chia, Y. C., Wan Sulaiman, W. A., Suppiah, S., Mohamed, M. H., & Veettil, S. K. (2018). Prevalence and risk factors of gestational diabetes mellitus in Asia: A systematic review and meta-analysis. *BMC Pregnancy and Childbirth*, *18*, 494. https://doi.org/10.1186/s12884-018-2131-4

Lenoir-Wijnkoop, I., van der Beek, E. M., Garssen, J., Nuijten, M. J. C., & Uauy, R. D. (2015). Health economic modeling to assess short-term costs of maternal overweight, gestational diabetes, and related macrosomia — A pilot evaluation. *Frontiers in Pharmacology*, *6*, 103. https://doi.org/10.3389/fphar.2015.00103

McIntyre, H. D., Catalano, P., Zhang, C., Desoye, G., Mathiesen, E. R., & Damm, P. (2019). Gestational diabetes mellitus. *Nature Reviews Disease Primers*, *5*, 47. https://doi.org/10.1038/s41572-019-0098-8

Pazoki, R., Vujkovic, M., Elliott, J., Evangelou, E., Gill, D., Ghanbari, M., van der Most, P. J., Pinto, R. C., Wielscher, M., Farlik, M., Zuber, V., de Knegt, R. J., Snieder, H., Uitterlinden, A. G., Lifelines Cohort Study, Lynch, J. A., Jiang, X., Said, S., Kaplan, D. E., … VA Million Veteran Program. (2021). Genetic analysis in European ancestry individuals identifies 517 loci associated with liver enzymes. *Nature Communications*, *12*, 2579. https://doi.org/10.1038/s41467-021-22338-2

Peracchi, A., & Polverini, E. (2022). Using steady-state kinetics to quantitate substrate selectivity and specificity: A case study with two human transaminases. *Molecules*, *27*, 1398. https://doi.org/10.3390/molecules27041398

Sakurai, Y., Kubota, N., Yamauchi, T., & Kadowaki, T. (2021). Role of insulin resistance in MAFLD. *International Journal of Molecular Sciences*, *22*, 4156. https://doi.org/10.3390/ijms22084156

Song, S., Zhang, Y., Qiao, X., Duo, Y., Xu, J., Peng, Z., Zhang, J., Chen, Y., Nie, X., Sun, Q., Yang, X., Wang, A., Lu, Z., Sun, W., Fu, Y., Dong, Y., Yuan, T., & Zhao, W. (2022). ALT/AST as an independent risk factor of gestational diabetes mellitus compared with TG/HDL-C. *International Journal of General Medicine*, *15*, 115–121. https://doi.org/10.2147/IJGM.S332946

Wang, H., Li, N., Chivese, T., Werfalli, M., Sun, H., Yuen, L., Hoegfeldt, C. A., Elise Powe, C., Immanuel, J., Karuranga, S., Divakar, H., Levitt, Na., Li, C., Simmons, D., Yang, X., & IDF Diabetes Atlas Committee Hyperglycaemia in Pregnancy Special Interest Group. (2022). IDF diabetes atlas: Estimation of global and regional gestational diabetes mellitus prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's criteria. *Diabetes Research and Clinical Practice*, *183*, 109050. https://doi.org/10.1016/j.diabres.2021.109050

Zhao, W., Zhang, L., Zhang, G., Varkaneh, H. K., Rahmani, J., Clark, C., Ryan, P. M., Abdulazeem, H. M., & Salehisahlabadi, A. (2020). The association of plasma levels of liver enzymes and risk of gestational diabetes mellitus: A systematic review and dose-response meta-analysis of observational studies. *Acta Diabetologica*, *57*, 635–644. https://doi.org/10.1007/s00592-019-01458-8

Zheng, X., Ke, Y., Feng, A., Yuan, P., Zhou, J., Yu, Y., Wang, X., & Feng, W. (2016). The mechanism by which amentoflavone improves insulin resistance in hepG2 cells. *Molecules (Basel, Switzerland)*, *21*, 624. https://doi.org/10.3390/molecules21050624

https://doi.org/10.1017/thg.2024.16 Published online by Cambridge University Press