Research Article

Insights Journal of Obstetrics and Gynecology

Homocysteine and the Risk of pregnancy: A Mendelian Randomization Study

Heng Fan and Jian-hua Zhu

Department of Intensive Care Unit, The First Affiliated Hospital of Ningbo University, Ningbo, Zhejiang Province, P.R China

Correspondence author:
Heng Fan,
Department of Intensive Care Unit, The First Affiliated Hospital of Ningbo University, No.59 Liuting road, Ningbo, Zhejiang Province, China
E-mail: peterbenny@163.com

Received Date: 22 June 2024
Accepted Date: 03 July 2024
Published Date: 10 July 2024

Citation:
Heng Fan. Homocysteine and the Risk of pregnancy: A Mendelian Randomization Study. Insights Journal of Obstetrics and Gynecology 2024.

1. Abstract

The current research results on the relationship between Hcy and high-risk of pregnancy are inconsistent. We aimed to explore the potential causal relationship between homocysteine (Hcy) levels and high-risk factors for pregnancy. We used a two sample Mendelian randomization (MR) analysis method, and used Hcy related single nucleotide polymorphisms (SNPs) as instrumental variables (IV) to analyze the causal impact of Hcy on high-risk of pregnancy. The IV weighted, weighted median, and MR Egger analysis all showed no causal relationship between Hcy and high-risk of pregnancy (P>0.05). MR Egger analysis showed that the directional multiple effects in the results was unlikely to lead to bias (P = 0.94). There was no heterogeneity between the IV estimates based on individual variation. The leave one out analysis indicated that there were no individual SNPs that could drive the estimation results. The results of MR studies indicate that there is no causal relationship between Hcy and high-risk of pregnancy.

Keywords:
Homocysteine, Pregnancy, Mendelian, Female fertility, Pregnancy loss, Offspring birthweight

2. Introduction

Homocysteine (Hcy) is a cytotoxic sulfur-containing amino acid that is an intermediate product of methionine metabolism. Hyperhomocysteinemia (HHcy) is a symptom of abnormal Hcy metabolism in the body. The metabolism of Hcy in the body mainly depends on two ways, among which regulating the folate Hcy metabolic cycle is the most fully studied and effective way to maintain the normal level of Hcy [1]. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, catalyzing the production of 5-methyltetrahydrofolate from 5,10-methylenetetrahydrofolate, which is a necessary condition for the conversion of Hcy to methionine. The increase of Hcy concentration in the perinatal period can lead to birth defects such as fetal neural tube malformation, congenital heart disease, Down's syndrome, cleft lip and palate, adverse pregnancy outcomes such as abortion, fetal intrauterine growth restriction, low birth weight, preterm delivery, hypertension during pregnancy, diabetes during pregnancy and other diseases [3]. In 2020, the "Consensus of Multidisciplinary Experts on Reasonable Folate Supplementation in Chinese Clinical Practice" clearly proposed that women with HHcy should receive folic acid supplementation. At the same time, for pregnant women with TT genotype at the MTHFR 677 locus in the folic acid metabolism pathway, it is necessary to increase the supplementation dose or extend the pre pregnancy supplementation time as appropriate [4].

MTHFR is a key rate limiting enzyme in the folate Hcy cycle. However, nearly 60% of individuals in the Chinese population carry MTHFR mutant alleles, especially homozygous TT genotype individuals, whose enzyme activity decreases to about 30%, seriously affecting the conversion of folate activity [5]. There are research reports that the MTHFR677C>T gene polymorphism is closely related to the occurrence of HDP and can be used to evaluate the prognosis of HDP [6]. Elevated Hcy levels and the MTHFR gene 677TT genotype are both considered risk factors for HDP. However, due to the small sample size of previous studies and occasional negative results, the correlation between Hcy, MTHFR, and the onset of HDP is not fully recognized [7]. At the same time, considering the significant differences in gene frequency of the MTHFR gene among different races and regions. The current research results on the relationship between Hcy and high-risk of pregnancy are inconsistent [8, 9]. Due to the susceptibility of observational epidemiological studies to causal reversal and various biases, the two sample Mendelian Randomization (MR) study is an analytical method that uses genetic variation data that affects exposure factors to estimate the causal relationship between exposure factors and outcome indicators [10]. Its analysis strategy is to obtain evidence of the association between genetic risk factors and genetic outcomes from different genome-wide association studies (GWAS) datasets. This study utilized a large-scale GWAS dataset of Hcy and pregnancy for MR analysis of two samples, with the aim of exploring the relationship between Hcy and high-risk of pregnancy.

3. Methods

3.1 Database selection

We used data from the currently publicly available GWAS Catalog (https://www.ebi.ac.uk/gwas/), the rapid UK Biobank GWAS (http://www.nealelab.is/uk-biobank/), the Early Growth Genetics (EGG) Consortium (www.EGG-Consortium.org) and the International consortium on production genes (ReproGen) consortium (https://www.reprogen.org/data_download.html). The search keywords we used include: "Homocysteine", "Pregnancy", "Superhomocysteinemia" and "Perinal period". We set the search deadline to April 30, 2024. All participants have signed an informed consent form. This study was approved by the Human Ethics Committee of the First Affiliated Hospital of Ningbo University (No. 2023-R028-01).

3.2 Data extraction

We extracted 13 genes from the GWAS Catalog, of which 18 single nucleotide polymorphisms (SNPs) involved targets related to homocysteine, with a total of 36201 Europeanparticipants. In the latest genetic data released by the Rapid UK Biobank GWAS, we extracted data regarding "female fertility", "offspring birthweight", and"pregnancy loss"from a total of 194174 participants. In the genetic data provided by the EGG Consortium, we obtained raw data on "offspring birthweight" from a total of 155202 participants. Finally, we extracted relevant raw data on "female fertility" from the ReproGen Consortium.

3.3 Preliminary analysis of data

We obtained SNPs closely related to Hcy concentration through the GWAS Catalog to construct instrumental variables (IV). The GWAS Catalog focused on Europeans and could provide meta-analysis data for scientific research and follow-up. The potential IV that the alliance could provide for effective prediction of Hcy includes 18 SNPs.The effect size and standard error (SE) data of each genetic variation on Hcy concentration in the summary data were also obtained from the GWAS study published previously in the GWAS Catalog.

3.4 MR analysis

We used the "Two Sample MR" from R (version 4.0.2) for analysis, and $P<0.05$ was considered statistically significant. We used aggregated data from four different databases as part of the IV assessment of the causal relationship between Hcy and pregnancy risk, and used three MR analysis methods [inverse variance weighted (IVW) , weighted median (WM) , and MR Egger] to analyze whether there was a causal relationship between Hcy and pregnancy risk. The IVW method and WM method were mainly used for MR analysis, while the MR Egger was mainly used for statistical testing of potential pleiotropic effects. Due to the random effects of IVW allowing each SNP to produce a different average effect, we used the random effects IVW analysis method to perform regression analysis on the correlation between Hcy and SNPs in pregnancy risk [11]. To ensure that the selected outcome variable IV did not affect pregnancy risk through biological pathways other than Hcy, we used MR Egger intercept testing to evaluate the pleiotropic relationship between IV and other potential confounding factors [12]. For each genetic variation, $P = 5\times10\text{-}8$ corresponded to $F > 30$ [13].

3.5 Heterogeneity and Sensitivity Analysis

We used Cochran's Q-test and funnel plot to evaluate the heterogeneity between SNPs [14]. To evaluate the unknown pleiotropy, we used the following analysis methods: (1) Sensitivity analysis used "leave one out" to explore the possibility of individual SNPs driving this causal association; (2) The MR Egger intercept test evaluated the pleiotropic association between gene variations and other potential confounding factors. After excluding known pleiotropic SNPs, we repeated the aforementioned MR analysis. We searched for possible pleiotropic effects of SNPs on the Phenoscanner website (http://www.phenoscanner.medschl. Cam. ac.uk/) [15].

4. Results

4.1 Mendelian randomization analysis results

We found that 18 SNPs could effectively predict Hcy at the whole genome level, and they could be used as potential IVs. Correlation data on Hcy with female fertility and offspring birthweight had been published in the GWAS Catalog, the EGG, and the ReproGen consortium. Among them, rs1801133 (MTHFR) was correlated with an increase in menopausal age ($\beta = 0.62$, 95%CI: 0.34 ~ 0.90, $P = 0.02$) and a decrease in offspring birthweight ($\beta = -0.07$, 95%CI: -0.10 ~ -0.03, $P = 0.01$), and Hcy was also associated with a decrease in offspring birthweight ($\beta = -0.04$, 95%CI: -0.07 ~ -0.01, $P = 0.02$). However, after data integration, there was no significant correlation between Hcy and any outcomes at high risk of pregnancy, including female fertility, pregnancy loss, and offspring birthweight.

4.2 Multiple methods analysis Results

We used IVW, WM, and MR Egger analysis methods for in-depth analysis, and our results suggested that there was no causal relationship between Hcy and pregnancy risk ($P> 0.05$). MR Egger analysis showed that the directional multiple effects in the results did not cause bias ($P = 0.94$).

4.3 Heterogeneity and Sensitivity Analysis Results

We conducted heterogeneity and sensitivity analysis on the data results. Our results suggested that there were significant differences in heterogeneity among individuals based on individual variation, but no single SNPs could affect the estimation of results. Our sensitivity analysis results were consistent with the IVW analysis results of 18 SNPs. Leave one out analysis indicated that an increase in Hcy concentration determined by rs1801133 was associated with high-risk factors for pregnancy, including increased menstrual suspension and decreased birth weight of offspring.

5. Discussion

Hcy accumulated in the body can reduce the methylation level of the

body, damage vascular endothelial cells, and is a risk factor leading to cardiovascular and nervous system disease diseases [16, 17]. Hcy can activate the generation of reactive oxygen species and oxygen free radicals produced by body oxidation, induce apoptosis of smooth muscle cells and umbilical vein endothelial cells, promote thrombosis, induce vasospasm, and cause hypertension [18]. Recent studies have found that there is a mild increase in Hcy in the blood of pregnant women with HDP, and the serum Hcy concentration of pregnant women is closely related to maternal and infant health [19-21]. This can provide reference for pre pregnancy health care and eugenics, and serve as an indicator for evaluating RBCF levels in pregnant women, as well as a predictive indicator for pregnancy related diseases and fetal birth defects. The important metabolic pathway of Hcy in the body is involved in the folate methionine cycle, which requires the participation of vitamins B12 and N5, N10-MTHFR. Folic acid can be successfully converted to 5-methyltetrahydrofolate through MTHFR normal metabolic enzymes, achieving regulatory effects on normal levels of Hcy [22]. The serum Hcy concentration is influenced by various factors such as age, gender, genetics, diet, medication, and disease status [23]. Pregnancy is a special physiological stage, and the sensitivity of Hcy concentration in pregnant women to vascular damage significantly increases.

In recent years, with the continuous application of molecular biology technology in clinical practice and the continuous progress of human genome research, exploring the genetic genes related to pregnancy etiology has become a research hotspot. As early as 1997, Japanese scholar Sohda et al. [24] found a close relationship between the MTHFR 677C>T gene polymorphism and the onset of preeclampsia in 67 HDP patients. Women with TT genotype have a three fold higher risk of developing HDP compared to women without TT genotype. Chedraui et al. [25] conducted MTHFR gene polymorphism analysis on 100 pregnant women, and the results showed that mutations in the 677C>T gene polymorphism and TT genotype were three times more common in the HDP placenta than in the control group placenta. However, due to the small sample size of individuals included in the study, there are significant racial differences in the MTHFR gene. The results of a meta-analysis by Buurma et al. [26] showed that the correlation between the 677C>T gene polymorphism and HDP was not difference, leading to significant controversy. In order to further determine whether there is a causal relationship between Hcy and high-risk factors for pregnancy, we used three different analysis methods for MR analysis. We found that the results obtained by the three methods were consistent, and they supported the causal relationship between Hcy and high-risk factors for pregnancy. After excluding potential pleiotropic SNPs, the results of the three MR analysis methods remained consistent. The results of MR analysis are easily influenced by pleiotropy [27]. Multiplicity refers to the possibility that genetic variation as type IV may be associated with multiple phenotypes, leading to bias in the results obtained from MR analysis [28]. Therefore, we used sensitivity analysis to further verify the conclusions. Firstly, we used 'leave one out' to analyze the likelihood of individual SNPs driving causal relationship results. Secondly, we used the weighted median method to decrease the impact of pleiotropy [29]. Furthermore, we used MR Egger method to evaluate the causal effects of exposure on outcomes. The calculation results of the weighted median method in this study are consistent with those of the IVW method. Our MR analysishas certain limitations:(1) We did not obtain more SNPs as IV, resulting in insufficient explanation of causal relationships in this study. (2) Potential confounding factors may lead to heterogeneity in the results of this study. (3) The population included in the study is mainly European, and due to the possibility that the causal analysis results may also be influenced by race, we need to further conduct similar MR studies in other races to verify this conclusion. (4) We are unable to obtain data for each patient, making it difficult to conduct further subgroup analysis in this study.

6. Conclusion

Our MR study showed no significant correlation between Hcy and high risk of pregnancy. However, the TT gene and HHcy have a synergistic effect on high-risk pregnancies, and special attention needs to be paid to this genotype. Timely identification of MTHFR genotypes will help in early assessment and effective prevention of high-risk pregnancies.

Acknowledgement

This research was supported by the Project of Ningbo Key R&D Plan and "Unveiling and Leading" under Grant No.2023Z174, Ningbo Clinical Research Center for Emergency and Critical Diseasesunder Grant No.2024L003, and the Project of Ningbo Leading Medical & Health Discipline under Grant No.2022-B04.

References

1. Mascarenhas M, Habeebullah S, Sridhar MG. Revisiting the role of first trimester homocysteine as an index of maternal and fetal outcome. J Pregnancy. 2014;2014:123024.
2. Tallova J, Tomandl J, Bicikova M, Hill M. Changes of plasma total homocysteine levels during the menstrual cycle. Eur J Clin Invest. 1999;29(12):1041-1044.
3. Dodds L, Fell DB, Dooley KC, Armson BA, Allen AC, Nassar BA, et al. Effect of homocysteine concentration in early pregnancy on gestational hypertensive disorders and other pregnancy outcomes. Clin Chem. 2008;54(2):326-334.
4. Hak AE, Polderman KH, Westendorp IC, Jakobs C, Hofman A, Witteman JC, et al. Increased plasma homocysteine after menopause. Atherosclerosis. 2000;149(1):163-168.
5. Hartwig FP, Davies NM, Hemani G, Davey Smith G. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. Int J Epidemiol. 2016;45(6):1717-1726.
6. Cueto HT, Riis AH, Hatch EE, Wise LA, Rothman KJ, Sørensen HT, et al. Folic acid supplement use and menstrual cycle characteristics: a cross-sectional study of Danish pregnancy planners. Ann Epidemiol.

2015;25(10):723-729.e1.

7. Cao Y, Xu J, Zhang Z, Huang X, Zhang A, Wang J, et al. Association study between methylenetetrahydrofolate reductase polymorphisms and unexplained recurrent pregnancy loss: a meta-analysis. Gene. 2013;514(2):105-111.
8. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. Nat Rev Cardiol. 2017;14(10):577-590.
9. Wu X, Zhao L, Zhu H, He D, Tang W, Luo Y. Association between the MTHFR C677T polymorphism and recurrent pregnancy loss: a meta-analysis. Genet Test Mol Biomarkers. 2012;16(7):806-811.
10. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. Am J Clin Nutr. 2016;103(4):965-978.
11. Dastani Z, Johnson T, Kronenberg F, Nelson CP, Assimes TL, März W; CARDIoGRAM Consortium; ADIPOGen Consortium; Richards JB. The shared allelic architecture of adiponectin levels and coronary artery disease. Atherosclerosis. 2013;229(1):145-148.
12. Bowden J, Del Greco M F, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. Stat Med. 2017;36(11):1783-1802.
13. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015;44(2):512-525.
14. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-Egger method. Eur J Epidemiol. 2017;32(5):377-389.
15. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 2016;40(4):304-314.
16. Larsson SC, Traylor M, Markus HS. Homocysteine and small vessel stroke: A mendelian randomization analysis. Ann Neurol. 2019;85(4):495-501.
17. Warrington NM, Beaumont RN, Horikoshi M, Day FR, Helgeland Ø, Laurin C, et al. Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. Nat Genet. 2019;51(5):804-814.
18. Yajnik CS, Chandak GR, Joglekar C, Katre P, Bhat DS, Singh SN, et al. Maternal homocysteine in pregnancy and offspring birthweight: epidemiological associations and Mendelian randomization analysis. Int J Epidemiol. 2014;43(5):1487-1497.
19. Van Meurs JB, Pare G, Schwartz SM, Hazra A, Tanaka T, Vermeulen SH, et al. Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. Am J Clin Nutr. 2013;98(3):668-676.
20. Lee HA, Park EA, Cho SJ, Kim HS, Kim YJ, Lee H, et al. Mendelian randomization analysis of the effect of maternal homocysteine during pregnancy, as represented by maternal MTHFR C677T genotype, on birth weight. J Epidemiol. 2013;23(5):371-375.
21. Davey Smith G, Paternoster L, Relton C. When Will Mendelian Randomization Become Relevant for Clinical Practice and Public Health? JAMA. 2017;317(6):589-591.
22. Borges MC, Hartwig FP, Oliveira IO, Horta BL. Is there a causal role for homocysteine concentration in blood pressure? A Mendelian randomization study. Am J Clin Nutr. 2016;103(1):39-49.
23. Benn M, Nordestgaard BG. From genome-wide association studies to Mendelian randomization: novel opportunities for understanding cardiovascular disease causality, pathogenesis, prevention, and treatment. Cardiovasc Res. 2018;114(9):1192-1208.
24. Sohda S, Arinami T, Hamada H, Yamada N, Hamaguchi H, Kubo T. Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J Med Genet. 1997;34(6):525-526.
25. Chedraui P, Andrade ME, Salazar-Pousada D, Escobar GS, Hidalgo L, Ramirez C, et al. Polymorphisms of the methylenetetrahydrofolate reductase gene (C677T and A1298C) in the placenta of pregnancies complicated with preeclampsia. Gynecol Endocrinol. 2015;31(7):569-572.
26. Buurma AJ, Turner RJ, Driessen JH, Mooyaart AL, Schoones JW, Bruijn JA, et al. Genetic variants in pre-eclampsia: a meta-analysis. Hum Reprod Update. 2013;19(3):289-303.
27. Chaudhry SH, Taljaard M, MacFarlane AJ, Gaudet LM, Smith GN, Rodger M, et al. The role of maternal homocysteine concentration in placenta-mediated complications: findings from the Ottawa and Kingston birth cohort. BMC Pregnancy Childbirth. 2019;19(1):75.
28. Timmermans S, Jaddoe VW, Hofman A, Steegers-Theunissen RP, Steegers EA. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. Br J Nutr. 2009;102(5):777-785.
29. Sleiman PM, Grant SF. Mendelian randomization in the era of genomewide association studies. Clin Chem. 2010;56(5):723-728.