Iran J Public Health, Vol. 54, No.2, Feb 2025, pp.361-369

Original Article

Exploring the Association between Childhood Asthma and Abnormal Spermatozoa: An Analysis Using Mendelian Randomization

Qian Zhang [1,2#], Yao Ge [1#], Yuan Chen [1#], Yun Zhang [1], Xiaoyan Xie [1], Jian Yu [1], Yunlei Bao [3], *Feng Jiang [3], *Chuyan Wu [1]

1. *Department of Rehabilitation Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China*
2. *Department of Child Health, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China*
3. *Department of Neonatology, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China*

[#]These authors contributed equally to this work

***Corresponding Authors:** Emails: chuyan_w@hotmail.com, dxyjiang@163.com

(Received 20 Aug 2024; accepted 11 Nov 2024)

Abstract

Background: Childhood asthma ranks among the prevailing chronic respiratory conditions affecting a significant number of individuals. The long-term hypoxic state and chronic inflammatory state caused by asthma could be linked with spermatozoa apoptosis. However, the correlation between childhood asthma and abnormal spermatozoa is currently unknown.

Methods: In our study, the method of two-sample Mendelian randomization (2SMR) was used by searching an appropriate European population genome-wide association studies (GWAS) database of childhood asthma and abnormal spermatozoa from the Ieu Open GWAS Project database. Sixteen related single-nucleotide polymorphisms (SNPs) were screened as instrumental variables (IV). Subsequently, we employ various statistical methods including inverse variance weighting (IVW), weighted median method (WME), MR-Egger regression, Simple mode, and Weighted mode to investigate the causal link between childhood asthma and the development of abnormal spermatozoa.

Results: Based on IVW results, childhood asthma is not an independent risk factor for abnormal sperm formation (P=0.14). Other statistical models such as WME, MR-Egger regression, Simple mode, and weighted mode also showed the same results. Leave-one-out sensitivity analysis and heterogeneity test were conducted and no horizontal pleiotropy was found.

Conclusion: There was no causal relationship between childhood asthma and abnormal spermatozoa formation at the genetic level.

Keywords: Asthma; Spermatozoa; Mendelian randomization; Genome-wide association study

Introduction

Asthma is a common chronic inflammatory airway condition that presents with varying degrees of recurrent attacks with reversible airflow restriction (1). Currently, approximately 300 million people worldwide are affected by asthma, and its incidence is increasing annually. Asthma not only affects normal respiratory function but also affects other physical systems, such as the genital system, recirculating system, and so on. Among them, the formation of sperm could be associated

with the pathophysiological mechanism of asthma. As an essential element of the male genital system, sperm quality is critical to the reproduction of offspring. The abnormal spermatozoa not only prolong the time to natural conception but also increase the risk of abortion (2).

Previous studies have attempted to investigate the correlation between asthma and abnormal sperm. Asthma leads to abnormal sperm through inducing hypoxia in the body. Asthma patients frequently show signs of chronic hypoxia, which increases the expression of IL-6 (3). IL-6 hurts the male reproductive system by reducing sperm penetration ability, impairing antioxidant activity, and causing chronic inflammation (4). Additionally, chronic inflammation can also induce the production of reactive oxygen species (ROS) (5, 6). Excessive production of ROS may lead to the degradation of DNA integrity in sperm nuclei and mitochondria, triggering various pathways that accelerate sperm apoptosis (6). However, studies conducted to date have not fully accounted for potential confounding factors such as lifestyle changes, exposure to chemical pesticides, and air pollution, which can independently affect sperm vitality. Therefore, the assertion of a direct correlation between childhood asthma and abnormal sperm formation remains influenced by these common factors (7, 8).

Whether there is a causal association between childhood asthma and abnormal sperm formation requires further study. Conventional retrospective studies, owing to their reliance on randomized controlled trial designs, often encounter challenges related to reverse causality and confounding factors. However, these issues can be effectively addressed through the implementation of Mendelian randomization (MR) analysis. MR analysis is a technique that utilizes genetic variation as an instrumental variable (IV) to evaluate the causal association between exposure factors and outcomes (9-11). The definition of two-sample MR (2SMR) is to measure the exposure and outcome data of different samples. We employed 2SMR to investigate if there exists a causal link between childhood asthma and the development of anomalous spermatozoa.

Materials and Methods

Study design

Utilizing the most extensive publicly accessible GWAS datasets, we conducted a two-sample MR study to examine the correlation between childhood asthma and the likelihood of developing anomalous spermatozoa. For the analysis to be conducted using 2SMR, genetic variants must satisfy the following conditions: a) Exhibit a robust correlation with childhood asthma, b) unrelated to any confounding factors of childhood asthma and abnormal spermatozoa, and c) exclusively influence the occurrence of abnormal spermatozoa through childhood asthma. (Fig. 1) (12, 13). To determine the most suitable genome-wide association study (GWAS) database, an extensive search was conducted within the Ieu Open GWAS project database (https://gwas.mrcieu.ac.uk/) to retrieve data on both Exposure and Outcome. For database selection, we used the GWAS database with a larger sample size to be able to include more single-nucleotide polymorphisms (SNPs) to ensure a higher credibility of our study. Moreover, to avoid stratification effects due to ancestry and population, both data sets were derived from European populations.

We obtained two sets of data with larger sample sizes through the Ieu Open GWAS project database. Childhood asthma-associated SNPs were obtained from the GWAS-ID "ukb-d-ASTHMA_CHILD" in the database, including 359,201 childhood asthma patients (<16 yr old) and 1,993 healthy controls. The GWAS-ID "finn-b-R18_ABNORMAL_ SPERMATOZ" within the database yielded genetic association data related to abnormal spermatozoa.

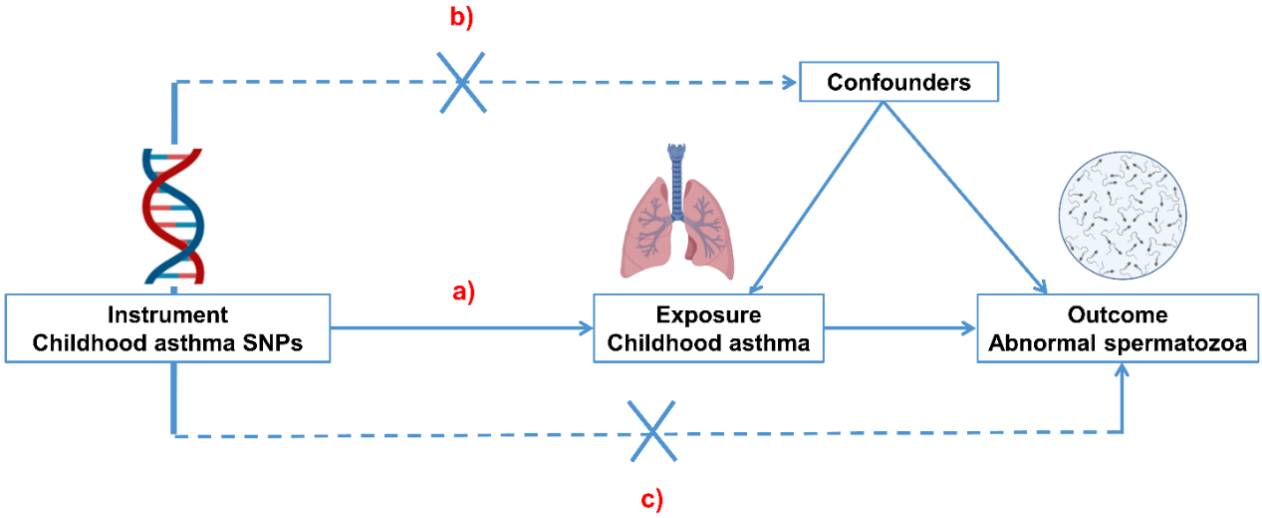

Fig. 1: Schematic Representation of 2SMR Study of the Association between Childhood Asthma and Abnormal Spermatozoa. The 2SMR analysis allows us to estimate the hypothesis that childhood asthma, as the exposure, is causally linked to the occurrence of abnormal spermatozoa, as the outcomes. To meet the necessary criteria, the genetic variants must fulfill the following conditions: a) exhibit a robust association with childhood asthma, b) have no connection to any confounding factors related to childhood asthma and abnormal spermatozoa, and c) exclusively influence the occurrence of abnormal spermatozoa through childhood asthma

This dataset comprised 915 individuals diagnosed with abnormal spermatozoa and 209,006 healthy controls, with both sets of data originating from European populations. To satisfy the condition: "the genetic variants used in the 2SMR analysis must be strongly associated with childhood asthma", SNPs significantly associated with exposure factors were selected in the whole of the genome threshold of P<5e-06. In addition, the strength of individual SNPs was evaluated by using the F statistic. When F was less than 10, the estimate of the causal effect could be heavily biased. As a result, to avoid possible weak instrumental variable bias, the corresponding SNPs were excluded (14).

Statistical analyses

Linkage disequilibrium assessment

To avoid bias in the analysis due to linkage disequilibrium (LD) among SNPs, the LD coefficient r^2 and the width of the LD region were calculated. When the r^2 was less than 0.001 and the region was 10000 kb, the relevant SNPs could be convinced to be independent of each other (15).

2SMR analysis

The primary approach employed in this study to estimate the causal relationship was inverse variance weighting (IVW) (16). The IVW method is a powerful tool utilized for identifying causal relationships, leveraging the instrumental variable nature of all genes. IVW is a more desirable estimation method in 2SMR analysis. In the context of 2SMR analysis, the IVW approach necessitates that the gene variants solely exert their influence on the studied outcomes through the specific exposures examined. While this study partially accounted for the influence of known confounding SNPs, numerous unidentified confounding factors could potentially introduce genetic polymorphisms that bias the estimation of effect values. As such, four supplementary techniques were employed to assess the reliability and consistency of the findings, including MR-Egger regression (17), weighted median method (WME)(18), Simple mode(19), and weighted mode (20). If the results obtained from the above five MR models are similar, then we can conclude that the causal link between childhood asthma and abnormal spermatozoa formation was credible.

Heterogeneity test

Because of the differences in experimental conditions, analytical platforms, enrolled populations, and SNPs, 2SMR analysis methods may still have heterogeneity that may bias the estimation of causal effects. Hence, Cochran's Q statistic and its corresponding *P*-value were utilized to assess the heterogeneity of the IVW analysis and MR-Egger regression (21). If the *P*-value exceeded 0.05, indicating the absence of significant heterogeneity among the included instrumental variables, the impact of heterogeneity on the estimation of causal effects was deemed negligible.

Pleiotropy test

Due to a fundamental assumption in MR analysis that instrumental variables affect the outcome solely through exposure, it is essential to validate the genetic pleiotropy underlying the association between exposure and outcome. To assess the bias introduced by genetic pleiotropy, MR-Egger regression analysis was utilized, with the regression intercept serving as an indicator of pleiotropy magnitude. A smaller intercept value indicates a lesser degree of genetic pleiotropy. In this study, the presence of genetic pleiotropy in the analysis was evaluated by examining the *P*-value of the genetic pleiotropy test. A *P*-value greater than 0.05 suggests weak evidence for the existence of genetic pleiotropy in the causal analysis, with negligible effects. Hence, we employed the *P*-value as a means to evaluate the impact of genetic pleiotropy in the causal analysis.

Sensitivity Analysis

Building upon this groundwork, along with the aforementioned four approaches (MR-Egger regression, WME, Simple mode, and Weighted mode), we employed the leave-one-out method as a sensitivity analysis to validate the reliability and stability of the model. We excluded each single nucleotide polymorphic site one by one and then evaluated the role of each monomorphic site in causal inference by analyzing the combined effect of the remaining polymorphic sites.

Results

SNP Selection and Validation

SNPs associated with childhood asthma were screened in the Ieu Open GWAS childhood asthma database according to the SNP screening criteria of *P*<5e-06 and linkage disequilibrium <0.001. Twenty SNPs were associated with childhood asthma. Since rs17612712, rs2066361, rs113498532, and rs11654153 were not included in the database of abnormal sperm, 16 other SNPs were included in the final study, and the F-statistics of them were all larger than 10. We searched the genes to which each SNP belonged and the data related to the genetic variation of SNPs were shown in Table 1, including the coding of SNPs, the frequency of effector genes, the standard error of effect values, the *P*-values for childhood asthma and the abnormal sperm.

MR assessment of the effect of childhood asthma on abnormal spermatozoa

To investigate the impact of childhood asthma on the risk of abnormal spermatozoa, our study employed five different 2SMR methods. The primary method, IVW, was utilized to evaluate the causal association between childhood asthma and abnormal spermatozoa. The consistent results of the five analyses, as depicted in Table 2 and Fig. 2, indicated a negligible causal relationship between childhood asthma and the formation of abnormal spermatozoa within the European population.

Table 1: Characteristics of SNPs for the analysis of childhood asthma in response to abnormal spermatozoa

Chr	SNP	EA	OA	EAF	Beta	SE	*P*-value
1	rs114820795	G	T	0.042617	0.00202	0.000431	2.77E-06
3	rs73148319	A	G	0.012559	0.003848	0.000806	1.80E-06
3	rs140066178	T	C	0.056954	0.001761	0.000379	3.45E-06
4	rs192613850	A	G	0.013896	0.003574	0.000779	4.50E-06
5	rs12055232	T	C	0.1589	0.001143	0.00024	1.83E-06
5	rs6894249	G	A	0.386603	0.000949	0.000179	1.17E-07
6	rs3129871	C	A	0.640946	0.001096	0.000182	1.68E-09
9	rs79872606	G	A	0.023078	0.00281	0.00058	1.28E-06
9	rs78327215	G	A	0.027305	0.002647	0.00055	1.48E-06
10	rs1444782	A	G	0.423243	-0.00102	0.000176	7.94E-09
12	rs4432080	G	T	0.342541	0.000854	0.000184	3.40E-06
13	rs72667528	T	C	0.018214	0.003292	0.000695	2.20E-06
18	rs34886811	T	C	0.1574	-0.00113	0.00024	2.50E-06
21	rs415597	A	G	0.322681	0.000943	0.000187	4.41E-07
22	rs16992028	C	T	0.0244	0.002652	0.00058	4.88E-06
22	rs137992872	A	G	0.062067	0.001762	0.000362	1.14E-06

Chr: chromosome; SNP: single nucleotide polymorphism; EA: Effect Allele; OA: Other Allele; EAF: effect allele frequency; SE: standard error

Table 2: 2SMR analysis of the association between childhood asthma and the risk of abnormal spermatozoa

Methods	Beta	SE	*P*-value
MR Egger	-18.074930	39.14	0.65
Weighted median	17.437266	21.46	0.42
Inverse variance weighted	24.820977	16.79	0.14
Simple mode	-2.515833	34.79	0.94
Weighted mode	16.897258	31.55	0.60

SE: standard error

Assessment of MR assumptions

To assess the robustness of the findings, a leave-one-out method was employed for sensitivity analysis in this study. No individual SNPs were identified to have a substantial impact on the overall effect of childhood asthma on the formation of abnormal spermatozoa (Fig. 3). Additionally, through heterogeneity analysis, no statistically significant heterogeneity was observed in any of the five-2SMR analyses (Table 3). Moreover, the results of the pleiotropy test showed that the intercept of the MR-Egger regression was 0.06, P=0.25, indicating that the results of the causal analysis are not affected by horizontal pleiotropy. The causality estimates between childhood asthma and abnormal spermatozoa formation were not affected by confounding factors and confirmed the robustness of the results.

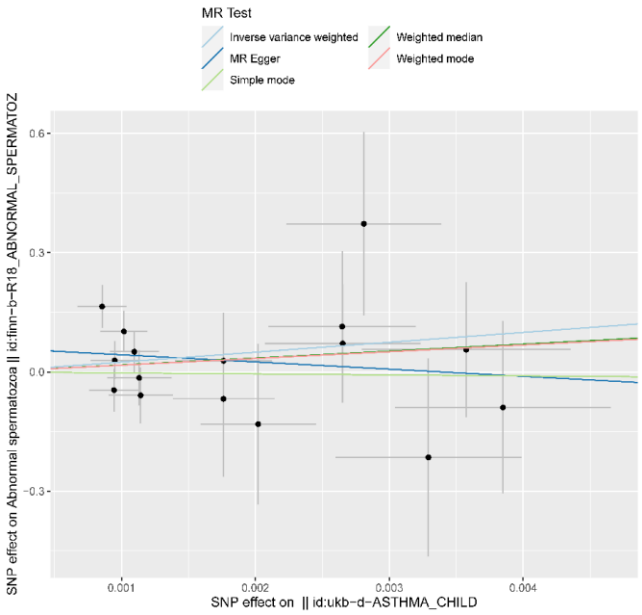

Fig. 2: The scatterplot depicts the distribution of individual rate estimates, displaying the relationship between childhood asthma and abnormal spermatozoa as an outcome. Scatterplots of five different 2SMR methods to show causality

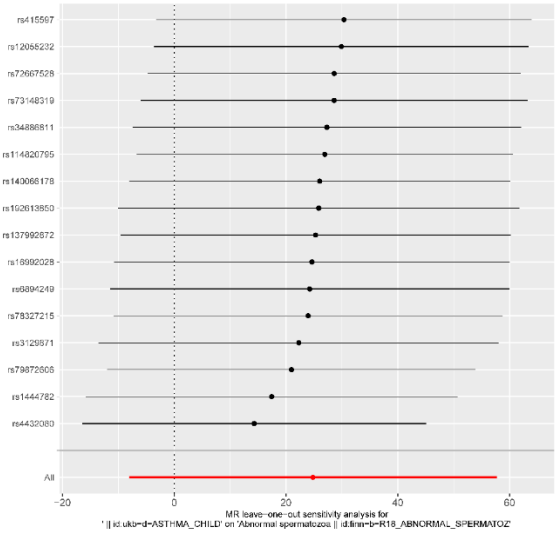

Fig. 3: Sensitivity analysis of the leave-one-out method for childhood asthma on abnormal spermatozo. Each black dot indicates the causal effect of IVW after removing the specific SNP on the left. The red line indicates the causal analysis of the total effect including all SNPs performed by IVW

Table 3: Heterogeneity statistics of two-sample Mendelian randomization analysis

Methods	Q value	Degrees of freedom	*P*-value
MR Egger	16.50	14	0.28
Inverse variance weighted	18.22	15	0.25

Discussion

In this study, we analyzed the correlation between childhood asthma and abnormal sperm by using a 2SMR study with a large sample size GWAS database. Our study revealed no evidence of a causal relationship between childhood asthma and the formation of abnormal spermatozoa in the European population.

Numerous human and animal studies have pointed out the correlation of asthma with abnormal male reproductive function. Asthma could promote human sperm apoptosis by up regulating the expression of the HIF-1 signaling pathway and eventually induce infertility. Another animal study also reported that the increased expression of the HIF-1 pathway, induced by a hypoxic response, could result in inflammatory cell infiltration and further conduce the epithelium of the spermatic cord to thin and the decline of sperm count (22, 23). Hypobaric hypoxia resulted in the deterioration of spermatocytes within the seminiferous tubules, distortion of the germinal epithelium, and folding of the basement membrane. These observed morphological alterations indicate that hypoxia hurts spermatogenesis. Meanwhile, hypoxia could lead to a significant increase in spermatocyte apoptosis, which results in a decrease in sperm production (24).

Hypoxia caused by asthma can result in endocrine dysfunction and have an impact on the vitality of sperm (25). Testosterone plays a crucial role in spermatogenesis. It specifically binds to the androgen receptor, maintains the spermatogenic process, and inhibits germinal apoptosis. Male rats showed a declined level of testosterone and sexual dysfunction after prolonged intermittent hypoxic exposure (26). Male subjects living at high altitudes would represent significantly lower testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) than subjects at sea level. Considering people who lived at high altitudes might tolerate a long-term hypoxic state, the hypoxia could suppress the hypothalamic-pituitary-testicular axis, leading to the decline of circulating testosterone (27). Intermittent hypoxia could improve the synthesis of testosterone in mesenchymal cells through the up-regulation of 17β-hydroxysteroid dehydrogenase, adenylyl cyclase, cyclic adenosine monophosphate (CAMP), and voltage-gated L-type calcium channels (28). Acute hypoxia could stimulate autophagy in rat mesenchymal cells and promote the secretion of testosterone by decomposing total cholesterol and intracellular lipid droplets (29).

In the above studies of asthma and abnormal spermatogenesis, the only parameters used to observe spermatozoa were density and motility. There was no clear description of sperm morphology or sperm motility parameters. A large cross-sectional study showed that although men with asthma had a later onset of puberty than non-asthmatic men had, there were few statistically significant differences in reproductive hormone levels and semen quality (30). Among men with asthma, there were no significant differences observed in terms of semen volume, sperm motility, and morphology when compared to men without asthma. However, lower levels of sperm concentration and total sperm count were noted in men with asthma. In the adjusted regression analysis, these differences remained statistically significant in total sperm count. In contrast, these differences were not statistically significant in sperm concentration. This might be consistent with our findings.

We have to consider that the inconsistencies in these studies may be attributed to confounding factors. At this point, variation reflects the main advantage of MR, that is, the elimination of confounding factors. There are some limitations to this study. First, this study only used data on European populations, which is not representative of all ethnic groups and regions; the sample size of the abnormal sperm we used may not be large enough to influence the model. Nevertheless, this constraint presents an important avenue for future research to elucidate further the causal asso-

ciation between childhood asthma and the development of abnormal spermatozoa.

Conclusion

Our two-sample Mendelian randomization analysis revealed no evidence of a causal relationship between childhood asthma and abnormal spermatozoa at the genetic level. However, further investigation utilizing larger GWAS databases and comprehensive studies is necessary to assess the causal link between childhood asthma and abnormal spermatozoa.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Availability of data and materials

The datasets analyzed during the current study are available in the European Bioinformatics Institute database (https://www.ebi.ac.uk/gwas/) and the FinnGen repository (https://www.finngen.fi/en).

Acknowledgements

This study was funded by the Jiangsu Province Hospital (the First Affiliated Hospital with Nanjing Medical University) Clinical Capacity Enhancement Project (Grant No. JSPH-MC-2022-19) and Medical Innovation Team (Grant No. CXZX202222). The funding bodies had no role in the study design, data collection, analysis, and interpretation of data.

Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Chakaya J, Ait-Khaled N (2022). Global Asthma Report 2022: A wake-up call to enhance care and treatment for asthma globally. *Int J Tuberc Lung Dis*, 26 (11): 999-1000.
2. Gill K, Jakubik J, Rosiak-Gill A, et al (2019). Utility and Predictive Value of Human Standard Semen Parameters and Sperm DNA Dispersion for Fertility Potential. *Int J Environ Res Public Health*, 16 (11): 2004.
3. Feng J, Tang Y, Yang Z, et al (2022). Explore the Effect of Asthma Regulating HIF-1 Pathway on Sperm Quality Based on Rat Model. *Biomed Res Int*, 2022:4194685.
4. Potashnik H, Elhija MA, Lunenfeld E, et al (2005). Interleukin-6 expression during normal maturation of the mouse testis. *Eur Cytokine Netw*, 16 (2): 161-165.
5. Aitken RJ, Gibb Z, Baker MA, et al (2016). Causes and consequences of oxidative stress in spermatozoa. *Reprod Fertil Dev*, 28 (1-2): 1-10.
6. Aitken RJ (2017). Reactive oxygen species as mediators of sperm capacitation and pathological damage. *Mol Reprod Dev*, 84 (10): 1039-1052.
7. Somers CM (2011). Ambient air pollution exposure and damage to male gametes: human studies and in situ 'sentinel' animal experiments. *Syst Biol Reprod Med*, 57 (1-2): 63-71.
8. Collins GG, Rossi BV (2015). The impact of lifestyle modifications, diet, and vitamin supplementation on natural fertility. *Fertil Res Pract*, 1: 11.
9. Burgess S, Butterworth A, Thompson SG (2013). Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol*, 37 (7): 658-665.
10. Birney E (2022). Mendelian Randomization. *Cold Spring Harb Perspect Med*, 12 (4): a041302.
11. Burgess S, Davey Smith G, Davies NM, et al (2023). Guidelines for performing Mendelian randomization investigations: update for summer 2023. *Wellcome Open Res*, 4: 186.
12. Bowden J, Davey Smith G, Burgess S (2015). Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*, 44 (2): 512-525.

13. Zheng J, Baird D, Borges MC, et al (2017). Recent Developments in Mendelian Randomization Studies. *Curr Epidemiol Rep*, 4 (4): 330-345.
14. Burgess S, Small DS, Thompson SG (2017). A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res*, 26 (5): 2333-2355.
15. Paternoster L, Tilling K, Davey Smith G (2017). Genetic epidemiology and Mendelian randomization for informing disease therapeutics: Conceptual and methodological challenges. *PLoS Genet*, 13 (10): e1006944.
16. Mounier N, Kutalik Z (2023). Bias correction for inverse variance weighting Mendelian randomization. *Genet Epidemiology*, 47 (4): 314-331.
17. Bowden J, Del Greco MF, Minelli C, et al (2016). Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. *Int J Epidemiol*, 45 (6): 1961-1974.
18. Bowden J, Davey Smith G, Haycock PC, et al (2016). Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol*, 40 (4): 304-314.
19. Hartwig FP, Davey Smith G, Bowden J (2017). Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol*, 46 (6): 1985-1998.
20. Emdin CA, Khera AV, Kathiresan S (2017). Mendelian Randomization. *JAMA*, 318 (19): 1925-1926.
21. Bowden J, Holmes MV (2019). Meta-analysis and Mendelian randomization: A review. *Res Synth Methods*, 10 (4): 486-496.
22. Ahmad T, Kumar M, Mabalirajan U, et al (2012). Hypoxia response in asthma: Differential modulation on inflammation and epithelial injury. *Am J Respir Cell Mol Biol*, 47 (1): 1-10.
23. Farias JG, Bustos-Obregon E, Reyes JG (2005). Increase in testicular temperature and vascularization induced by hypobaric hypoxia in rats. *J Androl*, 26 (6): 693-697.
24. Liao W, Cai M, Chen J, et al (2010). Hypobaric hypoxia causes deleterious effects on spermatogenesis in rats. *Reproduction*, 139 (6): 1031-1038.
25. Sun S, Chen Y, Hu R (2020). Aquatic hypoxia disturbs oriental river prawn (Macrobrachium nipponense) testicular development: A cross-generational study. *Environ Pollut*, 266 (Pt 3): 115093.
26. Wilson EN, Anderson M, Snyder B, et al (2018). Chronic intermittent hypoxia induces hormonal and male sexual behavioral changes: Hypoxia as an advance of aging. *Physiol Behav*, 189: 64-73.
27. Ayman AH, Anas AO, Yasser AA, et al (2021). Levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone and Prolactin at Moderate Altitude Inhabitant Male. *Pak J Biol Sci*, 24 (2): 188-192.
28. Raff H, Hong JJ, Oaks MK, et al (2003). Adrenocortical responses to ACTH in neonatal rats: Effect of hypoxia from birth on corticosterone, StAR, and PBR. *Am J Physiol Regul Integr Comp Physiol,* 284 (1): R78-85.
29. Ma Y, Zhou Y, Zhu YC, et al (2018). Lipophagy Contributes to Testosterone Biosynthesis in Male Rat Leydig Cells. *Endocrinology*, 159 (2): 1119-1129.
30. Pedersen MK, Brauner EV, Hansen AH, et al (2023). Self-Reported Asthma Is Associated with Reduced Sperm Count-A Cross-Sectional Study of More than 6000 Young Men from the General Population. *Life (Basel)*, 13 (2): 278.