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REVIEW

# A Systematic Review of Mendelian Randomization in Spontaneous Miscarriage

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**Abstract:** Spontaneous miscarriage (SM) is a common pregnancy complication. Although clinical factors are associated with SM, establishing causality is challenging. Mendelian randomization (MR) helps evaluate the causal effects of exposure variables. This study systematically reviewed 31 MR studies performed in SM, identifying causal relationships between SM and smoking, obesity, insomnia, rheumatoid arthritis, and immune-related factors. Smoking initiation and insomnia were identified as risk factors for SM. Coffee consumption showed no causal association with SM risk. Inconsistent evidence was reported for alcohol intake, BMI, depression, and RA regarding their causal relationships with SM. Smoking initiation, specific cytokines (eg, IL-12, TNF-β), and immune cells (eg, CD4+ T cells) demonstrated causal associations with the number of SM. Notably, key SNPs like rs13261666 and rs7127595 played significant roles in MR analyses due to their strong genetic associations with risk factors. Future research should further investigate the mechanistic pathways linking these genetic variants to SM, aiming to provide precise guidance for clinical prevention and treatment. Additionally, inconsistencies in MR results may stem from differences in data sources, SNP selection criteria, and statistical methodologies, indicating the importance of improving data consistency and standardizing analytical approaches in future research.

**Keywords:** Mendelian randomization, spontaneous abortion, epidemiology, single nucleotide polymorphism

## Introduction

Spontaneous miscarriage (SM) refers to the natural loss of a pregnancy occurring before 12 weeks (early SM) or between 12 to 24 weeks (late SM).[1] It is clinically detected in approximately 10% to 15% of pregnancies and recurs in 5% of subsequent pregnancies.[2] SM can lead to complications such as excessive bleeding, infection, anxiety, depression, infertility, and an increased lifetime risk of cardiovascular disease.[3]

Previous studies have linked modifiable risk factors with SM, including alcohol,[4] smoking,[5] and coffee.[6] However, these associations are inconsistent and lack causality evidence.[7] Clarifying the impacts of behaviors, comorbidities, and biomarkers on SM is crucial for advancing personalized prevention and treatment interventions.

Mendelian randomization (MR) is a research method using observational data to study causal relationships. By analyzing whether target variable-related genetic variations influence outcomes (eg, SM),[8] it can overcome the primary limitations of observational studies, such as unmeasured confounding factors, ascertainment bias, and small sample sizes.[9] For instance, conventional epidemiological techniques may find an association between alcohol consumption and SM,[10] but this association could be affected by confounding factors. In contrast, MR utilizes genetic data determined at conception, which is less susceptible to these influences, thus avoiding reverse causality and confounding. Due to these advantages, MR studies have garnered widespread attention in recent years as a high-quality method for identifying disease risk factors.

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MR study uses genome-wide association studies (GWAS) data to identify single nucleotide polymorphisms (SNPs) associated with specific risk factors. These SNPs are then used in an independent GWAS dataset to assess outcomes, determining the relationship between exposure and outcome through two-sample MR. MR analysis relies on three key assumptions: (i) SNPs are strongly associated with exposure variables; (ii) SNPs influence the outcome only through the exposure variable, minimizing horizontal pleiotropy; (iii) SNPs are unrelated to confounding factors, which is confirmed through sensitivity analysis.[11]

In this review, we have identified 31 MR studies related to SM. Our goal is to review MR results to identify risk factors and biomarkers causally related to SM or its comorbidities.

## Included Studies

This review systematically searched PubMed, Embase, and Web of Science on June 28, 2024. Two medical subject heading terms were utilized for this search. Term A was 'spontaneous abortion' OR 'miscarriage' OR 'spontaneous miscarriage' OR 'natural miscarriage'. Term B was 'Mendelian randomization' OR 'Mendelian randomisation' OR 'MR analysis'. The inclusion criteria were: (i) employed an MR study design; (ii) used SNP data related to SM. The following types of studies were excluded: non-original research, non-human studies, duplicate studies, study protocols, letters, and conference proceedings.

Our literature search identified 134 studies. In the abstract screening phase, some studies were excluded for the following reasons: non-SM study population (n = 16); non-original research, letters, or conference proceedings (n = 10); and duplicate studies (n = 73). Consequently, 35 studies proceeded to full-text screening, where exclusions occurred due to conference proceedings (n = 1), unclear statements on MR use (n = 1), and preprints with causal inference (n = 2). Ultimately, our review included 31 studies. Tables 1 and S1 present the main results and methodology checklist for all 31

**Table 1** Mendelian Randomization (MR) Studies of Exposure Variables Associated With Spontaneous Miscarriage (SM)

| Reference | Exposure Variable(s) | Outcome Variable(s) | Significant OR (95% CI) |
|---|---|---|---|
| [12] | Smoking initiation (ever smoked regularly), alcohol use (drinks per week), BMI. | SM | Smoking initiation: 1.16 (1.11–1.22). |
| [13] | Smoking initiation, alcohol drinking, coffee consumption. | SM | Smoking initiation: 1.31 (1.25–1.37). |
| [14] | Alcohol consumption, problematic alcohol use. | SM | Alcohol consumption: 1.35 (1.11–1.64). |
| [15] | Coffee consumption | SM | Not significant |
| [16] | BMI, WHR, WHR adjusted for BMI, predicted visceral adipose tissue mass, WC, hip circumference, waist-specific WHR, hip-specific WHR, leptin, fasting blood insulin, insulin sensitivity. | SM | BMI: 1.06 (1.01–1.12) |
| [17] | Arm fat percentage (left), leg fat percentage (left), WC, body fat percentage, smoking initiation, smoking/smokers in household, AFS, age at menarche, age at first birth, etc. | SM | **UVMR**:<br>WC: 0.795 (0.676–0.935).<br>Body fat percentage: 1.126 (1.010–1.255).<br>Arm fat percentage: 1.220 (1.014–1.467).<br>Leg fat percentage: 1.228 (1.047–1.441).<br>Smoking/smoker in household: 5.154 (1.061–25.045).<br>Smoking initiation: 1.141 (1.035–1.258).<br>Age at menarche: 1.063 (1.005–1.124).<br>Age at first birth: 0.926 (0.885–0.969).<br>AFS: 0.728 (0.650–0.816).<br>**MVMR**:<br>Smoking initiation: 1.472 (1.048–2.067).<br>AFS: 0.802 (0.661–0.973).<br>WC: 0.813 (0.681–0.971).<br>Leg fat percentage: 4.446 (1.045–18.920). |

(*Continued*)

**Table 1** (Continued).

| Reference | Exposure Variable(s) | Outcome Variable(s) | Significant OR (95% CI) |
|---|---|---|---|
| [8] | Physical condition: overall health rating, etc. Basic condition: birth weight, years of schooling. Physical activity: sedentary, etc. Lifestyle habits: smoking initiation, etc. | SM | Overall health rating: 1.68 (1.34–2.13).<br>Sedentary: 1.56 (1.20–2.01).<br>Smoking initiation: 1.26 (1.16–1.38).<br>Birth weight: 0.70 (0.58–0.85).<br>Years of schooling: 0.64 (0.55–0.73). |
| [18] | Anxiety disorder, broad depression, major depression disorder, bipolar disorder, insomnia | SM, RSM | SM:<br>Anxiety disorder: 1.230 (1.063–1.420).<br>Major depression disorder: 1.690 (1.239–2.307).<br>Bipolar disorder: 1.110 (1.052–1.170).<br>Insomnia: 1.292 (1.052–1.588). |
| [19] | Depression/dysthymia | SM | Not significant |
| [20] | Sleep duration | SM | Not significant |
| [21] | Insomnia | SM | 1.60 (1.18–2.17). |
| [22] | Systemic lupus erythematosus, RA | SM | Not significant |
| [23] | RA | SM | 1.13 (1.00–1.27). |
| [24] | Periodontitis (acute, chronic) | SM | Chronic periodontitis: 0.921 (0.875–0.969) |
| [25] | Asthma | SM | 1.092 (1.017–1.174) |
| [26] | Kidney damage: estimated glomerular filtration rate. | SM | 2.63 (1.269–5.450). |
| [27] | COVID-19 | SM | Not significant |
| [28] | 41 inflammatory cytokines: IL-12 TNF-β, etc.<br>731 immune cell traits: switched memory B-cell absolute count, IgD+ CD24+ B cell absolute count, CD39+ resting CD4 regulatory T cell absolute count, activated and resting CD4 regulatory T cell %CD4 regulatory T cell, CD45RA+ CD28- CD8+ T cell %CD8+ T cell, etc. | RSM | IL-12: 1.78 (1.25–2.55).<br>TNF-β: 0.75 (0.56–0.99).<br>Switched memory B-cell RSM count: 0.66 (0.49–0.87).<br>IgD+ CD24+ B cell absolute count: 0.69 (0.53–0.88).<br>CD39+ resting CD4 regulatory T cell absolute count: 0.86 (0.78–0.95).<br>Activated & resting CD4 regulatory T cell %CD4 regulatory T cell: 0.89 (0.82–0.97).<br>CD45RA+ CD28- CD8+ T cell %CD8+ T cell: 0.99 (0.98–1.00). |
| [29] | 47 inflammatory cytokines: MCSF, sICAM-1, etc. | SM | MCSF: 1.15 (1.06–1.24).<br>sICAM-1: 1.09 (1.04–1.15). |
| [30] | Inflammatory cytokines: IL-1β, etc. | SM | IL-1β: 1.13 (1.02–1.25) |
| [31] | Plasma/serum cytokines circulation levels: MCP1, MCP3, IL-17, IL-1β, IL-8, RANTES, etc. | SM | **UVMR**:<br>MCP1: 0.93 (0.87–1.00).<br>MCP3: 1.05 (1.00–1.11).<br>IL-17: 1.11 (1.01–1.21).<br>IL-1β: 1.17 (1.03–1.33).<br>**MVMR**:<br>MCP1: 0.90 (0.83–0.97).<br>IL-8: 1.18 (1.07, 1.31).<br>RANTES: 1.10 (1.02–1.18). |
| [32] | Follistatin | SM | 0.999 (0.997–0.999). |
| [33] | Anti-*Helicobacter pylori* IgG level | SM | Not significant |
| [34] | Telomere length | SM | **UVMR**: 0.815 (0.714–0.930).<br>**MVMR**: 0.867 (0.763–0.985). |
| [35] | Histo-blood group ABO system transferase protein levels. | SM | Not significant |

(*Continued*)


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**Table 1** (Continued).

| Reference | Exposure Variable(s) | Outcome Variable(s) | Significant OR (95% CI) |
|---|---|---|---|
| [8] | 25OHD, vitamin D deficiency. | SM, number of SM | Not significant |
| [36] | Chronic periodontitis | Number of SM | Not significant |
| [37] | Homocysteine concentrations | Number of SM | Not significant |
| [38] | 731 immunophenotypes: FSC-A on CD4+ T cell, CD8 on HLA DR+ CD8+ T cell, HLADR on CD33dim HLA DR+ CD11b−, HLA DR+ T cell absolute count, HLA DR+ T cell absolute count, HLA DR+ T cell % lymphocyte, HLA DR+ T cell % T cell, HLA DR+ CD4+ T cell % lymphocyte, HLA DR on B cell, CD19 on naive-mature B cell, CD28 on CD39+ CD4+ T cell | Number of SM, RSM | **Number of SM**:<br>CD4+ Tcell: 0.9503 (−0.085, −0.017).<br>CD8 on HLA DR+ CD8+ Tcell: 0.961 (−0.067, −0.014).<br>HLA DR on CD33dim HLA DR+ CD11b−: 0.979 (−0.036, −0.005).<br>HLA DR+ T cell absolute count: 1.022 (0.006–0.037).<br>HLA DR+ T cell % lymphocyte: 1.026 (0.010–0.041).<br>HLA DR+ T cell % T cell: 1.023 (0.007–0.039).<br>HLA DR+ CD4+ T cell % lymphocyte: 1.034 (0.007–0.060).<br>HLA DR on B cell: 1.012 (0.003–0.021). CD28 on CD39 + CD4+ T cell: 1.011 (0.001–0.022).<br>CD28 on CD39+ CD4+ Tcell: 1.212 (1.024–1.434).<br>**Number of RSM**:<br>HLA DR on B cell: 0.854 (0.757–0.964).<br>CD19 on naive-mature B cell: 4.595 (1.674–12.617). |
| [39] | COVID-19 | Number of SM | Not significant |
| [40] | Health behaviors: smoking initiation, etc. | Number of SM | Smoking initiation: −0.123 (−0.182, −0.064) |

**Notes**: This table summarizes the exposure variable(s), outcome variable(s), description of the dataset(s) used, number of SNPs used as instrumental variables, MR analysis techniques, ORs, and *P*-values used in all the 31 MR studies. Studies are organized into sections (smoking, alcohol, and coffee consumption; dietary intake and physical/emotional conditions; sleep; other medical conditions; inflammatory cytokines; other serum biomarkers) and ordered alphabetically within sections by the first author. Only exposure–outcome pairings with significant ORs results are explicitly listed in the rightmost columns; nonsignificant exposure/outcome pairings are not listed. Only results from the primary mode of MR analysis technique (eg, inverse variance weighted [IVW]) are described in the OR columns.

**Abbreviations**: BMI, body mass index; UVMR, univariable MR; MVMR, multivariable MR; WHR, waist-to-hip ratio; WC, waist circumference; AFS, age first had sexual intercourse; IL, interleukin; TNF-β, tumor necrosis factor-beta; OR, odds ratio; 95% CI, 95% confidence interval; SNP, single nucleotide polymorphism; 25OHD, 25-hydroxyvitamin D; RA, rheumatoid arthritis; RSM, recurrent spontaneous miscarriage; MCSF, macrophage colony-stimulating factor; sICAM-1, soluble intercellular adhesion molecule 1; MCP, monocyte chemoattractant protein; RANTES, regulated upon activation normal T cell expressed and secreted.

studies. The distinction lies in that Table 1 categorizes information by reference, while Table S1 details each study with its exposure types. Additionally, Table S2 contains all significant SNPs. It should be noted that the populations included in the 31 studies were primarily of European descent.

# Results

## Smoking, Alcohol, and Coffee Consumption

Four MR studies have assessed the association between smoking and SM,[8,12,13,17] with the odds ratio for smoking initiation ranging from 1.14 to 1.31, indicating smoking initiation as a consistent risk factor. However, the smoking behaviors evaluation is incomprehensive. For example, quantity (cigarettes per day), cessation (current or former smoker), lifetime use (about population-based averages), and smoking/smokers in the household were not fully considered. Only one study reported the impact of smoking/smokers in the household, which was significant in univariable MR analysis but not in multivariable MR analysis.[17] One study on MR reported 116 significant SNPs, such as rs3001723, rs12356821 andrs12474587 (additional details are available in Table S2).

Four MR studies investigated the role of alcohol consumption in SM.[3,8,13,14] Two found no significant link,[3,13] while the other indicated a positive correlation (OR: 1.35–1.74).[8,14] Inconsistent results were possibly caused by differences in

data sources, SNP selection criteria sample sizes, multiple testing correction methods (unknown vs Bonferroni), and MR analysis methods used. One MR study reported significant SNPs (199 in total) (see Table S2 for details).[41]

Three MR studies evaluated the causal relationship between coffee consumption and SM.[8,13,15] All studies utilized data from European populations and selected SNPs significantly associated with coffee intake ($P < 5\times10^{-8}$), applying various MR analysis methods (such as IVW, weighted median, MR-Egger, etc). However, none of the studies found a significant association between coffee intake and SM.

## Depression, and Insomnia

Currently, two MR studies have evaluated the causal relationship between depression and SM, with neither finding a significant causal relationship.[18,19] However, one of these identified a significant causal relationship between anxiety disorder and major depression disorder with SM.[18] This study also reported significant SNPs (244 in total), such as rs12958048, rs4799092, and rs12135727 (see Table S2 for details). Two MR studies reported that insomnia significantly increases the risk of SM (OR: 1.18–1.29),[18,21] and both of them reported significant SNPs (rs113851554, rs7337692, rs10947428, etc).

## Medical Comorbidities

Two MR analyses investigated the association between rheumatoid arthritis (RA) and SM.[22,23] One study found a causal association between RA and SM susceptibility (OR = 1.13, 95% CI, 1.00–1.27), but not in SM and RA.[23] This study reported 47 significant SNPs, such rs2476601, rs7574865, rs10821948. The second MR study found no causal relationship between RA and SM in either the forward or reverse study after Bonferroni correction.[22] This bidirectional MR analysis also showed no relationship between systemic lupus erythematosus and SM.[22]

A bidirectional two-sample MR study found a null association between acute periodontitis and SM, while chronic periodontitis was identified as a protective factor for SM (OR = 0.921, 95% CI, 0.875–0.969).[24] However, the results from MR Egger, weighted median, simple mode, and weighted mode analyses were not significant[24] According to an MR study, asthma is a pathogenic risk factor for SM in the European population (OR = 1.092).[25] Although some studies suggest that asthma may increase the risk of SM, the overall evidence remains inconsistent, and further research is necessary. Furthermore, MR evidence does not support COVID-19 as a pathogenic risk factor for SM in the European population.[27]

## Serum Biomarkers

In our review, seven cytokines were found to have a causal relationship with SM: interleukin (IL)-12 (OR = 1.78), tumor necrosis factor-beta (TNF-β) (OR = 0.75),[28] macrophage colony-stimulating factor (MCSF) (OR = 1.15), soluble intercellular adhesion molecule (sICAM-1) (OR = 1.09),[29] IL-1β (OR = 1.13),[30] monocyte chemoattractant protein (MCP)-1 (OR = 0.93), and regulated upon activation normal T cell expressed and secreted (RANTES) (OR = 1.10).[31] Among these, IL-12 and TNF-β were found to have a causal relationship with recurrent spontaneous abortion.[28] MR also identified causal relationships between immune cells and SM, such as switched memory B-cell absolute count (OR = 0.66) and IgD+ CD24+ B-cell absolute count (OR = 0.69).[28] In these immune-related MR studies, particularly those on cytokines, the selection of exposure SNPs varied, with *P*-values typically ranging from $5*10^{-6}$ to $1*10^{-5}$.

MR analysis revealed that telomere length is associated with an increased risk of SM.[34] Additionally, MR analysis indicated a significant causal relationship between low follistatin levels and SM ($P = 0.03$).[42] Meanwhile, specific cytokines and immune cells were also found to exhibit causal associations with SM development. Other serum indicators such as anti-*Helicobacter pylori* IgG levels,[33] homocysteine concentrations,[37] tissue blood group ABO system transferase protein levels,[35] 25OHD,[8] and vitamin D deficiency[8] do not increase the risk of SM.

## Body Mass Index (BMI)

Four MR investigations examined causal relationships between body weight/fat distribution metrics and SM.[3,8,16,41] Two analyses revealed a positive causal association of elevated BMI with SM risk (OR range: 1.06–1.10),[8,16] while one study showed non-significant findings.[3] Regarding regional adiposity measures, waist circumference (WC) demonstrated

protective effects against SM (OR = 0.795, 95% CI, 0.676–0.935), contrasting with positive correlations observed for whole-body fat (OR = 1.126), left arm fat mass (OR = 1.220), and left leg fat mass (OR = 1.228). Multivariable MR analysis identified differential causal patterns: WC maintained an inverse association (OR = 0.813), whereas leg fat percentage exhibited a substantially elevated risk (OR = 4.446) for SM.[41] Two studies on MR reported significant SNPs (265 vs 1001),[16,41] such as rs10938397, rs1801282 and rs2785988 (additional details are available in Table S2).

## Number of SM

Six studies evaluated the causal relationship between exposure factors and the number of SM. These exposure factors included smoking initiation,[40] 25OHD,[8] vitamin D deficiency,[8] chronic periodontitis,[36] COVID-19,[39] immune markers,[38] and homocysteine concentrations.[37] Among these, only the smoking initiation and some immune markers were related to the number of SM (OR = −0.123).[40]

# Discussion

This systematic review of 31 MR studies evaluated causal risk factors for SM. Smoking initiation and insomnia were robustly identified as independent risk factors, while coffee consumption exhibited no causal association. Inconsistent evidence emerged regarding alcohol intake, BMI, rheumatoid arthritis, specific immune markers. Key SNPs (eg, rs13261666, rs7127595) demonstrated pivotal roles in MR analyses due to their strong genetic associations with SM-related traits. Future investigations should prioritize elucidating biological pathways connecting these genetic variants to SM pathogenesis to inform targeted preventive and therapeutic strategies.

## Causal Factors Associated With SM

### Smoking

This study systematically synthesized four MR investigations examining the causal relationship between smoking behaviors and SM.[3,8,13,41] The pooled analyses demonstrated a significant positive association, with smoking initiation exhibiting combined OR estimates ranging from 1.14 to 1.31 (95% CIs excluding unity). Notably, smoking/smokers in household exposure manifested an elevated effect size of 5.15 (95% CI, 1.06–25.05), consistent with observational evidence.[43]

One study reported 179 genome-wide significant SNPs for smoking initiation, with the top-ranked variants (*P*-value hierarchy) including rs13030994, rs3001723 (*PTPRF*), rs12356821 (*WBP1L*), rs12474587 (*SLC4A10*), rs13261666 (*TOX*), rs4044321 (*TENM2*), rs7921378 (*ARID5B*), rs4236259 (*ELFN1*), rs10001365 (*TTC29*), and rs1899896. Twenty smoking/smoker in household-associated SNPs were reported, exemplified by *EPB41L3* (rs7127595) and *TTC21A* (rs60429130).

The identified loci potentially mediate SM pathogenesis through regulatory effects on critical biological pathways. *PTPRF* encodes a receptor-type tyrosine-protein phosphatase modulating cellular proliferation and differentiation.[44] *TOX* participates in immune cell development, potentially influencing immune homeostasis at the maternal-fetal interface.[45] *ARID5B* regulates mitochondrial function and interferon-γ production in oxidative metabolism.[46] Mutations in *TTC29*[47] and *TTC21A*[48] have been implicated in spermatogenic defects through structural sperm abnormalities. These mechanisms may collectively impair embryonic viability, maternal immune tolerance, or placental function - pivotal processes in pregnancy maintenance - thereby potentially mediating the causal pathway between smoking exposure and SM risk.

## Insomnia

This study consolidates genetic evidence indicating a significant positive association between sleep disorders (particularly insomnia) and SM risk. Two independent investigations employing bidirectional two-sample MR analyses (Bonferroni-corrected) on European population GWAS datasets jointly validated this association 18.20. A total of 149 genome-wide significant SNPs were identified, including pivotal loci: rs113851554 (*MEIS1*, 2p14), rs10947428 (*ITPR3*, 6p21), rs28576953 (*KMT5A*, 5q23), rs77641763 (*EXD3*, 14q24), rs4073582 (*CNIH2*, 11p13), and rs3774751 (*SEMA3F*, 3p21).

*MEIS1* demonstrates hematopoietic stem/progenitor cell-specific expression, with murine knockout models exhibiting embryonic lethality characterized by hematopoietic defects, megakaryocyte/platelet deficiency, and vascular abnormalities [49]. *ITPR3* functions as an endoplasmic reticulum-mitochondrial $Ca^{2+}$ release channel, regulating calcium signaling cascades involved in cellular proliferation, migration, and apoptosis [50]. *KMT5A* modulates angiogenesis through H4K20me1 methylation, with experimental evidence confirming its regulatory role in human umbilical vein endothelial cell vascularization.[50] *SEMA3F* mediates axonal guidance and cellular polarity establishment during cell migration processes.[51]

These candidate genes influence biological pathways encompassing hematopoietic development, calcium homeostasis, epigenetic modifications, and cellular motility. Given the critical roles of placental vascularization, trophoblast invasion, and decidualization in pregnancy maintenance, functional perturbations in these genes may elevate SM risk via disruption of embryo implantation, placental development, and maternal-fetal interface homeostasis. These findings collectively suggest molecular mechanisms potentially underlying the association between insomnia and adverse pregnancy outcomes.

## The Causal Relationship With SM Is Unclear

The MR analyses investigating alcohol, BMI, RA, and cytokines in the current study exhibited inconsistencies in their respective findings.

Inconsistent findings in MR studies may arise from multiple factors, including variations in exposure variable definitions, measurement approaches, data source heterogeneity, SNP selection criteria, statistical methodologies, and multiple testing correction strategies.

First, data source heterogeneity represents a factor contributing to MR result discrepancies. The heterogeneity is reflected in the differences in data extraction time and data sources. For instance, in BMI-focused MR studies, one investigation employed the 2018 UK Biobank release while another utilized 2015 data.[3,8] Regarding the data source, one study used data from multiple sources,[3] including 23andMe, Avon Longitudinal Study of Parents and Children (ALSPAC), Atherosclerosis Risk in Communities Study (ARIC), Estonian Genome Center, University of Tartu (EGCUT), Framingham Heart Study (FHS), which covered individuals of European, African, Asian, and Latin American descent, while another study only used European data from the UK Biobank.[8] Such differences in data sources could affect the statistical power, genetic variation distribution, and environmental factors, thereby influencing MR analysis results.

Additionally, variations in SNP selection criteria could also lead to differences in MR findings. For example, the study by Venkatesh et al[16] applied an SNP selection criterion of $P < 5\times10^{-9}$, while the study by Tong et al[8] used a more lenient criterion of $P < 5\times10^{-8}$, with clumping distance of 10,000 kb, linkage disequilibrium $r^2 < 0.001$, and minor allele frequency $\geq 0.01$. Stricter or more lenient SNP selection criteria affect the strength and validity of instrument variables, thereby influencing the final causal inferences.

Next, different multiple testing correction strategies also play a role. For example, in MR studies related to BMI, one study applied a false discovery rate of 10% and Bonferroni correction,[3] while another study did not apply any correction.[41] Different correction methods affect the significance thresholds, thereby influencing the number of significant SNPs and the final causal conclusions.

Finally, differences in MR analysis methods can also cause inconsistency in results. For example, one BMI-related MR study used a range of methods,[41] including IVW, MR-Egger, weighted median, MR-PRESSO, and MVMR (IVW, MR-LASSO, MR-Egger, multivariable median), while another study used IVW, weighted median, MR-Egger, and hypothesis-generating MR phenome-wide (PheWAS) analysis.[3] Different methods have varying abilities to control for horizontal pleiotropy and multi-allelic SNPs, which may result in different causal conclusions across studies.

In summary, the inconsistency in MR study results is mainly influenced by factors such as the timing of data extraction, heterogeneity in data sources, SNP selection criteria, statistical analysis methods, and multiple testing correction strategies. To address these issues, future research should focus more on ensuring data consistency.

## Number of SM

The number of SM is linked to reproductive and other health problems. Women who have experienced multiple SM have an increased recurrence risk as the number rises. Patients with a history of three or more consecutive SM have a recurrence rate of 40% to 80% in subsequent pregnancies.[52] A history of multiple SM is a significant predictor of future SM, potentially due to factors such as uterine environment, immune responses, and genetic factors. Moreover, the number of SM is significantly associated with an increased risk of pregnancy complications. MR analysis has found that smoking initiation and certain immune cells (such as CD4+ T cells and CD8+ on HLA DR+ CD8+ T cells) are causally linked to SM,[38] although the specific mechanisms remain unclear. Future studies will aim to clarify these mechanisms further and explore potential preventive measures.

# Conclusion

This systematic review of 31 MR studies identifies causal factors for SM, with smoking initiation and insomnia consistently emerging as significant independent risk factors. Genetic variants linked to smoking and sleep disorders play pivotal roles in influencing biological pathways critical to pregnancy maintenance, such as immune homeostasis. In contrast, coffee consumption showed no causal association, and inconsistent findings were observed regarding alcohol intake, BMI, RA, and immune markers, highlighting the complexity and variability in MR analysis results. These discrepancies are likely due to variations in data sources, SNP selection, and statistical methodologies. Moving forward, more robust research is needed to clarify the mechanisms linking genetic variants to SM pathogenesis and explore targeted interventions that may help reduce the risk of miscarriage. Understanding the role of genetic factors in SM will be essential for developing personalized preventive and therapeutic strategies to improve reproductive health outcomes.

# Abbreviations

SM, spontaneous abortion; MR, Mendelian randomization; GWAS, genome-wide association studies; SNPs, single nucleotide polymorphisms; OR, odds ratio; 95% CI; 95% confidence interval; UVMR, univariable MR; MVMR, multivariable MR; 25OHD, 25-hydroxyvitamin D; WC, waist circumference; RSM, recurrent spontaneous miscarriage; RA, rheumatoid arthritis; IL, interleukin; TNF-β, tumor necrosis factor-beta; MCSF, macrophage colony-stimulating factor; sICAM-1, soluble intercellular adhesion molecule; MCP, monocyte chemoattractant protein; RANTES, regulated upon activation normal T cell expressed and secreted; BMI, Body mass index; IVW, inverse variance weighted; AFS, age first had sexual intercourse.

# Data Sharing Statement

The data analyzed in this study can be obtained from the main text.

# Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the *Journal of Multidisciplinary Healthcare*; and agree to be accountable for all aspects of the work.

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