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Research Article

The impact of obesity and sexual behavior on prostate cancer risk is mediated by testosterone levels: a mendelian randomization study and mediation analysis

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ABSTRACT

Background: The relationship between obesity, sexual behavior, and prostate cancer (PCa) has been widely debated, contributing to a lack of understanding of its potential mechanisms and hindering the development of effective prevention measures.
Purpose: The aim of this study was to examine the causal effect of body mass index (BMI), age at first sexual intercourse (AFS), and bioavailable testosterone levels on PCa while also quantifying the potential roles of mediators.
Method: We conducted a Mendelian randomization (MR) study using summary statistics from genome-wide associations of BMI (152,893 European males), AFS (182,791 European males), bioavailable testosterone (184,205 European males), and PCa (79,148 cases, 61,106 controls, European ancestry). Inverse-variance weighted method, weighted median method, MR-Egger regression, Least Absolute Shrinkage and Selection Operator (LASSO), and outlier test were used for MR analyses. Reverse MR and mediation analysis were performed. Data analyses were conducted from December 2022 to July 2023.
Results: The results showed that genetic liability to BMI was protective of PCa (OR, 0.82; 95% CI: 0.74-0.91; $P = 3.29 \times 10^{-4}$). Genetic liability to later AFS (OR, 1.28; 95% CI: 1.08-1.53; $P = 5.64 \times 10^{-3}$) and higher bioavailable testosterone levels (OR = 1.11, 95% CI: 1.01−1.24, $P = 0.04$) were associated with an increased risk of PCa. All of these potential causal effects could only be forwarded and were not affected by prostate specific antigen (PSA) screening. After controlling for bioavailable testosterone levels, the causal impact of BMI and AFS on PCa was no longer significant. The mediation analysis suggested that the causal influence of AFS/BMI on PCa relied on bioavailable testosterone levels.
Conclusion: In conclusion, the difference between the univariable and multivariable MR results suggested that the causal influence of BMI and AFS on PCa relied on bioavailable testosterone levels. Further work is needed to identify other risk factors and to elucidate the specific mechanisms that underlie this causal pathway.

1. Introduction

Prostate cancer (PCa) is one of the most commonly diagnosed hormone-related malignancies globally, causing significant clinical and socioeconomic burdens. According to the Cancer Statistics, 2024 published by the American Cancer Society, it is estimated that there will be 299,010 new cases of PCa in 2024, representing the highest number of new cancer cases in men at 29%.[1] Despite advancements in PCa screening, treatment, and monitoring in recent years,[2–5] the annual incidence of PCa continues to escalate.[6,7] The

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incidence of PCa increased by 2−3% annually between 2015 and 2019.[1] Therefore, it is imperative to gain a more profound understanding of the underlying causes of PCa as a fundamental step toward developing effective primary prevention strategies.

PCa is a multifactorial and complex disease influenced by various environmental, physiological, immunological, and genetic factors. Age, race, and family history are recognized as established risk factors, while the influence of other factors remains uncertain.[8−10] The rapidly growing public health concern of obesity has emerged as a significant contributing factor in cancer development, playing important roles in driving PCa aggressiveness and increased mortality. However, the association between obesity and the incidence of PCa remained controversial.[11] A 7.4-year follow-up study of about six thousand individuals found an association between body mass index (BMI) and an increased risk of PCa (HR: 1.78, 95% CI: 1.04-3.04, $P = 0.035$).[12] In contrast, the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which followed 141,896 adult men in Europe for 13.9 years, reported the opposite result.[13] A similar situation exists regarding the relationship between sexual behavior and PCa. Available evidence suggests that sexual activity might play a role in PCa pathogenesis.[14] A recent meta-analysis indicated that the risk of PCa was decreased by 4% for every 5-year delay in age at first intercourse (OR 0.96, 95% CI: 0.92-0.99)[15]; however, consensus on this matter has proven elusive.[16,17]

The association between testosterone levels and PCa has been a topic of interest among physicians for decades.[18−21] Increasing evidence supports the link between obesity and the dysregulation of hormonal pathways, particularly those involving testosterone, the primary circulating androgen in males.[22] Moreover, sex steroids, including testosterone, have long been closely linked to PCa. Investigating and controlling modifiable risk factors like BMI and testosterone levels for PCa can yield clinically actionable insights and hold significant public health relevance. However, there is still a dearth of research investigating the potential multivariate relationship between obesity, sexual behavior, testosterone levels, and PCa. Here, leveraging population-scale human genetics data from genome-wide association studies (GWASs), we used the Mendelian randomization (MR) framework with genetic variants as instrumental variables to (1) estimate the effect size for associations between BMI, age at first sexual intercourse (AFS), bioavailable testosterone levels, and PCa; (2) perform multivariate MR (MVMR) to estimate the corrected causal effect sizes after controlling for potential confounders (PSA screening) and mediators; (3) conduct reverse MR and mediation analysis which may help to understand the pathway from AFS/BMI to PCa; (4) make a preliminary estimate of the causal relationship between other sexual activity (lifetime number of sexual/same-sex sexual partners and ever had same-sex intercourse) and PCa; (5) interpret and extrapolate of the MR results based on potential mechanisms/additional MR analysis.

2. Materials and methods

2.1. General methodology

In recent times, significant advancements have been made in genomic research through large-scale GWASs. These studies have identified genetic loci associated with various factors such as serum testosterone levels, BMI,[23] sexual behavior,[24] and PCa.[25] Moreover, these studies have provided gender-stratified data for further analysis.[23,24] Building upon these findings, we conducted MR analyses to explore potential effects and pathways, utilizing testosterone hormone levels as a mediator. The MR framework utilizes the natural randomization of genetic variation during conception, which helps mitigate confounding risks commonly encountered in other observational methods.[26] Through the random distribution of genetic variants in the population, analogous to randomization in a clinical trial, potential biases arising from environmental confounding and reverse causality are minimized.[26,27] By satisfying specific assumptions (Fig. 1), the MR framework improves the precision of quantifying potential differences between exposure-outcome pairs.[27] It also allows for the consideration of joint exposures, enabling the estimation of the direct effects of each exposure on the outcome of interest, akin to mediation analysis.[28] In this study, both univariable and multivariable MR analyses were carried out within a two-sample MR framework, utilizing summary statistics from the IEU Open GWAS project.[29,30] The study adhered to the STROBE-MR guidelines,[31] and a checklist is provided in Supplementary Table S13. Since the study utilized publicly available summary statistics data, no additional ethics approval was necessary. A comprehensive outline of the study design is depicted in Figs. 1 and 2, while the summary of each genome-wide association study and univariable MR results is presented in Table 1.

2.2. Study populations

European men were the subjects of this MR study. We access the data through the OpenGWAS database application programming interface (API).[29,30] To minimize exposure sample heterogeneity, mixed-gender datasets were not included in the MR analysis. AFS was treated as a continuous variable, with individuals considered eligible if they had given a valid answer to the question "What was your age when you first had sexual intercourse?" (Sexual intercourse includes vaginal, oral, or anal intercourse), and ages <12 years old were excluded.[24,32] Since AFS had a non-normal distribution, an inverse rank normal transformation was applied.[24] PCa data for MR analysis were obtained from the GWAS study published in *Nature Genetics* by Schumacher et al which included a sample size of 79,148 cases and 61,106 controls.[25]

2.3. Statistical analysis

In the MR analysis, the single nucleotide polymorphisms (SNPs) used as genetic instruments must satisfy three main assumptions,[9,26,31] as depicted in Fig. 1. Here, we described how we met each of the three main assumptions of MR. Here is a breakdown of how each assumption was addressed.

1. The genetic variant is associated with the risk factor. To meet this assumption, we extracted variants that reached genome-wide significance ($P < 5 \times 10^{-8}$). Clumping was performed with a linkage disequilibrium (LD) threshold (r^2) < 0.001 and a distance >10,000 kb. The 1000 Genomes Project European sample was used as the LD reference panel. The instrument's strength was evaluated by assessing the association between the genetic variants and the exposure. F-statistics were calculated for each exposure, where F > 10 was considered indicative of adequate instrument strength (33). The F-statistic is derived from the variance explained ($R^2 = 2 \times (1 - MAF) \times MAF \times \beta^2$) by SNPs for each exposure by $F = \left(\frac{R^2}{1-R^2}\right)\left(\frac{n-k-1}{k}\right)$, where k is the number of SNPs and n the sample size.[9,33]
2. The genetic variant influences the outcome only through the risk factor. Fulfill assumption 2: manual filtering based on the p-values from the outcome GWAS was not performed. Instead, the authors employed MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO), a method for detecting outliers and assessing pleiotropy, to perform downstream analysis. This approach utilizes a sophisticated approach to outlier detection.[9]
3. The genetic variant is not associated with confounders. To address this assumption, we searched the PhenoScanner

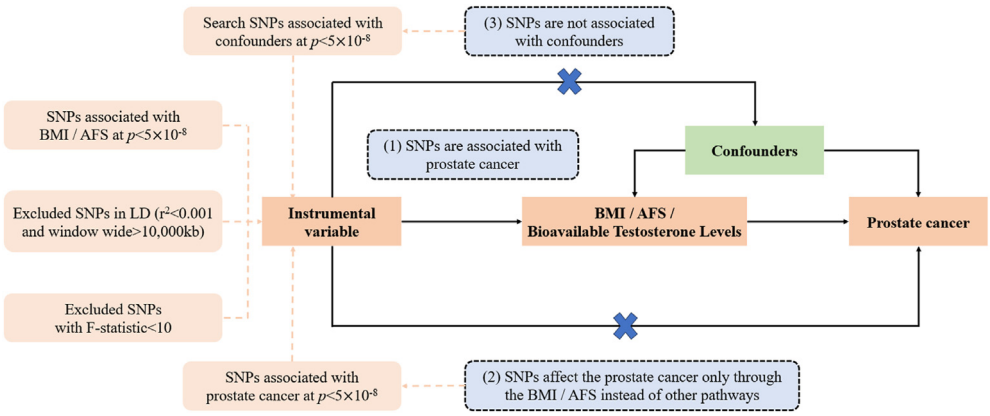

Fig. 1. An overview of the study design and the core three instrumental variable assumptions.

database[34,35] for previously reported associations between the instrumental SNPs and potential confounding factors. If any genome-wide significant associations ($P < 5 \times 10^{-8}$) were found with common causes of exposures and the outcome, a multivariable MR analysis was performed to account for indirect pathways and correlated pleiotropy.[9] Associations of instrumental variables with obesity-related traits were identified through the PhenoScanner database search.

The analysis was conducted using the "Mendelian randomization" package in R, specifically version 0.7.0. By following these steps, we aimed to fulfill all three assumptions in order to obtain valid and reliable results in their MR analysis.

In this study, univariable MR analyses were performed to estimate the effects of BMI, AFS, and serum bioavailable testosterone levels on PCa.[25] To ensure the correct harmonization of alleles, SNPs were oriented in such a way that the effect variants of the exposure and outcome corresponded to the same allele.[30] The total effect of an exposure on an outcome can be broken down into indirect effects (mediated through a causal intermediate) and direct effects (not mediated through the mediator).[36] In this study, multivariable MR analyses were used to estimate the direct effect of each exposure on PCa, specifically focusing on the effects that were not mediated through any intermediate variables. Two-sample MR analyses were conducted using the "TwoSampleMR" package (https://github.com/MRCIEU/TwoSampleMR) in R, with version 0.5.7 of the package being utilized. This package allows for MR analyses using summary data from different datasets or studies. The data analyses were conducted between December 2022 and July 2023.

In interpreting the results of the MR analysis in this study, the following principles were applied.[37]

I. When there was no heterogeneity or pleiotropy, the results using the inverse-variance weighted (IVW) method were prioritized. Fixed effect IVW (IVW-FE) assumes all instruments are valid (no horizontal pleiotropy), whereas multiplicative random effects IVW (IVW-MRE) can account for heterogeneity due to balanced pleiotropy.[38]
II. If there was only heterogeneity without pleiotropy, the weighted median method results were prioritized while also utilizing the IVW random effects model. The weighted median allows for up to 50% of the weights in the estimator to be from invalid instruments.[39]
III. In scenarios that involved horizontal pleiotropy, the MR-Egger method results were preferred. MR-Egger can provide unbiased estimates when the instrument strength

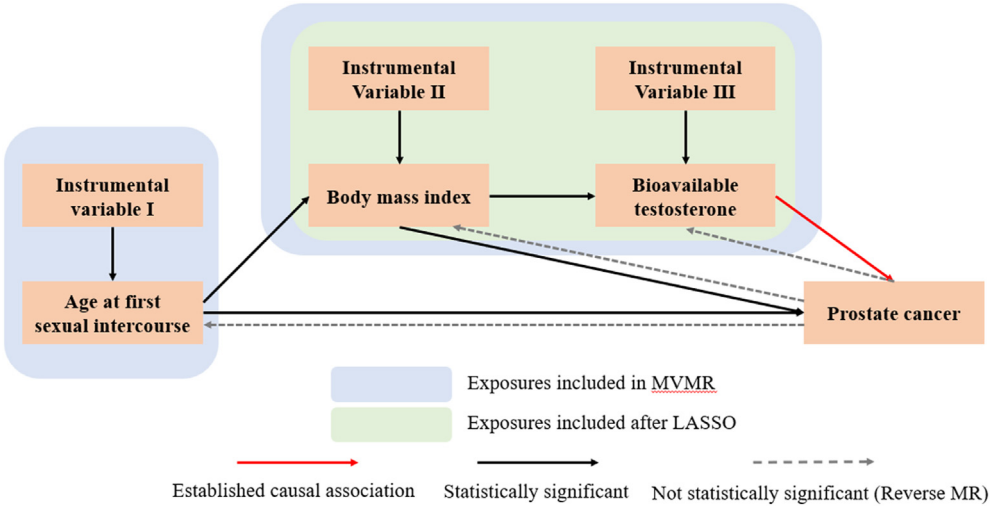

Fig. 2. The causal relationship between exposures and outcomes.

Table 1
Summary of each genome-wide association study and univariate MR results

Exposure (Sample size)	Outcome (Sample size)	R^2	F	P_h	Egger intercept	$P_{intercept}$	Method	OR (95% CI)	P
Body mass index (23) (152,893 individuals)	Prostate cancer (25) (140,254 individuals)	1.55%	77.52	0.090	<0.001	0.889	IVW	0.82 (0.74, 0.91)	<0.001
							Weighted median	0.82 (0.71,0.95)	0.007
Age at first sexual intercourse (24) (182,791 individuals)	Prostate cancer (25) (140,254 individuals)	N/A	N/A	0.023	0.013	0.085	IVW	1.21 (1.06, 1.38)	0.006
							Weighted median	1.28 (1.08, 1.52)	0.004
Bioavailable testosterone (184,205 individuals)	Prostate cancer (25) (140,254 individuals)	3.98%	91.17	<0.001	0.002	0.626	IVW	1.18 (1.07, 1.29)	<0.001
							Weighted median	1.12 (1.01, 1.24)	0.036

IVW, inverse variance weighted method; MR Mendelian randomization. OR, 95% CI, and P values were for the respective MR analysis. P_h, P value for heterogeneity; $P_{intercept}$, P value for intercept of MR-Egger regression. N/A: effect allele frequency not available for PCa to allow estimation of r^2 and F-statistic.

independent of direct effect (InSIDE) assumption holds.[40] The strengths and weaknesses of each MR method are provided in Supplementary Table S14.[9]

2.4. Sensitivity analyses

In the study, several sensitivity analyses were conducted to evaluate the robustness of the MR results. These analyses included the following:

i. **Heterogeneity test**:
   - Cochran's Q test: This test was utilized to assess heterogeneity using the Two Sample MR package. A *P*-value of less than 0.05 was considered indicative of heterogeneity, while the opposite suggested homogeneity within the data.

ii. **Pleiotropy test**:
   - MR Egger method intercept term: The MR Egger method intercept term was employed to assess pleiotropy. A *P*-value of less than 0.05 suggested the presence of pleiotropy, while the converse indicated its absence.
   - Global test of the MR-PRESSO package: The global test of the MR-PRESSO package was also applied to identify horizontal pleiotropy.

iii. **Distortion test**:
   - Leave-one-out analysis: This analysis was conducted to evaluate the influence of individual SNPs on the estimates.
   - MR-PRESSO outlier test: The MR-PRESSO outlier test and distortion test were employed to identify and remove any outlier SNPs. Subsequent analysis was then carried out after removing the identified outliers.

iv. **Least Absolute Shrinkage and Selection Operator (LASSO) feature selection**:
   - LASSO feature selection method: The LASSO feature selection method was applied to the multivariable MR (MVMR) data object. This method aids in identifying the most relevant features in the analysis.

By implementing these sensitivity analyses, the study aimed to thoroughly assess the robustness of the MR results by examining heterogeneity, pleiotropy, potential distortion by individual SNPs, and employing feature selection to identify the most relevant features in the MVMR analysis.

3. Results

3.1. Causal effects of body mass index and age at first sexual intercourse on PCa risk

Two-sample univariable and MVMR analyses were conducted to explore the causal effects of body mass index and age at first sexual intercourse on prostate cancer risk. Using twenty-eight SNPs robustly and independently associated with BMI (Supplementary Table S1), there was evidence of a protective effect of increased BMI on PCa (IVW, OR = 0.82, 95% CI: 0.74, 0.91, $P < 0.001$) (Fig. 3; Supplementary Figure S1–S3; Supplementary Table S2). There was no statistically significant heterogeneity ($P = 0.09$) or horizontal pleiotropy ($P = 0.89$) in the association (Supplementary Tables S5–S6). The MR-PRESSO test showed no outliers, and the leave-one-out analysis did not suggest that the causal estimates were driven by a particular SNP. Using a set of 52 SNPs that were both robust and independent in their association with AFS (Supplementary Table S1), our results provided strong evidence for a causal relationship between later AFS and PCa (weighted median, OR = 1.28, 95% CI: 1.08, 1.53, $P < 0.01$), which was consistent with the findings of the IVW method (OR = 1.20, 95% CI: 1.06, 1.37, $P < 0.01$) (Fig. 3; Supplementary Figures S5–S7; Supplementary Table S2). There was significant heterogeneity ($P = 0.02$) in the MR estimates for the SNPs but no evidence of horizontal pleiotropy ($P = 0.09$) (Table 1). The MR-PRESSO test showed no outliers, and the leave-one-out analysis did not suggest that the causal estimates were driven by a particular SNP. The results of the sensitivity analysis are presented in Supplementary Figures S4 and S8.

In total, 81 SNPs for bioavailable testosterone (Supplementary Table 1) were selected as the genetic instruments. The results demonstrated a strong causal relationship between high bioavailable testosterone levels and prostate cancer (Weighted Median, OR = 1.12, 95% CI: 1.01, 1.24, $P = 0.04$; IVW, OR = 1.18, 95% CI: 1.07, 1.29, $P < 0.001$), thereby indicating that bioavailable testosterone levels were a risk factor for PCa risk (Supplementary Figures S9-S10; Supplementary Table S2). There was significant heterogeneity ($P = 3.21 \times 10^{-12}$) but no evidence of horizontal pleiotropy ($P = 0.63$). The MR-PRESSO results indicated the presence of significant horizontal pleiotropy ($P < 0.001$) with five outliers. After removing SNPs, the results showed a higher level of statistical significance (Raw: $P = 7.3 \times 10^{-4}$; outlier-corrected: $P = 2.1 \times 10^{-4}$). No significant distortion was observed in the causal estimates before and after outlier removal (MR-PRESSO distortion test, $P = 0.162$). In all the analyses, the MR-Egger intercept was close to zero with a narrow 95% CI (BMI, intercept = 7.94×10^{-4}, $P = 0.89$; AFS, intercept = 0.01, $P = 0.09$; bioavailable testosterone, intercept = 1.72×10^{-3}, $P = 0.63$).

To investigate the role of PSA screening in these associations, we performed MVMR on BMI and AFS sequentially, using time since the last PSA test as an additional exposure. The MVMR results showed that the potential causal relationship between BMI, AFS, and PCa remained statistically significant after controlling for the PSA test (BMI, $P = 0.0001$; AFS, $P = 0.003$) (Supplementary Table S3). The PhenoScanner search detected associations of IVs with obesity-related traits, which had been chosen as an exposure for MVMR. In the multivariable MR analysis, after controlling for bioavailable testosterone levels, the

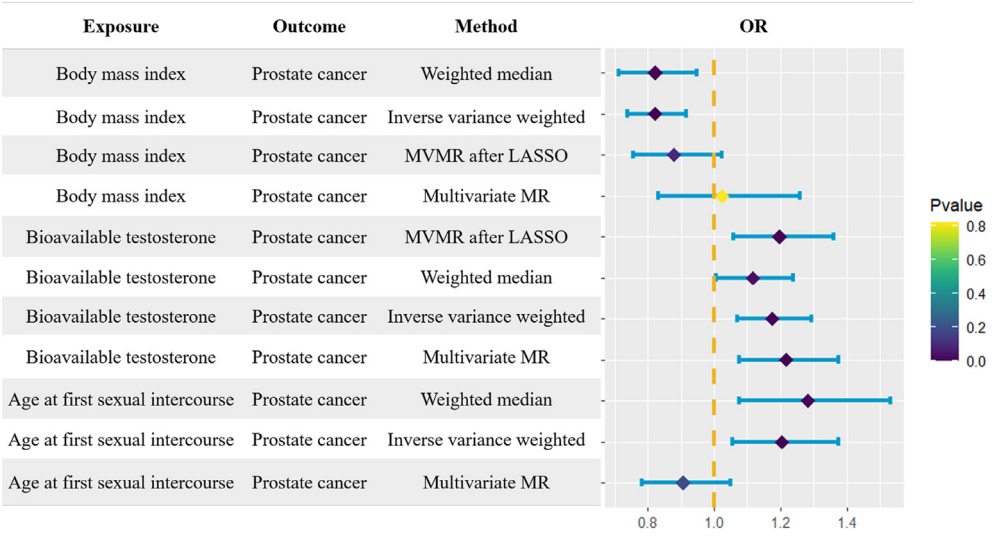

Fig. 3. Forest plot for the effect of BMI, AFS, and bioavailable testosterone levels on PCa. AFS, age at first sexual intercourse; BMI, body mass index; PCa, prostate cancer.

causal impact of BMI ($P = 0.19$) and AFS ($P = 0.82$) on PCa was no longer significant (Fig. 3). The great difference between the univariable and MVMR results suggested that the causal influence of AFS and BMI on prostate cancer could rely on bioavailable testosterone levels.

3.2. Reverse Mendelian randomization findings and mediation analysis

To further explore the causality in the association between exposure and PCa, we also performed reverse MR analyses to assess the effect of PCa on AFS, BMI, and bioavailable testosterone level. The univariable MR results indicated no evidence of a causal effect of PCa on AFS (weighted median, OR = 1.00, 95% CI: 0.99, 1.02, $P = 0.45$), BMI (Weighted Median, OR = 1.01, 95% CI: 0.99, 1.04, $P = 0.19$), or bioavailable testosterone level (weighted median, OR = 1.00, 95% CI: 0.99, 1.00, $P = 0.63$) (Fig. 3; Supplementary Table S2). In all the analyses, there was significant heterogeneity but no evidence of horizontal pleiotropy. The forward and reverse MR analyses demonstrated the unidirectional causal effects of AFS, BMI, and bioavailable testosterone levels on prostate cancer. Based on these findings, we further conducted a mediation analysis to investigate the etiological mechanisms underlying prostate cancer. In the multivariable IVW analysis, after only controlling for BMI, the causal impact of AFS on PCa was also no longer statistically significant (OR = 1.06, 95% CI: 0.90, 1.25, $P = 0.49$). While there is strong evidence that AFS has a protective effect on BMI (weighted median, OR = 0.78, 95% CI: 0.68, 0.89, $P = 3.73 \times 10^{-4}$). The sensitivity analysis revealed significant heterogeneity ($P = 3.26 \times 10^{-5}$) but no evidence of horizontal pleiotropy ($P = 0.26$). Therefore, the impact of AFS on PCa is mediated by BMI. Similarly, there is strong evidence that BMI has a protective effect on bioavailable testosterone (weighted median OR = 0.88, 95% CI: 0.83, 0.94, $P = 1.51 \times 10^{-4}$). The sensitivity analysis revealed significant heterogeneity ($P = 1.07 \times 10^{-15}$) but no evidence of horizontal pleiotropy ($P = 0.81$). In MVMR analysis, after applying LASSO feature selection (AFS was removed), the results showed that the causal effect of BMI on PCa was no longer statistically significant ($P = 0.10$). AFS can be deemed an irrelevant feature as there was collinearity between AFS and other exposures during the MVMR analysis. Drawing from the univariate and MVMR analyses, it was found that the impact of AFS on PCa was mediated by BMI and that the impact of BMI on PCa was mediated by bioavailable testosterone levels. The causal relationship between exposures and outcomes is summarized in Fig. 2.

4. Discussion

In this study, we performed univariable and multivariable MR analyses using genetic variants as unconfounded proxies to explore the causal effects of obesity and sexual behavior on prostate cancer. We found evidence that the genetic liability to lower body mass index, later age at first sexual intercourse, and higher bioavailable testosterone levels was causally associated with an increased probability of prostate cancer. However, in the MVMR analyses controlling for bioavailable testosterone levels, the effect was abolished. The differences estimated by univariable and multivariable analyses indicated that the influence of BMI and AFS on PCa was largely because of the risk factor of bioavailable testosterone levels rather than their direct impacts. The reverse MR analysis showed that this potential causal effect could only be forward rather than bidirectional. In summary, this MR analysis investigated the potential causal effects and pathways of BMI and AFS on PCa, by incorporating bioavailable testosterone levels as a mediator.

4.1. Comparison to previous studies

- Comparison to previous MR studies in PCa. MR has been previously utilized to investigate potential risk factors for PCa that have been reported in several observational studies, including coffee intake,[41,42] smoking,[43,44] alcohol consumption,[43,45] and vitamin E.[46,47] However, the MR results for these factors have always been negative.[48–51] After reviewing these MR studies, we discovered that some did not stratify the exposed population by sex or ancestry. This can result in population heterogeneity, which may cause instability in the results, particularly for two-sample MR, a frequently used method in current MR research.

Furthermore, this is the first study to utilize mediator analysis to investigate risk factors of PCa, which can enhance the understanding of the pathway linking exposure to PCa.

- Comparison to previous findings in risk factors of PCa. Sexual behavior has been proposed to impact the development of prostate cancer through several mechanisms. On one hand, increased sexual activity has been hypothesized to be related to higher androgenic activity, potentially indicating an increased risk of PCa.[15] On the other hand, sexual behavior also increases the likelihood of exposure to sexually transmitted infections, which have been shown to be linked to higher androgenic activity, potentially indicating an elevated risk of PCa. Additionally, another hypothesis, known as the prostate stagnation hypothesis, exists. It suggests that a higher frequency of ejaculation can lower the concentration of carcinogenic substances in prostatic fluid. Therefore, reduced erectile function may be considered a potential risk factor for the development of PCa.[52] The main characteristics of sexual behavior studied are the number of sexual partners, sexual orientation, ejaculation frequency, and age at first intercourse.[14] Regarding the effect of the age of first sexual intercourse on PCa, there has been controversy for a long time.[15,53] Additionally, we conducted a univariate MR analysis on the lifetime number of sexual partners/same-sex sexual partners who ever had same-sex intercourse, but the results did not reach statistical significance (Supplementary Table S7–S9). It is worth noting that the exposure sample exhibited significant heterogeneity, as we were unable to obtain sex-stratified data from available GWASs. We found evidence that the genetic liability to lower BMI was causally associated with an increased probability of PCa. Although the results did not reach statistical significance ($P = 0.10$) using the MVMR method, BMI still exhibited a protective effect for PCa (OR = 0.88). However, previous evidence suggested that the greatest protection against PCa was associated with moderate to high intensities of physical activity.[14] Hence, a decrease of adiposity may also be involved in potential protective mechanisms.[14] What's more, there is growing evidence of the importance of adipose tissue-derived cytokines to the microenvironment, favoring tumor growth.[14,54,55] Thus, more research is necessary to elucidate the relationship between obesity and prostate cancer.

4.2. Interpretation and extrapolation

As noted above, previous studies on the relationship between obesity and PCa incidence are inconsistent. In part, this inconsistency may be due to the differential effect of obesity on low-grade and high-grade cancer. Compared with men with BMI <25, obese men (BMI $\geq$ 30) had an 18% (OR 0.82, 95% CI 0.69,0.98), decreased risk of low-grade PCa (Gleason <7) and a 29% (OR 1.29, 95% CI 1.01,1.67), increased risk of high-grade PCa (Gleason $\geq$ 7) or, alternatively, a 78% (OR 1.78, 95% CI 1.10-2.87) increased risk, defining high-grade cancer as Gleason sum 8 to 10.[56] In other words, obesity increases the risk of high-grade PCa but decreases the risk of low-grade PCa.[56] Additionally, when stratified based on BMI, metabolically obese patients showed a significantly higher hazard ratio than metabolically healthy patients.[57] Metabolically obese individuals typically demonstrate elevated levels of insulin-like growth factor 1 (IGF-1), which has been associated with a higher incidence of PCa. Furthermore, some studies have demonstrated that IGF-1 receptors can transform androgen-sensitive PCa cells into androgen-insensitive ones, facilitating the proliferation and invasion of cancer cells.[58] Miles et al reported that increased serum 25(OH)D was associated with increased PCa risk among men with higher circulating levels of IGF-2 in the placebo arm of the PCa prevention trial study.[59] Therefore, we carried out an additional MR analysis to determine the causal impact of serum 25-hydroxyvitamin D levels on PCa. Unfortunately, the results did not yield statistical significance (Supplementary Materials Tables S10–S12), potentially due to heterogeneity in the study population. Furthermore, adipose tissue dysfunction in obese individuals can disrupt the secretion of adipocytokines and hormonal biosynthesis. One such adipokine, adiponectin, is produced by adipose tissue and released into the circulation, where it acts to inhibit the growth-promoting effects of dihydrotestosterone-activated cancer cells.[60] However, the results did not yield statistical significance in the MR analysis (Supplementary Tables S10–S12). Given the discrepancy between MR analysis and previous clinical observations, it is essential to consider its extrapolation, which will be discussed in the following section.

4.3. Strengths and limitations

The key strength of this study is the ability of MR to provide causal estimates. In the context of mediation, MR provides further robustness to non-differential measurement error in the mediator.[36] MVMR accommodates the joint effects of multiple mediators even in the presence of bidirectional relationships.[36] There are limitations in the study. MVMR analyses have the potential to provide informative insights, but they are prone to bias due to the asymmetric instrument strength. For instance, when there is a substantial disparity in the number and combined strength of instruments for one trait compared to another, the adjustment for the inadequately instrumented trait may yield minimal influence on the MVMR results. Conversely, the opposite scenario may also hold true. Thus, it becomes evident that statistical significance should not be solely relied upon to infer causality, further underscoring the need for caution in interpreting the results. To minimize heterogeneity, we selected SNPs that closely matched the baseline characteristics of our study population, rather than focusing solely on the largest SNPs. The existence of twenty-eight independent instruments for BMI in European males may be considered a relatively low number compared to the recent identification of a larger set of independently associated SNPs through GWAS.[61] Data concerning sexual behaviors may be subject to significant influence from social desirability and reporting biases, resulting in elevated levels of measurement error. Among the subset of individuals who consent to answering questions regarding sexual behavior, bias was expected to be present, as previous research has demonstrated the potential impact of participation bias on GWAS outcomes.[62,63] Additionally, similar to traits heavily shaped by social and cultural factors, sexual behaviors were prone to assortative mating, a phenomenon that may weaken to some extent the fundamental assumption of randomly segregating alleles in MR. Assortative mating may also pose a concern for BMI, but to a lesser degree.[64] This limited number of instruments is likely to introduce bias and explain a small percentage of the variation in BMI. As a result, the instrumental variables may lack statistical power compared to larger SNPs and fail to provide more informative MR analysis. A primary source of potential bias in MR studies is horizontal pleiotropy. We addressed this issue by utilizing a variety of MR methods that consistently yielded results congruent with the primary analysis. Moreover, as previously indicated, we reiterated that the assumptions underlying a causal relationship may not be applicable to specific genetic variants.[9] In practical studies, genetic variants may violate the assumption of having no direct effect on the outcome (Hypothesis 2) or being unassociated with confounding

risk factors (Hypothesis 3), potentially leading to erroneous conclusions due to breaches in internal validity. The importance of considering the external validity of MR in addition to its key aspects should not be overlooked. Unlike randomized trials, MR raises concerns about external validity, which may lead to nonequivalent estimated effects for several reasons, including differences in time scale, canalization, and alternative pathways. The primary influence of genetic variation on average exposure levels is of particular interest to epidemiologists focusing on disease prevention. Given the impracticality of conducting randomized trials that impact everyday exposure levels over a lifetime, MR plays a distinct role in this context. However, it is important to note that MR typically does not provide insight into how individuals respond to acute stimuli, such as sudden and substantial increases in inflammatory biomarkers. While the long-term average exposure levels may not impact prognosis, acute responses can have an effect, indicating that MR methods are not well-suited to evaluate the causal effects of short-term, targeted interventions aimed at specific pathological exposure levels. Additionally, MR may not adequately capture the time-varying nature of exposures, as a single BMI measurement may not accurately reflect an individual's BMI over their lifetime. Furthermore, genetic variation and the intervention typically do not operate via the same mechanism of effect on exposure. For example, while prostate cancer may be linked to obesity, genetic variations in factors such as FTO can impact satiety, thus influencing BMI. Consequently, the impact of an intervention might extend beyond the targeted risk factor, making it challenging to determine the direct causal relationship between BMI and prostate cancer. Moreover, even if both genetic modifications and interventions target exposure specifically, it is plausible that they impact outcomes differently and to varying degrees as they operate within different biological, biochemical, or physiological pathways.[9]

5. Conclusion

In summary, our results suggested a significant influence of bioavailable testosterone levels in the BMI/AFS–PCa pathway. Further work is needed to identify other risk factors and to elucidate the specific mechanisms that underlie this causal pathway.

Author contribution

H.J.D., Y.W., J.Y.W, J.Y.W, Y.L. and F.H.S contributed to the study conception and design, drafted the manuscript and analyzed the data. All authors critically revised the manuscript for important intellectual content, provided administrative, technical, or material support and approved the final version. H.J.D. and Y.W. contributed equally to this article.

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Data availability statement

The data that supports the findings of this study are publicly available GWAS summary data and are available in the supplementary materials of this article.

Conflicts of interest

The authors declare no competing interests.

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The outcomes data for this study were sourced from the IEU OpenGWAS database. The data were downloaded from the following website: https://gwas.mrcieu.ac.uk/. The authors would like to express their gratitude to the individual patients who provided the samples that made the data accessible. Without their contributions, this study would not have been feasible.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.prnil.2024.03.003.

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