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Research article

Insulin-related traits and prostate cancer: A Mendelian randomization study

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ABSTRACT

Investigating the causal relationship between insulin secretion and prostate cancer (PCa) development is challenging due to the multifactorial nature of PCa, which complicates the isolation of the specific impact of insulin-related factors. We conducted a Mendelian randomization (MR) study to investigate the associations between insulin secretion-related traits and PCa. We used 36, 60, 56, 23, 48, and 49 single nucleotide polymorphisms (SNPs) as instrumental variables for fasting insulin, insulin sensitivity, proinsulin, and proinsulin in nondiabetic individuals, individuals with diabetes, and individuals receiving exogenous insulin, respectively. These SNPs were selected from various genome-wide association studies. To clarify the causal relationship between insulin-related traits and PCa, we utilized a multivariable MR analysis to adjust for obesity and body fat percentage. Additionally, two-step Mendelian randomization was conducted to assess the role of insulin-like growth factor 1 (IGF-1) in the relationship between proinsulin and PCa. Two-sample MR analysis revealed strong associations between genetically predicted fasting insulin, insulin sensitivity, proinsulin, and proinsulin in nondiabetic individuals and the development of PCa. After adjustment for obesity and body fat percentage using multivariable MR analysis, proinsulin remained significantly associated with PCa, whereas other factors were not. Furthermore, two-step MR analysis demonstrated that proinsulin acts as a negative factor in prostate carcinogenesis, largely independent of IGF-1. This study provides evidence suggesting that proinsulin may act as a negative factor contributing to the development of PCa. Novel therapies targeting proinsulin may have potential benefits for PCa patients, potentially reducing the need for unnecessary surgical treatments.

1. Introduction

Prostate cancer (PCa) poses a significant global health concern and ranks as the second leading cause of cancer-related deaths in men worldwide. The American Cancer Society reported an estimated 1,414,259 new cases of PCa and 375,304 deaths from the disease worldwide in 2020 [1]. Insulin and insulin-like growth factor 1 (IGF-1) play pivotal roles in prostate carcinogenesis by stimulating cell growth and proliferation through activation of the insulin receptor and the IGF-1 receptor. This activation triggers downstream signaling pathways, such as the phosphoinositide 3-kinase (PI3K) pathway [2–4]. The recruitment of PI3K occurs subsequent to the activation of the insulin receptor substrate, which results from insulin binding to the insulin receptor and facilitates cell survival and proliferation [5,6]. In vitro studies have reported an association between the overexpression of the insulin receptor and cell proliferation, as well as decreased apoptosis [7–9]. In the case of androgen-independent prostate cancer, combined inhibition of PI3K and the androgen receptor, as well as direct inhibition of the IGF-1 receptor, has shown significant effectiveness [10,11]. Preclinical studies have suggested that targeting the insulin/IGF-1 signaling

Abbreviations: MR, Mendelian randomization; PCa, prostate cancer; IGF-1, insulin-like growth factor 1; HOMA-IR, homeostasis model assessment of insulin resistance; MAGIC, Glucose and Insulin-related traits Consortium; FI, fasting insulin; ISI, insulin sensitivity; nonDM, non-diabetic; GWAS, genome-wide association studies; BMI, body mass index; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; PRACTICAL, Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome; IVs, instrumental variables; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; IVW, inverse variance weighted.

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pathway could be a potential therapeutic strategy for PCa [4].

Investigating the causal relationship between insulin secretion and PCa development is challenging due to the multifactorial nature of PCa, which complicates the isolation of the specific impact of insulin-related factors. The majority of studies are observational and susceptible to bias and confounding factors [12–14]. Insulin levels can be influenced by various factors, such as diet, physical activity, and body weight, which can confound the association between insulin and PCa, posing challenges in establishing causality [12]. Additionally, variability in measuring insulin resistance using surrogate markers, such as the homeostasis model assessment of insulin resistance (HOMA-IR), can be influenced by various factors [15,16]. Despite these challenges, several observational studies have suggested a potential link between insulin-related factors and PCa. However, further research is needed to establish causality and identify potential therapeutic targets [2,3,13,14].

Mendelian randomization is a method that employs genetic variants as instrumental variables to investigate causal relationships in observational studies. This approach offers a natural experiment capable of establishing causality and overcoming the limitations of observational studies, including confounders and bias. Furthermore, it can explore the effects of exposures that may be challenging to manipulate in a clinical setting and identify potential therapeutic targets [17]. The counterfactual framework inherent in Mendelian randomization enables the estimation of causal effects by comparing observed outcomes among individuals with different genetic variants. Fig. 1 depicts the workflow of this study.

2. Methods

2.1. Data sources for exposure

Genetic variants associated with insulin production were extracted from the largest genome-wide meta-analysis to date conducted by the

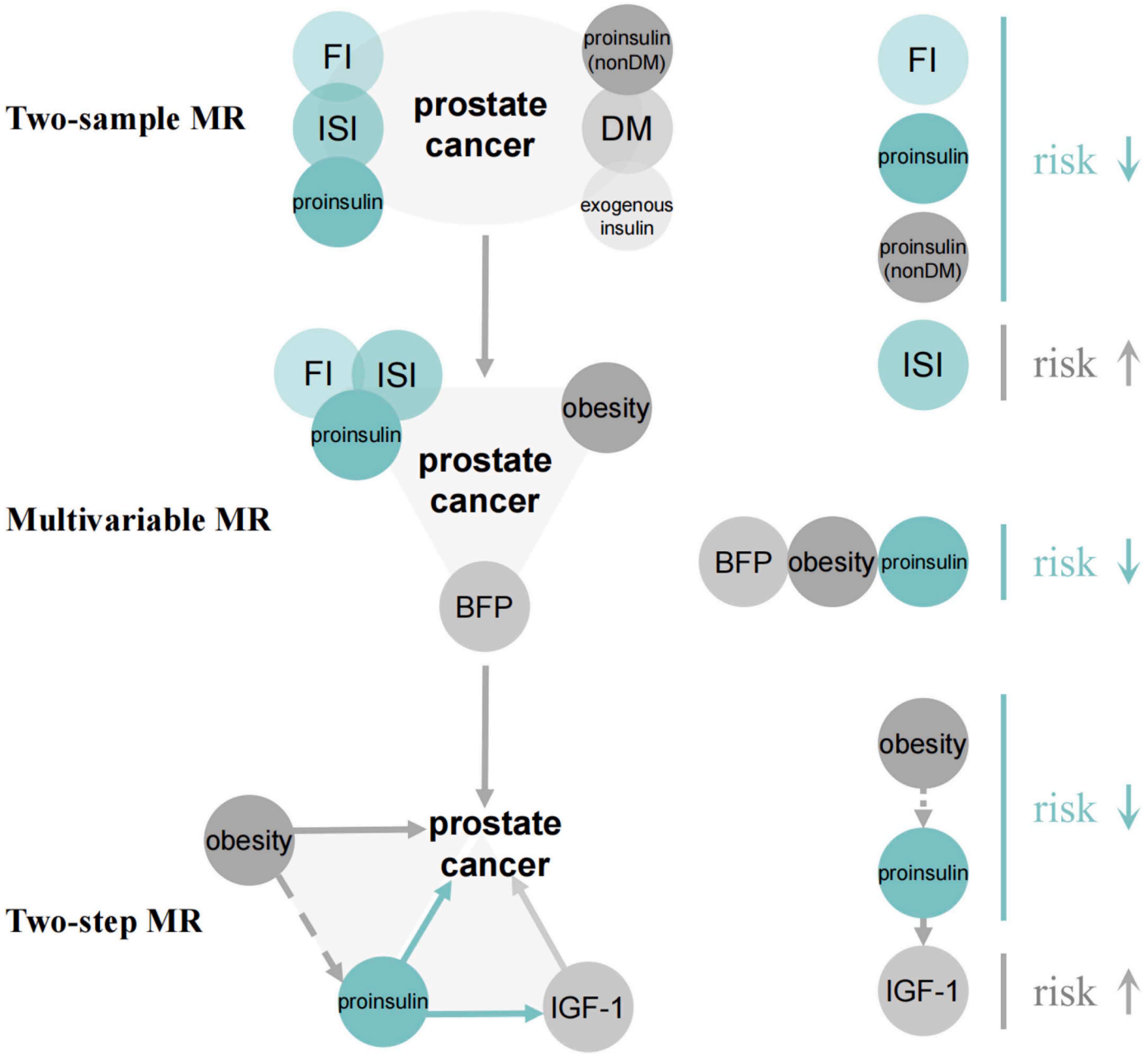

Fig. 1. Workflow diagram. FI: fasting insulin; ISI: insulin sensitivity; BFP: body fat percentage; IGF-1: insulin-like growth factor 1.

Glucose and Insulin-related Traits Consortium (MAGIC) [18]. The MAGIC genome-wide association studies (GWAS) meta-analyses included 151,013 participants for fasting insulin (FI) concentration, 16,735 participants for insulin sensitivity (ISI) evaluation, 45,861 participants for proinsulin concentration, and 10,701 nondiabetic individuals for the proinsulin concentration (nonDM) trait. All participants had European ancestry. The GWAS for the proinsulin trait (nonDM) included only nondiabetic participants. The FI levels were adjusted for body mass index (BMI), study-specific covariates, and principal components [18]. The GWAS for ISI analyzed the combined effects of genotype, adjusted for BMI, and the interaction effect between genotype and BMI on the ISI following the method developed by Manning *et al.* [19]. Proinsulin values underwent natural logarithm transformation and were regressed with age, sex, and population structure [20]. Proinsulin values from nondiabetic individuals were adjusted for fasting insulin, age, sex, and study-specific covariates [21]. Supplemental analyses on diabetes mellitus (DM) and exogenous insulin were derived from the GWASs conducted by the Neale laboratory, which included 55,495 and 62,295 individuals from the UK Biobank, respectively [22]. This study adheres to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (**S1 checklist**).

2.2. Data sources for outcome

The Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome (PRACTICAL) Consortium provided GWAS summary level data for PCa risk, which comprised a total of 79,148 cases and 61,106 controls. All of the individuals in these studies were males of European descent [23]**.** Genotyping was performed using a custom array, namely, OncoArray.

2.3. Selection of genetic instruments

Instrumental variables (IVs) included in the Mendelian randomization study had to demonstrate a significantly strong correlation with exposure. For the analysis of the FI trait, the limitation was defined as $p < 5 \times 10^{-8}$. To ensure that a sufficient number of instrumental variables were present, $p < 1 \times 10^{-5}$ was deemed acceptable when selecting the IVs of the ISI, proinsulin, and proinsulin (nonDM) traits. The necessity of guaranteeing a certain number of IVs stems from the avoidance of bias in parameter estimates due to problems such as weak instruments and low statistical power [24]. Moreover, this study also adopted the MR—Egger method to test potential horizontal pleiotropy due to the loose of screening thresholds for IVs to ensure compliance with the exclusivity constraints of the three principles of the MR method [25].

The threshold of r^2 for clumping was fixed at 0.001 in order to eliminate the IVs with linkage disequilibrium (LD). The F-statistic was utilized to evaluate the significance of the selected instrumental variables in the study, and the F value calculation formula has been validated by previous studies [26].

2.4. Statistical analysis

2.4.1. Heterogeneity and pleiotropy analysis

Cochran Q statistics were employed to quantify the heterogeneity of independent single nucleotide polymorphism (SNP) effects. Directional pleiotropy was identified by MR—Egger, which is the regression of genetic associations with the outcome on genetic correlations with the exposure based on the InSIDE assumption [27,28]. The fact that the MR—Egger intercept was not null or that the P value was less than 0.05 indicated pleiotropy. The results of scanning the IVs selected for this study in the Phenoscanner database revealed that there were associations of IVs with obesity and body fat percentage ($p < 5 \times 10^{-8}$). Therefore, multivariable MR analyses were conducted to examine the causal effect of insulin production-related traits on PCa after adjusting for putative pleiotropy. To analyze the insulin secretion-related traits from a genetic correlation perspective, this study utilized the MTAG method. The MTAG is designed to enhance statistical power in detecting genetic associations by jointly analyzing GWAS results for multiple related traits [29].

2.4.2. Two-sample MR analysis

Two-sample Mendelian randomization is a method for estimating the association of genetic instruments with exposure and outcome based on two sets of GWAS that require their populations to be of the same ancestry and have similar traits [24]. MR is based on three assumptions to ensure the validity of the method, and two-sample MR, as a basic MR method, also follows these three assumptions [25]. First, any of the instrumental variables are strongly associated with the exposure trait under study, which is called the correlation assumption. Second, none of the instrumental variables are associated with traits other than exposure, which is called the independence assumption. Third, genetic variation can only affect the outcome through exposure, which is called the exclusivity constraints assumption.

Common two-sample Mendelian randomization (MR) methods include the random effects inverse variance weighted (IVW) method, MR—Egger, and weighted median estimator. The IVW method served as the primary statistical analysis technique in this study, while MR—Egger and the weighted median estimator were employed as validation methods.

2.5. IVW method

The principles underlying the IVW technique involves weighting each SNP's estimated effect by its precision, which is inversely proportional to its variance. This approach allows the method to generate more precise estimates of causal influence by considering the quality of evidence from each variant. The weighted effect estimates are subsequently merged using a random effects model, which considers heterogeneity. Consequently, the overall effect estimate provided by the random effects IVW method is more robust than that provided by fixed effects models [30,31].

2.6. MR-Egger

In this study, the MR—Egger method was employed to detect the pleiotropy of IVs. It is important to note that the MR—Egger method does not completely eliminate the potential for bias due to horizontal pleiotropy and should be used in conjunction with other MR methods, such as IVW, to obtain a more comprehensive assessment of the causal effect of exposure on an outcome [27,28].

2.7. Weighted median estimator

The weighted median estimator is used to calculate a summary statistic for the effect of a genetic variant on the exposure and outcome. The method involves weighting each SNP by its effect size and using the median of the weighted effects as a summary statistic. This approach is more robust to outliers than other methods that use mean statistics, such as IVW methods [32].

2.8. Multivariable MR analysis

To avoid confounding the causal relationship between insulin-related traits and PCa, we applied a multivariable IVW approach to adjust for obesity and body fat percentage, which were prompted by the Phenoscanner to be associated with IVs. A GWAS involving 18,953 European participants from the FinnGen consortium was used to select the IVs of the obesity trait [33]. In addition, the IVs of body fat percentage were gathered from 354,628 European participants in the UK Biobank

project [22].

Compared to two-sample MR, multivariable MR has three underlying assumptions about instrumental variables that need to be applied to each exposure [34,35]. When a set of SNPs is screened as instrumental variables for multivariable MR, each exposure in the estimation should be strongly predicted by the set of instrumental variables; otherwise, there is weak instrumental bias due to multicollinearity [34]. Multivariable MR avoids the pleiotropic effects that occur in two-sample MR caused by overlap in instrumental variables between one exposure and other exposures by incorporating multiple exposures into the same model for analysis; thus, multivariable MR itself is considered a method for pleiotropy adjustment [34]. However, when there are genetic variants violating the exchangeability assumptions through unknown pleiotropic pathways, multivariable MR—Egger is considered a method that can be used to estimate consistent causal effects [36]. And MVMR-robust which is a new MVMR method that retains correct type I error rates despite the presence of horizontal pleiotropy in certain instrumental variables has been employed in this study to further supplement the validation of MVMR results [37].

2.9. Two-step MR analysis

To better understand the involvement of proinsulin, an insulin-related trait, in prostate carcinogenesis, we conducted two two-step Mendelian randomization analyses. Initially, our goal was to assess whether proinsulin acted as a mediator in the association between obesity and prostate cancer, where obesity served as the exposure, proinsulin served as the mediator, and PCa served as the outcome. Subsequently, we conducted a second two-step Mendelian randomization analysis to investigate whether IGF-1 acted as a mediator in the relationship between proinsulin and PCa, where proinsulin was considered the exposure, IGF-1 was considered the mediator, and PCa was considered the outcome. The methodology for two-step Mendelian randomization and the relevant formulae are detailed in the Supplementary Material.

3. Results

3.1. Selection of instrumental variables and F-test

A total of 36, 60, 56, 23, 48, and 49 SNPs were selected as instrumental variables for fast insulin, insulin sensitivity, proinsulin, proinsulin (nonDM), diabetes, and exogenous insulin, explaining 1.3%, 5.8%, 9.5%, 9.3%, 6.4%, and 5.9% of the phenotypic variance, respectively, with total F-statistics of 55.25, 17.01, 84.08, 47.48, 78.90, and 79.75, respectively.

3.2. Detection of heterogeneity and directional pleiotropy

The sources of all exposure and outcome data are listed in Table S1. Table S2 presents the SNPs included in the two-sample Mendelian randomization of this study, as well as their descriptive statistics in the exposure and outcome GWAS. MR—Egger intercept analysis did not demonstrate multidirectionality in any of the analyses (Table S3). The p values of the intercepts for fasting insulin, insulin sensitivity, proinsulin, proinsulin (nonDM), diabetes, and exogenous insulin were 0.476, 0.433, 0.995, 0.717, 0.057 and 0.376, respectively. None of the p values obtained based on MR—Egger intercept analysis were statistically significant, suggesting the absence of pleiotropy. Using Cochran Q statistics for heterogeneity identification, proinsulin and proinsulin (nonDM) analyses revealed the presence of heterogeneity. The IVW approach has been used as the main analytical approach because of its strength in dealing with heterogeneity between IVs when assessing causality.

3.3. Two-sample MR analysis

Two-sample Mendelian randomization studies have revealed strong associations between genetically predicted fasting insulin levels, insulin sensitivity, proinsulin levels in individuals with diabetes mellitus (DM), and proinsulin levels in nondiabetic patients and the development of PCa (Fig. 2). Fasting insulin, insulin sensitivity, proinsulin, and proinsulin in nondiabetic patients increased by one SD, and the integrated ORs for PCa were 0.781 (IVW 95% CI: 0.627–0.972; P = .026), 1.008 (IVW 95% CI: 1.000–1.016; P = .037), 0.941 (IVW 95% CI: 0.887–0.999; P = .048), and 0.927 (IVW 95% CI: 0.860–0.999; P = .046), respectively. MR—Egger and the weighted median were used to further validate the findings of the univariate MR analysis, and the results are presented in the supplementary tables (Table S4) alongside the IVW results. The beta values from the MR—Egger and weighted median results agreed with the IVW results, indicating a positive or negative association between exposure and outcome, although some of the results were not statistically significant.

To further assess the impact of diabetes and insulin levels on the occurrence of PCa, we examined the causal relationships among diabetes, exogenous insulin, and PCa occurrence. Our investigation revealed that none of these relationships were present, as detailed in the supplementary material (Table S4).

3.4. Multivariable MR analysis

Phenoscanner was applied to detect the traits associated with the selected IVs, and obesity and body fat percentage were identified as possible variables confounding the association between exposure and outcome in the present study. The associations between fasting insulin, insulin sensitivity, and PCa were not significant in multivariable IVW analyses after adjusting for obesity alone, body fat percentage alone, or both obesity and body fat percentage (Table S5). However, proinsulin remained significantly associated with all three conditions, adjusting for obesity alone, body fat percentage alone, or both obesity and body fat percentage, with corresponding composite ORs for PCa of 0.931 (IVW 95% CI: 0.882–0.984; P = .012), 0.947 (IVW 95% CI: 0.903- 0.994; P = .026), and 0.934 (IVW 95% CI: 0.891–0.979; P = .005), respectively (Figs. S1–3**)**. MVMR-robust, as a complementary validation method, also found proinsulin as a protective factor for PCa when performing multivariate analyses of proinsulin, obesity and body fat percentage, but the results were not statistically significant (Table S6). The MR—Egger approach confirmed that this association was significant without horizontal pleiotropy (Table S7).

Correlations between the three traits associated with insulin were present that were validated by MTAG (Table S8). To further validate the causal role of proinsulin in prostate carcinogenesis, a multivariable Mendelian randomization analysis of fasting insulin, insulin sensitivity, and proinsulin was performed. After adjusting for two other indicators related to insulin secretion, proinsulin remained significantly causally associated with prostate carcinogenesis, with an OR of 0.919 (IVW 95% CI: 0.868–0.972; P = .003) (Fig. 3). The MVMR-robust findings for fasting insulin, insulin sensitivity, and proinsulin indicate that while proinsulin is still regarded as a protective factor for PCa, there is no statistically significant relationship (Table S6).

3.5. Two-step MR analysis

We investigated the causal relationship between obesity and PCa with proinsulin as a mediator and between proinsulin and PCa with IGF-1 as a mediator. This approach allowed us to explore the mechanism linking obesity to prostate carcinogenesis and the involvement of IGF-1 in the causal pathway between proinsulin and PCa risk.

According to the two-step Mendelian randomization with obesity as the exposure, proinsulin as the mediator, and PCa as the outcome, we found a significant negative association between trait obesity and PCa

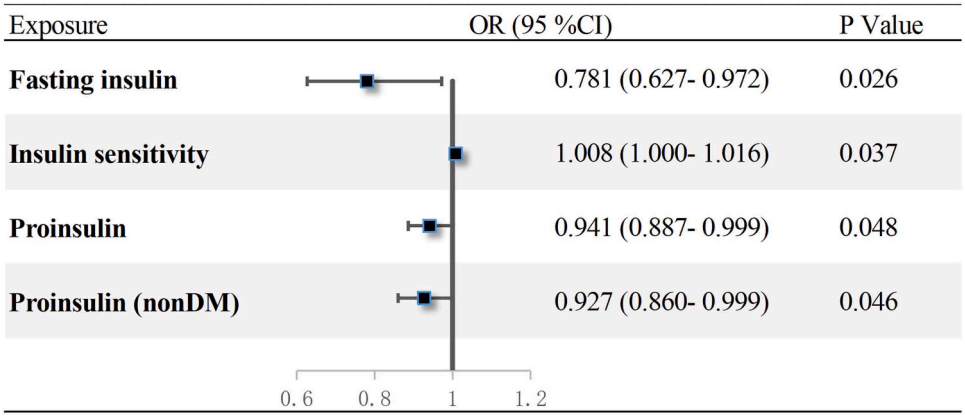

Fig. 2. Estimates for the association of fasting insulin, insulin sensitivity, proinsulin, and proinsulin (nonDM) with risk of prostate cancer. Odds ratios (OR) per SD increment in the exposure from inverse variance weighted analysis.

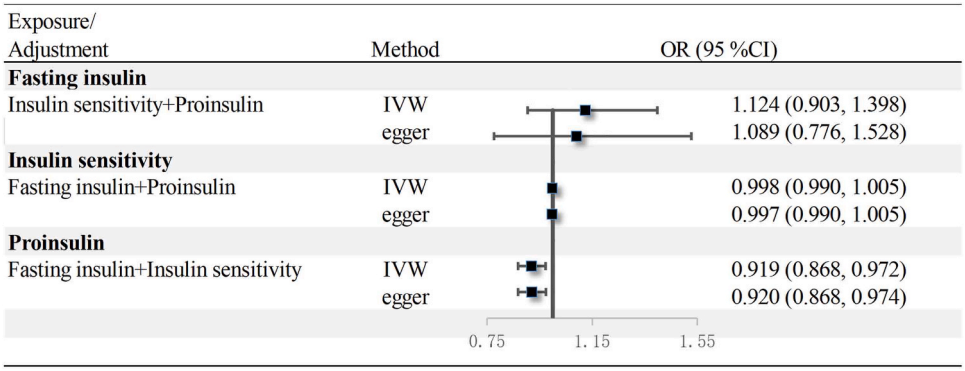

Fig. 3. Multivariable MR analysis results of fasting insulin, insulin sensitivity, and proinsulin. Odds ratios (OR) per SD increment in the exposure from inverse variance weighted analysis or egger analysis. IVW: Inverse variance weighted analysis; egger: Mendelian randomization egger analysis.

risk (OR: 0.937; IVW 95% CI: 0.881–0.997; P = .040) and a significant negative association between proinsulin levels and PCa risk (OR: 0.941; IVW 95% CI: 0.887–0.999; P = .040). However, no association between obesity and proinsulin levels was confirmed (OR: 1.029; IVW 95% CI: 0.965–1.097; P = .376). Therefore, proinsulin did not play a mediating role in the association between obesity and PCa risk (P = .394) (Fig. 4).

According to the two-step Mendelian randomization with proinsulin as the exposure, IGF-1 as the mediator, and PCa as the outcome, a significant negative association was found between proinsulin and PCa risk (OR: 0.941; IVW 95% CI: 0.887–0.999; P = .040), and a significant positive association was found between IGF-1 and PCa risk (OR: 1.012; IVW 95% CI: 1.000–1.024; P = .043). An exploration of the associations between exposure factors and mediators indicated a significant positive association between proinsulin and IGF-1 (OR: 1.218; IVW 95% CI: 1.015–1.460; P = .034). After calculating the total effect of proinsulin in leading to a reduced risk of PCa as 1, the mediating effect of IGF-1 between proinsulin levels and PCa risk was − 0.042, and the direct effect of proinsulin was 1.042 (P = .044) (Fig. 5), with the opposite signs indicating the opposite direction of the two effects. Specifically, proinsulin has a negative effect on PCa, as one of its protective factors, and a positive effect on IGF-1, which in turn has a positive effect on PCa, as one of its facilitators.

4. Discussion

Employing a two-sample Mendelian randomization approach, we found that fasting insulin levels, proinsulin levels, and proinsulin levels in nondiabetic individuals were inversely associated with prostate

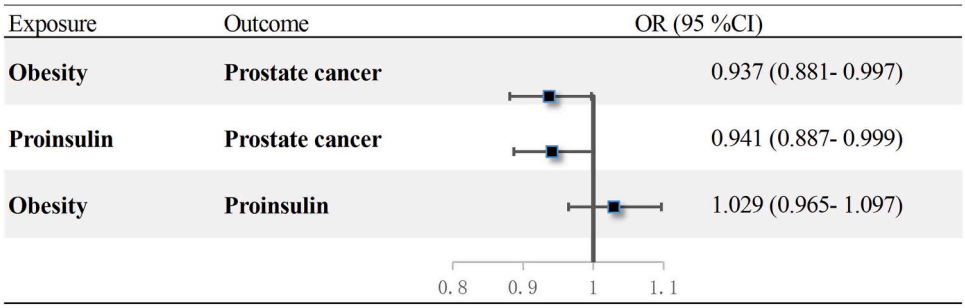

Fig. 4. The results of two-step Mendelian randomization with obesity as exposure, proinsulin as mediator, and prostate cancer as outcome. Odds ratios (OR) per SD increment in the exposure from inverse variance weighted analysis.

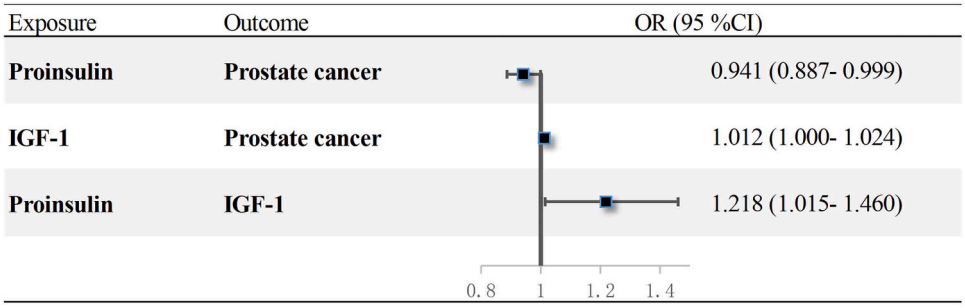

Fig. 5. The results of two-step Mendelian randomization with proinsulin as the exposure, IGF-1 as the mediator, and prostate cancer as the outcome. Odds ratios (OR) per SD increment in the exposure from inverse variance weighted analysis. IGF-1: insulin-like growth factor 1.

cancer (PCa) risk, while insulin sensitivity exhibited a positive association. However, upon conducting multivariable Mendelian randomization adjusted for obesity and body fat percentage, the significance of the associations between all exposure factors and PCa disappeared, except for proinsulin. Moreover, in multivariable Mendelian randomization analyses involving fasting insulin, insulin sensitivity, and proinsulin, proinsulin retained a significant causal association with PCa risk despite adjusting for the other two indicators. Subsequently, two-step Mendelian randomization revealed that proinsulin does not mediate the relationship between obesity and PCa development. Conversely, IGF-1 acts as a mediator in the causal pathway between proinsulin and prostate carcinogenesis, exhibiting an opposing effect on the association between proinsulin and prostate cancer. Proinsulin was negatively associated with PCa risk, while IGF-1 was positively associated with PCa risk. Notably, the effect of proinsulin was significantly stronger than that of IGF-1.

Several experimental and observational studies have examined the association between insulin secretion-related characteristics and PCa. Insulin and IGF-1 are closely related ligands that bind to the insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) families. Signaling occurs via the PI3K-AKT-mTOR and RAS-MAPK pathways upon ligand activation of insulin receptor and IGF-1R, ultimately resulting in cell proliferation and migration and the inhibition of apoptosis [38,39]. Mutations in the PI3K-AKT and RAS-MAPK pathways are prevalent in cancer, and insulin and IGF signaling can activate other pathways and promote cell proliferation [40]. In vitro experiments revealed that high-grade prostate tumors had more insulin receptors on their cell membranes [41], especially the IR-A subtype [42,43], which is consistent with findings from a number of other tumors [44].

Current epidemiological studies indicate that the association between insulin and PCa is ambiguous. In an early retrospective study, insulin levels among PCa patients and the general population were not substantially different, but elevated serum insulin was found in PCa patients older than 65 years of age [45]. The results of our study are consistent with these arguable findings, except that our study focused on the phenomenon of high insulin levels as a protective factor against prostate cancer in a wide range of populations. In a prospective study conducted in 2021, 5929 incident cases of total PCa and 667 cases of fatal PCa were tracked for 28 years, and the results indicated that a hyperinsulinemic diet was not related to total PCa. The connection between a hyperinsulinemic diet and advanced and fatal PCa was only identified after adjusting for variables such as race, height, BMI, and smoking status (HR: 1.07; 95% CI: 1.01–1.15) [46]. Although this phenomenon may conflict with our findings, it is difficult to determine the relationship between insulin-related factors and prostate cancer development through dietary patterns alone, given the relationship between a hyperinsulinemic diet and inflammation development that existed in this study, as well as many other potential confounding factors.

The relationship between insulin sensitivity and PCa was also investigated in this study. Insulin sensitivity was computed utilizing a model created by Manning and colleagues based on the Stumvoll insulin sensitivity index and adjusted for BMI [19,47]. Insulin sensitivity, as measured by the insulin resistance index (HOMA-IR), was thought to be negatively associated with nonaggressive PCa in earlier epidemiological research [48]. This is consistent with the findings of our investigation. According to the univariate Mendelian randomization analysis, there was a significant negative causal link between fasting insulin and PCa. However, the causal relationship disappeared when the two components of obesity and body fat percentage were included together. In the study of insulin sensitivity, its significant causal relationship with PCa similarly disappeared after applying a multivariable Mendelian randomization approach to adjust for two factors, obesity and body fat percentage. This indicates that the effect of insulin on prostate carcinogenesis is regulated by a multitude of other variables.

In our study, proinsulin was examined as a distinct indicator. Based on previous in vitro studies, proinsulin is a prohormone with minimal biological activity that exerts biological effects by binding and activating IR isomers. Proinsulin was discovered to have a greater affinity for the IR-A subtype, a low-specificity receptor with a greater affinity for both insulin and IGF-II, than for the IR-B subtype [49]. The binding of proinsulin to IR-A induces its phosphorylation, leads to sustained ERK1/2 activation and increases the rate of ERK1/2-Akt activation, and exerts a biological effect comparable to that of insulin in promoting mitosis and cell migration. Additionally, experiments conducted on a PCa PC3 cell line revealed that proinsulin and insulin were equally effective at stimulating all downstream kinases. Moreover, the highest absolute levels of IR-A were detected in PCa cell lines compared to breast and smooth muscle tumor cell lines, and in terms of biological effects, the difference in efficacy between proinsulin and insulin in promoting cell mitosis was minimal [49]. This provides more evidence that proinsulin can have an impact comparable to that of insulin in altering the mitosis of tumor cells in prostate carcinogenesis by activating the receptor for IR-A. In this research, proinsulin was found to have a statistically significant causal relationship with PCa in all main analyses, demonstrating its importance in the development of the disease. The IVs associated with proinsulin are also worthy of further investigation. The top SNPs with significant correlation and high statistical power with proinsulin were rs7109575 and rs10501320, whose chromosomal positions involved the genes ARAP1 and MADD, respectively. Previous studies have confirmed the role of ARAP1 and MADD in the regulation of proinsulin secretion [50–52]. Additionally, ARAP1 is also involved in promoting the formation of a special ring-shaped membrane structure rich in F-actin in breast cancer, contributing to tumor migration [53]. In a recent study, MADD was found to bind to the lactylated NCL and potentiate intrahepatic cholangiocarcinoma pathogenesis via the MAPK pathway [54]. The role of these genes in other malignant tumors may provide insights into the mechanisms underlying the causal relationship between proinsulin and prostate cancer.

This study represents an inaugural Mendelian randomization investigation into the correlation between insulin secretion-related traits and PCa. The multifaceted nature of insulin involvement in tumorigenesis is

well recognized. Employing a multivariable Mendelian randomization approach, this research amalgamates interconnected traits in a unified analysis, elucidating their contribution to the relationship between insulin-related characteristics and PCa. Following adjustment for obesity and body fat percentage, two variables were speculated to potentially affect the exploration of the causal link between insulin and PCa, with proinsulin emerging as a trait significantly associated with PCa.

Proinsulin has received less attention than IGF-1 in the past decade. However, our study suggested that proinsulin may constitute a novel and potentially significant factor in prostate carcinogenesis, which is supported by previous in vitro findings. Our subsequent investigations confirmed this assertion. Employing a two-step Mendelian randomization approach, we found that proinsulin does not mediate the causal relationship between obesity and prostate cancer (PCa); rather, it exerts a distinct influence. Conversely, IGF-1 serves as a mediator between proinsulin and PCa, albeit with minimal impact, underscoring the strong association between proinsulin and prostate carcinogenesis.

The findings of this study may have value for drawing attention to proinsulin in subsequent clinical applications and suggesting a potential preventive role for drugs that regulate insulin production and proinsulin levels in the development of prostate cancer. Lifestyle changes or insulin sensitizers, which may influence the level and sensitivity of individuals to insulin, have the potential to reduce the risk of prostate cancer.

There are several limitations to our study. The validity of the MR method is contingent upon its three underlying assumptions. Despite the use of multivariable Mendelian randomization and MR—Egger methods to assess and avoid the presence of IV level multiple effects and heterogeneity, respectively, to satisfy the second and third assumptions, there is no universally acknowledged method that guarantees complete avoidance. Second, our study sample consisted solely of Europeans and was not validated in other populations. Under the assumption that PC or insulin-related traits are regulated by different genetic patterns in different populations, the results of this study can be generalized to the entire spectrum of ethnic groupings. On the one hand, repeating our experimental analyses in populations based on other races is an approach that can address racial heterogeneity. On the other hand, comparing the pathogenic gene patterns of different racial populations through genomic approaches can also address the underlying racial heterogeneity at a fundamental level. In addition, past epidemiological research has revealed a correlation between stratified insulin levels and distinct PCa grades, suggesting the possibility of a threshold effect. Because our investigation was based on summary GWAS results, we were unable to precisely define insulin levels, which may have obscured the causal association between insulin and prostate development.

5. Conclusions

We initiated the first Mendelian randomization (MR) study to explore the link between insulin secretion-related traits and prostate cancer. Two-sample MR analysis revealed a positive association between insulin sensitivity and prostate cancer risk. However, upon adjusting for two confounding factors, namely, obesity and body fat percentage, these associations lost significance, except for proinsulin. Multivariable MR analysis demonstrated that proinsulin remained significantly correlated with prostate cancer risk, even after accounting for fasting insulin and insulin sensitivity. Two-step MR analysis indicated that IGF-1 mediated the causal relationship between proinsulin and prostate carcinogenesis. Our findings suggest that proinsulin may act as a restraint factor in prostate cancer development. Targeting proinsulin with novel therapies could hold promise for prostate cancer patients, potentially reducing the need for unnecessary surgical interventions.

Ethics approval and consent to participate

In each of the studies that provided data for the GWAS meta-analyses, informed consent was obtained from all participants. Our own study adhered to all applicable ethical guidelines, including the Declaration of Helsinki. The local ethics committees responsible for each study included in the GWAS provided ethical clearance for data collection and analysis. Notably, ethical approval was not deemed necessary for our study given the publicly accessible nature of the GWAS data.

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Author Statement

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Guihua Chen: Writing – original draft, Validation, Methodology, Data curation. **Xiang Wang:** Resources, Project administration. **Yi Wang:** Writing – review & editing, Validation, Software, Methodology, Data curation.

Declaration of Competing Interest

We certify that the submission is an original work and this paper has not been published elsewhere in whole or in part. All authors have read and approved the content, and agree to submit it for consideration for publication in your journal. We declare that there are no ethical/legal conflicts involved in the article.

Data availability

The data that underpins the outcomes of this investigation is accessible in both the main article and its supplementary information documents.

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Consent for publication

Not applicable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2024.05.034.

References

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer J Clin 2021;71(3):209–49.
[2] Knuppel A, Fensom GK, Watts EL, Gunter MJ, Murphy N, Papier K, Perez-Cornago A, Schmidt JA, Smith Byrne K, Travis RC, et al. Circulating insulin-like growth factor-i concentrations and risk of 30 cancers: prospective analyses in UK biobank. Cancer Res 2020;80(18):4014–21.
[3] Travis RC, Appleby PN, Martin RM, Holly JMP, Albanes D, Black A, Bueno-de-Mesquita HBA, Chan JM, Chen C, Chirlaque MD, et al. A meta-analysis of

individual participant data reveals an association between circulating levels of IGF-I and prostate cancer risk. Cancer Res 2016;76(8):2288–300.
[4] Chakraborty G, Nandakumar S, Hirani R, Nguyen B, Stopsack KH, Kreitzer C, Rajanala SH, Ghale R, Mazzu YZ, Pillarsetty NVK, et al. The impact of PIK3R1 mutations and insulin-PI3K-glycolytic pathway regulation in prostate cancer. Clin Cancer Res: J Am Assoc Cancer Res 2022;28(16):3603–17.
[5] Li Y, Jiang J, Yang J, Xiao L, Hua Q, Zou Y. PI3K/AKT/mTOR signaling participates in insulin-mediated regulation of pathological myopia-related factors in retinal pigment epithelial cells. BMC Ophthalmol 2021;21(1):218.
[6] Akhtar A, Sah SP. Insulin signaling pathway and related molecules: role in neurodegeneration and Alzheimer's disease. Neurochem Int 2020;135:104707.
[7] Ahearn TU, Peisch S, Pettersson A, Ebot EM, Zhou CK, Graff RE, Sinnott JA, Fazli L, Judson GL, Bismar TA, et al. Expression of IGF/insulin receptor in prostate cancer tissue and progression to lethal disease. Carcinogenesis 2018;39(12):1431–7.
[8] Heidegger I, Kern J, Ofer P, Klocker H, Massoner P. Oncogenic functions of IGF1R and INSR in prostate cancer include enhanced tumor growth, cell migration and angiogenesis. Oncotarget 2014;5(9):2723–35.
[9] Heidegger I, Ofer P, Doppler W, Rotter V, Klocker H, Massoner P. Diverse functions of IGF/insulin signaling in malignant and noncancerous prostate cells: proliferation in cancer cells and differentiation in noncancerous cells. Endocrinology 2012;153(10):4633–43.
[10] Wissmiller K, Bilekova S, Franko A, Lutz SZ, Katsburg M, Gulde S, Pellegata NS, Stenzl A, Heni M, Berti L, et al. Inceptor correlates with markers of prostate cancer progression and modulates insulin/IGF1 signaling and cancer cell migration. Mol Metab 2023;71:101706.
[11] Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, Arora VK, Le C, Koutcher J, Scher H, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 2011;19(5):575–86.
[12] Di Sebastiano KM, Pinthus JH, Duivenvoorden WCM, Mourtzakis M. Glucose impairments and insulin resistance in prostate cancer: the role of obesity, nutrition and exercise. Obes Rev: J Int Assoc Study Obes 2018;19(7):1008–16.
[13] Rowlands MA, Gunnell D, Harris R, Vatten LJ, Holly JM, Martin RM. Circulating insulin-like growth factor peptides and prostate cancer risk: a systematic review and meta-analysis. Int J Cancer 2009;124(10):2416–29.
[14] Rowlands MA, Holly JM, Gunnell D, Donovan J, Lane JA, Hamdy F, Neal DE, Oliver S, Smith GD, Martin RM. Circulating insulin-like growth factors and IGF-binding proteins in PSA-detected prostate cancer: the large case-control study ProtecT. Cancer Res 2012;72(2):503–15.
[15] Kang ES, Yun YS, Park SW, Kim HJ, Ahn CW, Song YD, Cha BS, Lim SK, Kim KR, Lee HC. Limitation of the validity of the homeostasis model assessment as an index of insulin resistance in Korea. Metab: Clin Exp 2005;54(2):206–11.
[16] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27(6):1487–95.
[17] Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. J Am Soc Nephrol: JASN 2016;27(11):3253–65.
[18] Chen J, Spracklen CN, Marenne G, Varshney A, Corbin LJ, Luan J, Willems SM, Wu Y, Zhang X, Horikoshi M, et al. The trans-ancestral genomic architecture of glycemic traits. Nat Genet 2021;53(6):840–60.
[19] Manning AK, LaValley M, Liu CT, Rice K, An P, Liu Y, Miljkovic I, Rasmussen-Torvik L, Harris TB, Province MA, et al. Meta-analysis of gene-environment interaction: joint estimation of SNP and SNP × environment regression coefficients. Genet Epidemiol 2011;35(1):11–8.
[20] Broadaway KA, Yin X, Williamson A, Parsons VA, Wilson EP, Moxley AH, Vadlamudi S, Varshney A, Jackson AU, Ahuja V, et al. Loci for insulin processing and secretion provide insight into type 2 diabetes risk. Am J Hum Genet 2023;110(2):284–99.
[21] Strawbridge RJ, Dupuis J, Prokopenko I, Barker A, Ahlqvist E, Rybin D, Petrie JR, Travers ME, Bouatia-Naji N, Dimas AS, et al. Genome-wide association identifies nine common variants associated with fasting proinsulin levels and provides new insights into the pathophysiology of type 2 diabetes. Diabetes 2011;60(10):2624–34.
[22] UK Biobank GWAS Results. [http://www.nealelab.is/uk-biobank].
[23] Schumacher FR, Al Olama AA, Berndt SI, Benlloch S, Ahmed M, Saunders EJ, Dadaev T, Leongamornlert D, Anokian E, Cieza-Borrella C, et al. Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci. Nat Genet 2018;50(7):928–36.
[24] Tin A, Köttgen A. Mendelian randomization analysis as a tool to gain insights into causes of diseases: a primer. J Am Soc Nephrol: JASN 2021;32(10):2400–7.
[25] Larsson SC, Butterworth AS, Burgess S. Mendelian randomization for cardiovascular diseases: principles and applications. Eur Heart J 2023;44(47):4913–24.
[26] Papadimitriou N, Dimou N, Tsilidis KK, Banbury B, Martin RM, Lewis SJ, Kazmi N, Robinson TM, Albanes D, Aleksandrova K, et al. Physical activity and risks of breast and colorectal cancer: a Mendelian randomisation analysis. Nat Commun 2020;11(1):597.
[27] Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44(2):512–25.
[28] Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. 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 2016;45(6):1961–74.
[29] Turley P, Walters RK, Maghzian O, Okbay A, Lee JJ, Fontana MA, Nguyen-Viet TA, Wedow R, Zacher M, Furlotte NA, et al. Multi-trait analysis of genome-wide association summary statistics using MTAG. Nat Genet 2018;50(2):229–37.
[30] Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. Stat Med 2017;36(11):1783–802.
[31] Bowden J, Del Greco MF, Minelli C, Zhao Q, Lawlor DA, Sheehan NA, Thompson J, Davey Smith G. Improving the accuracy of two-sample summary-data Mendelian randomization: moving beyond the NOME assumption. Int J Epidemiol 2019;48(3):728–42.
[32] Minelli C, Del Greco MF, van der Plaat DA, Bowden J, Sheehan NA, Thompson J. The use of two-sample methods for Mendelian randomization analyses on single large datasets. Int J Epidemiol 2021;50(5):1651–9.
[33] Kurki M.I., Karjalainen J., Palta P., Sipilä TP, Kristiansson K., Donner K., Reeve M. P., Laivuori H., Aavikko M., Kaunisto M.A. *et al*: FinnGen: Unique genetic insights from combining isolated population and national health register data. 2022: 2022.2003.2003.22271360.
[34] Sanderson E. Multivariable mendelian randomization and mediation. Cold Spring Harb Perspect Med 2021;11(2).
[35] Sanderson E, Davey Smith G, Windmeijer F, Bowden J. An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings. Int J Epidemiol 2019;48(3):713–27.
[36] Rees JMB, Wood AM, Burgess S. Extending the MR-Egger method for multivariable Mendelian randomization to correct for both measured and unmeasured pleiotropy. Stat Med 2017;36(29):4705–18.
[37] Grant AJ, Burgess S. Pleiotropy robust methods for multivariable Mendelian randomization. Stat Med 2021;40(26):5813–30.
[38] Gallagher EJ, LeRoith D. Hyperinsulinaemia in cancer. Nat Rev Cancer 2020;20(11):629–44.
[39] Arcidiacono B, Iiritano S, Nocera A, Possidente K, Nevolo MT, Ventura V, Foti D, Chiefari E, Brunetti A. Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms. Exp Diabetes Res 2012;2012:789174.
[40] Werner H, Maor S. The insulin-like growth factor-I receptor gene: a downstream target for oncogene and tumor suppressor action. Trends Endocrinol Metab: TEM 2006;17(6):236–42.
[41] Cox ME, Gleave ME, Zakikhani M, Bell RH, Piura E, Vickers E, Cunningham M, Larsson O, Fazli L, Pollak M. Insulin receptor expression by human prostate cancers. Prostate 2009;69(1):33–40.
[42] Perks CM, Zielinska HA, Wang J, Jarrett C, Frankow A, Ladomery MR, Bahl A, Rhodes A, Oxley J, Holly JM. Insulin receptor isoform variations in prostate cancer cells. Front Endocrinol 2016;7:132.
[43] Aleksic T, Verrill C, Bryant RJ, Han C, Worrall AR, Brureau L, Larré S, Higgins GS, Fazal F, Sabbagh A, et al. IGF-1R associates with adverse outcomes after radical radiotherapy for prostate cancer. Br J Cancer 2017;117(11):1600–6.
[44] Forest A, Amatulli M, Ludwig DL, Damoci CB, Wang Y, Burns CA, Donoho GP, Zanella N, Fiebig HH, Prewett MC, et al. Intrinsic resistance to cixutumumab is conferred by distinct isoforms of the insulin receptor. Mol Cancer Res: MCR 2015;13(12):1615–26.
[45] Albanes D, Weinstein SJ, Wright ME, Männistö S, Limburg PJ, Snyder K, Virtamo J. Serum insulin, glucose, indices of insulin resistance, and risk of prostate cancer. J Natl Cancer Inst 2009;101(18):1272–9.
[46] Fu BC, Tabung FK, Pernar CH, Wang W, Gonzalez-Feliciano AG, Chowdhury-Paulino IM, Clinton SK, Folefac E, Song M, Kibel AS, et al. Insulinemic and inflammatory dietary patterns and risk of prostate cancer. Eur Urol 2021;79(3):405–12.
[47] Walford GA, Gustafsson S, Rybin D, Stančáková A, Chen H, Liu CT, Hong J, Jensen RA, Rice K, Morris AP, et al. Genome-wide association study of the modified stumvoll insulin sensitivity index identifies BCL2 and FAM19A2 as novel insulin sensitivity loci. Diabetes 2016;65(10):3200–11.
[48] Stocks T, Lukanova A, Rinaldi S, Biessy C, Dossus L, Lindahl B, Hallmans G, Kaaks R, Stattin P. Insulin resistance is inversely related to prostate cancer: a prospective study in Northern Sweden. Int J Cancer 2007;120(12):2678–86.
[49] Malaguarnera R, Sacco A, Voci C, Pandini G, Vigneri R, Belfiore A. Proinsulin binds with high affinity the insulin receptor isoform A and predominantly activates the mitogenic pathway. Endocrinology 2012;153(5):2152–63.
[50] Huyghe JR, Jackson AU, Fogarty MP, Buchkovich ML, Stančáková A, Stringham HM, Sim X, Yang L, Fuchsberger C, Cederberg H, et al. Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion. Nat Genet 2013;45(2):197–201.
[51] Li LC, Wang Y, Carr R, Haddad CS, Li Z, Qian L, Oberholzer J, Maker AV, Wang Q, Prabhakar BS. IG20/MADD plays a critical role in glucose-induced insulin secretion. Diabetes 2014;63(5):1612–23.
[52] Kulzer JR, Stitzel ML, Morken MA, Huyghe JR, Fuchsberger C, Kuusisto J, Laakso M, Boehnke M, Collins FS, Mohlke KL. A common functional regulatory variant at a type 2 diabetes locus upregulates ARAP1 expression in the pancreatic beta cell. Am J Hum Genet 2014;94(2):186–97.
[53] Qin X, Zhang Y, He Y, Chen K, Zhang Y, Li P, Jiang Y, Li S, Li T, Yang H, et al. Shear stress triggered circular dorsal ruffles formation to facilitate cancer cell migration. Arch Biochem Biophys 2021;709:108967.
[54] Yang L, Niu K, Wang J, Shen W, Jiang R, Liu L, Song W, Wang X, Zhang X, Zhang R, et al. Nucleolin lactylation contributes to intrahepatic cholangiocarcinoma pathogenesis via RNA splicing regulation of MADD. J Hepatol 2024.