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# Proteome-wide Mendelian randomization and therapeutic targets for bladder cancer

Meng-Hua Wu[1], Min-Heng Zhang[2], Xiao-Dong Hu[3] and Hai-Xia Fan[3*]

## Abstract

**Objective** To identify therapeutic protein targets for bladder cancer (BCa) using Mendelian randomization (MR) and assess potential adverse effects of these targets.

**Methods** A proteome-wide MR study was conducted to determine causal relationships between plasma proteins and BCa risk. In the discovery stage, the plasma proteins (Exposure) were sourced from the R10 of Finnish database, Olink (619 samples across 2925 proteins) and SomaScan (828 samples across 7596 proteins), and Iceland database. In the replication stage, plasma proteins (Exposure) were sourced from the UK-Biobank-PPP database (54,219 participants and 2940 proteins). Summary-level data for BCa (Outcome) were obtained from the UK Biobank (UKB-SAIGE: cancer of bladder) in the discovery phase and the FinnGen consortium (FinnGen R11: cancer of bladder) in the replication phase. Colocalization and fix-effect meta-analyses were performed to validate MR findings. Finally, phenome-wide association study (Phe-WAS) was conducted to explore the side effects of druggable proteins utilizing UKB-SAIGE encompassing 783 phenotypes.

**Results** The MR analysis identified PSCA, LY6D, and SLURP1 as proteins with a genetic association to BCa risk. SLURP1 was confirmed in the replication phase, with a meta-analysis showing an odds ratio of 1.50 (95% CI: 1.30–1.74, $P < 0.001$). Phe-WAS indicated potential side effects for these targets.

**Conclusion** This study provides insights into the causal relationships of plasma proteins with BCa, identifying PSCA, LY6D, and SLURP1 as potential therapeutic targets, with implications for future BCa treatment strategies.

**Keywords** Plasma proteins, Mendelian randomization, Bladder cancer, Drug target, Meta-analysis, Phenome wide association study

## Introduction

Bladder cancer (BCa), which affects the cells that line the urinary bladder, is the tenth most commonly diagnosed cancer globally. In 2020, it accounted for approximately 573,000 new cases and 213,000 deaths. It disproportionately affects men, being the sixth most diagnosed cancer and the ninth leading cause of cancer-related deaths [1]. BCa is commonly identified by the hallmark symptom of painless hematuria, alongside urinary symptoms such as urgency, frequency, and nocturia. Advanced stages can bring pelvic or lower abdominal pain and urinary retention [2]. Current treatments—surgery, chemotherapy, and immunotherapy—target tumor removal and symptom management but lack universal effectiveness and can have severe side effects, failing to prevent recurrence or address the disease's underlying causes [2]. There is an urgent need for more effective strategies to alleviate symptoms, understand the pathophysiology of BCa, and

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ultimately prevent relapse, reduce complications, and improve patients' quality of life.

Recently, Mendelian randomization (MR) has gained prominence as a powerful tool for drug repurposing and the discovery of new therapeutic targets [3]. By leveraging genome-wide association studies (GWAS), single nucleotide polymorphisms (SNPs) influencing protein expression have been pinpointed, termed protein quantitative trait loci (pQTL) [4]. MR employs these pQTL as instrumental variables to explore the potential causality between exposures and outcomes, facilitating the identification of drug targets and biomarkers [5]. Compared to traditional observational studies, MR effectively minimizes the impact of confounding factors, thus providing a more robust evaluation of causal relationships. Conducting a meaningful Mendelian randomization study necessitates careful consideration and interdisciplinary collaboration across various research domains [6]. Additionally, the integration of phenome-wide association studies (PheWAS) enables the prediction of adverse effects related to these targets, further enhancing the precision and safety of therapeutic interventions [6, 7].

Plasma proteins play a crucial role in a vast array of biological processes, including signal transduction, transport, growth, repair, and immune defense [8]. Their dysregulation is a common hallmark in the BCa, underscoring their significance as targets for pharmaceutical intervention. In light of this, we embarked on a proteome-wide Mendelian randomization study aimed at uncovering potential therapeutic targets for BCa [9].

## Methods

### Study design

The study was divided into two phases: a discovery phase to identify plasma proteins linked to BCa and a replication phase for validation, as depicted in Supplementary Fig. 1.

### Plasma Protein Quantitative Trait Loci (pQTLs)-Exposure

Plasma pQTLS were obtained from the following database:

#### *Discovery stage*

FinnGen Database, DF10 v1 proteomics QTL results: SomaScan (828 samples across 7596 proteins) and Olink (619 samples across 2925 proteins) (https://r10.finngen.fi/); Iceland Database: 4907 aptamers in 35,559 Icelanders (PMID: 34857953;).

#### *Replication stage*

UK Biobank-PPP database (54,219 UK Biobank participants and 2490 proteins).

pQTLs are categorized based on their proximity to the corresponding gene. A pQTL near its cognate gene is termed a "cis-pQTL," whereas those exerting effects on other chromosomes or distant loci are "trans-pQTL" [4]. Various studies use different distance thresholds to distinguish cis-pQTLs from intrachromosomal trans-pQTLs, with common thresholds being 500 kb or 1000 kb. In the proteome-wide MR study focused on drug targets, we opted for pQTLs as instrumental variables, applying specific selection criteria. SNPs within ± 1 Mb of the gene region were defined as cis-pQTLs. The 1 Mb threshold is a commonly used standard that balances capturing relevant regulatory variants near the gene while maintaining statistical power. Smaller thresholds could increase specificity, whereas larger ones might capture distal elements. If missing data were present, multiple imputation by chained equations (MICE) with 20 iterations was used, incorporating key variables (SNP genotypes, plasma protein levels, and covariates). Sensitivity analyses comparing original and imputed datasets indicated that the findings were likely robust to missing data handling. These are outlined as the following criteria:

a. SNPs within ± 1 Mb of the gene region (cis- pQTLs);
b. SNPs with plasma proteins with a genome-wide significant threshold of $P < 5^{*}10^{-8}$;
c. A threshold of 0.001 for the linkage disequilibrium parameter (LD) ($r^2$) and a genetic distance of 10,000 kb;
d. F-value > 10 to avoid the bias of instrumental variants [10, 11].

### GWAS statistics of BCa-outcome

Genetic association data for BCa (Outcome) were sourced from:

#### *Discovery stage*

UKB-SAIGE.189.2: 2427 cases and 404,796 controls(https://www.leelabsg.org/resources) (PMID:33,568,819);

#### *Replication stage*

FinnGen R11–Cancer of Bladder: 2574 cases and 345,118 controls (https://r10.finngen.fi/).

### MR analysis (discovery stage and replication stage)

Two-sample MR analysis was conducted by using plasma proteins as the exposure and BCa as the outcome. When only on SNP was available for a particular protein, we applied the Wald ratio method. Conversely, if there were two or more SNPs available, the inverse variance

weighted (IVW) method was employed [12, 13]. False Discovery Rate (FDR) method was used to correct *P*-values, with statistical significance set at *P*_fdr < 0.05 [14]. To control the FDR, we used the Benjamini-Hochberg (BH) procedure, a widely adopted method for multiple hypothesis testing in high-throughput studies [15]. The R package "TwoSampleMR" V.0.5.6 was to perform the MR analysis.

#### Sensitivity analysis

To verify the robustness of the findings, a sensitivity analysis was carried out to assess pleiotropy and heterogeneity. Initially, Cochran's Q test was applied to identify potential heterogeneity. A *P*-value < 0.05 indicated that Cochran's Q statistic reflected heterogeneity among genetic variants. The outcomes were illustrated using funnel plots. Next, MR-Egger intercept tests were utilized to evaluate horizontal pleiotropy, with a *P*-value < 0.05 indicating the presence of pleiotropy in the results.

#### Colocalization analysis

After the MR analysis, a colocalization analysis was conducted to determine whether shared causal variants exist between the exposure (proteins) and the outcome (BCa), thereby supporting the robustness of the MR findings. We analyzed SNPs within a ± 1 Mb range around the gene regions (cis-pQTLs) for colocalization with BCa [16]. Examination on genes with a combined posterior probability of association (PPH3 + PPH4) was equal to or greater than 0.8. Colocalization was examined for genes with a combined posterior probability of association (PPH3 + PPH4) equal to or greater than 0.8, as recommended in recent studies [17–19].This threshold has been used to indicate a relatively strong likelihood of shared causal variants and has been demonstrated to provide reliable colocalization conclusions.

#### Meta-analysis

To combine the results of discovery (the FinnGen consortium) and replication stage (UKBiobank), the fixed-effect meta-analysis was performed. FDR correction was applied to adjust for multiple testing, with significance determined at *P*-value < 0.05. We evaluated the evidence based on the statistical significance, result consistency (FinnGen consortium and UK Biobank), and the effect estimates of meta-analyses [20].

#### Phenome-wide association study

Phe-WAS, also known as reverse GWAS, was employed to investigate associations between genetic variants and a wide range of phenotypes [21]. This method is particularly useful for identifying potential adverse effects related to drug targets [22]. We used the same instrumental variable selection criteria as before and sourced phenotypic data from the UKB database, covering 783 phenotypes (SAIGE). Statistical significance was defined as *P*_fdr < 0.05. To account for potential confounding factors and pleiotropy, we applied several steps to adjust for shared pathways between phenotypes. Cochran's Q test was applied to identify potential heterogeneity. Next, MR-Egger intercept tests were utilized to evaluate horizontal pleiotropy [23].

#### Go-enrichment and KEGG pathway analysis

We performed Gene Ontology (GO) enrichment and KEGG pathway analysis to evaluate the biological significance of PEC subpopulation-specific markers. Using the runGSAhyper function from the piano package (v2.6.0) in R, we applied Fisher's exact test for enrichment analysis. The results were filtered to show the top 20 enriched GO terms and KEGG pathways, focusing on the biological process category.

## Results

#### MR analysis for these plasma proteins associated with BCa

##### *Discovery stage*

Upon strict adherence to the instrumental variables screening criteria in this study, eventually a total of 6018 plasma proteins were included in the MR analysis of the discovery stage. Relevant SNP information can be found in Supplementary Table 1. It is worth noting that, among the subset of three plasma proteins (SLURP1, LY6D, PSCA), the MR analysis, based on IVW or Wald ratio results (*P*_fdr < 0.05), revealed positive causal associations with BCa. (As shown in Supplementary Table 3) The volcano plot (Fig. 1) and forest plot (Fig. 2) showed the visualized details of MR results.

##### *Replication stage*

In the replication stage, we identified one plasma protein (SLURP1), the MR analysis, based on IVW results (*P*_fdr < 0.05), revealed positive causal associations with BCa. Relevant SNP information can be found in Supplementary Table 2 and MR analysis results is shown in Supplementary Table 4.The heterogeneity tests were shown in Supplementary Table 5, indicated minimal heterogeneity across the included SNPs (Cochran's Q test, *P* > 0.05), suggesting that the genetic instruments used in our analysis were sufficiently homogeneous and appropriate for inference.

#### Meta-analysis

We performed meta-analysis of MR results from two sources to further confirm the significant impact of SLURP1, PSCA AND LY6D on BCa. The meta forest figure is shown in Fig. 3. The results indicated significant

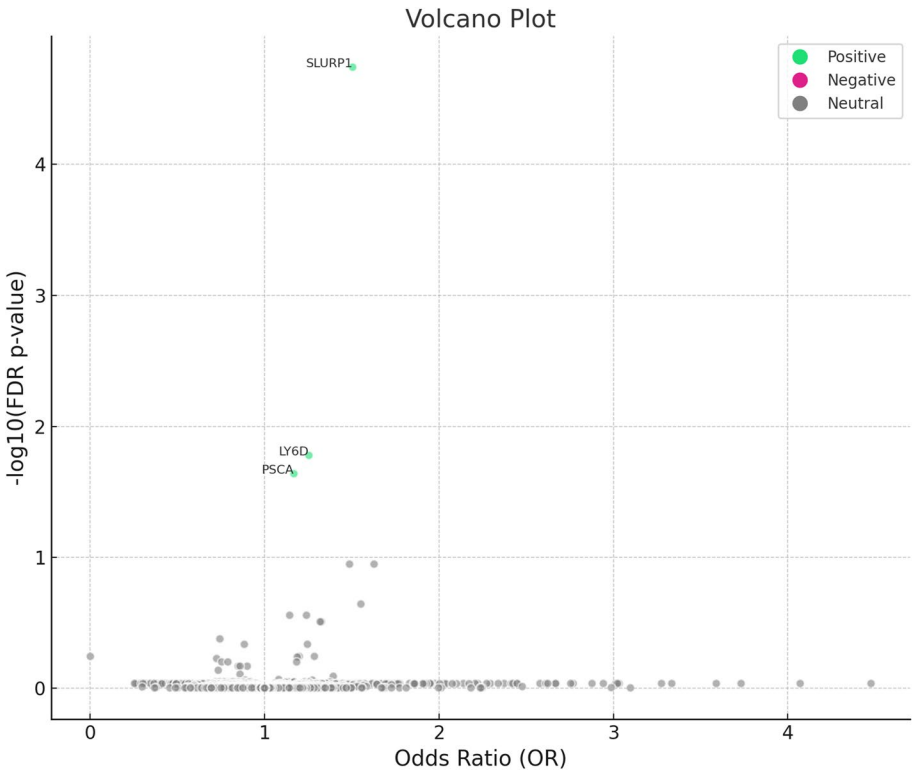


**Fig. 1** Volcano plot of MR results: Causal relationship between plasma proteins and BCa

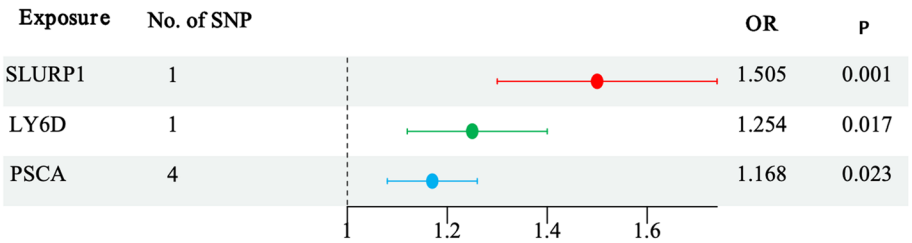


**Fig. 2** Forest plot of the MR results: Effects of plasma proteins on BCa. CI: confidence interval; OR: odds ratio

associations for all three proteins, with SLURP1 showing the strongest association (OR = 1.53, *P* = 5.66e-14), followed by LY6D (OR = 1.31, *P* = 2.35e-04) and PSCA (OR = 1.16, *P* = 1.22e-05). The heterogeneity tests showed minimal variability across the studies for all proteins, suggesting consistency in the findings. These results support the potential causal role of these proteins in BCa risk.

### Sensitivity analysis for these plasma proteins associated with BCa

MR-Egger intercept tests for horizontal pleiotropy, shown in Supplementary Table 6, suggested no strong evidence of pleiotropy ($p > 0.05$), indicating that the observed associations are less likely to be substantially influenced by horizontal pleiotropy, thus supporting the validity of our causal estimates. Additionally, leave-one-out analyses, presented in Supplementary Table 7, showed that the overall results appeared consistent, with no single SNP showing a notable influence. These results from different sensitivity analyses suggest that our findings may be robust and reliable, providing some support for the inferred causal relationships.

Gene colocalization analysis for these 3 plasma proteins within a range of ± 1 MB upstream and downstream of their respective genes was performed to explore potential association with BCa. SLURP1, LY6D and PSCA may share a causal variant with BCa in this region (PPH3 + PPH4 ≥ 0.8). Supplementary Table 8 showed the colocalization analysis results and

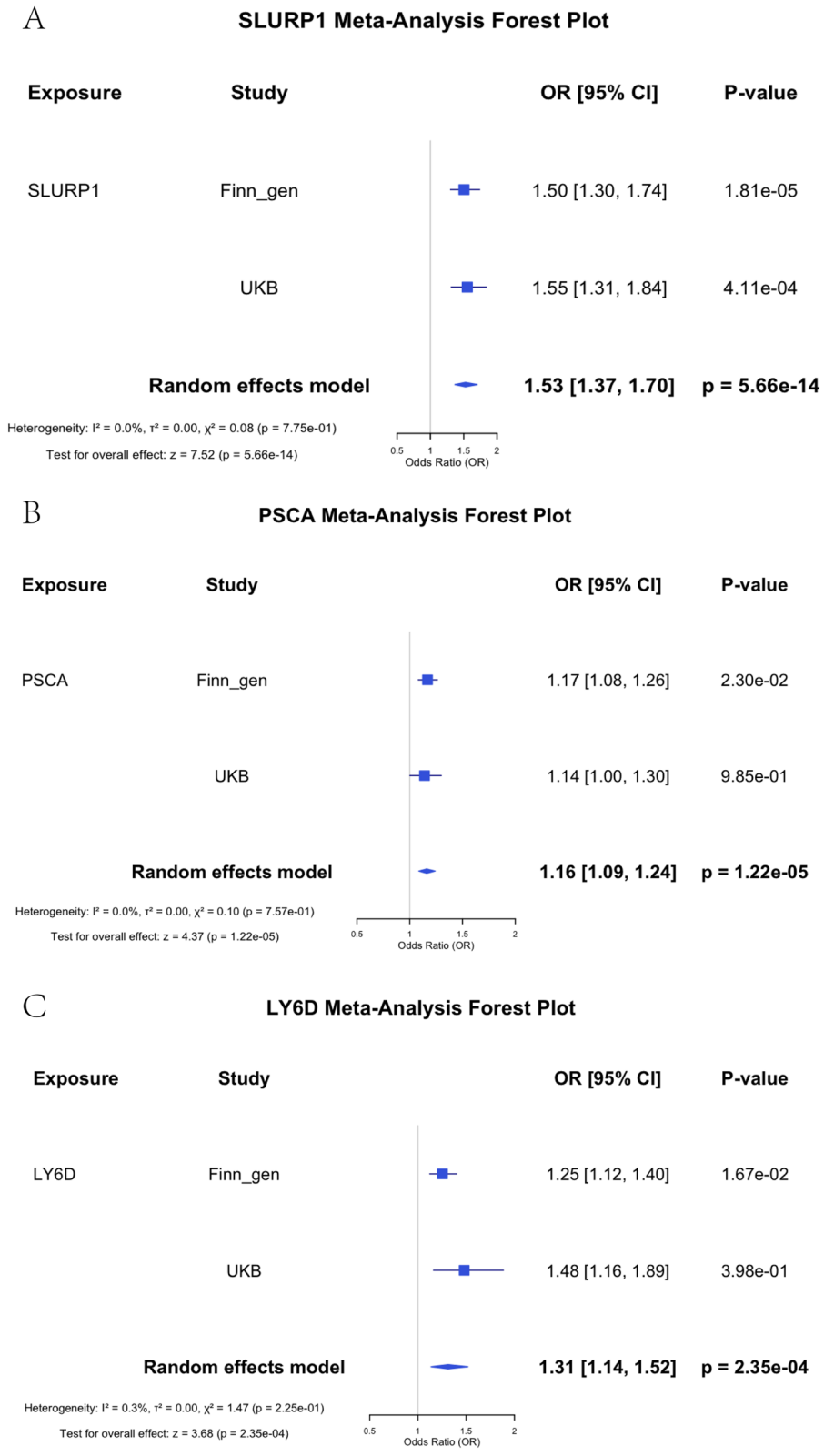


**Fig. 3** Meta-analysis: **A** SLURP1, **B** PSCA, **C** LY6D. OR: odds ratio

suggested that these 3 plasma proteins may serve as potential targets for treating BCa.

### Phenome-wide associations analysis for three plasma proteins linked to BCa

To evaluate the potential beneficial or deleterious effects of the 3 plasma proteins associated with BCa on other phenotypes, we conducted a phenome-wide association analysis, screening 783 phenotypes from UK-Biobank database (SAIGE). We observed significant causal associations between SLURP1 and phenotypes ($P$_fdr < 0.05), and LY6D phenotypes across 4 categories ($P$_fdr < 0.05) and PSCA and 14 phenotypes across 9 categories ($P$_fdr < 0.05). Manhattan plot was shown in Fig. 4, detailed data was shown in Supplementary Table 9. The heterogeneity of Phe-WAS and MR-Egger intercept tests for pleiotropy were shown in Supplementary Table 10 and 11. These phenotypes may be therapeutic objects or deleterious effects for the target protein.

### GO-enrichment and KEGG pathways analysis

The GO-enrichment analysis revealed that the top enriched terms were related to immune regulation, xenobiotic metabolism, and defense response, suggesting that SLURP1, LY6D, and PSCA may play roles in immune modulation and protective cellular responses (Fig. 5). Specifically, the enriched GO terms included "defense response," "xenobiotic metabolic process," and "response to Gram-negative bacterium," indicating potential involvement in cellular defense mechanisms.

The KEGG pathway analysis identified several significant pathways, including "bladder cancer," "Jak-STAT signaling pathway," and "cytokine-cytokine receptor interaction," which suggest potential contributions of these genes to cancer-related mechanisms and immune signaling. Additionally, pathways such as "endocytosis" indicate roles in cellular uptake and signaling. The top 20 enriched GO terms and KEGG pathways are summarized in Supplementary Tables 12 and 13, providing a detailed overview of the key biological processes and pathways associated with these genes.

## Discussion

Bladder cancer(BCa) ranks as the second most prevalent urological malignancy worldwide, with an annual incidence of 549,000 new cases and approximately 200,000 deaths [24]. The advent of new treatment modalities for BCa, notably monoclonal antibodies that target specific

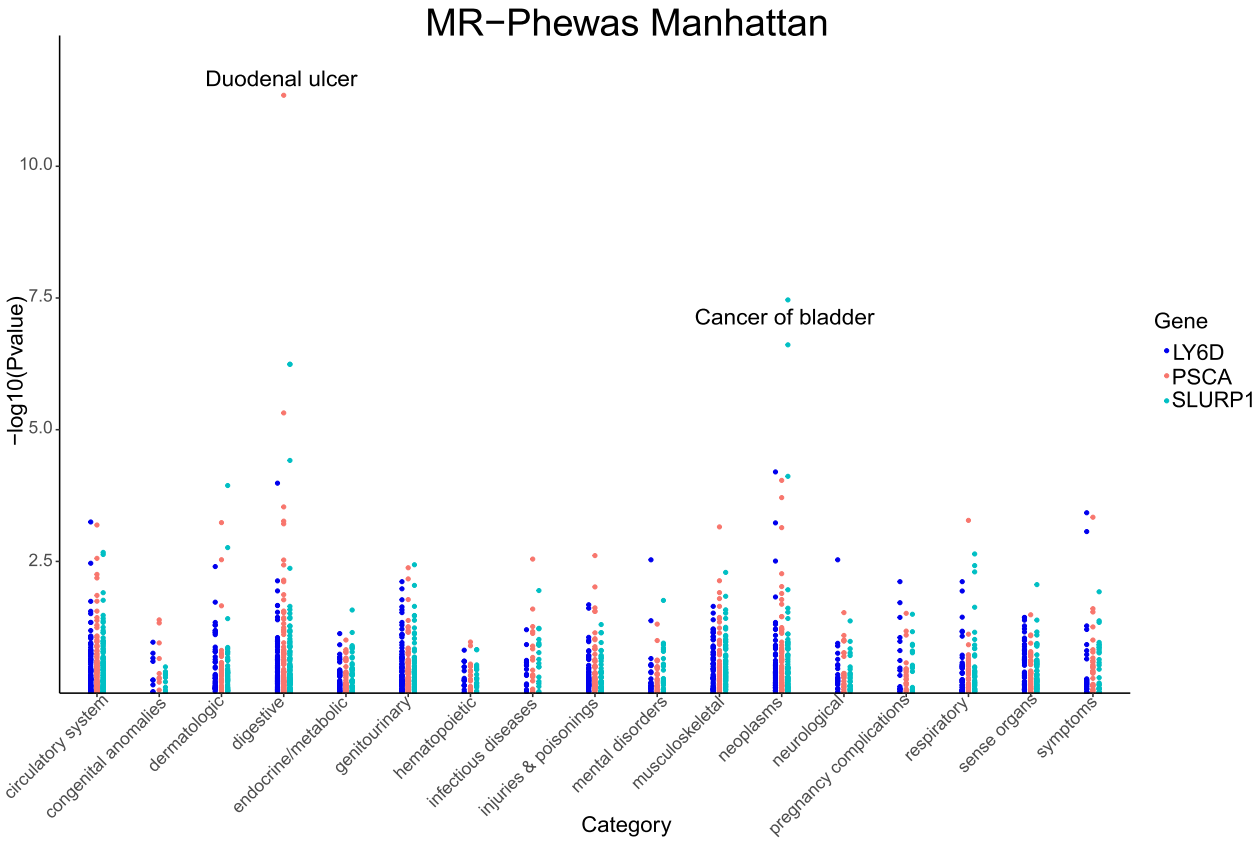


**Fig. 4** Manhattan plot of result of PheWAS analysis of associations between BCa plasma proteins and other disease outcomes

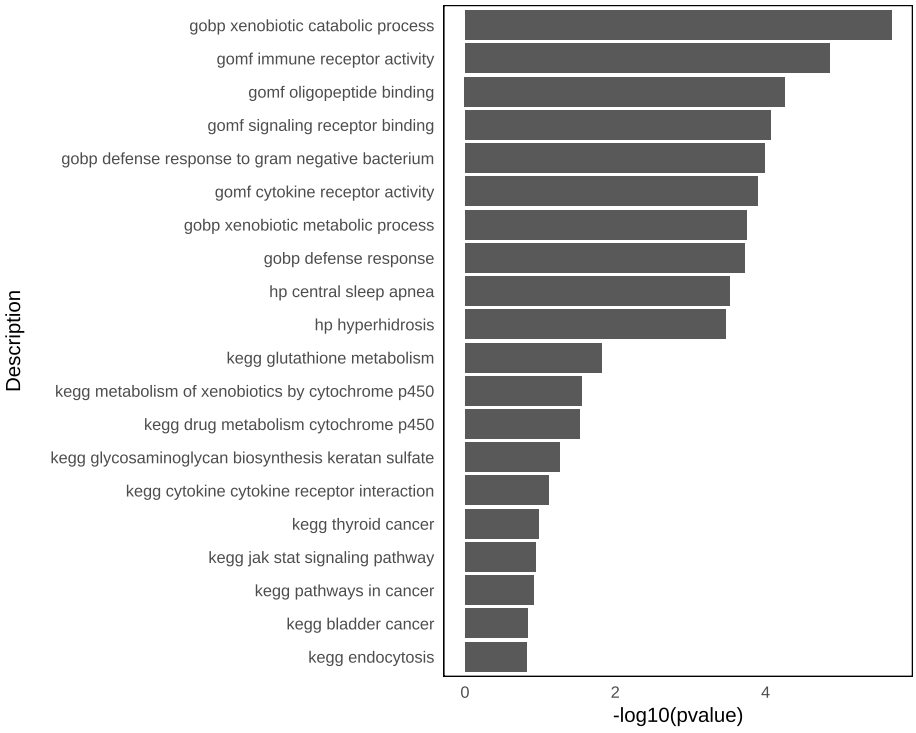


**Fig. 5** Go-enrichment and KEGG pathways analysis

tumor markers, has uncovered key pathological pathways. Yet, less than half of the patients see significant improvement with these targeted therapies. Given that BCa remains incurable, most patients need continuous treatment, which can have side effects, to manage symptoms. Thus, discovering new therapeutic targets is crucial for enhancing patient outcomes.

Plasma proteins with positive correlation with BCa in this study were SLURP1, LY6D and PSCA. SLURP1 (Secreted Ly6/urokinase-type plasminogen activator receptor-related protein 1), as an antagonist of the α7 nicotinic acetylcholine receptor (α7-nAChR), is an immunomodulatory protein associated with inflammation. It mitigates cellular proliferation via receptor interaction and may exert regulatory effects on inflammatory pathways in colon cancer [25], suggesting a conceivable therapeutic relevance in the management of BCa. Our research indicates that SLURP1 is overexpressed, which could serve as a potential biomarker for BCa. In the replication stage, although PSCA and LY6D did not yield significant results, a subsequent meta-analysis suggested that PSCA and LY6D may also show positive significance. Therefore, PSCA and LY6D were included in the PheWAS analysis.

LY6D, a cell surface protein from the Ly6 family, is involved in cell recognition, proliferation, differentiation, and apoptosis. It has been associated with resistance to androgen deprivation therapy in prostate cancer, particularly in LY6D + cells [26]. This resistance is hypothesized to stem from their stem-like characteristics and activation of survival and proliferation pathways like MAPK in the absence of androgens. Targeting LY6D could pave the way for novel treatments to combat prostate cancer cells resistant to existing therapies [27].

Prostate Stem Cell Antigen (PSCA), a Thy-1/Ly-6 family member, is a cell surface protein overexpressed in prostate cancer and implicated in cell adhesion, proliferation, and survival [28]. PSCA is considered a promising biomarker and therapeutic target for prostate cancer due to its high expression levels in the disease [29]. PSCA is also found in bladder tissues, with higher levels in tumors, and its mRNA expression is upregulated in various BCa types, correlating with tumor grade and differentiation, indicating its prognostic significance [30]. Higher PSCA expression correlates with higher tumor grade and poor differentiation, suggesting its potential as an independent prognostic factor for tumor recurrence. The PSCA rs2294008 T polymorphism has been identified as a risk factor for increased BCa susceptibility, with studies showing its association in both Asian and Caucasian populations [31]. Furthermore, the rs2978974 variant is linked to poorer recurrence-free survival in post-surgery bladder and prostate cancer patients, highlighting the

need for further research into its prognostic implications [32].

This proteome-wide Mendelian randomization (MR) analysis, which integrates pQTL and BCa GWAS data, strengthens the causal relationship between plasma proteins and the risk of BCa, offering new perspectives on its treatment. The study's key strengths include the use of MR design to minimize potential confounders and reverse causality bias, the incorporation of cis-pQTLs to enhance the level of evidence (cis-pQTL > trans-pQTL > eQTL), and gene colocalization analyses to improve statistical power, thereby increasing the reliability of the findings. A comprehensive phenotypic association analysis allowed for a deeper investigation into the side effects of potential drug targets. We also employed the PheWAS approach to further explore the potential side effects of drug targets, enriching the field's knowledge base.

Recent advances in multi-omics approaches for BCa, such as integrating DNA methylation, copy number alteration, and RNA-seq data for TMB classification, demonstrate the value of combining data types to refine patient stratification and treatment [33]. Previous studies have also demonstrated the importance of genomic targets, which aligns with the focus of our MR analysis to explore these targets further, particularly involving targets like FGFR3, can provide insights into the progression mechanisms of NMIBC to MIBC and highlight the potential for these targets in therapeutic interventions [34]. Similarly, our MR analysis identified potential drug targets—SLURP1, LY6D, and PSCA—important for BCa progression and treatment. Integrating multi-omics and genetic analysis opens new opportunities for personalized therapies and deeper understanding of BCa biology.

This study aims to offer insights into potential therapeutic targets for bladder cancer (BCa) using Mendelian randomization (MR), though we recognize that further research is needed to validate and expand upon these findings. We acknowledge the five challenges outlined by Burgess et al. [6, 35], which have guided our approach while highlighting areas for improvement. Our research question was carefully defined, though we see the potential to refine it further by exploring specific BCa subtypes in future studies. While we made efforts to select appropriate instruments, we understand that the limited representation of European populations may affect generalizability, and we hope to address this limitation in future work. Although we conducted sensitivity analyses to ensure robustness, we acknowledge that more advanced methods could strengthen our findings. We also interpreted the results with caution, knowing that experimental validation is necessary to confirm causal relationships. Finally, while we reviewed relevant literature, we recognize that a deeper engagement with the broader body of research could enhance the interpretation of our findings. This guidance have been helpful in shaping our study and will continue to guide the design and interpretation of future MR research.

However, the study has several limitations. First, the GWAS data were derived exclusively from individuals of European ancestry, which may limit the generalizability of the findings to other populations [36]. The power of colocalization analysis was also constrained, focusing only on cases where the joint posterior association probability (PPH3 + PPH4) exceeded 0.8 [37]. Practical limitations prevented the incorporation of additional cell-based and animal model experiments at this stage, but we plan to address this in future work or through collaborations [38]. While this study used MR to explore causal relationships between plasma proteins and BCa, we did not employ a genetics-driven drug target prioritization approach, such as the Priority Index (PI), which could uncover additional underexplored targets. Besides, pleiotropy remains a concern, as genetic variants can influence multiple traits, potentially confounding results. While sensitivity analyses, including Cochran's Q test and MR-Egger intercept tests, were conducted, more advanced methods, like multivariable MR, could better address pleiotropy [35]. Finally, incorporating clinical and environmental factors, along with deeper engagement with existing literature, will further refine our understanding and improve the development of targeted therapeutic interventions.

## Conclusion

The statement outlines a research investigation into the causal relationship between three plasma proteins (SLURP1, LY6D, PSCA) and BCa. The goal of this research is to identify new targets for the treatment of BCa. Further studies will be performed deeper into the mechanisms by which these proteins contribute to BCa and how they can be targeted for treatment.

### Abbreviations

| | |
|---|---|
| BCa | Bladder cancer |
| MR | Mendelian randomization |
| IVW | Inverse variance weighted |
| Phe-WAS | Phenome-wide association study |
| GWAS | Genome-wide association studies |
| SNPs | Single nucleotide polymorphisms |
| pQTL | Protein quantitative trait loci |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12894-024-01677-4.

Supplementary Material 1:Supplementary Table 1. SNPs in the discovery stage. Supplementary Table 2. SNPs in the replication stage. Supplementary Table 3. MR analysis results (discovery stage). Supplementary Table 4.

MR analysis results (replication stage). Supplementary Table 5. Heterogeneity tests using Cochran's Q method. Supplementary Table 6. MR-Egger intercept tests for horizontal pleiotropy. Supplementary Table 7. Leave-one-out analyses. Supplementary Table 8. Results of gene co-localization analysis between 3 plasma proteins. Supplementary Table 9. Phe-WAS for Manhattan plot statistics. Supplementary Table 10. The heterogeneity of Phe-WAS using Cochran's Q method. Supplementary Table 11. MR-Egger intercept tests for pleiotropy of Phe-WAS. Supplementary Table 12. Top 20 Go-Enrichment terms. Supplementary Table 13. Top 20 KEGG pathways.

Supplementary Material 2: Supplementary Fig. 1. Flowchart of the study design.

Supplementary Material 3.

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**Declaration of generative AI and AI-assisted technologies in the writing process**
*Statement*: During the preparation of this work the author(s) used ChatGPT 4.0 in order to improve the readability and language of the manuscript in the writing process. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

**Authors' contributions**
Responsibility for the idea's development, data analysis, and manuscript production fell to Meng-Hua Wu. To carry out the experiment, Min-Heng Zhang, Xiao-Dong Hu collaborated. The manuscript was written by Hai-Xia Fan and Meng-Hua Wu, then reviewed by Haixia Fan. The article's submission was reviewed and approved by all authors.

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**Data availability**
The original data that support this paper's conclusions will be provided by the writers in in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

## Declarations

**Ethics approval and consent to participate**
The study involving humans did not necessitate ethical approval in accordance with local legislation and institutional requirements. Participants or their legal guardians/next of kin were not required to provide written informed consent to participate in this study, in line with national legislation and institutional requirements. The study protocol and details were not pre-registered.

**Consent for publication**
Not Applicable.

**Competing interests**
The authors declare no competing interests.

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