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ORIGINAL

CAUSAL INFERENCE OF TESTOSTERONE'S ROLE IN THE ONSET OF MEMBRANOUS NEPHROPATHY: IMPLICATIONS FOR RENAL FUNCTION, PHYSICAL PERFORMANCE, AND SPORTS MEDICINE

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ABSTRACT

Background: Membranous nephropathy (MN) is a chronic glomerular disease characterized by immune complex deposition and thickening of the glomerular basement membrane, leading to renal dysfunction, fluid imbalance, and potential limitations in physical activity and athletic performance. Testosterone, a key androgenic hormone, plays a crucial role in muscle strength, metabolic regulation, and cardiovascular function, but its influence on renal pathology and sports-related physiological adaptations remains unclear. This study aims to investigate the causal relationship between testosterone levels and the onset of MN using a two-sample Mendelian randomization (MR) approach, providing new insights into the intersection of endocrine function, renal health, and physical performance. **Methods:** Publicly available genome-wide association study (GWAS) datasets were analyzed, incorporating case-control data on testosterone levels and MN incidence. Single nucleotide polymorphisms (SNPs) significantly associated with testosterone concentrations were selected as instrumental variables (IVs). Two-sample Mendelian randomization analyses, including Inverse Variance Weighted (IVW) and MR-Egger methods, were used to assess the potential causal effect of testosterone on MN risk. Sensitivity analyses, including heterogeneity tests, pleiotropy assessments, and stepwise elimination procedures, were conducted to ensure the robustness of findings. **Results:** A significant causal association between testosterone levels and MN development was observed. IVW analysis yielded an odds ratio (OR) of 3.02,

indicating that higher testosterone levels are associated with an increased risk of MN, with consistent results across multiple analytical models. Sensitivity analysis confirmed no evidence of heterogeneity or pleiotropic bias, reinforcing the stability of these findings. Furthermore, reverse causality testing revealed that MN does not have a causal effect on testosterone levels, suggesting that the endocrine influence is unidirectional. **Conclusion:** This study provides genetic evidence supporting a causal role of testosterone in the development of MN, highlighting potential implications for renal function, metabolic balance, and physical performance in active individuals. Given the crucial role of testosterone in muscle physiology, recovery, and exercise capacity, understanding its influence on renal health and metabolic adaptation is essential for optimizing training protocols, athlete health monitoring, and prevention strategies in sports medicine. Future research should explore testosterone regulation, exercise interventions, and renal protective strategies to mitigate the impact of hormonal fluctuations on kidney function and athletic performance.

KEYWORDS: Bidirectional, Testosterone, Membranous nephropathy, Two-Sample Mendelian Randomization Study.

1. INTRODUCTION

Testosterone is a crucial androgenic hormone that plays a fundamental role in muscle growth, metabolic regulation, cardiovascular health, and overall physical performance. It is widely recognized for its effects on exercise capacity, recovery, and neuromuscular function, making it a key focus in sports medicine and physical activity research. However, while testosterone is primarily associated with enhanced strength and endurance, emerging evidence suggests that hormonal imbalances may contribute to metabolic and renal dysfunctions, potentially impacting an individual's ability to engage in high-intensity physical activity. One such condition linked to endocrine regulation is membranous nephropathy (MN), a chronic glomerular disease characterized by subepithelial immune complex deposition and thickening of the glomerular basement membrane, leading to proteinuria, kidney dysfunction, and fluid imbalances (Tharakan & Seethalekshmy, 2022; Wu et al., 2021). Given the close relationship between renal function, metabolic homeostasis, and physical performance, understanding how testosterone influences the development of MN is crucial for both athletic health management and clinical nephrology. MN is one of the leading causes of nephrotic syndrome, which can result in systemic inflammation, oxidative stress, and reduced exercise tolerance due to chronic protein loss, muscle wasting, and electrolyte imbalances. Given that testosterone plays a pivotal role in maintaining muscle integrity and metabolic efficiency, any causal link between testosterone levels and MN onset could have significant implications for athletes (Limin et al., 2023), physically active individuals, and patients undergoing exercise-based rehabilitation programs.

While previous studies have explored the role of sex hormones in immune regulation and renal function, there remains a gap in understanding the causal relationship between testosterone and MN pathogenesis (Obana et al., 2006; Ronco et al., 2021). This study aims to bridge that gap by utilizing a two-sample Mendelian randomization (MR) approach to assess whether testosterone has a direct causal influence on the initiation of MN and to explore potential mechanisms through which hormonal variations may contribute to renal dysfunction and its subsequent impact on physical performance. Mendelian randomization (MR) is a genetic epidemiology approach that uses genetic variants as instrumental variables (IVs) to assess causal relationships between exposure (testosterone levels) and outcome (MN development). Unlike observational studies, which may be affected by confounding variables and reverse causation, MR offers a robust method to infer causality by leveraging genome-wide association study (GWAS) data. In this study, we employ a two-sample MR framework, analysing publicly available GWAS datasets on testosterone levels and MN incidence to determine whether testosterone plays a direct role in MN pathogenesis. By integrating genetic insights with clinical and sports medicine perspectives, this research provides a novel understanding of the potential risks associated with hormonal regulation in physically active populations (Auerbach & Khera, 2022; Fehmi et al., 2023). Understanding the relationship between testosterone and renal health is particularly relevant to sports science and exercise physiology, as hormonal fluctuations impact athletic performance, recovery, and overall metabolic resilience. If testosterone is found to have a causal role in MN, it could have significant implications for the management of hormone therapy, performance-enhancing strategies, and renal monitoring in athletes (Hreha et al., 2020; Kanda et al., 2017). Additionally, given that renal dysfunction can limit endurance, hydration control, and muscle function, this study may provide actionable insights for developing targeted interventions to mitigate kidney-related risks in physically active individuals. However, the specific relationship between testosterone and membranous nephropathy and its mechanism of action are not fully understood. To further investigate the effect of testosterone on the pathogenesis of membranous nephropathy, we used the Two-sample Mendelian Randomization (TSMR) method (Gu et al., 2021; Harris et al., 2020). Mendelian randomization employs genotype as an instrumental variable to deduce the causal linkage between exposure factors and outcomes (Li et al., 2022). Due to the random allocation of genetic variations and their independence from confounding factors, they allow for a more accurate inference of the impact of biological factors on diseases. Thus, by selecting gene variants associated with testosterone levels as instrumental variables, we could more accurately assess the causal effect of testosterone on the pathogenesis of membranous nephropathy, thereby avoiding potential confounding factors and reverse causality that might exist in traditional observational studies. Utilizing the two-sample Mendelian randomization

approach, this study seeks to leverage extensive genome-wide association study (GWAS) data, along with bioinformatics and statistical methods, to delve into the possible causal connection between testosterone levels and the development of membranous nephropathy. We hypothesize that if testosterone levels have a causal effect on the onset of membranous nephropathy, then gene variations related to testosterone levels should be significantly associated with the risk of membranous nephropathy. By means of this methodology, we anticipate offering fresh perspectives on the pathogenesis of membranous nephropathy, as well as presenting scientific support for future preventative and therapeutic approaches.

2. Materials and Methods

2.1 Study Reporting Guidelines and Study Design

Publicly available datasets and two-sample MR were utilized to explore the impact of testosterone on membranous nephropathy. The study adheres to the Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization Guidelines, widely recognized as the STROBE-MR Statement, in presenting its findings (Skrivankova et al., 2021). A schematic representation of the study's design is depicted in Figure 1.

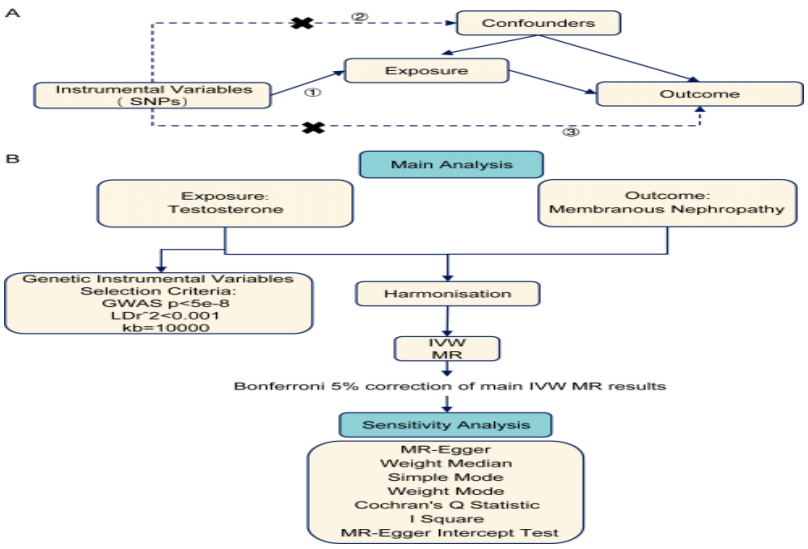


Figure 1: Flow Chart of Mendelian Randomization Analysis.

A. Mendelian randomization analysis rests on several fundamental assumptions: ① representing the association assumption, ② representing the exclusivity limitation; and ③ representing the independence assumption. B. Analytical methods flowchart for this study.

2.2 Data Sources

Testosterone GWAS data: Testosterone (ukb-d-30850_irnt) GWAS data from Neale GWAS analysis Lab, raw data derived from the British bank (UK

Biobank, https://www.nealelab.is/uk-biobank). GWAS data for Membranous Nephropathy: GWAS data for membranous nephropathy (ebi-a-GCST010005) were obtained from a GWAS analysis of 7979 Europeans by Xie J et al. Among them, 2150 membranous nephropathy cases and 5829 control cases were included (Yıldız, 2020).

2.3 Instrumental Variable Selection

A valid genetic variation instrumental variable must satisfy three core assumptions: Firstly, the hypothesis of association, that is, the selected instrumental variable must be significantly related to the exposure factor. Secondly, independence assumption, that is, the instrumental variable must not be significantly related to potential confounders that might affect the exposure or outcome. Thirdly, exclusivity limitation, that is, the instrumental variable could only affect the outcome through the path of "instrumental variable → exposure → outcome". In this study, the criteria for screening instrumental variables for exposure were: SNPs with a P-value less than 5×10^{-8} in GWAS served as the primary screening criterion. SNPs in linkage disequilibrium (defined as SNPS with r^2 less than 0.001 and a physical distance greater than 10000 kb between each pair of genes) were excluded. The selected SNPS were then used to extract instrumental variables from the GWAS of outcome data.

2.4 MR Causal Effect Estimation

Multiple two-sample Mendelian randomization techniques, including Inverse-Variance Weighted (IVW), Mendelian Randomization-Egger (MR-Egger), Weight Median, Simple Mode, and weight mode, were employed to evaluate the causal relationships between exposures and outcomes. Certain studies indicate that under specific conditions, the IVW method exhibits slightly superior performance compared to the others (Bowden et al., 2016); The IVW method is characterized by its exclusion of the intercept term in regression analysis and the utilization of the inverse of the outcome variance as the fitting weight. As a result, in scenarios where pleiotropy is absent, regardless of heterogeneity, IVW serves as the primary MR analysis, complemented by four additional methods. In cases of heterogeneity, the IVW random effects model is applied. In the presence of pleiotropy, the MR-Egger method was employed to compute the results. Additionally, this method was also used to assess reverse causality, exploring potential causal effects of outcomes on exposure.

2.5 Sensitivity Analysis

Sensitivity analysis of the results was conducted through multiple methods, including heterogeneity testing, pleiotropy assessment, and one-by-one exclusion testing, as described below: Firstly, heterogeneity was assessed using the Cochran Q test for SNP estimate variations. A significant Q

test result points to notable heterogeneity, but doesn't reveal its distribution. Hence, to quantify heterogeneity among instrumental variables, the I^2 statistic was used. I^2 values indicate heterogeneity levels: 0 means none, 0-25% is mild, 25-50% is moderate, and over 50% is high. The calculation formula is:

$$I^2 = \frac{Q - df}{Q} \times 100\%$$

Secondly, for the pleiotropy test, the MR-Egger method was utilized to assess the pleiotropy of instrumental variables. Thirdly, a Leave-One-Out test was performed, excluding each SNP in turn and recalculating MR results to assess the impact of individual SNPs on the testosterone-membranous nephropathy association. Significant disparities between MR Effect and overall effect estimations after excluding a specific SNP suggest sensitivity to that SNP.

2.6 Statistical Analysis

All data computations and statistical evaluations were executed utilizing R programming (accessible at https://cran.r-project.org/, version 4.2.2). For Mendelian randomization analysis, the Two Sample MR package was employed (Hemani et al., 2018), the Cochran Q test and Leave-One-Out analysis were employed to assess the durability and dependability of the findings, while the MR-Egger intercept method was utilized for genetic pleiotropy testing. The assessment criteria consisted of Odds Ratio (OR) and 95% Confidence Interval (95% CI). All statistical P-values were bilateral. For SNPs originating from GWAS studies, statistical significance was established at $P < 5\times10^{-8}$. In other statistical evaluations, a P-value less than 0.05 was deemed statistically significant.

3. Results

3.1 Instrumental Variable Screening

Based on our screening criteria, SNPs with linkage disequilibrium were excluded. SNPs associated with testosterone and aligned with GWAS data on membranous nephropathy were selected as instrumental variables. Table 1 shows the number of these variables, emphasizing significant indicators (P. Adj < 0.05) from the MR Analysis.

Table 1: Selection of Instrumental Variables for Testosterone and Membranous Nephropathy

EXPOSURE	NUMBER OF SNPS	MEDIAN OF F	MINIMUM OF F	MAXIMUM OF F
TESTOSTERONE	46	45.28	29.73	1620.05

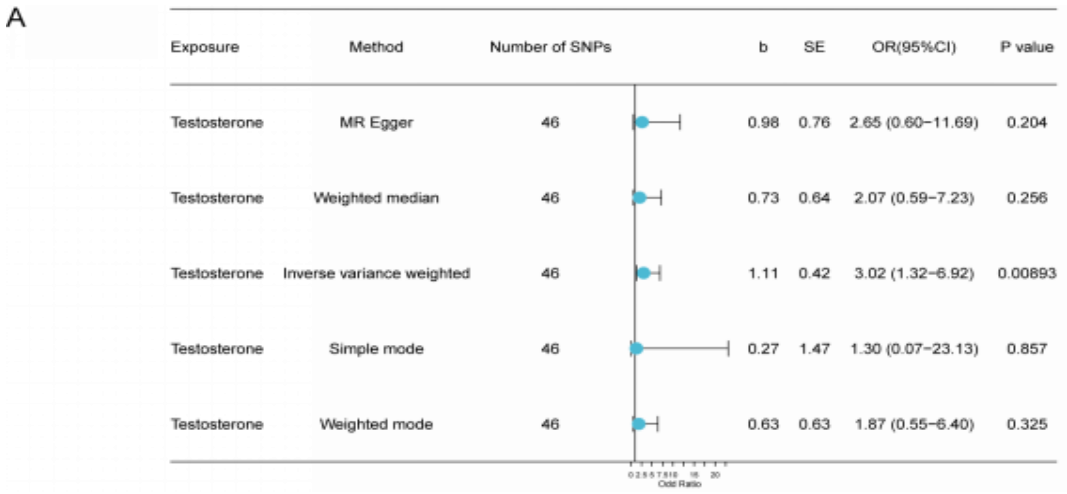
F, F Statistics.

3.2 MR Causal Effect Estimates

A total of 5 models, including MR Egger, Weighted Median, IVW, Simple Mode (SM), and Weighted Mode, were utilized for the analysis. According to the IVW model's findings on the impact of Testosterone on membranous nephropathy (Table 2 and Figure 2A), there exists a significant causal link between Testosterone (OR = 3.02) and membranous nephropathy. Drawing the scatter plots of the five models of testosterone on membranous nephropathy (Figure 2B), it can be seen that the direction of the scatter plot fitting curve of the five models is basically the same, the slopes of most models are relatively consistent, and the intercept of the IVW model is close to 0.

Table 2: Estimates of Mendelian Randomized Causal Effects of Testosterone and Membranous Nephropathy

EXPOSURE	METHOD	NUMBER OF SNPS	BETA.	STANDARD ERROR	P VALUE	OR
TESTOSTERONE	MR Egger	46	0.967	0.757	0.204	2.65
TESTOSTERONE	Weighted median	46	0.725	0.639	0.256	2.07
TESTOSTERONE	Inverse variance weighted	46	1.110	0.423	0.00893	3.02
TESTOSTERONE	Simple mode	46	0.266	1.470	0.857	1.30
TESTOSTERONE	Weighted mode	46	0.626	0.628	0.325	1.87



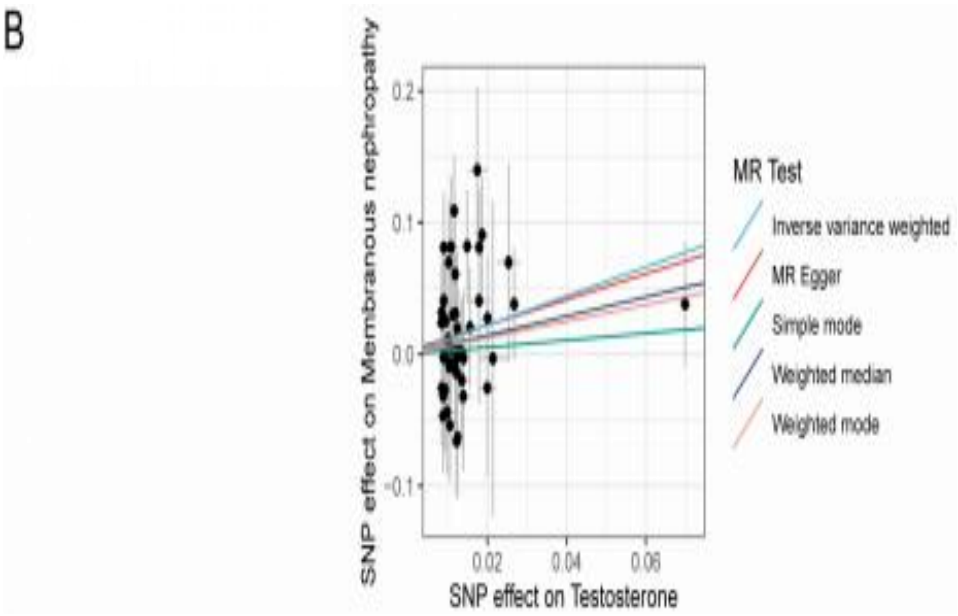


Figure 2: IVW model analysis results for Mendelian randomization analysis of testosterone and membranous nephropathy.

The results of the causal association analysis between testosterone and membranous nephropathy by the IVW Mendelian randomization model are shown in the forest plot. The estimated effect is represented by OR and 95% CI. Additionally, the number of instrumental variables incorporated in each model, along with the determined Beta value and standard error, are displayed. B. A scatter plot illustrates the causal connection between testosterone and membranous nephropathy, where in the slope of the plotted line signifies the strength of the causal association anticipated by various models. b, beta; SE, Standard Error; CI, Confidence Interval.

3.3 Sensitivity Analysis

The heterogeneity of the significant findings was examined using the Cochran Q test and I^2 statistic, as detailed in Table 3. The results showed that there was no high heterogeneity in the MR Results of Testosterone (ukb-d-30850_irnt) for membranous nephropathy (Cochran Q P Value > 0.05, I^2 < 50%). MR-Egger regression was utilized to assess the horizontal pleiotropy of instrumental variables.

The statistical hypothesis test revealed a P-value greater than 0.05 for the intercept term of each index, and the intercept was nearly zero. This suggests that the causal inference in our study was not influenced by horizontal pleiotropy (Table 4). Sensitivity analysis of the results with the use of one-by-one exclusion tests did not show any change in the significance of the testosterone effect estimates, suggesting stability of the results.

Table 3: Mendelian Randomization Analysis Heterogeneity Test for the Association Between Testosterone and Membranous Nephropathy

EXPOSURE	METHOD	Q	Q DF	COCHRAN Q P VALUE	I^2 (%)
TESTOSTERONE	MR Egger	42.5	44	0.573	0
TESTOSTERONE	Inverse variance weighted	42.5	45	0.578	0

Q, Cochran's Q Test Statistic; Q DF, Degrees of Freedom for the Q Test.

Table 4: Mendelian Randomization Analysis of Horizontal Pleiotropy for the Association Between Testosterone and Membranous Nephropathy

EXPOSURE	MR-EGGER INTERCEPT	STANDARD ERROR	P VALUE
Testosterone	2.55×10^{-3}	0.0123	0.837

3.4 Reverse MR Analysis

To evaluate the reverse causal relationship, membranous nephropathy was considered as the exposure variable, while testosterone served as the outcome. SNPS exhibiting linkage disequilibrium were excluded based on the screening criteria established in this study for instrumental variables. The reverse causal MR Analysis results (Figure 3) indicate that membranous nephropathy does not have a significant causal impact on testosterone levels (P value > 0.05). These findings are summarized in Table 5.

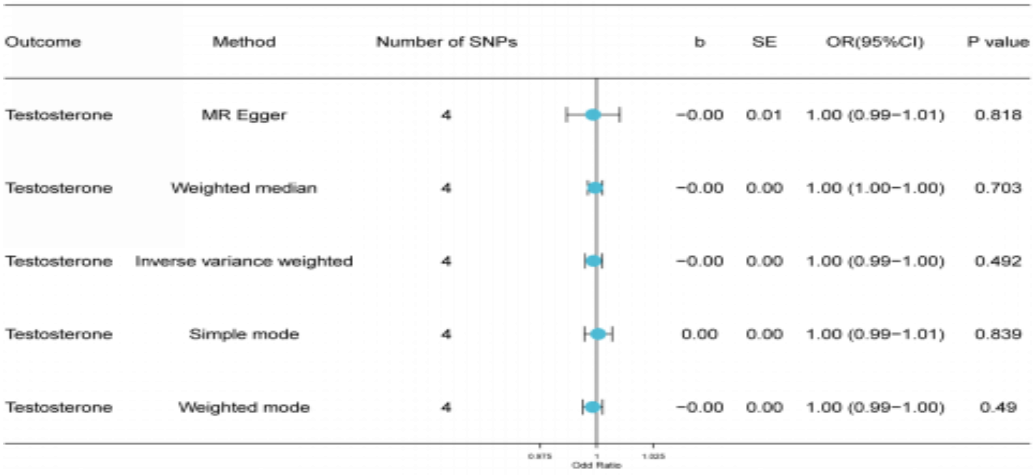


Figure 3: IVW model analysis results pertaining to the reverse causality Mendelian randomization analysis of testosterone.

The forest plots depict the outcomes of the reverse causal association analysis conducted using various Mendelian randomization models on membranous nephropathy and testosterone. The estimated effects are expressed as OR and 95% CI, while the number of instrumental variables incorporated in each model, along with the computed Beta values and standard errors, are also provided.

Table 5: Findings from the Reverse Causal Mendelian Randomization Analysis Examining the Relationship Between Membranous Nephropathy and Testosterone

OUTCOME	EXPOSURE	METHOD	NUMBER OF SNPS	BETA.	STANDARD ERROR	P VALUE
TESTOSTERONE	Membranous nephropathy	MR Egger	4	1.57×10^{-3}	6.01×10^{-3}	0.818
TESTOSTERONE	Membranous nephropathy	Weighted median	4	6.33×10^{-3}	1.66×10^{-3}	0.703
TESTOSTERONE	Membranous nephropathy	Inverse variance weighted	4	1.32×10^{-3}	1.92×10^{-3}	0.492
TESTOSTERONE	Membranous nephropathy	Simple mode	4	6.88×10^{-4}	3.11×10^{-3}	0.839
TESTOSTERONE	Membranous nephropathy	Weighted mode	4	1.75×10^{-3}	2.23×10^{-3}	0.490

4. Discussion

Membranous nephropathy is a severe glomerular disease with epidemiological characteristics showing a rising incidence year by year, affecting 140 million patients annually. Notably, it predominantly occurs in middle-aged and elderly individuals and clinically manifests as nephrotic syndrome, including massive proteinuria, hypoalbuminemia, edema, etc. Furthermore, it is prone to complications such as thromboembolism, thereby posing a significant threat to patients' health. Current treatment options mainly include immunosuppressive agents and symptomatic treatment, which can alleviate the condition, but long-term use of immunosuppressive agents may cause severe side effects, and the efficacy is unstable. Moreover, at present, the precise etiology and pathogenesis of membranous nephropathy are still not completely understood, particularly the influence of testosterone levels and other endogenous factors on its development, which requires further investigation.

Utilizing a two-sample Mendelian randomization method, the aim of this study is to seeks to precisely evaluate the causal association between testosterone and the development of membranous nephropathy through a genetic lens. This will not only help reveal the pathogenesis of the disease but also provide new ideas for clinical prevention and treatment. Considering the limitations of existing research and the urgent need for a deeper understanding of the disease, conducting this study is particularly important. It will provide a scientific basis for precision medicine and individualized treatment strategies for membranous nephropathy, potentially reducing disease incidence and improving patients' quality of life, thus having profound academic and clinical significance.

Ultimately, through this study, we hope to bring new breakthroughs in the diagnosis and treatment of membranous nephropathy, offering new hope to the medical community and patients. In this study, multiple model analyses consistently showed that increased testosterone levels significantly increased the risk of membranous nephropathy. Sensitivity analysis further validated the stability and reliability of the findings by eliminating the impact of confounding variables and instrumental factors. The reverse MR analysis indicated that membranous nephropathy does not have a significant causal impact on testosterone levels, which strengthened the view that testosterone is a pathogenic factor of membranous nephropathy. These findings provide a new perspective for the pathogenesis of membranous nephropathy and emphasize the importance of testosterone levels in the prevention and treatment of membranous nephropathy. According to strict screening criteria, this study selected SNPs that are significantly correlated with testosterone levels as instrumental variables. These instrumental variables have a strong association with testosterone levels and meet the core assumptions of Mendelian randomization, which means that they have no significant correlation with potential confounding factors and only affect membranous nephropathy through testosterone levels. The effectiveness of instrumental variables is the key to MR analysis, and the 46 SNPs selected in this study are shown to have a solid foundation for causal inference (Bowden & Holmes, 2019). The IVW model analysis revealed a significant association between elevated testosterone levels and an increased risk of membranous nephropathy (OR = 3.02, 95%CI: 1.32~6.92, P=0.00893). This finding is statistically significant and further backed by the results obtained from four other MR models, including MR Egger (OR =3.65, P=0.204), weighted median (OR = 2.07, P=0.256), simple model (OR = 1.30, P=0.857), and weighted model(OR = 1.87, P=0.325). Although the effect estimates of these models vary slightly, the overall trend is consistent, which increases our confidence in the causal relationship between testosterone and membranous nephropathy (Birney, 2021). Sensitivity analysis is an important step in evaluating the reliability of MR research results. In this study, various sensitivity analysis methods were used, including heterogeneity testing, level pleiotropy testing, and one-by-one exclusion testing. The heterogeneity test results showed that there is no high heterogeneity in the association between testosterone and membranous nephropathy, which increases the robustness of the results; the level pleiotropy test results showed that our causal inference is not affected by the level pleiotropy of instrumental variables; in addition, the one-by-one exclusion test also did not find significant changes in the effect estimates of testosterone, further supporting the stability of the results (Larsson et al., 2023). In epidemiological studies, reverse causality is a common challenge. When an association between two variables is observed, it is difficult to determine whether one variable causes the change in the other variable or whether both variables are jointly affected by a third

unknown factor (Ho et al., 2022). By conducting a reverse Mendelian randomization analysis, it is possible to assess whether membranous nephropathy might lead to changes in testosterone levels, thereby eliminating this potential reverse causality. The results of the reverse Mendelian randomization analysis showed that membranous nephropathy had no significant causal effect on testosterone levels (P value ＞0.05), indicating that although there is a significant association between testosterone levels and membranous nephropathy, this association is unlikely to be caused by changes in testosterone levels due to membranous nephropathy; instead, it is more likely that abnormal increases in testosterone levels directly affect the risk of developing membranous nephropathy. This conclusion provides strong support for previous direct causal effect analysis results and enhances our confidence in the causal relationship between testosterone and membranous nephropathy (Ference et al., 2021; Guo et al., 2022). Testosterone, as the main male sex hormone, plays an important role in maintaining male physiological characteristics, promoting protein synthesis, and bone growth. However, recent studies have shown that abnormal testosterone levels might be associated with the occurrence and development of various chronic diseases (Morgentaler & Traish, 2020). The low testosterone status might increase the risk of cardiovascular disease by affecting blood lipid metabolism, vascular endothelial function and promoting the formation of atherosclerosis. Testosterone supplementation might help improve cardiovascular health indicators (Di Lodovico et al., 2022); Abnormal testosterone level is also closely related to metabolic syndrome and diabetes. Metabolic syndrome is a group of metabolic disorders characterized by insulin resistance, hypertension, hyperglycemia, hypertriglyceridemia and abdominal obesity (Kelly & Jones, 2013); Low testosterone levels might promote metabolic syndrome and increase diabetes risk by affecting insulin sensitivity, fat metabolism, and insulin secretion (Wittert & Grossmann, 2022; Zolla, 2022). In addition, low levels of testosterone are also associated with an increased risk of osteoporosis; testosterone has a protective effect on bone health, promoting bone formation and inhibiting bone resorption (Dorr et al., 2023). In kidney-related diseases, testosterone deficiency is one of the main endocrine complications of chronic kidney disease, which has profound effects on patients with end-stage renal disease and those undergoing kidney transplantation (Deebel et al., 2024; Romejko et al., 2022). A study on women of childbearing age showed that although testosterone levels cannot independently predict vascular function, the level of testosterone relative to estradiol is associated with vascular relaxation function, which might affect endothelial function in high-risk populations of women with chronic kidney disease (CKD) (Gulamhusein et al., 2024). The testosterone level in patients with CKD is significantly lower than that in non-CKD populations, and patients with low testosterone levels in CKD have a poor prognosis. Timely correction of testosterone levels could prevent and treat the progression of CKD (Leśniak

et al., 2022). Basic research has shown that testosterone could promote the proliferation and differentiation of renal tubular epithelial cells and maintain the integrity of the glomerular filtration membrane; in addition, testosterone could also regulate intracellular signaling pathways in kidney cells, affecting cellular metabolism and immune function (Garibotto et al., 2021; Gryzinski & Bernie, 2022). A 2023 study indicated that androgen testosterone may be a factor that makes men more susceptible to kidney diseases. The study uncovered the mechanism by which androgen testosterone drives renal differences between male and female mice, finding that reducing testosterone levels (through castration or androgen receptor knockout) can "feminize" kidney organs and improve their "elasticity," suggesting a correlation between testosterone levels and kidney disease risk (Du & Xiong, 2023). Additionally, from a clinical practice perspective, incorrect use of testosterone supplements or abnormally elevated testosterone levels may cause a range of side effects, including renal failure, further corroborating the potential connection between testosterone levels and kidney disease (Iglesias et al., 2012). Membranous nephropathy is a glomerular disease characterized by the deposition of immune complexes on the glomerular basement membrane, leading to thickening of the basement membrane (Huang et al., 2022). However, there are currently no studies on the relationship between testosterone levels and model nephropathy. This study is the first to discover that elevated testosterone levels might be an independent risk factor for the development of membranous nephropathy, which has important clinical implications for the prevention and treatment of membranous nephropathy. This is because testosterone, as a sex hormone, has a certain impact on the immune system (Ketchem et al., 2023). Membranous nephropathy is often associated with abnormal immune responses, especially the formation and deposition of immune complexes (Alsharhan & Beck Jr, 2021). By regulating testosterone levels, it may be possible to modulate the immune response, reduce the production and deposition of immune complexes, thereby mitigating kidney damage. Furthermore, testosterone possesses certain anti-inflammatory properties. In membranous nephropathy, the kidneys undergo inflammatory reactions due to immune system attacks. Adjusting testosterone levels may help alleviate such inflammatory reactions, protecting kidney function (Robinson et al., 2022). Some studies suggest a correlation between abnormal sex hormone levels and kidney function damage. Adjusting testosterone levels may contribute to improving kidney function and delaying the progression of membranous nephropathy (Deebel et al., 2024; Lucas-Herald et al., 2017). However, these hypotheses still require clinical validation in the future. For patients with abnormal high testosterone levels, especially male patients, regular renal function tests might help to detect and treat membranous nephropathy early. In addition, decreasing testosterone levels might become a potential treatment strategy for membranous nephropathy.

5. Conclusion

This study provides genetic evidence supporting a potential causal relationship between testosterone levels and the development of membranous nephropathy (MN), highlighting important implications for renal function, metabolic balance, and physical performance. By utilizing a two-sample Mendelian randomization (MR) approach, we identified a significant association between elevated testosterone levels and an increased risk of MN, suggesting that hormonal regulation may influence immune responses, glomerular integrity, and renal health. These findings contribute to a deeper understanding of how endocrine factors impact kidney function, with direct relevance to sports medicine, exercise physiology, and athletic health monitoring. Testosterone plays a pivotal role in muscle growth, endurance, and post-exercise recovery, making it a key determinant of physical performance in athletes and physically active individuals. However, the potential link between testosterone fluctuations and renal dysfunction introduces new considerations for hormone therapy, performance-enhancing drug use, and athlete health management. Given that MN can lead to proteinuria, fluid imbalances, and systemic inflammation, individuals with high testosterone levels—either naturally or through supplementation—may be at an increased risk of renal complications that could impair exercise capacity, hydration regulation, and overall well-being. Understanding these risks is essential for developing targeted strategies to optimize hormone balance while ensuring renal health and athletic performance.

From a sports science and rehabilitation perspective, this study underscores the importance of renal function monitoring in athletes, particularly those undergoing testosterone therapy or supplementation. Routine screening for kidney health markers, hydration status, and metabolic function should be integrated into sports medicine protocols to prevent potential complications related to hormone-induced nephropathy. Additionally, tailored exercise programs and nutritional strategies should be designed for individuals at risk of renal dysfunction to maintain optimal performance without compromising kidney health. Future research should focus on longitudinal studies assessing the impact of testosterone variations on renal adaptation in physically active populations. Exploring how different training modalities, dietary interventions, and recovery strategies influence testosterone-induced metabolic changes could provide practical insights for sports professionals, nephrologists, and endocrinologists. Additionally, further investigation into sex-based differences, genetic predisposition, and the role of immune modulation in hormone-related renal dysfunction will be crucial for refining precision medicine approaches in sports health and exercise rehabilitation. By bridging the fields of genetic epidemiology, endocrinology, and sports medicine, this study contributes to a growing body of research that emphasizes the need for individualized approaches to hormone management and renal protection in athletes and

physically active individuals. Addressing the balance between hormonal optimization and kidney health will be key in ensuring that individuals can safely maintain physical performance, prevent renal complications, and sustain long-term well-being.

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