Submitted: 07 March, 2024 Accepted: 12 April, 2024 Published: 30 October, 2024 DOI:10.22514/jomh.2024.174

ORIGINAL RESEARCH

JOMH Journal of Men's Health

Genetic associations of plasma amino acid levels with male hypogonadism, erectile dysfunction and infertility

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Abstract

Amino acids are involved in male reproductive health. However, a comprehensive survey exploring the role of amino acids in male hypogonadism, erectile dysfunction and male infertility is still lacking. To address this concern, summary-level statistics of 20 amino acids in plasma, testosterone, sex hormone binding globulin, bioavailable testosterone, erectile dysfunction and male infertility were obtained from previously published genome-wide association studies. The genetic correlation between amino acids and male reproductive health was determined by linkage disequilibrium score regression. An inverse variance weighting estimator was employed toprovide causal inference, supplemented by Mendelian Randomization (MR)-Egger, Weighted median, Maximum likelihood, MR. robust adjusted profile score and MR- Pleiotropy Residual Sum and Outlier estimators for sensitivity analyses. The unit of serum amino acids is 1-standard deviation of log-normalized values. The inverse variance weighting estimator found that as per unit increase in serum isoleucine, the concentration of bioavailable testosterone decreased by 0.013 standard deviations (95% confidence interval (CI) = 0.024–0.002, p = 0.021). There was a significant genetic correlation between serum lysine and erectile dysfunction (rg = −0.484, p = 0.025). A one-unit increase in serum lysine corresponded to a 0.93-fold risk of erectile dysfunction (95% CI = 0.88–0.99, p = 0.015). One unit increase in genetically predicted serum levels of isoleucine, leucine and tyrosine corresponded to a 1.08-fold (95% CI = 1.01–1.15, p = 0.030), 1.09-fold (95% CI = 1.02–1.17, p = 0.008), and 1.09-fold (95% CI = 1.01–1.18, p = 0.033) increased risk of male infertility, respectively. Sensitivity analyses confirmed these results. No pleiotropy and heterogeneity were identified. These findings highlight the risky role of plasma amino acids in the occurrence of male hypogonadism, erectile dysfunction and male infertility. Early identification and intervention for plasma amino acids overload or deficiency may be beneficial to male reproductive health.

Keywords

Amino acids; Erectile dysfunction; Hypogonadism; Infertility; Mendelian randomization

1. Introduction

Erectile dysfunction (ED), as well as hypogonadism and infertility, has long been a focal point of concern in male sexual health [1]. Studies indicate a rising trend in ED prevalence with increasing age, affecting more than 70% of men aged over 70 years [2, 3]. Notably, rates of ED surpass 5%, even among men younger than 40 years old [2, 3]. In China, the reported prevalence of ED ranges from 19.5–28.5% in the general population [4–6], while a nationwide survey in Singapore reported a prevalence rate of 77% among males aged >70 years [7]. Age-related hypogonadism lacks a definitive definition, with some defining it based on symptomatic presentation and low testosterone levels [8, 9]. The frequency of hypogonadism also increases with advancing age, ranging from 6.0% to 38.7% in men aged 40 and older [1]. Specifically, in Asian nations, the prevalence of hypogonadism, defined by total testosterone levels below 11 nmol/L, has been reported to range from 18.2% to 19.1% [10]. These figures are poised to witness an upward trend as the global population continues to age. Infertility affects approximately 12% of men, and sexual dysfunction is a common issue among men of reproductive age, contributing to infertility in a subset of cases [11]. Among infertile men, low libido and lack of sexual satisfaction are the most prevalent sexual dysfunctions, with a prevalence ranging from 8.9% to 68.7% [11]. ED and/or premature ejaculation affect one-sixth of infertile males, while orgasmic dysfunction affects one-tenth of them [11].

It is evident that ED [1], hypogonadism [1] and infertility [11] negatively impact male health. Research conducted in Asian countries indicates that ED is linked to diminished

quality-of-life, particularly in the mental and vitality domains [1]. Moreover, ED is intricately linked to an array of physical comorbidities, including but not limited to diabetes mellitus, cardiovascular diseases, obesity and prostate hyperplasia, as well as psychological issues such as depression [12]. In infertile men, ED isas a separate risk factor contributing to a reduction in the frequency of sexual intercourse, thereby negatively impacting fertility [11]. However, the incidence of ED increases with age along with its associated comorbidities such as hypogonadism, making it challenging to independently illustrate a distinct correlation between ED and infertility [11]. While data on hypogonadism are limited, available evidence suggests its association with various aspects of quality-of-life scores and depression [1]. Male infertility is also associated with a decline in overall health [13]. In fact, males in infertile couples have a higher prevalence of both malignant [14–17] and non-malignant [13, 18] diseases than age-matched males without couple infertility. Thus, identifying the risk factors for ED, hypogonadism, and male infertility remains requisite and may be beneficial for the prevention and treatment of male reproductive-related disorders.

Amino acids are essential constituents of proteins and play versatile roles in regulating cellular processes [19]. They serve as substrates for protein synthesis, act as precursors for various metabolic pathways, and participate in signaling cascades that govern cell proliferation, differentiation and growth [20]. These functions are exerted either directly or through their derivatives, and are intricately linked to male reproductive health [20, 21]. For instance, arginine, the sole physiological substrate of nitric oxide (NO) synthase, facilitates penile arterial vasodilation by increasing the release of NO, thereby contributing to penile erection and serving as a potential therapeutic alternative for ED [21, 22]. Normal sperm morphology and vitality are pivotal factors in assessing male infertility, with studies revealing a positive correlation between these parameters and certain amino acids in seminal plasma (SP) [23]. Notably, Xu *et al.* [24] identified a positive association between abnormal sperm morphology and the levels of leucylproline, valine, glutamyl-arginine and hypoxanthine. Similarly, research by Amirjannati *et al.* [23] highlighted a positive correlation between amino acids, including proline, phenylalanine and leucine and slow progressive sperm motility. Additionally, the total antioxidant capacity (TAC) of SP plays a significant role in male infertility. Studies have shown a positive correlation between tyrosine and TAC [25]. Regarding sperm concentration, studies have found a negative correlation between glycine, serine, isoleucine and lysine, while certain proteinogenic amino acids, including tryptophan, phenylalanine and alanine, are positively correlated with sperm concentration [23].

While some studies hint at links between amino acids and male reproductive health, research has focused primarily on semen rather than plasma levels [23]. However, plasma amino acid concentrations more accurately reflect overall metabolic and nutritional status and can influence reproductive health more broadly [20, 26]. Nevertheless, comprehensive research on the exact roles of these amino acids in conditions such as male hypogonadism, ED and male infertility is lacking. Novel methodologies are required to thoroughly elucidate the intricate relationship between plasma amino acids and male reproductive health. Although randomized controlled trials are regarded as the gold standard for establishing causality, their execution is often impeded by exorbitant costs or ethical constraints [27]. Recently, numerous robust causal inference techniques have been developed to address the constraints inherent in observational studies. Among these methods, linkage disequilibrium score regression (LDSC) stands out as a prominent approach for genetic correlation analysis [28]. LDSC enables the assessment of genetic correlations between plasma amino acids and conditions (*i.e.*, hypogonadism), although determining causal relationships remains challenging. Mendelian randomization (MR) offers another valuable tool for inferring causality by leveraging genetic variants strongly linked to an exposure variable as proxies, thereby estimating their effects on an outcome variable [29]. This approach allows researchers to better control for confounding factors and reduces the risk of reverse causality [30]. Sensitivity analyses can be employed to evaluate potential horizontal pleiotropy, where genetic variants directly impact the outcome rather than through the exposure [31].

Based on summary-level statistics from Genome Wide Association Study (GWAS), we explored the genetic associations of plasma amino acids with male hypogonadism, ED and male infertility. The findings of this study are expected to fill existing knowledge gaps and provide new scientific evidence and clinical guidance for the prevention and treatment of male reproductive-related disorders.

2. Materials and methods

2.1 Research design and study samples

To discern the genetic associations of plasma amino acid levels with hypogonadism, ED and male infertility, we employed summary-level statistics of traits for further MR and genetic correlation analyses. As shown in Fig. 1, the genetic variants should satisfy three basic assumptions of MR, which is a precondition for yielding solid causal inferences. First, it is imperative that the derived instrumental variables (IVs) exhibit a strong correlation with the traits. Only then can it be used to substitute the exposures (*i.e.*, plasma amino acids) and outcomes (*i.e.*, hypogonadism). Second, IVs can only affect outcomes *via* exposure rather than other traits. Finally, it is crucial that the IVs remain uncorrelated with any confounders, thereby mitigating bias arising from pleiotropy.

The genetic associations of plasma amino acid levels were obtained from Chen Y *et al.* [32]. The plasma was obtained from 8299 European individuals. Individuals with first- or second-degree relatives were removed to reduce bias. The levels of circulating plasma amino acids were measured by the Metabolon HD4 platform. The genotypes of the included individuals were sequenced *via* the Affymetrix Axiom genotyping platform. The plasma amino acid levels underwent a natural logarithmic transformation, followed by standardization to a mean of 0 and a standard deviation (SD) of 1. Detailed information on these data sources can be found in Table 1.

Summary-level statistics for hypogonadism (total testosterone, bioavailable testosterone (BAT), and sex

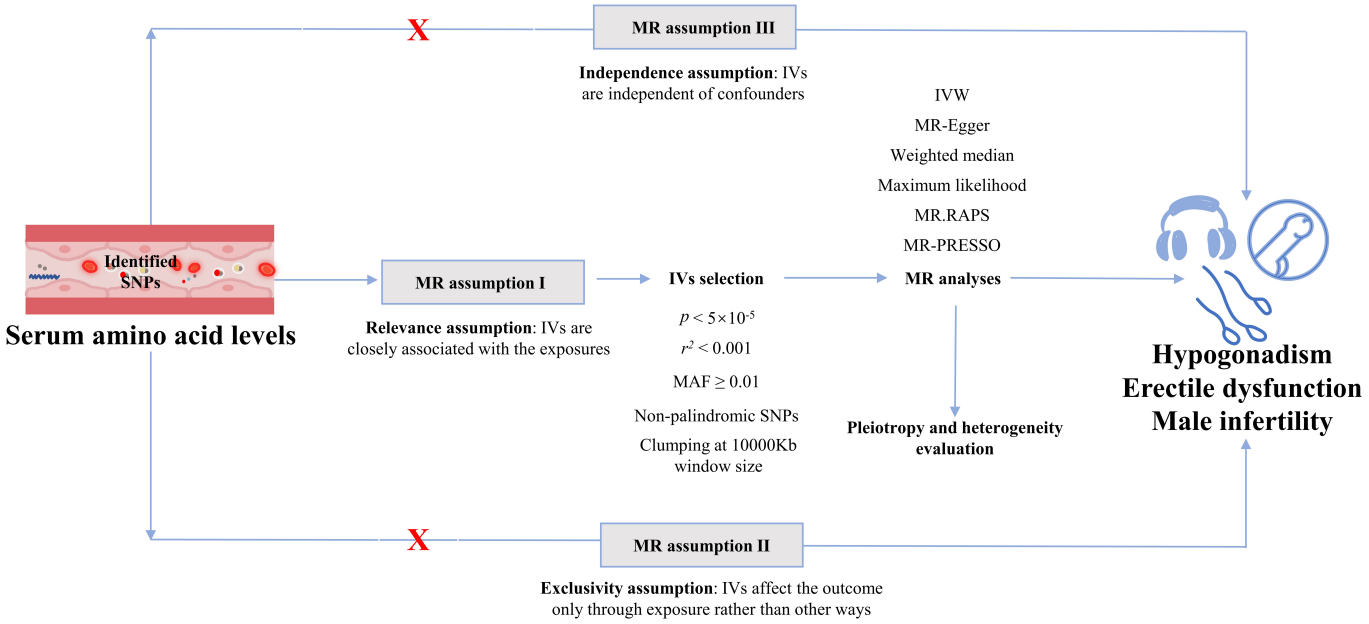


FIGURE 1. Overview of the study design and analysis strategy. IVW: inverse variance weighting; IV: instrumental variables; MR: Mendelian randomization; MR-PRESSO: MR Pleiotropy Residual Sum and Outlier; MAF: minor allele frequency; RAPS: robust adjusted profile score; SNP: single nucleotide polymorphism.

TABLE 1. Baseline characteristics of included GWASs.

Traits	Sample size	Consortium/authors	SNPs	Descent	PMID	Unit
Exposures						
Isoleucine	8255	Chen Y *et al.* [32]	14,380,329	European	36635386	1 SD of log-normalized values
Lysine	8250	Chen Y *et al.* [32]	14,379,670	European	36635386	1 SD of log-normalized values
Leucine	8252	Chen Y *et al.* [32]	14,380,367	European	36635386	1 SD of log-normalized values
Tyrosine	8252	Chen Y *et al.* [32]	14,380,370	European	36635386	1 SD of log-normalized values
Outcomes						
Testosterone	199,569	Ruth KS *et al.* [33]	12,321,875	European	32042192	SD
SHBG	185,221	Ruth KS *et al.* [33]	12,321,875	European	32042192	SD
BAT	184,205	Ruth KS *et al.* [33]	12,321,875	European	32042192	SD
ED	223,805 (6175 cases and 217,630 controls)	Bovijn J *et al.* [34]	9,310,196	European	30583798	logOR
Male infertility	131,568 (1429 cases and 130,139 controls)	FinnGen	2,1277,625	European	36653562	logOR

BAT: bioavailable testosterone; ED: erectile dysfunction; SNP: Single nucleotide polymorphism; SHBG: Sex hormone binding globulin; SD: standard deviation; PMID: PubMed Identifier; OR: odds ratio.

hormone binding globulin (SHBG)) were sourced from the work of Rebecca Richmond *et al.* [33]. All the participants were European individuals from the UK biobank. Testosterone levels, measured in nmol/L, were determined *via* a one-step competitive analysis on a Beckman Coulter Unicel Dxl 800 using blood samples. SHBG was quantified through a two-step sandwich immunoassay analysis on the same device. The BAT was computed using the Vermeulen equation, which is based on testosterone, SHBG and albumin levels. The original study provides a comprehensive outline of the inclusion and exclusion criteria [33]. A total of 199,569 males were included for measuring total testosterone, 185,221 males for SHBG and 184,205 males for BAT.

GWAS of ED was obtained from Bovijn J *et al.* [34]. The included samples were from the UK biobank, the Estonian Genome Center of the University of Tartu cohort, and the

hospital-recruited Partners HealthCare Biobank cohort. The cases were defined as patients with the ICD-10 codes (F52.2 or N48.4), or oral medications like sildenafil or surgical intervention for ED, or self-report from the respondents. This dataset included 223,805 European individuals (6175 cases and 217,630 controls).

The genetic association estimates for male infertility were extracted from FinnGen (https://r10.finngen.fi/) [35]. The definition of male infertility was based on the ICD codes (N46 for the ICD-10 and 606 for the ICD-8 and ICD-9). There were 152 cases excluded from the endpoint due to the possibility of inaccurate diagnosis. In addition, males with diseases of genital organs were excluded from the controls. Ultimately, 1429 cases and 130,139 controls were included in the final GWAS. Participants were genotyped utilizing Illumina and Affymetrix chip arrays. Detailed information pertaining to the phenotype, imputation, and quality control procedures is available on the official website (https://finngen.gitbook.io/documentation/).

2.2 Genetic instruments selection

All the IVs of the twenty amino acids reached the set significance threshold ($p < 5 \times 10^{-5}$). To obtain independent genetic variants, the retrieved genetic variants were further clumped based on the 1000 G phase III reference genome ($r^2 < 0.001$ at a window size of 1 Mb). To avoid reverse causality, the Steiger-MR method was used to calculate the variance explained by the SNPs. The residual Single Nucleotide Polymorphisms (SNPs) accounted for greater variance in exposures than in outcomes, suggesting a reduced probability of reverse causality. To reduce bias from pleiotropy, we further adopted radial-MR and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) approaches to identify pleiotropic outliers, which were then excluded from analysis if detected. To mitigate bias from weak IVs, we computed the F statistic using the following formula: F statistic = $(\beta/\mathrm{SE})^2$. All resulting F statistics exceeded 10, indicating a reduced probability of weak IV bias. In addition, we used the robust adjusted profile score (MR.RAPS) method to combine the effect sizes of the IVs, minimizing the bias from weak IVs.

2.3 Statistical analyses

The primary technique for combining the effect size of IVs was the inverse variance weighting (IVW) method, which assumes the validity of all IVs and thus offers superior statistical power. Given the possible weak IVs and pleiotropic outliers, we utilized additional estimators for sensitivity analyses, including MR-Egger, Weighted median, Maximum likelihood, MR.RAPS and MR-PRESSO. The MR-Egger method is capable of providing consistent causal inference even in the presence of entirely invalid IVs [36]. Furthermore, the intercept term in the regression function serves as a detector for pleiotropy. Analogous to the MR-Egger estimator, the weighted median method can yield consistent estimates even when up to 50% of the IVs are invalid [37]. Maximum likelihood estimator displays higher statistic power in datasets with limited sample size [38]. MR.RAPS is capable of generating robust causal inference in the presence of weak IVs and both systematic and idiosyncratic pleiotropy [38]. MR-PRESSO was utilized for the detection of pleiotropy and the elimination of pleiotropic outliers, subsequently combining the effect size of IVs using an approach akin to the IVW estimator [39]. Furthermore, LDSC regression was employed to investigate the genetic correlation between traits [40].

All computations were performed using R 4.0.2 software (R Foundation for Statistical Computing, Vienna, Austria), employing the "TwoSampleMR", "RadialMR", "mr.raps" and "forestplot" packages for analysis and figure generation. A two-sided p-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1 Causal associations of plasma amino acids with hypogonadism

The genetic associations of 20 amino acids with testosterone, BAT and SHBG are shown in **Supplementary Tables 1,2,3**, respectively. Among them, only genetically proxied serum isoleucine levels were causally associated with BAT levels (Fig. 2A). Specifically, as shown in Fig. 2B, the IVW estimator disclosed that, per unit increase in serum isoleucine, the concentration of BAT decreased by 0.013 SD. The unit of serum isoleucine is 1 SD of log-normalized values. Notably, the MR-Egger estimator found a significant pleiotropy (intercept term = 0.003, p = 0.015, Table 2), suggesting biased results in the IVW estimator. However, the MR-Egger estimator still identified significant associations of isoleucine with BAT (β = −0.032, p = 0.001). The causal estimates of Maximum likelihood, MR.RAPS and MR-PRESSO were −0.014 (95% CI = −0.024 to −0.003, p = 0.012), −0.017 (95% CI = −0.028 to −0.005, p = 0.005), and −0.013 (95% CI = −0.024 to −0.002, p = 0.023), respectively. No heterogeneity was detected in the IVs ($p > 0.05$, Fig. 2C).

TABLE 2. Strength and pleiotropy of instrumental variables.

Exposures	Outcomes	Number of SNPs	F statistics	Intercept for Egger regression	p for Egger	p for Global test
Isoleucine	BAT	92	18.52	0.0032	0.015	0.127
Lysine	ED	97	20.96	−0.0023	0.790	0.382
Isoleucine	Male infertility	138	18.62	−0.0167	0.124	0.876
Leucine	Male infertility	148	18.54	0.0090	0.363	0.613
Tyrosine	Male infertility	137	19.16	−0.0105	0.276	0.405

F statistics = $(\beta/Se)^2$. BAT: bioavailable testosterone; ED: erectile dysfunction; SNP: Single nucleotide polymorphism.

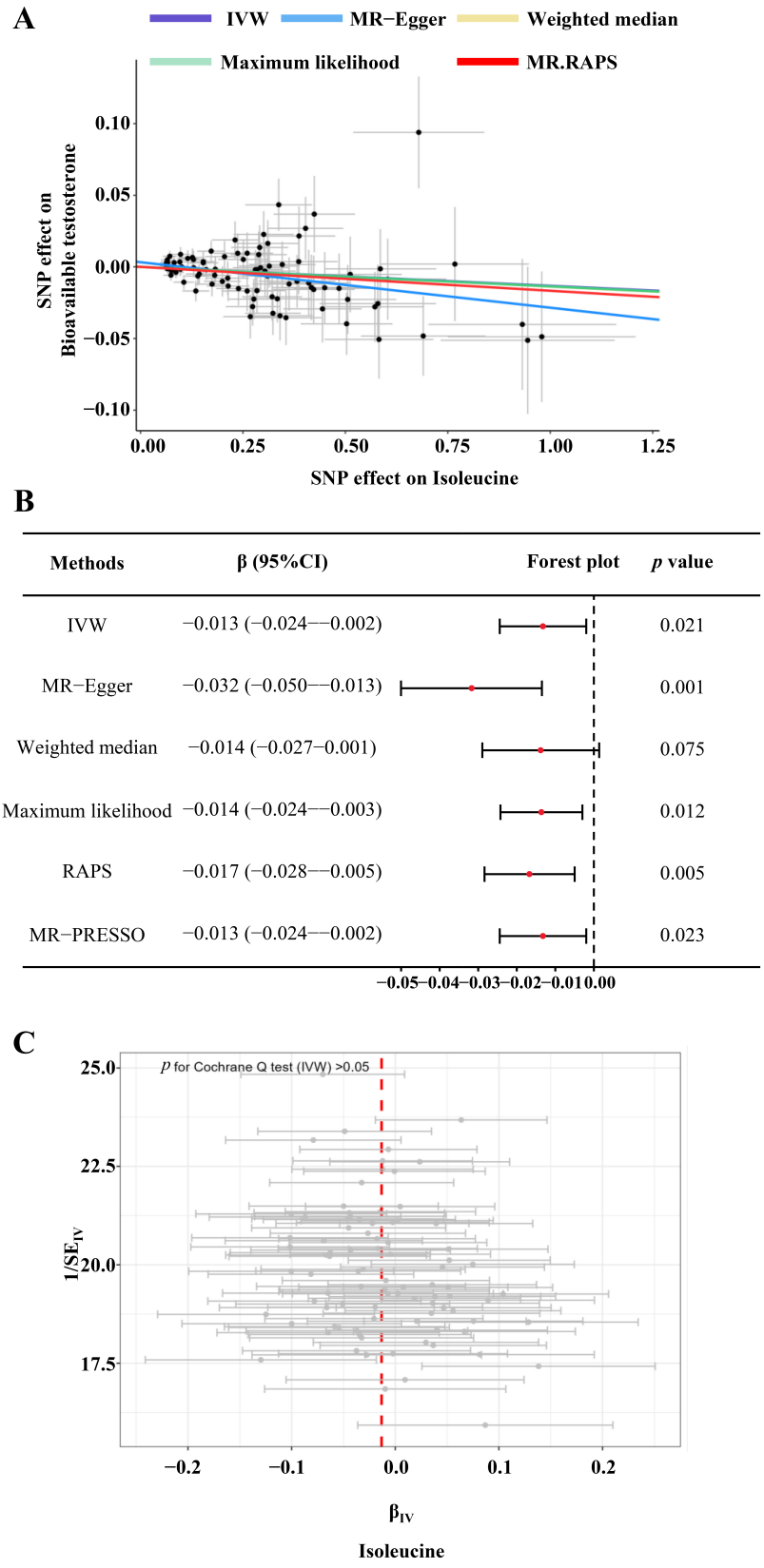


FIGURE 2. Causal estimates of genetically proxied isoleucine with bioavailable testosterone. (A) scatter plot visualizing the SNP effect on isoleucine and bioavailable testosterone, respectively; (B) forest plot showing the causal estimates between genetically proxied isoleucine and bioavailable testosterone; (C) funnel plot visualizing the heterogeneity in the instrumental variables. IVW: inverse variance weighting; IV: instrumental variable; MR: Mendelian randomization; RAPS: robust adjusted profile score; SNP: single nucleotide polymorphism; CI: confidence interval; SE: standard error.

3.2 Causal associations of plasma amino acids with ED

Among the 20 amino acids, only genetically proxied lysine was found to exert protective effects on ED (**Supplementary Table 4** and Fig. 3A). LDSC also revealed a notable genetic correlation between lysine and ED, with a correlation coefficient of −0.484 (p = 0.025). The IVW estimator indicated that a one-unit increase in serum lysine corresponded to a 0.93-fold increase in the risk of ED (95% CI = 0.88–0.99, p < 0.05, Fig. 3B). Fig. 3B presents causal estimates of 0.93 (95% CI = 0.88–0.99, p = 0.014) for the Maximum Likelihood method, 0.92 (95% CI = 0.86–0.98, p = 0.006) for the MR.RAPS method, and 0.93 (95% CI = 0.88–0.99, p = 0.016) for the MR-PRESSO method. Notably, the MR-Egger and Weighted Median estimators aligned with the IVW method directionally (Odds ratio (OR) = 0.94 for MR-Egger and 0.92 for Weighted Median), albeit without statistical significance, potentially due to their limited statistical power. Neither the MR-Egger regression nor the Global test from MR-PRESSO detected pleiotropy (both $p > 0.05$, Table 2). Fig. 3C confirms the absence of heterogeneity in the IVs ($p > 0.05$).

3.3 Causal associations of plasma amino acids with male infertility

Three amino acids were found to be involved in the elevated risk of male infertility (**Supplementary Table 5** and Fig. 4A–C). No pleiotropy or heterogeneity was found in the IVs (Fig. 4D–F and Table 2). Fig. 5A shows that a one-unit increase in genetically proxied isoleucine was associated with a 1.08-fold increase in the risk of male infertility (95% CI = 1.01–1.15, p = 0.030). The results were further verified by MR-Egger (OR = 1.12, 95% CI = 1.03–1.21, p = 0.009), Weighted median (OR = 1.16, 95% CI = 1.04–1.30, p = 0.006), Maximum likelihood (OR = 1.08, 95% CI = 1.01–1.15, p = 0.030), MR.RAPS (OR = 1.09, 95% CI = 1.01–1.17, p = 0.024), and MR-PRESSO (OR = 1.08, 95% CI = 1.01–1.14, p = 0.021) estimators.

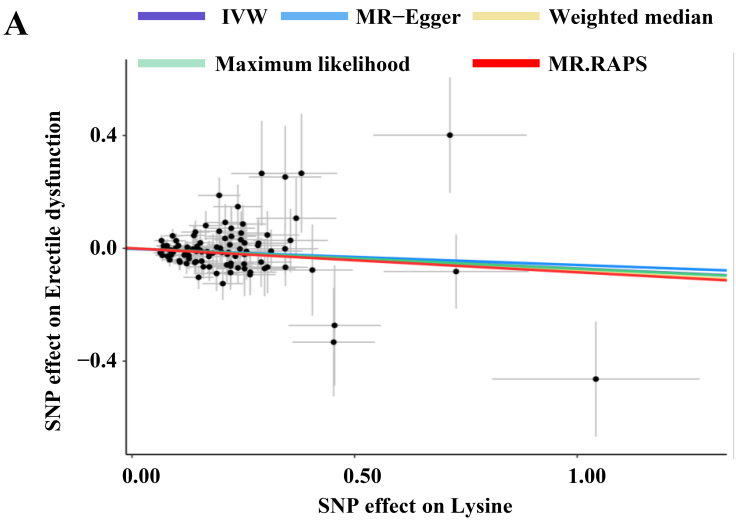
In addition, the IVW model found that a genetically predicted one-unit increase in the serum leucine concentration was associated with a 1.09-fold increase in the risk of male infertility (95% CI = 1.02–1.17, p = 0.008, Fig. 5B). The ORs were 1.10 (95% CI = 1.02–1.17, p = 0.009) for the Maximum likelihood method, 1.08 (95% CI = 1.01–1.17, p = 0.034) for the MR.RAPS method, and 1.09 (95% CI = 1.02–1.17, p = 0.008) for the MR-PRESSO method. Although the results from the MR-Egger and Weighted Median estimators were not statistically significant (both $p > 0.05$), their direction was consistent with that of the IVW estimator, reinforcing the causal link between leucine and male infertility. Similarly, no pleiotropy or heterogeneity was found in the IVs (Fig. 4E and Table 2).

The results also supported a causal association of tyrosine with male infertility (Fig. 5C). According to the IVW method, a one-unit increase in serum tyrosine corresponded to a 1.09-fold increased risk of male infertility (95% CI = 1.01–1.18, p = 0.033). This observation was consistent with the results from the MR-Egger (OR = 1.14, 95% CI = 1.02–1.26, p = 0.022), Weighted Median (OR = 1.15, 95% CI = 1.01–1.31, p = 0.035), Maximum Likelihood (OR = 1.09, 95% CI = 1.01–1.18, p = 0.030), MR.RAPS (OR = 1.09, 95% CI = 1.01–1.19, p = 0.036), and MR-PRESSO (OR = 1.09, 95% CI = 1.01–1.18, p = 0.034) estimators. Both MR-Egger regression and Global test indicated no pleiotropy (both $p > 0.05$, Table 2). The funnel plot in Fig. 4F shows a symmetric distribution of IVs, revealing no heterogeneity.

4. Discussion

To our knowledge, this study represents the inaugural utilization of LDSC regression and MR analysis to probe the genetic correlations and causal implications between plasma amino acid levels and male reproductive health parameters, including hypogonadism, ED and male infertility. Within this genetic association inquiry, we discerned a causative linkage between elevated serum isoleucine levels and diminished BAT concentrations. Likewise, genetically elevated levels of isoleucine, leucine and tyrosine were found to correspond with escalated male infertility risk. Conversely, a noteworthy negative genetic correlation emerged between lysine levels and ED. Collectively, these findings underscore distinct patterns between plasma amino acid profiles and male reproductive health.

To date, no studies have reported direct associations between plasma amino acid levels and male reproductive health. However, several investigations have documented relationships between SP amino acid concentrations and male infertility. Amino acids function as vital constituents of sperm proteins and exhibit unique roles, such as preserving sperm viability by binding to toxic heavy metals, mitigating free radical damage, safeguarding cellular integrity, providing oxidative substrates for sperm, and acting as buffering agents in SP [41]. For instance, previous research has elucidated the free radical scavenging properties of amino acids such as proline, histidine, lysine and arginine [42–45]. Amino acids such as cysteine and methionine have been shown to chelate a wide range of heavy metals, thereby counteracting their reproductive toxicity [46]. Glycine, cysteine and glutamate are utilized in the synthesis of the tripeptide glutathione, which enhances cellular antioxidant defense mechanisms [47]. Amino acids such as proline, histidine, taurine and glycine have been found to improve functional parameters of reproductive system mitochondria and alleviate mitochondria-mediated reactive oxygen species (ROS) generation [47–50]. Papp *et al.* [51] elucidated the significance of amino acids in male reproductive processes. Their study delved into changes in SP levels of Arg, Orn and total amino acids across various types of pathospermia. Arg and Orn were identified as the most severely depleted amino acids, and rectification of their levels may contribute to improving the fertility potential of spermatozoa. Additionally, Nissen *et al.* [52] documented markedly reduced SP concentrations of Asp, Glu, Thr, Gly, Ala and Ile among men experiencing infertility. Zhang *et al.* [53] identified methionine and glutamine as significant biomarkers in the SPs of patients with asthenozoospermia. In summary, several studies support the concept of an association between SP amino acid levels and male infertility. However, further research is required to validate our findings regarding the relationship be-



B

Methods	OR (95% CI)	Forest plot	*p* value
IVW	0.93 (0.88−0.99)		0.015
MR−Egger	0.94 (0.84−1.07)		0.357
Weighted median	0.92 (0.85−1.01)		0.057
Maximum likelihood	0.93 (0.88−0.99)		0.014
RAPS	0.92 (0.86−0.98)		0.006
MR−PRESSO	0.93 (0.88−0.99)		0.016

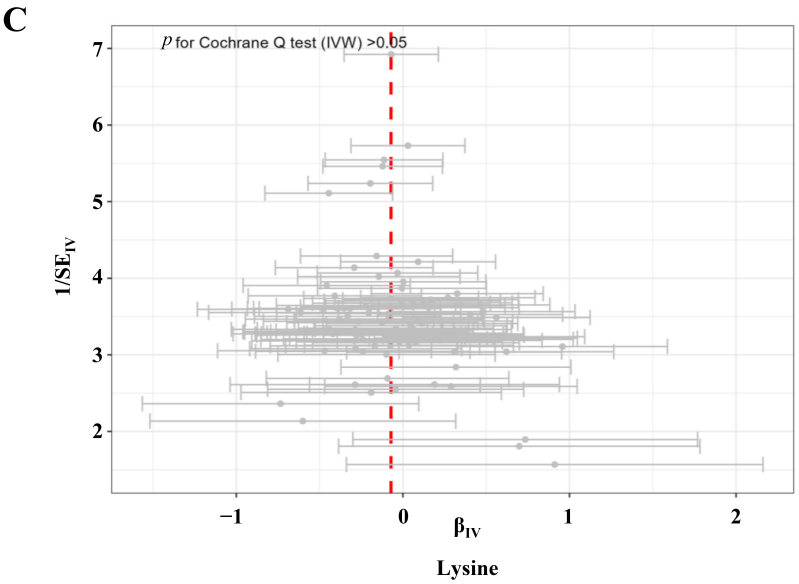


FIGURE 3. Causal estimates of genetically proxied lysine with erectile dysfunction. (A) scatter plot visualizing the SNP effect on lysine and erectile dysfunction, respectively; (B) forest plot showing the causal estimates between genetically proxied lysine and erectile dysfunction; (C) funnel plot visualizing the heterogeneity in the instrumental variables. IVW: inverse variance weighting; IV: instrumental variable; MR: Mendelian randomization; RAPS: robust adjusted profile score; SNP: single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval; SE: standard error.

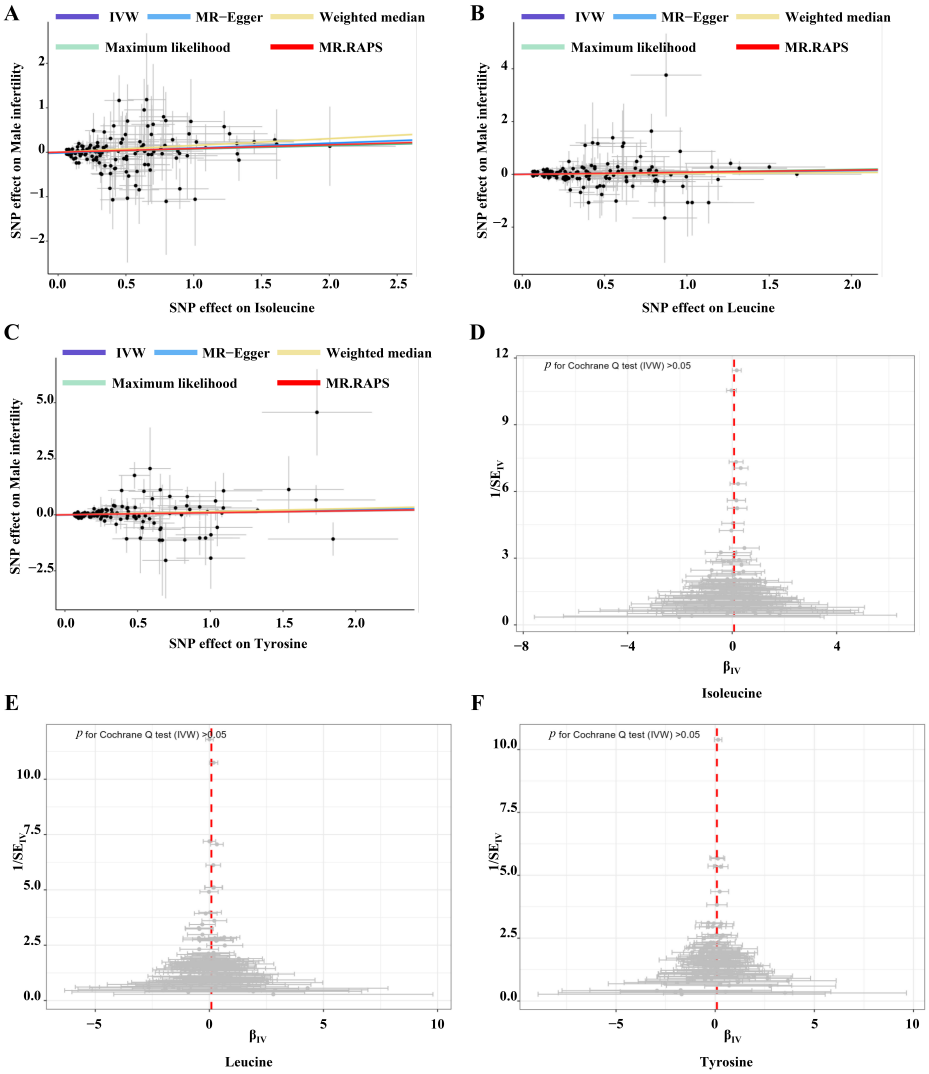


FIGURE 4. Scatter and funnel plots in the associations of isoleucine, leucine, tyrosine with male infertility. (A–C) scatter plots visualizing the SNP effect on isoleucine, leucine, tyrosine and male infertility, respectively; (D–F) funnel plots showing the heterogeneity in the instrumental variables of isoleucine, leucine and tyrosine. IVW: inverse variance weighting; IV: instrumental variable; MR: Mendelian randomization; RAPS: robust adjusted profile score; SNP: single nucleotide polymorphisms; SE: standard error.

tween plasma amino acid levels and male reproductive health. Additionally, dietary and lifestyle modifications may promote beneficial epigenetic alterations, thereby ameliorating male reproductive health-related disorders. Studies have indicated that supplementing the diet with arginine leads to an 18% increase in sperm count and a 7.6% improvement in sperm motility [54]. The mechanisms underlying these effects may involve the enhanced synthesis of NO and polyamines, both of which are crucial for spermatogenesis and sperm viability [54].

Notably, there is a causal relationship between heightened isoleucine levels and diminished BAT levels. Consistent with our observations, Amirjannati *et al.* [23] noted a negative correlation between isoleucine and sperm concentration [55]. Similarly, Bahadorani *et al.* [56] reported that excessive supplementation of branched-chain amino acids (BCAA), including leucine, isoleucine and valine, leads to a reduction in testicular hormone levels. This outcome may be associated with oxidative stress and mitochondrial dysfunction in the testicular endocrine environment [56]. A study by Zhenyukh *et al.* [57] demonstrated that BCAA can induce ROS production by activating NADPH oxidase (NOX), serving as the primary source of ROS generation in human sperm. Additionally, BCAA can induce mitochondrial dysfunction and ROS production [57]. Consequently, heightened isoleucine levels may indirectly modulate the regulation of BAT concentration.

Our research findings indicate a significant negative genetic correlation between lysine and ED. Lysine plays a role in various biological activities within the body, including nitrogen metabolism, protein synthesis and the synthesis of NO [58]. NO serves as a critical vasodilator essential for erectile function [59]. Therefore, a reduction in lysine levels could influence the generation of NO, consequently affecting erectile function.

Increased concentrations of genetically proxied isoleucine, leucine and tyrosine are associated with an elevated risk of

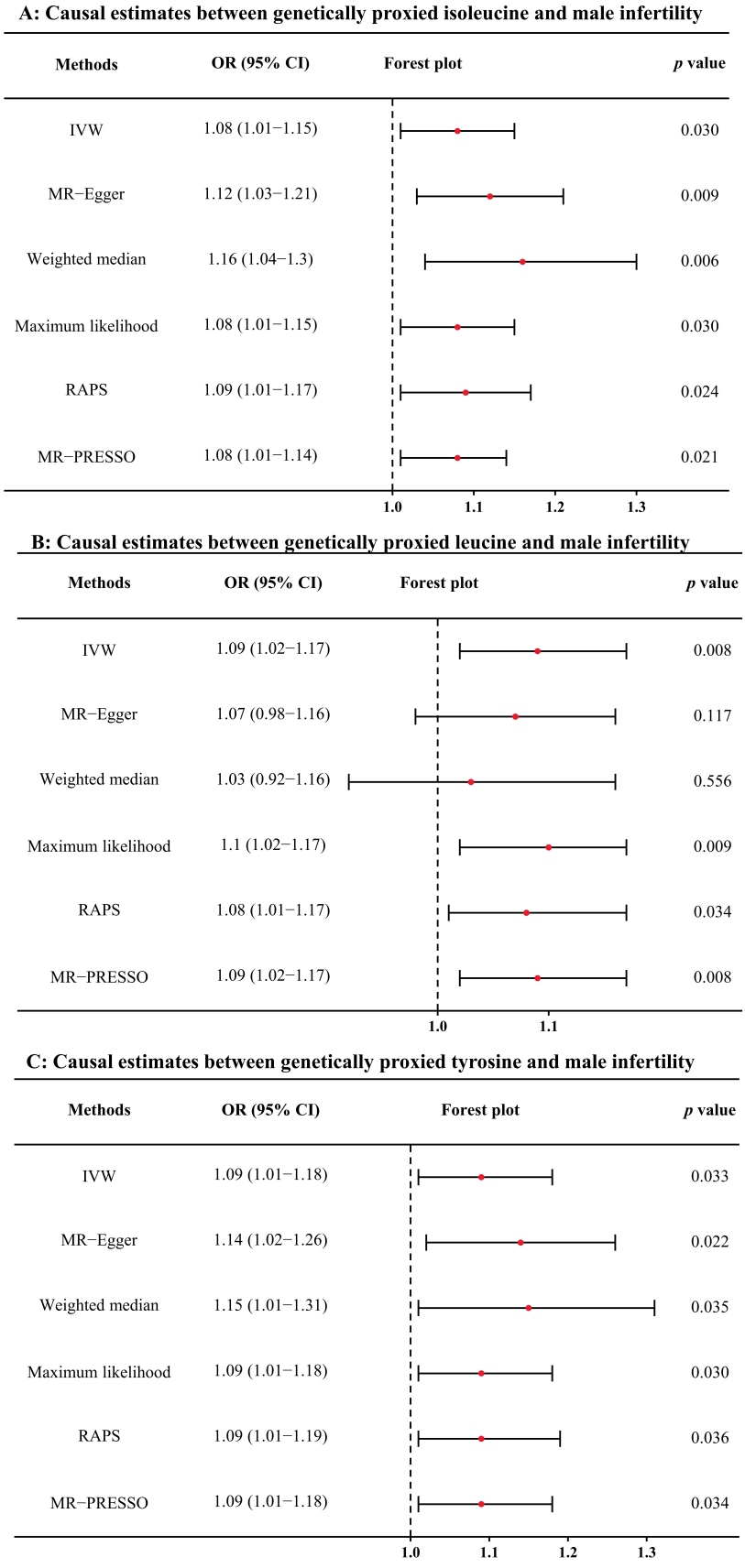


FIGURE 5. Causal estimates of genetically proxied isoleucine, leucine, tyrosine with male infertility. (A–C) forest plot showing the causal estimates between genetically proxied isoleucine, leucine, tyrosine and male infertility, respectively. IVW: inverse variance weighting; MR: Mendelian randomization; RAPS: robust adjusted profile score; SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.

male infertility. The TAC of SPs is known to significantly influence male infertility [60, 61]. It is widely recognized that infertile men often exhibit an imbalanced TAC, leading to oxidative stress, which is associated with male infertility [62]. Isoleucine and leucine, as BCAAs, can directly promote oxidative stress and inflammatory processes when their concentrations increase. This may occur through the generation of ROS by NOX5 and mitochondrial dysfunction, thereby affecting the normal functioning of the reproductive system [56]. Therefore, supplementation with BCAAs alone is not recommended for infertile men [56]. However, contrary to our findings, Amirjannati *et al.* [23] reported a positive correlation between tyrosine ($rs = 0.79$) and TAC, while van Overveld *et al.* [25] demonstrated that tyrosine is a significant contributor to the antioxidant capacity of SP. Notably, these mechanisms may interact with each other or be influenced by other factors. Therefore, additional investigations are warranted to determine the exact mechanisms driving the causal link between plasma amino acid levels and male infertility.

This study has several strengths. First, this is the first investigation to report an association between plasma amino acid levels and male reproductive health. Second, our study is grounded in extensive summary-level GWAS data. Moreover, the implementation of MR analysis facilitated the generation of virtually unbiased causal estimates, circumventing the challenges associated with confounder identification and reverse causality inherent in traditional observational and longitudinal studies [63]. It is crucial to note that the validity of causal effect estimates in MR analysis hinges upon adherence to three fundamental assumptions. Deviation from these assumptions could introduce significant bias into the estimates. To guarantee the reliability of our MR results, we utilized six distinct MR methodologies.

However, our study has several limitations. First, we lacked access to individual-level data, precluding us from conducting stratified analyses based on potential influencing factors. Second, due to the scarcity of GWASs focused on plasma amino acid levels and male reproductive health, we were unable to access additional source datasets to validate our findings. Third, the signaling pathway involved in mediating plasma amino acids and male reproductive health still needs further exploration. In addition, to mitigate potential bias resulting from population stratification, our study exclusively utilized data from individuals of European ancestry. Consequently, further investigations are warranted to ascertain the generalizability of our results across diverse populations. Last, given the absence of comparable studies, additional research is indispensable to corroborate our conclusions.

5. Conclusions

According to summary-level statistics, increased serum isoleucine levels are causally associated with decreased BAT concentrations. In addition, there was a significant negative genetic correlation between lysine and ED. Increased serum lysine was linked to a reduced risk of ED. For male infertility, genetical proxies of increases in the concentrations of isoleucine, leucine and tyrosine can increase the risk of male infertility. These findings highlight the risky role of plasma amino acids in the occurrence of male hypogonadism, ED and male infertility. Early identification and intervention for plasma amino acids may be beneficial for the prevention or therapy of these conditions.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

XH and LZ—Conceptualization; XH and CCZ—Data curation; XH—Formal analysis; XH and THW—Writing-original draft; XH, THW and LZ—Writing-review & editing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

We sincerely thank Kun Han for his technique support.

FUNDING

This work was supported by the Frontiers Medical Center, Tianfu Jincheng Laboratory Foundation (Project No. TFJC2023010001).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.jomh.org/files/article/1851525199081422848/attachment/Supplementary%20material.docx.

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How to cite this article: Xing Hu, Congcong Zou, Tianhong Wang, Leng Zhou. Genetic associations of plasma amino acid levels with male hypogonadism, erectile dysfunction and infertility. Journal of Men's Health. 2024; 20(10): 146-157. doi: 10.22514/jomh.2024.174.